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Review

An Overview of the Latest Developments in the Electrochemical Aptasensing of Neurodegenerative Diseases

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Abstract: Neurodegenerative diseases (NDs) are becoming a major global health problem. They constitute an incurable diverse group of disorders characterized by the progressive degeneration of the structure and function of the central or peripheral nervous system. There is an enormous unmet demand worldwide for methods for the early detection of ND biomarkers. The complexity of the molecular mechanisms underlying neuronal degeneration as well as the inhomogeneity of the patient population pose a great challenge for the development of early diagnostic tools. Various analytical technologies have been developed to meet this challenge. Among the various approaches reported so far, biosensors are powerful analytical implements that have been applied to detect biomarkers of NDs. Over the past decade, electrochemical aptasensors have been at the forefront of this development not only thanks to their low cost and simple design but also due to advances in nanomaterials modifying the surface of the transducers involved. The design of electrochemical aptasensors for the detection of ND biomarkers such as α-synuclein, amyloid β peptide, tau protein and human cellular prion protein were summarized and compared. Innovative strategies for increasing their sensitivity and selectivity were also pointed out. Undoubtedly, there is still a need for low-cost, fast and easy-to-use systems for the early detection of NDs.

Keywords: aptasensor; aptamer; neurodegenerative disease biomarkers; electrochemical detection

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1. Introduction

Each year, tens of millions of people around the world are diagnosed with NDs including Alzheimer's disease (AD), Parkinson's disease (PD) or prion diseases. These are known as "protein misfolding disorders" and are caused by proteins that are prone to aggregation. In NDs, there is a slow, progressive loss of the structure or dysfunction of neurons in the central nervous system, leading to deficits in specific brain functions. They are considered incurable due to the lack of a known way to reverse the progressive degeneration of neurons. Most NDs begin years before symptoms are present, and the first symptoms appear when a significant number of neurons are damaged or the damage affects a specific part of the central nervous system. For many of these illnesses, diagnosis and treatment are limited. Early diagnosis of age-related disorders is an increasingly pressing health challenge worldwide and is crucial in the management of the disease and determines the patient's living conditions [1,2]. The detection of NDs can be accomplished by determining the concentration of specific biomarkers including α-synuclein (α-syn), amyloid β peptide (Aβ), tau protein or human cellular prion protein (PrPC) in body fluids.

Therefore, non-invasive, simple, fast and real-time methods of detecting the biomarkers of NDs are particularly sought after. To fulfill these requirements, electrochemical biosensors offer potential diagnostic and theranostic applications.

Biosensors are analytical devices that convert the result of a biological reaction into an analytical signal. Each biosensor consists of two basic components: an analytically active layer (the receptor part) and a transducer. The recognition elements include molecules

constituting biological material, e.g., nucleic acids (DNA or RNA aptamers), proteins, enzymes, antibodies or whole cells, immobilized on a suitable carrier. In the analytically active layer, the process of intermolecular recognition (receptor–analyte) takes place. During this process, a physicochemical signal is generated, and a transducer converts it into an analytically useful signal. The most commonly used transducers are optical, piezoelectric or electrochemical systems [3].

In the last few years, special emphasis has been placed on aptamers as the molecular recognition elements in electrochemical biosensors (aptasensors), which has been confirmed by the rapid increase in the number of scientific papers in this field [4–7]. According to Web of Science, publications concerning electrochemical aptamer-based biosensors represent about 28% of the total number of publications on electrochemical biosensors [3].

Aptamers are short, single- or double-stranded DNA or RNA nucleotides or peptides [8]. They have emerged as good alternatives to antibodies in the design of electrochemical biosensors. They are distinguished by a low immunogenicity, longer shelf life, easy storage and the possibility of transport at ambient temperature. The advantages of aptamer manufacturing processes include their lower cost, batch-to-batch variability, simple upscaling and purification. These processes do not require experiments with animals or cells. Additionally, they can be selected under non-physiological conditions [9–11]. They also exhibit a high binding affinity and specificity for a broad range of targets (such as proteins, amino acids, peptides, drugs, small metal ions and small organic molecules, as well as bacteria and viruses and even whole cells) due to their specific three-dimensional structures [12–16]. Moreover, aptamers display a specificity for some targets (e.g., small molecules or ions) that cannot be recognized by antibodies [17]. They are also able to distinguish forms and isoforms of the same protein, which is an important advantage for biomarker detection. Furthermore, it is possible to manipulate the binding reaction conditions and optimize the aptamers' recognition sequences to fine-tune their affinities [18]. Aptamers are also good candidates for conjugation, labeling or functionalization, which is often achieved in the step of aptamer synthesis [19,20]. However, there are several issues regarding the use of aptamers in sensory bioassays that are still under investigation and need to be solved. The main issue is the lack of developed universal procedures for the use of aptamers in biosensing. In contrast, in the case of antibodies, these procedures are now well established [21,22].

With each new aptamer–protein couple, a systematic analysis of their binding process, conditions and structure of the created complexes, as well as their interfacial behavior, is necessary [22,23].

In this framework, a suitable method for aptamer immobilization on a solid surface is critical for the successful elaboration of the biosensor. The selection of the aptamer deposition strategy strongly relies on the modification of the aptamer and the physicochemical features of the electrode surface [8,23].

The electrode material used for the deposition of aptamers should allow the precise control of the packing density and spatial orientation of the aptamers in the deposited layer via their controlled immobilization on the surface. This bind should be strong and stable, providing the conformational flexibility required for the process of the specific recognition of the analyte. The aptamer immobilization procedure should ensure the formation of a stable, reproducible receptor layer and the sensitive detection of the analyte, and it should limit the non-specific adsorption of matrix components. An important issue to be addressed in the development of protein aptasensors is the occurrence of false-positive signals due to the non-specific adsorption of proteins found in biological fluids [24].

The most frequently described methods of assembly of aptamers on solid surfaces are physisorption, chemisorption and affinity conjugation (avidin–biotin). Physical absorption based on electrostatic interactions is the simplest method for depositing of aptamers on solid surfaces. However, the low stability of the electrostatically deposited layer caused by the rapid desorption of the aptamers from the surface is problematic in this method. The solution may be additional stabilization by covalent bonding [24]. The second very common

method of attaching aptamers to solid surfaces is chemisorption. Aptamers are covalently deposited on the surfaces of the solid electrodes depending on the type of substrate and its functionalization. However, they do not spontaneously form covalent bonds with the electrode surfaces. Therefore, it is necessary to functionalize either the aptamer or the electrode surface [25]. Possibly the most popular approach is the chemisorption of a thiol-modified aptamer on a gold surface [26,27]. However, this method is unstable over time. Generally, aptamers are co-immobilized on the gold electrode surface with alkanethiols [28,29], dithiols [30,31] or anti-biofouling agents [32,33] to fill unmodified areas. One of the most effective approaches to aptamer immobilization is based on the strong affinity of biotin to avidin [34], streptavidin [35] and neutravidin [36]. Compared to other aptamer immobilization methods, the high affinity of biotin to streptavidin increases the potential number of aptamers that can be deposited on the surface of the detection platform. This is because each streptavidin molecule can bind to two biotinylated aptamers. This implies a reduction in non-specific adsorption and an improvement in the signal-to-noise ratio (S/N) of the biosensor [37].

The appropriate preparation of the detection platform ensures the low sensitivity and high selectivity of the constructed aptasensor. Therefore, it is important to implement a signal amplification strategy through the use of functional nanomaterials in the detection layer [38,39]. A wide range of nanomaterials, including silica nanoparticles [40] and precious metals [41,42], carbon nanomaterials [43,44], nanocomposites and nanohybrids [45,46], polymers [47,48] and metal oxides [49], can be used in the construction of electrochemical aptasensors for biomedical applications. Advances in electrochemical aptasensors have resulted not only from the use of nanomaterials but also from the interdisciplinary combination of nanotechnology, engineering, nanomaterial chemistry and related fields [3,50–55].

Recently, there is a huge demand for new portable analytical devices with better selectivity and specificity for health-care applications, mainly for addressing the point-of-care (POC) detection of biomarkers for chronic and emergent diseases such as cancer, neurodegenerative disorders, cardiovascular diseases or chronic respiratory diseases [3]. This represents the main reason for the development of innovative techniques for the modification and immobilization of aptamers as biorecognition molecules with unique properties in the field of electrochemical aptasensors [56,57]. Nevertheless, their numerous assets and significant advances have not yet resulted in their entry to the market of electrochemical aptasensors [58–60].

Electrochemical aptasensors are widely used in the detection of NDs biomarkers due to their numerous advantages such as their speed of analysis, low cost, easy operation and high miniaturization potential.

This paper presents electrochemical platforms based on aptamers for the detection of biomarkers of NDs and for monitoring the progression of these diseases. We reviewed the most important clinical biomarkers for NDs, focusing on their physiological function and role in the disease. A current evaluation of electrochemical aptasensors for the detection of specific and quantitative ND biomarkers in clinical and synthetic samples for the preparation of a detection platform were presented. Particular emphasis was placed on the recent advances in the modification of electrochemical aptasensor surfaces and signal amplification strategies. Future alternatives to novel electrochemical aptasensors including ideal platforms for point-of-care (POC) diagnostics were also discussed.

2. Neurodegenerative Diseases Biomarkers

A measurable change arising in biological surroundings such as human tissues or body fluids is defined as a biomarker. This change should arise from the pathological condition or response of the body to treatment when assessing the effectiveness of pharmacological therapy [61,62]. Thus, biomarkers are compounds that need to reflect the basic neuropathological features of a disease, and their determination should be conducted by means of a safe, easy and quick diagnostic test [63].

Neurodegenerative diseases such as AD, PD or prion diseases are complex disorders of the central nervous system that cause progressive neuronal loss. In recent years, the prevalence of NDs has been increasing rapidly; therefore, early and accurate diagnosis of these diseases is an important challenge. Such a diagnosis enables the implementation of more effective therapies. Unfortunately, neuropathological diagnosis can only usually be carried out in the form of an autopsy after the death of the patient [64]. Earlier and more accurate diagnoses, as well as the effective monitoring of the rate of disease progression or the response to treatment, is possible thanks to the use of biomarkers [65].

Therefore, there has recently been great progress in the determination of biomarkers aimed at detecting these diseases in the phase preceding the appearance of distinctive clinical symptoms [66,67]. Enzyme-linked immunosorbent assay (ELISA) [68–70], magnetic resonance imaging (MRI) [71], manganese-enhanced magnetic resonance imaging (MEMRI) [72], mass spectrometry (MS) [73,74], flexible multi-analyte profiling (xMAP) [75], positron emission tomography (PET) [76,77], surface plasmon resonance [78], Western blotting [79,80], scanning tunneling electron microscopy [81], immunohistochemistry [82–84] and fluorescence [85–87] are some of the major techniques used for the detection of biomarkers of NDs. Despite their numerous advantages, these methods are relatively expensive, time-consuming and difficult to access, and they often require specialized equipment, qualified personnel and large volumes of samples. Moreover, they are not yet suitable for POC diagnostics.

The development of easy-of-use electrochemical aptamer-based platforms with a high sensitivity and specificity is probably one of the most promising methods to solve some of the problems regarding fast and cost-effective measurements appropriate for POC diagnostics due to their potential in terms of their portability and novel microfabrication technologies [88–91].

The biomarkers that play an important role (such as α-syn, Aβ, tau protein and PrPC) in the clinical practice of NDs such as Alzheimer's, Parkinson's and prion diseases are displayed below. Recently developed sensing platforms for their electrochemical detection are also presented. All of the electrochemical aptasensors described in this review are summarized in Table 1.

2.1. α-Synuclein

α-syn is an amyloid-forming protein with a high tendency to form toxic aggregates. This protein plays a key role in the pathogenesis of synucleinopathies such as multiple system atrophy or PD, and it has been investigated as a biomarker in plasma, serum and cerebrospinal fluid (CSF) [92–94]. According to the literature data, α-syn oligomers exhibit many toxic effects, such as inflammation, synaptic dysfunction or impaired degradation of intracellular proteins [95–97]. It has been suggested that the transformation of α-syn from soluble monomers to aggregated, insoluble forms is a significant event in the pathogenesis of PD and other synucleinopathies. Contrary to amyloid beta plaques, Lewy bodies and aggregates of α-syn are well correlated with cognitive impairment in PD, as well as in AD, indicating α-syn to be a primary candidate for a biomarker of NDs [98–101].

Several electrochemical aptasensors for the detection of α-syn have been reported. In the first example, a voltammetric aptasensor based on a methylene blue (MB) redox probe, terminal deoxynucleotidyl transferase (TdT) and exonuclease I (Exo I) was proposed [102]. The working principle of the presented assay is showed in Figure 1. Briefly, depending on the presence or absence of α-syn oligomers (analyte) on the sensing platform, different amounts of the MB redox probe are accumulated. This effect generates signals that differ in current intensity (Figure 1A,B). Additionally, the introduction of TdT and Exo I into the system increases the sensitivity of the elaborated aptasensor. This device displays a high selectivity in a concentration range from 60 pM to 150 nM with a limit of detection (LOD) of 10 pM (Table 1). Moreover, it has also demonstrated a high repeatability and precision in detecting the analyte in serum samples.

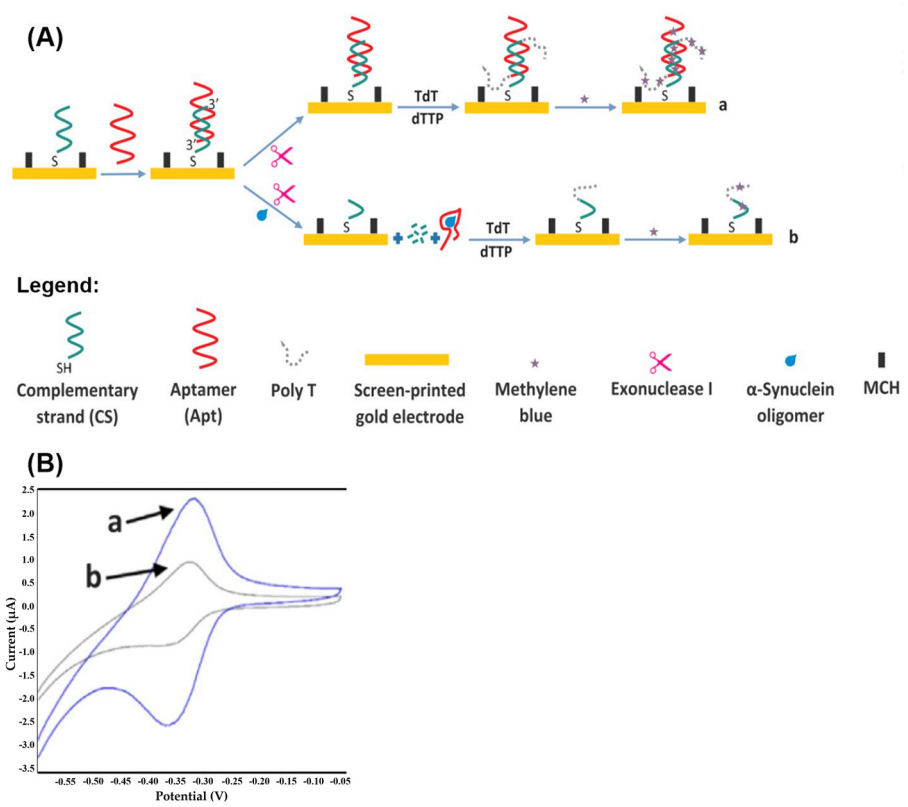

Figure 1. (**A**) Schematic illustration of voltammetric detection of α-syn oligomers. (**B**) CV curves obtained for the sensing platform (a) in the absence and (b) in the presence of α-syn oligomers. Measurements were carried out in PBS solution, scanning from −0.6 V to −0.05 V at a scan rate of 100 mV/s. Reprinted with permission from Ref. [102].

Sun and co-workers elaborated a simple-in-design and highly selective impedimetric aptamer-based biosensor that could draw upon the co-immobilization of thiolated DNA aptamers and 6-mercaptohexanol (MCH) on a gold electrode surface [103]. Gold nanoparticles (AuNPs) were used for the signal amplification. The described aptasensor was applied for the label-free detection of α-syn oligomers with a high sensitivity in the presence of interferents including α-syn monomers and fibrils in phosphate buffer saline (PBS) and human serum samples (HS). This assay provided an LOD of 1 pM in PBS (Table 1).

Another sensitive voltammetric aptasensor was proposed by Jang and co-workers [104]. In this approach MB-tagged aptamers were adsorbed on an electrochemically reduced graphene-oxide (rGO)-modified glassy carbon electrode (GCE). Using the developed aptasensor, α-syn oligomers were determined in a concentration range from 1 fM to 1 nM with an LOD of 0.64 fM (Table 1). The rGO/MB-Apt-based aptasensor demonstrated a reasonable suitability (stability and high reproducibility) for the detection of α-syn oligomers in human blood serum samples.

Among the approaches described in this section, the impedimetric aptasensor proposed by Tao and co-workers showed the best sensitivity to α-syn oligomers [105]. It was designed based on GCE using polythionine (pThi) and gold nanostars (AuNSs). This sensing platform improved the sensitivity of the assay due to its superior electrical conductivity and fast electron transfer ability. The aptasensor operated well, with negligible interferences from glucose (Glu), ascorbic acid (AA), L-Cysteine (L-Cys) and tau441 protein. It was able to detect the analyte in PBS buffer and in 1:4000 diluted HS with extremely low LODs of 0.07 aM and 0.09 aM, respectively.

The last example of an aptasensor was intended for the detection of total α-syn. You et al. described a voltammetric system based on Apt–AuNPs conjugates immobilized on an integrated electrode (IDE) [106]. In order to attach the Apt–AuNPs conjugate, the electrode

surface was functionalized with (3-aminopropyl) triethoxysilane. A linear relationship between the detected voltammetric currents and the total α-syn concentration was observed for a range from 10 pM to 1 µM, with an LOD of 10 pM. Tau protein and Aβ did not interfere with the aptasensor's response.

2.2. Amyloid β Peptide

In AD, the toxic effect of aggregated forms of Aβ is the reason for cell death [107]. Notably, the oligomeric forms of Aβ display a neurotoxic action. Elevated Aβ levels can be observed even many years before the onset of the disease [108,109]. Observing the plasma changes in the level of Aβ1-40 and 1-42 is extremely important for the early diagnosis of AD. This can provide an assessment of Alzheimer's disease risk. The physiological concentration of Aβ presented in CSF as well as in human plasma ranges from 0.1 to 0.5 nM [110]. In the asymptomatic preclinical phase of the disease, elevated values of the concentration of total Aβ are observed [111]. Aβ levels decrease to normal as the disease progresses, and a gradual build-up of amyloid, in particular Aβ1-42, forms in the brain. In summary, the essential biochemical processes relevant to this disease can be observed in the blood [112,113].

A highly sensitive and extremely simple voltammetric aptasensor for the detection of Aβ was demonstrated recently by Negahdary and co-workers [114]. This approach was based on a gold electrode modified with a fern-leaf-like gold nanostructure (FLGN), an RNA aptamer and MCH (Figure 2). The binding of Aβ by the RNA aptamers was observed in a concentration range from 0.002 to 1.28 ng/mL with an LOD of 88.6 amol/L (0.4 pg/mL) by using ferro/ferricyanide as a redox marker (Table 1). Additionally, the described aptasensor was easily applied for the detection of Aβ in human blood serum and CSF samples.

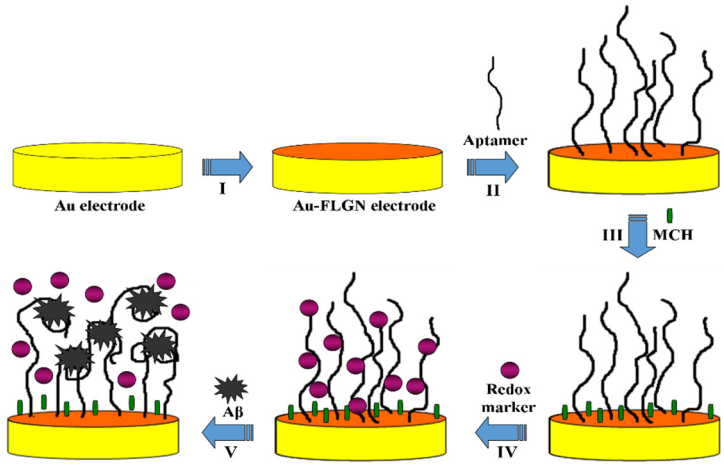

Figure 2. The scheme of fabrication of the aptasensor and Aβ detection: I—electrode position of FLGN, II—aptamer immobilization, III—MCH immobilization, IV—Aβ incubation. Adapted with permission from Ref. [114].

Another development in this field was a sensitive and selective antibody–aptamer sandwich assay for the electrochemical detection of Aβ oligomers (AβOs) [115]. In this electrochemical platform, the antibodies of AβOs were applied as the recognition elements, and a nanocomposite of AuNPs with an aptamer and Thi as the detection probe were used. The electrochemical signal of Thi reduction could provide measurable electrochemical signals. Moreover, the amplification of the signal was achieved due to the high loading of Thi on the AuNPs. Finally, this antibody–aptamer sandwich assay was efficiently applied for the estimation of AβOs in artificial CSF (aCSF) samples with a low LOD (100 pM).

The next-developed platform for the electrochemical detection of AβOs employed metal–organic frameworks (MOFs) as the signal probes [116]. In this case, aptamer-tethered

gold–nanoflower conjugates (AuNFs) were electrodeposited on the surface of a GCE in order to obtain a higher electronic conductivity and surface area. This approach allowed the self-assembly of more mercapto-modified aptamer molecules immobilized by Au-S bonding. In order to construct the composites, the AuNPs were loaded on a Cu/MOF layer. Next, the aptamers were conjugated with the AuNPs/Cu/MOFs, which acted as secondary aptamer bioconjugates. Finally, a sandwich aptasensor was formed from the Apt/AuNPs/Cu/MOF bioconjugates and self-assembling aptamers. AβOs were captured between them (Figure 3). This approach improved the sensitivity as well as the immobilization of the first layer due to the modification of the electrode by the aptamer-tethered AuNFs. Moreover, the Cu^{2+} from the Cu/MOFs, which played the role of a signal probe, could be directly detected. This strongly simplified the detection steps and highly decreased the detection time. The developed aptasensor displayed a low LOD of 0.45 nM and a wide linear range from 1 nM to 2 μM (Table 1).

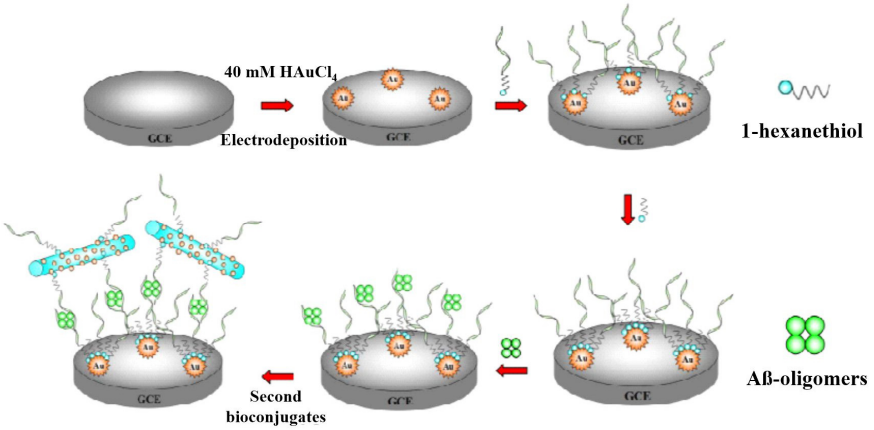

Figure 3. Schematic illustration of the fabrication of the aptasensor for AβOs detection. Reprinted with permission from Ref. [116].

The third aptasensor, developed by the same research group, had the lowest LOD of 0.35 pM. The sensing platform was composed of AuNPs and a vertical graphene (VG) film on carbon cloth (CC) [117]. The aptamer modified with poly T-templated copper nanoparticles (CuNPs) was used as a redox probe. To improve the specificity of the system, the sandwich strategy consisted of specific recognition elements comprising the aptamers, and residues of 95–110 of PrPC was proposed. The developed aptasensor exhibited a high sensitivity for detecting AβOs with an LOD of 3.5 pM along with a high specificity, good stability and good reproducibility. Aβ monomers (AβMs), Aβ fibrils (AβFs), tumor necrosis factor-alpha (TNF-α), insulin and C-reactive protein (CRP) in serum were tested as the interferents. In order to verify the applicability of the elaborated sensing platform, the practical application of an electrochemical aptasensor for Aβ detection in human serum was successfully investigated. To sum up, this flexible electrochemical sensor has the potential to be used as a high-performance tool in clinical analysis.

A different approach to the construction of aptasensors was proposed by Liao et al. [118]. This strategy was based on a hybridization chain reaction (HCR), which induced polyadenine to absorb silver nanoparticles (AgNPs). A "capture probe" immobilized on a gold electrode surface via polyadenine was used to attach aptamer 1. Aptamer 2 and AβOs were then deposited. The HCR process was initiated by a fragment of aptamer 2 used as a primer that produced a large number of long DNA sequences containing adenines. In the next step, the obtained HCR product could absorb the AgNPs. This label-free electrochemical aptasensor was able to detect AβOs in a concentration range from 1 pM to 10 nM, with an LOD of 0.43 pM. In order to assess the selectivity of this sensor towards AβOs in the presence of interfering substances, L-Cys, bovine serum albumin (BSA), immunoglobulin G (IgG), AβMs and AβFs were used. Additionally, the authors investigated whether other

components of the serum sample such as potassium cations (K^+), chloride anions (Cl^-), uric acid (UA), dopamine (DA) and Glu could interfere with the evaluation of the aptasensor. The obtained results confirmed that the tested interferents did not affect the detection of AβOs. The fabricated device could determine AβOs in the clinical serum samples.

Recently, Zhang and co-workers described a simple label-free aptasensor for the specific determination of AβOs [119]. The aptamer was immobilized on a gold-rod electrode through Au-S bonding. This label-free aptasensor was applied for the detection of AβOs using the EIS technique. In this case, the signal generation was based on the monitoring of the change in the charge transfer resistance of the $[Fe(CN)_6]^{3-/4-}$ redox couple. The proposed aptasensor showed an LOD of 30 pM and a wide linear concentration range from 0.1 to 500 nM (Table 1). The presented device also fulfilled its role in CSF with a satisfactory accuracy and could be used to study the aggregation of Aβ. Due to its effective regeneration and simple fabrication, the designed aptasensor could become an analytical tool suitable for the early detection of AD and drug development through the observation of their influence on Aβ plaque degradation.

Another aptasensor based on stem–loop probes for the amperometric detection of AβOs was reported by Zhang and co-workers [120]. In their previous work [119], the proposed aptasensor was characterized by an LOD of 30 pM due to the application of an impedimetric transducer. The optimization of the ACV frequency could influence the decrease in the LOD of the aptasensor, although at the cost of a restricted detection range. In order to improve the sensitivity of the detection of AβOs, in this study authors described a molecular-beacon-based aptasensor for the amperometric detection of AβOs. As a biorecognition element, the stem–loop probes with redox active moieties were applied, which were immobilized on a gold electrode surface. The stem–loop probes were modified by labelling with a thiol group at the proximal end and a Fc redox group at the distal end for signal generation. These structures were also improved by considering different Fc terminals, aptamer stem lengths and spacers. The mechanism of the electrochemical signal generation relied on redox Fc reporting via charge transfer in a biorecognition event influencing a conformational change in the molecular beacon. In summary, this novel aptasensor exhibited a high reproducibility, selectivity, sensitivity and detection limit down to the sub-picomolar range by the adequate selection of the ACV signal recording parameter (a detection range from 0.1 pM to 1500 nM with an LOD of 0.002 pM), which may contribute to an improvement in the diagnostic and pharmacological research of AD.

The same research group performed another study, this time on an aptasensor designed for the simultaneous determination of two biomarkers: AβOs and adenosine triphosphate (ATP) [121]. To our knowledge, this is the only example of a multisensor that can be found in the scientific literature. The developed device was a chip with microelectrode arrays (MEA) modified with dual AβOs and ATP aptamers. On this MEA chip, 3D gold nanostructures (3D-AuNS) were electrodeposited. Then, the 3D-GNS/MEA was modified with AβOs and ATP Fc-labelled aptamers. AβOs and ATP were detected simultaneously in the same analyte solution by the same chip. The addition of analytes generated a signal-off and a signal-on response mechanism for AβOs and ATP, respectively. The elaborated aptasensor showed a linear detection range from 1 pM to 200 nM and from 0.01 nM to 1000 nM for the detection of AβOs and ATP, respectively. The LODs were calculated to be 0.3 pM and 0.2 pM for AβOs and ATP, respectively. In order to evaluate the selectivity of the duosensor, the interference of AβMs and AβFs was successfully tested. In addition, an evaluation of the simultaneous detection of AβOs and ATP in aCSF was performed, where both biomarkers were effectively tested on the same chip.

In a further example, an analytical signal was amplified by including three-dimensional carbon nanomaterials (rGO and multiwalled carbon nanotubes (MWCNTs)) in a sensing platform [122]. AβOs were detected using a voltammetric aptasensor based on an rGO-MWCNT nanocomposite functionalized with thionine (Thi). The NH_2-terminated aptamer was covalently bound via an EDC/NHS coupling reaction. This assay worked over a wide range of concentrations between 0.0443 pM and 443 pM and with an LOD of 10 fM. AβMs,

AβFs, α-syn oligomers and tau protein were used as the interferents. The recovery of the aptasensor was implemented with a good selectivity, stability and reproducibility. The obtained results confirmed the outstanding performance of the developed assay. Moreover, this aptasensor was able to detect AβOs in HS.

The next aptasensor developed for the detection AβOs, characterized by a lower LOD (0.5 fM) than those previously described [91], was based on a triple-helix aptamer switch (THAS) [123]. A gold electrode was modified with a thiolated aptamer possessing two symmetrical arm segments via a covalent bond. Next, AuNPs-labelled signal transduction probes (AuNPs-STPs) were attached to the aptamer-modified platform. A rigid THAS was created on the electrode surface as a result of the simultaneous hybridization of both segments of the aptamer arm with the AuNPs-STPs. Subsequently, the AuNPs were captured with trithiocyanuric acid (TA), resulting in the formation of an AuNPs-TA network and simultaneously generating a strong DPV signal response (switch "on"). In the presence of AβOs, the AuNPs-STP was released from the THAS, causing a significant decrease in the DPV signal response (switch "off"). The elaborated device showed a wide linear range from 1 fM to 10 pM with a low LOD of 0.5 fM and was successfully applied for the determination of AβOs in twenty serum samples with satisfying recovery rates. Additionally, the selectivity of the aptasensor was successfully verified using BSA, hemoglobin (Hb) and thrombin (Thr).

The same research group also developed a second switchable voltammetric aptasensor for the detection of AβOs [124]. In this case, palladium nanoparticles (PdNPs), acting as signal transductors, were electrolytically deposited on a GCE. In the next step, an aptamer and MCH were co-immobilized on the GCE/PdNPs. Then, MOFs carrying AuNP nanocomposites (AuNPs@CuMOFs) functioning as label-signaling displaced-probe (SD) were attached to the GCE/PdNPs/Apt + MCH layer. The label-free aptamer formed a triple-helix switch (THS) in the hybridization process. As a result of the specific binding of the aptamer to the AβOs, the THS structure was destroyed and the AuNPs@CuMOFs/SD was released. The switchable "on-off" mode significantly enhanced the intensity change in the DPV signal and improved the sensitivity of AβOs detection with a linear range from 0.5 fM to 500 fM and a low LOD of 0.25 fM. The specificity and stability of the obtained device was also remarkable. The results also showed that the presence of biological components such as AβMs and AβFs, as well as Hb, Thr and BSA, had a negligible effect on the detection of AβOs at a low-level. The usability of the aptasensor was assessed by detecting AβOs in aCSF samples, and the results obtained were comparable to ELISA tests.

The application of antibodies (Ab) in the processes of molecular recognition has disadvantages such as low stability, difficulty in terms of modification and high cost. Thus, the development of alternative antibody-mimicking recognition elements to overcome the shortcomings of the current experimental strategies is of great importance. Recently, molecularly imprinted polymers (MIPs) have shown radical improvements in their molecular recognition properties for biosensing applications. They can act as an artificial Ab because they possess recognizable sites in their matrix that are functionally and structurally complementary to the analyte biomolecule. In this study, a voltammetric MIPs-Apt sandwich assay detected AβOs with an LOD of 1.22 pg/mL [125]. In the first step, GCE was modified with an AuNPs-GO layer to enhance the surface-to-volume ratio and the electrical conductivity. The MIPs were then immobilized on the GCE/AuNPs-GO surface. Afterwards, the AβOs captured by the MIFs from the analyzed samples were bound to the aptamers immobilized on the SiO_2NP/AgNPs. The voltammetric signal came from the creation of a sandwich structure on the MIP film. A high sensitivity of the sandwich assay was obtained by utilizing only small amount of AβOs to obtain an abundant amount of electrochemically active NPs. The biosensor showed linearity in a concentration range from 5 pg/mL to 10 ng/mL. Moreover, the described assay could be applied to detect other protein biomarkers using adequate aptamers and MIPs, which may be a new approach to employing aptamers and MIP antibodies in biomarker determination.

Another study developed a voltammetric aptasensor using a nanocomposite of superhydrophobic carbon fiber paper (CFP) and an Au-Pt alloy as a signal amplifier [126]. This aptasensor could detect AβOs in a linear range from 0.5 to 10,000 pg/mL, while the reported LOD was 0.16 pg/mL. The selectivity of this device was effectively verified using human serum albumin (HSA), neurofilament light protein (NFL), tau441 protein, AβMs(40) and AβMs(42). In addition, the reported assay showed excellent antifouling properties, a good selectivity and a high sensitivity with an LOD of 0.9 pg/mL in serum samples.

In this example, the authors used free-standing vertically aligned tin disulphide nanosheets (SnS_2 NSs) to increase the analytical performance of the impedimetric aptasensor [127]. The aptamer was covalently immobilized on a self-assembled layer of 3-mercaptopropyltrimethoxysilane (MPTMS)–SnS_2. This aptasensor displayed a good stability, selectivity and interference resistance. Successful results were obtained with selectivity-interfering substances including AβFs(42), AβMs(40), AβOs(40), AβFs(40), HSA, DA, Glu, NaCl and KCl. The responses of the interfering substances such as AβFs(42), AβOs(40) and Dop were slightly higher than those of the other interferents, probably because of a possible non-specific binding interaction. The elaborated aptasensor was effectively tested for the detection of AβOs in real blood serum samples from healthy mice and mice with AD. This device was characterized by a lower LOD in HS (56.9 fg/mL) than in a physiological buffer (238.9 fg/mL).

Deng and co-workers developed a new procedure for the voltammetric detection of AβOs(40) using an Fc-labelled aptamer located on streptavidin-modified AuNPs [128]. The obtained NPs were linked to the Au electrode via the connection of double-stranded DNA. The detection of AβOs(40) caused the mechanical stretching of the dsDNA and an exponential change in the charge transport through the dsDNA. This influenced the decrease in the signal of the Apt-Fc [129–131]. The possible interfering analogues (e.g., AβOs(42) AβMs(42)) had a negligible impact on the detection of Aβ40. The electrodes after the modification were stable, and the obtained LOD of AβOs(40) was 93 pM (linear range from 0.100 nM to 1.00 μM) (Table 1).

2.3. Tau Protein

Tau protein is another important biomarker that is especially associated with AD. The aggregation of tau into neurofibrillary tangles within brain tissues is related to AD pathology. This pathology proceeds in precisely designated stages based on the conception that neurofibrillary tangles form from the accumulation of abnormal tau proteins [132]. High levels of tau in the CSF of AD patients may reflect the intensity of neuronal damage and brain degeneration [133]. Phosphorylated tau (p-tau) and total tau (t-tau) levels are increased in AD patients compared to cognitively normal individuals [134]. The cut-off values for p-tau181 and t-tau are 350 pg/mL (7.6 pM) and 80 pg/mL (1.7 pM), respectively [135].

One of the widely applied ways to enhance the sensitivity of detection is to amplify the signal with the use of nanomaterials based on, e.g. gold or carbon. Due to their excellent electrical conductivity, high adsorption capacity, biocompatibility and specific surface area, gold- and carbon-based nanomaterials are suitable candidates for biosensors. Recently, Tao and co-workers developed an amplification procedure for the detection of tau-381. In this assay, the aptamer was immobilized on a carboxyl graphene (CG)/Thi/AuNPs-modified GCE [136]. The CG was electrostatically adsorbed onto the GCE surface. Then, Thi was attached to the GCE/CG layer by condensation interactions and π-stacking. In the next step, the AuNPs were electrodeposited, and an aminated aptamer was coupled to the modified CG/Thi/AuNPs layer (Figure 4). Thi played a double role, both as a molecular connector between the AuNPs and CG and as a redox probe, which provided an electrical signal. The detection of tau-381 protein was monitored by DPV. The obtained signal increased linearly with the logarithm of the analyte concentration in a range from 1.0 pM to 100 pM with an LOD of 0.70 pM (Table 1). The tau protein was precisely determined with no significant

interference from AA, L-Cys, Glu and tau441. Moreover, the presented aptasensor was also successfully applied in AD patients' sera.

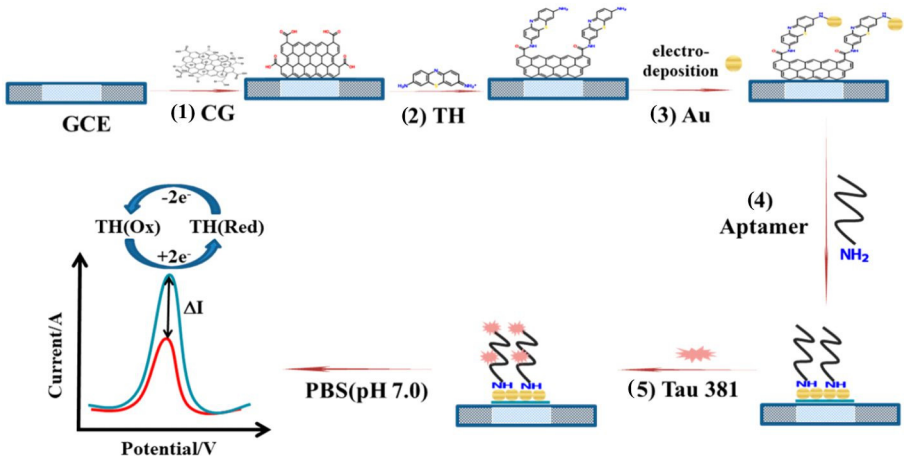

Figure 4. Schematic representation of tau-381 detection with the aptamer-based biosensor: (1) Doped CG on a GCE surface. (2) The incubation with Thi. (3) Electrodeposition and reduction of $HAuCl_4 \cdot 3H_2O$ to AuNPs. (4) Immobilization of aptamer. (5) The detection of tau-381 levels in HS by using DPV technique. Reprinted with permission from Ref. [136].

In this study, an Ab-Apt sandwich assay, with AuNPs enhancement, detected tau protein with an LOD of 0.42 pM [137]. The analyte was trapped between the anti-tau Ab immobilized onto the 3-mercaptopropionic acid (MPA)/AuE and the aptamer-reporter-bearing cysteamine (Cys)-modified AuNPs tags (Figure 5). This device showed a linear response to tau protein in a concentration range from 0.5 pM to 100 pM (Table 1). Moreover, this device was validated in HS from patients with AD. This biosensor has great potential and is a promising approach as a reliable analytical tool for the early diagnosis of AD.

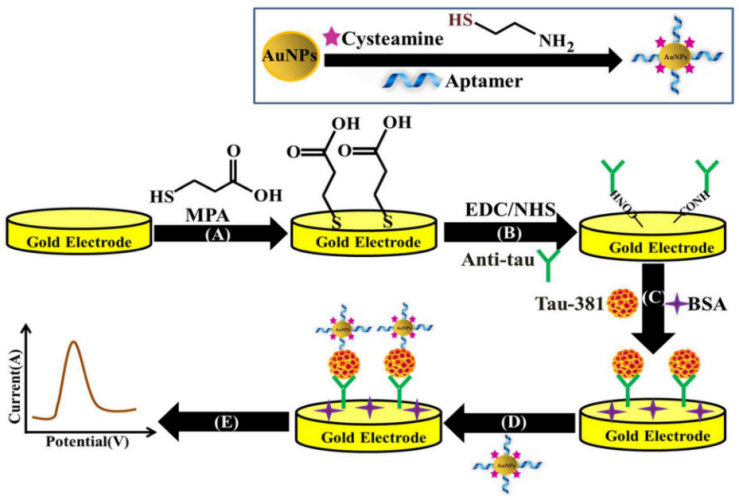

Figure 5. Preparation of Ab-Apt sandwich biosensor for detection of tau-381 protein: (A) Self-assembly of MPA at AuE. (B) EDC/NHS activation and anti-tau antibody immobilization. (C) Free sites blocked with BSA followed by immunoreaction with tau-381. (D) Affinity interaction the aptamer-Au-Cys bioconjugate. (E) Detection of tau-381 levels by using DPV. Reprinted with permission from Ref. [137].

The recently reported application of MWCNTs for the highly efficient immobilization of aptamers has been described by Yin and co-workers [138]. The electrode surface was functionalized with MWCNTs via the 3-(aminopropyl) triethoxysilane (APTES) connector.

Then, the aptamer was attached to the carboxylated MWCNTs. In the last step, polyethylene glycol (NH_2-PEG) was used as a blocking agent to coat the unmodified MWCNTs. Studies on the interaction of aptamers with tau protein were performed using EIS with an LOD of 1 fM. The presented aptasensor specifically recognized tau protein in control experiments performed with (i) a complementary aptamer instead of an aptamer, (ii) CSF instead of tau protein and (iii) albumin instead of tau protein. In addition, the described device was effectively tested in HS spiked with tau protein.

Another example of signal amplification with nanomaterials concerns a combination of integrated VG and AuNPs (VG@AuNPs) [139]. Aptamers were attached to a VG@AuNPs-modified paper-based surface via Au-S covalent bonding. The aptamer–tau protein binding followed by DPV in the presence of $[Fe(CN)_6]^{3-/4-}$ allowed the detection of tau down to a concentration of 34 fg/mL with a linear range from 0.1 pg/mL to 1 ng/mL (Table 1). AA, Glu, HSA and Aβ did not interfere with the tau protein detection, and the aptasensor operated well in clinical serum samples. The obtained results were comparable to a professional blood-tests for AD (Quanterix Co., Ltd. (Billerica, MA, USA)). It should also be mentioned that this portable aptasensor was able to transmit signals to a smartphone via Bluetooth, and the user could see the results in the smartphone application [139]. This makes the described tool very promising for clinical applications.

2.4. Human Cellular Prion Protein

Prion diseases (Creutzfeldt–Jakob disease, fatal familial insomnia and others) are mortal neurodegenerative diseases induced by the creation and propagation of misfolded proteins known as prions in the brain [140,141]. According to the protein hypothesis, the crucial step in prion propagation is the conformational change from PrPC into its infectious isoform (PrPSc) [142,143]. The plasma level of PrPC and total prion protein (t-PrP) are elevated in Creutzfeldt–Jakob disease patients compared to healthy controls [144,145]. Moreover, elevated levels of plasma t-PrP are also found in other neurological conditions with blood–brain barrier impairment such as AD and other neurodegenerative diseases [146]. However, the accurate detection of PrPSc is difficult due to its very-low blood concentration (down to picomolar) [142,143]. Therefore, the development of easy-to-use platforms for simple and sensitive prion protein determination is extremely necessary for the early diagnosis of prion diseases.

During the last decade, few tools for the detection of prion diseases have emerged in the field of electrochemical aptasensors. Miodek and co-workers used polypyrrole (PPy) modified with redox dendrimers to design a voltammetric aptasensor for PrPC determination [147]. The Au electrodes were modified with a conductive layer (PPy film coupled with fourth-generation polyamidoamine dendrimers (PAMAM G4)). Next, the amine groups of the modified surface were utilized for the covalent attachment of redox markers (ferrocenyl groups). Finally, the aptamers sensitive to prion proteins [148] were immobilized on the modified surface via biotin–streptavidin coupling. The applied aptamer sequence, selected by Takemura and co-workers, [149] was extended with a 15-mer thymine spacer, which provided a greater flexibility for the aptamer to anchor to the layer. The authors showed that the interaction between PrPC and the aptamer, monitored by the DPV and CV techniques, led to variations in the electrochemical signal of the ferrocenyl group. The conjugation of the redox dendrimers with the conducting PPy resulted in a high sensitivity of PrPC determination with an LOD of 0.8 pM (Table 1). The designed biosensor was validated by the determination of analytes in spiked human blood plasma, exhibiting an LOD of 1 pM [149].

The second example of a voltammetric aptasensor elaborated by Miodek's team used MWCNT–PAMAM G4 conjugates as a platform for the immobilization of biotinylated DNA aptamers [150]. Ferrocenyl redox markers were incorporated between the dendrimer and aptamer interlayers. The MWCNTs–PAMAM G4 conjugates provided a higher surface area for aptamer immobilization due to their electrical properties and nanostructural organization. The electrochemical signal of the ferrocenyl group was changed due to the

aptamer–PrPC recognition process. It was measured by CV with a linear range of detection from 1 pM to 10 µM. Moreover, the elaborated aptasensor displayed a low LOD of 0.5 pM. This device was also validated in spiked human blood plasma. The obtained results proved the possibility of the application of the designed platform for the practical detection of PrPC in real samples (Table 1).

Another development in this field was a ratiometric electrochemical platform based on protein biogates, which controlled the host–guest interaction between β-cyclodextrin (β-CD) and two competitive redox probes (Rhodamine B (RhB) and ferrocenecarboxylic acid (FcA)) (Figure 6) [142]. For this purpose, GCE modified with an MWCNT-β-CD composite was used. RhB-Apt was introduced into the MWCNT-β-CD composite by means of the host–guest interaction between the β-CD and RhB. In the absence of prions, the RhB-Apt conjugate could be displaced by the FcA due to its stronger binding affinity to β-CD. However, in the presence of prions, specific aptamer interactions led to the formation of a biogate protein that sealed the β-CD cavity, making it difficult for the FcA to displace the RhB guest. The developed aptasensor showed a good response to the prion protein (PrP) with a low LOD of 1.6×10^{-13} M. In order to investigate the selectivity of the developed aptasensor, control tests with interfering substances including pepsin, fibrin, IgG, Thr, transferrin and lysine(Lys) were performed. The obtained results confirmed the high selectivity of the analyzed system—the interferences generated insignificant current changes. Moreover, samples of normal human blood serum were successfully applied to assess the analytical suitability of the developed method.

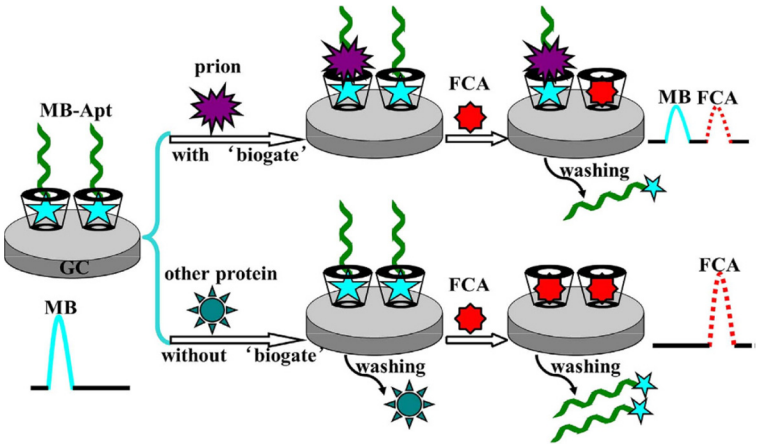

Figure 6. Schematic illustration of the voltammetric aptasensor for prion protein detection. Reprinted with permission from Ref. [142].

The last example of a PrPC detection tool was developed by the same research group. It concerned a label-free and three-level cascaded dual-signaling amplified voltammetric strategy [151]. As in the previous paper [142], the fabricated platform was based on protein biogates, which regulated the host–guest competitive interaction between β-CD and two redox probes (FcA and Rhodamine B (RhB)). It was based on an ordered mesoporous carbon probe (OMCP), exonuclease III (Exo III)-assisted signal amplification and competitive β-CD–guest interaction. The recognition layer consisted of double-stranded DNA containing a PrPC-binding aptamer (DNA1), its partially complementary DNA (DNA2) and OMCP received by sealing electroactive FcA into its internal pores and then by means of single-stranded DNA (DNA3) as a gatekeeper. In the presence of PrPC, the DNA1 could bind it and release DNA2. The authors used the same interferents in these selectivity studies as in the previous publication [142,151]. It is worth emphasizing that the developed aptasensor was highly selective and sensitive. The obtained LOD of 7.6 fM (Table 1) makes it the most sensitive tool for detection of PrPC described in this review. Researchers have shown its usefulness in samples of normal human blood serum, confirming that the developed system holds great promise for future clinical analysis.

Table 1. Specifications of electrochemical aptasensors for the detection of ND biomarkers.

Biomarker	Biorecognition Element	Techniques	Linear Range of Detection	LOD	Ref.
α-syn oligomer	DNA Apt	DPV	6×10^{-11}–1.5×10^{-7} M	10^{-11} M	[102]
	SH-DNA Apt	EIS	–	10^{-12} M	[103]
	DNA Apt-MB	DPV	10^{-15}–10^{-9} M	6.4×10^{-16} M	[104]
	NH_2-DNA Apt	EIS	10^{-19}–10^{-14} M	7×10^{-20} M (buffer) 9×10^{-20} M (plasma)	[105]
Total α-syn	DNA Apt-AuNPs conjugate	Vm	10^{-11}–10^{-6} M	10^{-11} M	[106]
Aβ	RNA Apt	DPV	0.002–1.28 ng/mL	0.4 pg/mL	[114]
AβOs	Ab-DNA Apt sandwich	DPV	–	10^{-10} M	[115]
	DNA Apt-AuNFs	DPV	10^{-9}–2×10^{-6} M	4.5×10^{-10} M	[116]
	DNA Apt	EIS	10^{-10}–5×10^{-7} M	3×10^{-11} M	[119]
	Apt-Poly T-CuNPs	DPV	10^{-11}–2.2×10^{-9} M	3.5×10^{-12} M	[117]
	DNA Apt1 Apt2	LSV	10^{-12}–10^{-8} M	4.3×10^{-13} M	[118]
	SH–stem-loop DNA Apt-Fc	ACV	10^{-12}–2×10^{-7} M	3×10^{-13} M	[121]
	NH_2-DNA Apt	DPV	4.43×10^{-14}–4.43×10^{-6} M	10^{-14} M	[122]
	DNA Apt–Fc	ACV	10^{-13}–1.5×10^{-6} M	2×10^{-15} M	[120]
	THAS	DPV	10^{-15}–10^{-11} M	5×10^{-16} M	[123]
	SH-DNA Apt	DPV	5×10^{-16}–5×10^{-13} M	2.5×10^{-16} M	[124]
	SiO_2Ag- DNA Apt bioconjugate	DPV	5 pg/mL–10 ng/mL	1.22 pg/mL	[125]
	SH-DNA Apt	DPV	0.5–10 pg/mL –	160 fg/mL (buffer) 900 fg/mL (serum)	[126]
	DNA Apt–SnS_2 NSs	EIS	10^{-4}–10^{3} ng/mL –	238.9 fg/mL (PB) 56.9 fg/mL (HS)	[127]
AβOs(40)	DNA Apt	SWV	0.100–1.00 μM	9.3×10^{-11} M	[128]
Tau-381 protein	NH_2-DNA Apt	DPV	1.0–10^{-10} M	7×10^{-13} M	[136]
	Anti-tau Ab + tau-381 DNA Apt	DPV	0.5–10^{-10} M	4.2×10^{-13} M	[137]
	NH_2-DNA Apt–MWCNTs	EIS	10^{-15}–1×10^{-9} M	10^{-15} M	[138]
	SH-DNA Apt–VG@Au	DPV	0.1 pg/mL^{-1}–1 ng/mL	0.034 pg/mL	[139]
PrPC	Biot DNA Apt with dT15 spacer	DPV	10^{-12}–10^{-9} M	8×10^{-13} M	[147]
	Biot DNA Apt with dT15 spacer	CV	10^{-12}–10^{-5} M	5×10^{-13} M	[150]
	MB DNA Apt and FcA	SWV	2×10^{-13}–10^{-5} M	1.6×10^{-13} M	[142]
	DNA1 Apt and DNA2 Apt	SWV	2×10^{-14}–2.8×10^{-13} M	7.6×10^{-15} M	[151]

Abbreviations: Apt—aptamer, EIS—electrochemical impedance spectroscopy, MB—methylene blue, DPV—differential pulse voltammetry, Vm—voltammetry, LSV—linear sweep voltammetry, AuNFs—gold nanoflowers, Ab—antibody, ACV—alternative current voltammetry, Biot—biotinylated, THAS—triple-helix aptamer switch, Poly T-CuNPs—poly(thymine) copper nanoparticles, MWCNTs—multiwalled carbon nanotubes, DNA Apt–SnS_2 NSs—DNA aptamer onto vertical aligned tin disulphide nanosheets, PB—physiological buffer, HS—human serum samples, VG@Au—vertical graphene modified with nanogold, CV—cyclic voltammetry, FcA—ferrocenecarboxylic acid.

3. Conclusions and Future Directions

This review focused on the recent developments in the field of electrochemical aptasensors for the biomarker detection of neurodegenerative diseases, which is an attractive topic in up-to-date medical diagnostics.

NDs are a serious global problem, not only from a medical point of view but also in social and economic terms. With the aging of the population, the prevalence rate of NDs such as Alzheimer's, Parkinson's or prion diseases is increasing. The solution to these problems is primarily an early, simple and at the same time precise diagnosis. Undoubtedly,

electrochemical aptasensors are prospective tools in the diagnosis of NDs due to their high sensitivity, low cost, simple design, easy operation, portability and high miniaturization potential. Thanks to their advantages, a combination of aptamer science and electrochemical biosensing seems to meet these requirements and is very promising.

The design and construction of biosensor platforms are crucial aspects in the development of electrochemical aptasensors for the early diagnosis of NDs using their specific biomarkers. They determine the sensitivity and selectivity of the device, as was highlighted in the review. In recent years, advances in nanoscience have allowed researchers to optimize such systems in order to obtain a detectable signal for proteins occurring in a very low concentration range [152,153]. In addition, improvements in the quality and sensitivity of these tools for biomarker detection are possible thanks to the development of techniques for the production of aptasensors and for the amplification of analytical signals. Additionally, the development of multichannel, multianalyte [121], electrode-array-based [154] and label-free detection systems [155] will probably be the direction of the improvement of aptasensors for biomarker detection in the near future.

Each technology has its pros and cons. This also applies to aptamers. There are several difficulties and challenges with the practical application of aptamers that are still under investigation. Thus, despite the advantages of aptamers over antibodies, further efforts are needed to spread aptamer-based technologies to medical assays. There are several challenges that need to be addressed in order to accelerate the development of aptasensor-based diagnostics. In diagnostics, one of the major challenges facing the development of the aptamer market is the lack of qualified and trained specialists to develop aptamers and aptamer-based products. Secondly, although aptamers can be generated for a large number of targets, antibodies are preferred because of the well-established confidence of researchers in immunological testing [58]. One of the challenges concerns the lack of a standard aptamer selection protocol for all kinds of targets. Aptamers generated for a single target in the same laboratory may vary in terms of their affinity, efficiency, sequence or structure. Hence, it can be concluded that one SELEX protocol cannot be used to select aptamers for another target [156,157].

A key challenge in the development of electrochemical aptasensors is the simultaneous detection of two or more analytes. Most of the research on aptasensors for the detection of ND biomarkers describes the detection of a single biomarker, and research into sensing platforms for the simultaneous detection of two or more biomarkers is severely limited. To our knowledge, there has only been one aptasensor developed for the simultaneous detection of two biomarkers, namely AβOs and ATP [121]. Therefore, more attention should be paid to this subject.

Despite the many well-described diagnostic methods, the incorporation of electrochemical aptasensors into diagnosis and clinical practice is still under study. The conversion of the developed miniaturized aptasensors to POC testing is an important challenge. However, there are still some limitations to overcome, such as the on-chip storage of reagents or the possible high costs of miniaturized biosensors. Most of the challenges associated with electrochemical biosensors and their ability to detect biomarkers outside the laboratory relate to the fabrication of reliable and durable chips that meet POC diagnosis requirements as well as traditional and commercial biosensor chips used in blood glucose meters. The use of rapid, low-cost and simple label-free transduction methods such as voltammetry and EIS, in the presence of an appropriate redox couple, make them extremely suitable for POC applications [158–160], which explains their boosting in recent years. Moreover, due to their attractive characteristics, biomolecular switches and aptamers are considered to be molecular recognition elements that are especially interesting for the development of POC electrochemical biosensors [159]. We hope that in the coming years, POC testing will use constantly evolving and advanced technologies and that we will have more devices capable of helping with early diagnosis, treatment monitoring and follow-up of neurodegenerative diseases.

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