



Advances in amyloid beta oligomer detection applications in Alzheimer's disease

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ABSTRACT

Changes in amyloid-beta-oligomer (A β O) peptides levels are considered one of the major neuropathologic biomarkers for Alzheimer's Disease (AD). A β O levels can be detected using a variety of techniques such as neuroimaging, single molecule detection, immunoassays, and polymerase chain reaction (PCR). However, such techniques are usually reserved for individuals with mild cognitive decline or symptoms of late onset AD often to the exclusion of those with subjective memory complaints and patients in the early presymptomatic stages of AD. Recently, many scientists have sought to address this issue by integrating these techniques onto a single device or inexpensive platform permitting routine measurements of A β O concentrations outside the clinic. In this review, we examine the different types of clinical A β O commercial assays and established analytical techniques currently employed in the diagnosis of AD as well as integrated optical and electrochemical sensing devices currently under development.

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1. Introduction

Alzheimer's disease is a multifactorial incurable disorder mainly characterised by the extracellular accumulation of amyloid- β protein (A β), and intracellular aggregation of hyperphosphorylated tubulin binding (tau) protein. Currently, there are only 3 clinical biomarkers used in AD diagnosis: those of neurodegeneration/injury, tau, and amyloid-beta(A β) protein. The polymerization, hyperphosphorylation and subsequent aggregation of said proteins in the frontal cortex and hippocampal areas of the brain strongly correlates with the clinical symptoms associated with AD [1–18]. Although all 3 are required for a diagnosis, amyloid beta is regarded as the primary biomarker for AD as compelling genetic evidence (mutations in genes encoding for the amyloid precursor protein, presenilin 1 and 2, and APOE4 (A β -clearance)) coupled with biochemical and histopathological observations suggests A β accumulation plays a key role in disease pathogenesis. On that evidence, the amyloid cascade theory (ACT) was formulated and until

recently thought best to explain AD pathogenesis. ACT posits that neuronal death in AD patients is a result of A β insoluble load (plaque burden) leading to memory loss and cognitive impairment. However, numerous reports show A β plaque burden poorly correlates with cognitive dysfunction in patients with advanced AD pathology [19].

Such theoretical inconsistencies can be accounted for by considering soluble amyloid-beta oligomers(A β O) and protofibril intermediates produced *en route* to plaque formation are highly neurotoxic, and better correlate with memory impairments in the early stages of AD [20]. In addition, the neurotoxicity of A β oligomers has been linked to tau hyperphosphorylation, seeding of α -synuclein (α -syn), tau and TAR DNA-binding protein (TDP-43) oligomers, providing further evidence of A β O's significance in AD pathology and its upstream role in ACT [21]. Moreover, Willbold et al. recently showed that elimination of A β O via oral administration of RD2 to transgenic mouse models of amyloidosis resulted in enhanced recognition, and deceleration of the early-onset phenotype in pyroglutamate-A β accumulating transgenic mice as well [22].

Such findings have accelerated the development of inexpensive A β O biomarkers, assays and devices that can aid in the diagnosis of the presymptomatic and prodromal stages of AD. (The various stages of AD and the current diagnostic tools used in their identification are shown in Fig. 1). The success of these devices will inevitably depend on the sensitivity and strength of the correlation

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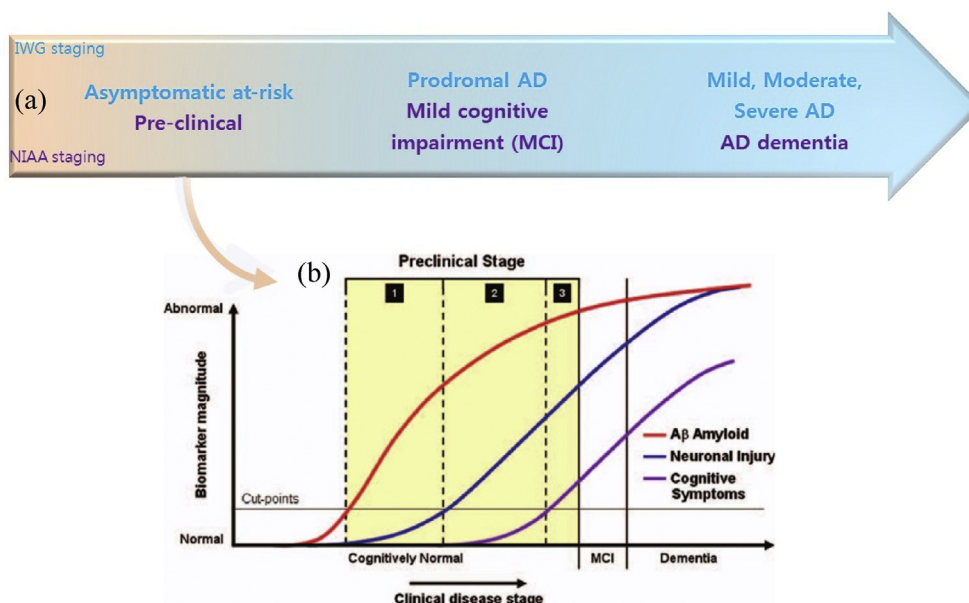


Fig. 1. (a) Clinical staging of Alzheimer's disease severity according to the International Working Group (IWG; blue) and the National Institute on Aging - Alzheimer's Association (NIAA; purple). Subjective cognitive decline is also considered as a useful biomarker in the pre-clinical stage. (b) Relation of A β concentrations to the various stages of the disease. The yellow areas indicate the three substages of the preclinical stage of AD. The A β biomarker is positron emission tomography (PET) imaging (red curve). Neuronal injury biomarkers are FDG-PET or atrophy measured by MRI (blue curve). Modified with permission from Wiley Science reference [23].

they exhibit compared with current clinical assays and techniques [23–31]. Once achieved, personalized monitoring of AD using cheaper non-invasive sampling procedures and diagnostics can begin in earnest. Given the numerous reports regarding AD biomarkers this review will primarily focus on the recent developments in AβO sensors and their contribution to AD diagnosis.

1.1. A β O production and toxicity

The production of A β O species starts with the neuronal expression (astrocytes and microglia) of precursor protein (APP) followed by its sequential reaction with either α -secretase, beta (β) and gamma (γ) secretase. The interaction of α -secretase at the A β domain within APP results in the secretion of soluble amyloid precursor protein- α (sAPP α) fragments. Whereas cleavage of APP via beta (β) and gamma (γ) secretase enzymes (amyloidogenic pathway) results in the formation of numerous peptides A β _{1–39}, A β _{1–40}, A β _{1–42}, and A β _{1–43} as well as amino-terminal truncated and pyro-Glu-A β species. The latter more neurotoxic than either A β _{1–40} or hydrophobic A β _{1–42}. A β _{1–40} is produced more frequently, is less neurotoxic A β _{1–42} and is found in larger quantities in brain interstitial fluid (ISF). These monomers can adopt different physiological states ranging from soluble dimers, oligomers (15–20 monomers long precursor), protofibrils (string of oligomers) to insoluble mature fibrils, and senile plaques in the brain, [32–35]. Hybrid oligomers composed of equimolar ratios of synthetic A β _{1–42} and A β _{1–40} are considered one of the most neurotoxic amyloid species due to their size (3–10 nm diameter), aggregation state and stability. Further studies have shown there are variety of other amyloid oligomeric species such A β ₅₆ and A β -derived diffusible ligands that are neurotoxic as well. However, not all A β O species are toxic. Non-toxic oligomers appear to be reactive with anti-fibril antibody OC, show structural similarities with amyloid plaques and are less than 50 kDa (low molecular weight, (LMW)) in size. On the other hand, toxic species are reactive to anti-A β O antibody NU4, anti-amyloid antibody A11 and greater than 50 kDa (high molecular weight) in size [36–38]. It should be noted the toxicity debate

regarding synthetic A β O species within the AD community is ongoing, with many voices calling for restricting toxicity analysis to brain derived A β Os due their ability to form highly neurotoxic oligomeric complexes with A β_{1-42} *in-vitro* which are absent in artificial samples.

Recent evidence suggests that A β oligomers or at least sub-fractions can reproduce in a prion like fashion, making the relationship between A β O and prion protein (PrP^C) very intriguing. Multiple studies have shown that suppression of long-term potentiation (LTP) and synaptic plasticity in mice by A β O is PrP^C dependent. PrP^C has a high affinity for A β O and activates the tyrosine protein kinase Fyn. Fyn kinase is a central player in signal transduction and is known to induce tau hyperphosphorylation in mouse neurons [39–47]. The mechanism by which PrP^C mediates A β O toxicity involves the molecular players metabotropic glutamate receptor 5 (mGluR5), N-methyl-D-Aspartate receptors (NMDARs) and protein tyrosine kinase 2 (Pyk2). Fig. 2 shows how PrP^C mediates A β O toxicity via mGluR5, Fyn kinase, and NMDARs.

2. Commercialized early-stage diagnostics and evolving analytical techniques

2.1. In-vivo brain imaging

As well as detecting changes in A β and tau levels, a positive AD diagnosis also requires a multi-layered (0.7 mm thickness/per layer) image of a patient's brain presenting significant A β plaque and NFT deposition. This can be achieved using various imaging modalities including positron emission tomography (PET), magnetic resonance imaging (MRI), and near infrared fluorescence (NIRF). The quality of the neurological image depends on several factors such as the resolution of the instrument used, the contrast/imaging agent's ability to transverse the BBB, and its specificity for A β diffuse, core, and intermediate plaque species [48–50]. Currently tomography (PET) has evolved as the primary imaging tool for the early detection of AD through visualization of abnormalities in brain structure, function, histopathology, deposits and

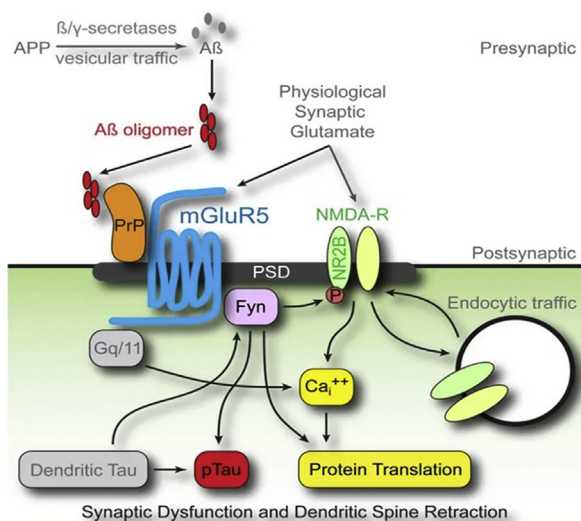


Fig. 2. PrPc mediates A β O toxicity through mGluR5, Fyn kinase, and NMDARs. Downstream consequences of the pathway include calcium dyshomeostasis, tau hyperphosphorylation, and synaptic dysfunction and loss. Reprinted from "Fyn kinase inhibition as a novel therapy for Alzheimer's disease" by Nygaard HB, van Dyck CH, and Strittmatter SM. This was published, under the terms of the Creative Commons Attribution License (CC BY) [47].

neuronal activity with the discovery of A β and glucose PET tracers respectively [51]. Several studies have shown that cerebral metabolic alterations precede the clinical manifestation of AD symptoms. Distinct patterns of cerebral glucose metabolism also help in differentiating AD from other causes of neurodegenerative diseases [52]. Since the development of the first A β radioligand 11C-ben-zothiazole and Pittsburgh compound B (PiB) [53], the US Food and Drug Administration (FDA) has approved three agents for imaging A β in the setting of AD, i.e. florbetapir [54,55] flutemetamol and florbetaben [56]. However, the high operational and analytical costs of PET have limited its wider clinical application in the broader community [57].

In addition to cerebral glucose patterns, research priority is given to the development and imaging of new soluble A β markers and their relationship to plaque stability. In this regard, fluorescent techniques using NIRF imaging probes have shown significant promise in transgenic AD mouse models [58]. Of note is the initial work by Zhang et al., involving the synthesis of curcumin analogues (CRANAD -58 & 17) for the *in-vivo* imaging of A β species [59]. *In-vivo* imaging with CRANAD-58 demonstrated its ability to differentiate 4-month old wt-mice from their AD counterparts signifying its potential as an early stage biomarker. Unfortunately, the slow washout time of CRANAD-58 has hindered its transfer to the clinical setting. Fast washout times are essential [60] but this often results in the inability of the probe to differentiate between soluble and insoluble A β species (insA β s). More recently, Li et al. reported on the application of 3 curcumin analogues namely CRANAD 102, 65, and 75 in the selective imaging of soluble forms of A β [61]. These analogues benefited from the introduction of a phenoxy-alkyl chain at the 4-position of the curcumin structure (see Fig. 3a) leading to an increase in their specificity for soluble A β species (sA β s). Investigations with CRANAD-65 and 75 showed a weak affinity for A β_{40} dimers and A β_{42} oligomers, and a strong affinity for A β_{42} monomers respectively. On the other hand, hydrophobic CRANAD-102 with its additional methyl groups (Fig. 3a) exhibited a moderate affinity for A β O's resulting in a 4.33-fold increase in fluorescent intensity. The titrated binding affinity for CRANAD-102 was 68 times higher for soluble A β s than for insoluble A β s. Further

investigations showed that CRANAD-102 easily transverse the BBB and is stable in the blood for more than an hour. In addition, *in-vivo* imaging of APP 12-month old mice (Fig. 3b) with CRANAD-102 exhibited a 1.89-fold difference in fluorescence 30 min after injection compared to age matched wt-mice. Finally, CRANAD-102 also demonstrated a fast wash out time (see Fig. 3c).

Realizing soluble A β imaging probes such as CRANAD-102 brings us every closer to a comprehensive clinical test for pre-symptomatic AD [62,63]. Li et al. recently sought to develop a highly specific imaging probe for soluble A β O's by fusing aminonaphthalene-2-cyanoacrylate (ANCA) to a spiropyran (SP) derivative resulting in the formation of AN-SP [64]. AN-SP exhibits 2 emission peaks at 557 and 701 nm respectively. Upon binding with A β O's the peak fluorescence increased 9.4-fold and was blue shifted by 20 nm. Additional investigations regarding the specificity of AN-SP for A β O's performed on 8-month-old APP/PS1 transgenic and wt-mice showed that AN-SP exhibited excellent colocalization with A β oligomer specific antibodies but not with conformational tau pathology. A summary of the current state of NIR imaging probes specific to A β can be found in a recent review by Peng et al. [65].

2.2. Enzyme-linked immunoassay (ELISA)

ELISA remains one of the most frequently used methods for detecting biochemical AD biomarkers. Substrates from pattern paper to gold coated plastic microwells have been used to detect A β levels in human plasma and serum [66,67]. Paper-based ELISA is the most common due to its low cost, high sensitivity, short assay time (<1 h) and compatibility with handheld scanners [68]. Lab-based A β ELISA kits with detection ranges from 10 to 1000 pg/mL are now readily available and capable of differentiating AD from healthy controls with good (80%) sensitivity and moderate (83.3%) specificity [69].

AD dependent buildup of A β O in human CSF occurs in the majority of confirmed AD patients. However, in the presymptomatic stages oligomer levels are so low, reliable measurement of CSF A β O levels using conventional ELISA is unreliable. To that end an attomolar sensitive nanoparticulate enhanced assay (BioBarcode) was developed, and when tested in 2005 showed median levels of A β O's (ADDL) in CSF from AD patients to be 30-fold higher than from non-demented individuals [70]. This elevation contrasts with monomeric A β levels, which decrease rather than increase as the disease develops [71]. At that time such assays were extremely costly (time and money) and confined to the research bench. Since then other groups such as Holtta et al. have developed a sandwich ELISA using the same N-terminally specific A β antibody as both capture and detection antibody to measure A β O's in CSF. The authors demonstrated that antibodies specific to the N terminus of A β have a higher affinity against fibrillar A β oligomers than antibodies with an epitope against the more C-terminal part of the A β sequence indicating that the N-terminal part of the A β sequence is the most likely one to be exposed in A β aggregates. By this approach, AD patients were consistently discriminated from controls [72]. More recently Savage et al. established an A β oligomer ELISA based on an oligomer-selective human antibody and with this demonstrated a significant 3–5-fold increase in A β oligomers in human AD CSF compared with controls. With 80% sensitivity and 88% specificity to detect AD, the authors suggest a reasonable discriminatory power for the AD state and the potential for utility as a diagnostic marker [73]. In 2015, Yang et al. reported achieving a similar degree of sensitivity and specificity with their sandwich immunoassay, but the signals in two cohorts of AD and age-matched control CSF samples were similar, while MCI subjects showed a trend towards having slightly higher levels of A β oligomers compared to control subjects [74].

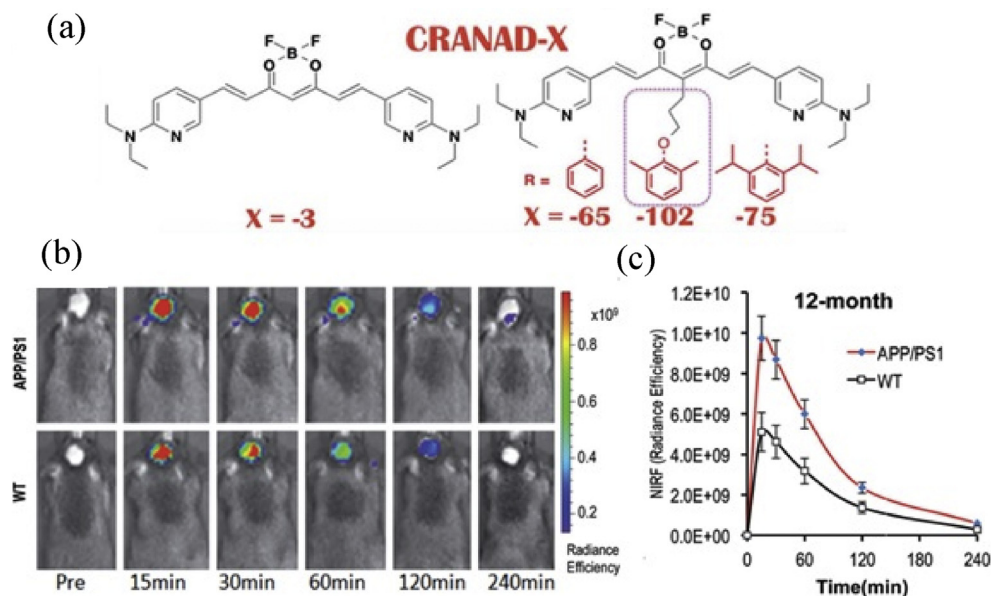


Fig. 3. (a) Structural tuning different CRANAD-X imaging probes to the accessibility of the A β species. (b) NIRF signal of APP and WT injected with CRANAL-102 over time. (c) Peak accumulation and clearance of CRANAD 102 in mice. Modified with permission from the Royal Society of Chemistry [61].

According to the National Institute on Aging (NIA) and the Alzheimer's Association (AA), (NIA-AA), accurately distinguishing A β O levels from normal controls is crucial in verifying AD onset. In 2010 An et al., employed an ELISA-based multimer detection system "Multimer Detection System-Oligomeric A β (MDS-OA β)" and successfully differentiated 24 AD patients from 37 cognitively normal controls based on their plasma A β O concentrations, demonstrating 83.3% sensitivity, 90.0% specificity and an AUC value of 0.8969 [75]. MDS-OA β is essentially an atypical ELISA that utilizes overlapping epitopes of capturing and detection antibodies to selectively measure for oligomerization [142]. It is one of these promising methods that exploits the oligomerization tendency of spiked synthetic A β in plasma to differentiate for AD. More recently, Wang et al. showed that A β O levels using MDS-OA β correlated well with other conventional AD biomarkers such as A β ₄₂, phosphorylated tau (p-tau), total tau(t-tau) levels in CSF, and Pittsburgh compound B (PIB) fluids [76]. In 2019, MDS-OA β also showed substantial correlation exists between amyloid β oligomerization in the blood and brain volume reduction characteristic of Alzheimer's disease [77]. More recently, Park et al. also demonstrated MDS-OA β was highly compatible with magnetized sample preparation procedures and droplet-based array technologies permitting 32 assays to be performed simultaneously in 1 h exhibiting a limit of detection (LOD) of approximately 3 pg/mL for A β O [78]. A modified schematic of the MDS-OA β droplet assay is shown in Fig. 4. In a blind study conducted by the Korean Ministry of Food and Drug Safety involving samples from 52 patients with AD and 52 age-matched normal controls in 2020, MDS exhibited a sensitivity of 100% and 92.31% specificity, respectively [143]. In another clinical study, strong correlations between MDS-OA β values and cognitive tests, such as MMSE, immediate recall, 5-min delayed recall, and 30-min delayed recall tests of the COMT, and ADAS-Cog scores were also demonstrated [144].

2.3. Early stage commercial platforms

Conventional ELISA assays and platforms are well suited to monomeric testing, however when concentrations of amyloids are extremely low such as in the early stages of the disease, single

molecule detection systems (SMDS) can be employed. Although significantly more expensive than conventional platforms, SMDS [79] can detect changes in patient serum proteins at sub-femtomolar concentrations. There are several SMDS platforms such as the single-molecule enzyme-linked immunosorbent array (SIMoA) and near field plasmonic fluorescent microscopy that can be used to detect single molecules of amyloid beta [80]. SIMoA makes use of arrays of femtoliter-sized reaction chambers and oil immersion microscopic methodologies to entrap and detect individual immunocomplexes formed on paramagnetic particles using standard ELISA reagents. Upon formation of the complex, each well/resonant cavity acts to localize and enhance the fluorescence signal. Recent work by Song et al. utilized SIMoA and antibodies 3D6 and 21F12 to measure A β ₄₂ levels in human plasma [81]. The assay demonstrated a limit of detection (LOD) of 2.8 pg/mL in human plasma. Janelidze and colleagues also employed SIMoA to assess the strength of the correlation between plasma and CSF levels for both A β ₄₂ and A β ₄₀ and plasma A β ₄₂ and neocortical amyloid deposition (measured with PET) in a cohort of 719 individuals. Using the platform, it was found that plasma levels of A β ₄₂ and A β ₄₀ were decreased in AD and MCI samples. In addition, reduced levels of A β ₄₂ and the A β ₄₂/40 ratio in plasma of patients with preclinical and prodromal AD (e.g. cognitively healthy individuals, subjective cognitive decline (SCD) and MCI patients with pathological CSF signature) were also detected [82]. The lower limit of detection (LLOD) for A β ₄₂ and A β ₄₀ in human plasma using SIMoA was 0.019 and 0.16 pg/mL respectively. In 2019, Wang et al. compared the A β O sensitivity of a SIMoA platform with a quartz crystal microbalance (QCM) using the same antibody. The authors found SIMoA to be 500 times more sensitive than the QCM technique to A β O in buffered solution demonstrating LODs of 0.22 125 nM respectively [83]. However, further testing using CSF was not undertaken as both techniques were not sufficiently sensitive in their adopted formats. Moreover, comparing SIMoA with other near field techniques rather than QCD might have been more appropriate.

Although SIMoA offers superior sensitivity compared to conventional microplate and paper A β ELISA assays, the current cost of the platforms and the associated optical instrumentation prevents

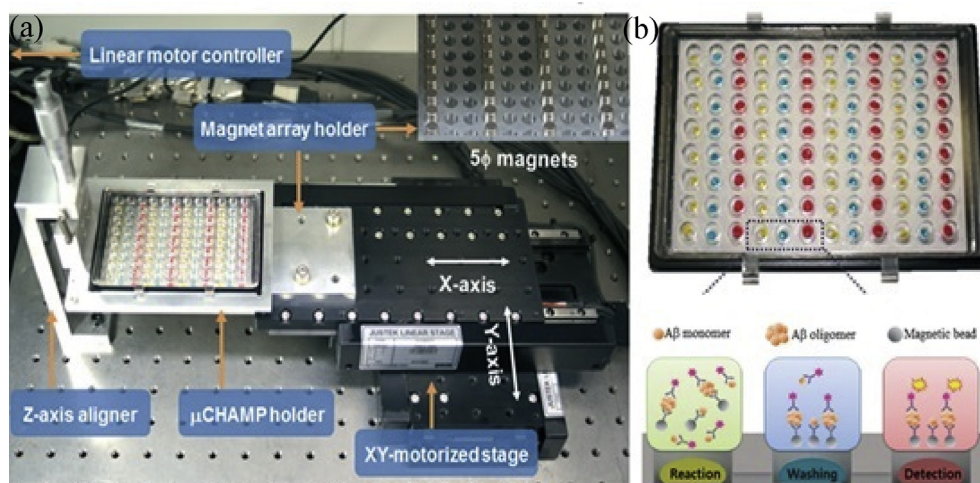


Fig. 4. (a) PDMS-μCHAMP plate attached to the XY-motorized magnet array stage. (b) A closer look shows how one set composed of three microwells are connected via a microchannel near the bottom of the walls that separates them. The microwells are filled with mineral oil and 30–50 μL of working buffer solutions. Each working buffer was dyed yellow, blue, and red to demonstrate stable droplet formation in each well (reaction, washing, and detection buffer droplets, respectively). Images modified with permission Royal Society of Chemistry, Ref [60].

its clinical application as a point of care device (POC), although this may change as integrated flat microscopes become more available [84]. Another single molecule detection system that uses ELISA is the single counting system developed by Singulex. Briefly, the system is based upon a paramagnetic microparticle-based immunoassay that uses single-photon fluorescence detection for analyte quantitation [85]. Recent work using the Singulex system identified neurogranin as a cerebrospinal fluid biomarker for AD. In addition, the Singulex ELISA platform was used to highlight a trend towards elevated orexin waking neuropeptides (OX-A) and A β O in subjects with insomnia versus those with a good sleep [86].

And last but not least, is a highly sensitive fluorescent A β platform based on surface fluorescent intensity analysis (sFIDA). sFIDA is a fluorescent immunoassay capable of quantifying single proteinaceous nanoparticles such as A β O in CSF. The advantage of this assay is that both immobilized and labelling antibodies recognize overlapping A β epitopes thereby rendering the assay insensitive to monomeric A β . Assay standardization is achieved via the employment of modified silica nanoparticles (SiNaP) with a narrow size range, specific to the aggregation region of the amyloid protein. Another advantage of said approach is that the diameter is independent of the aggregation conditions [87,88]. Initial studies in 2013 by Wang-Dietrich et al. showed that sFIDA could reliably distinguish A β O content in CSF samples taken from 14 AD patients and 12 age matched controls. Since then sFIDA-A β O detection has been automated, adapted to blood based analysis and employed in a significant number of suitable calibration standards [89].

2.4. Evolving analytical techniques

It is now well accepted decreasing A β_{1-42} /A β_{1-40} ratios in the CSF of MCI patients is a predictive marker for AD. However, the current gold standard for early diagnosis of AD requires the quantitative ratio of A β_{1-42} , A β_{2-40} and A β_{2-42} to be determined from a single sample. Such requirements usually involve the employment of multiple A β specific ELISA tests followed by an additional oligomeric assay. To circumvent this laborious and costly approach, researchers [90] have sought to integrate many of these steps into single analytical platform. A good attempt was the initial work by Smadja and Taverna's group in which a multi-step process involving sample pretreatment, thermal elution, and

simultaneous detection of all 3 peptides was performed on-chip. For the pre-treatment step, the authors used magnetic beads pre-conjugated with fluorescently labeled (FP-488) antibodies specific to different lengths of A β (A β_{38} , -40, and -42). This step resulted in a significant improvement in the signal-to-noise ratio of the eluate, allowing for lower concentrations of the peptide to be detected with capillary electrophoresis-laser induced fluorescence (CE-LIF). However, even with inclusion of a single pre-concentration step, the sensitivity of the device could not reach that of ELISA. In 2018, the same group integrated a digitized droplet sample procedure with a same pre-treatment step and a miniaturized detection system, permitting simultaneous quantification of 3 A β species in 45 min using a sample volume <1 μL [91]. Unfortunately, the sensitivity of their approach was an order of magnitude lower than ELISA.

Finally, in late 2018, Smadja and Taverna's group achieved the gold standard for all 3 peptides (LOD of 0.05 nM) using only FP-488 and no antibodies. This was accomplished by initially pre-concentrating multiple samples using a combination of multiple pressure-assisted large-volume sample stacking with an electro-osmotic flow pump (M-PA-LVSEP) in polyvinyl alcohol (PVA) coated microcapillaries. Sample integration with CE-LIF followed, resulting in pre-symptomatic A β levels in CSF from AD patients and healthy controls [108] being detected < 15 min. A schematic of their approach is shown in Fig. 5 [92]. A potential disadvantage of this approach is the requirement of labeled CSF samples to undergo numerous filtration steps on membranes (3–10 kDa in size) prior to preconcentration, which may result in a loss of detectable amyloid peptide and lower LOD values compared to ELISA. That said, the running cost of this technique are significantly less than conventional ELISA requiring no additional antibodies or conjugation steps or recourse to mass spectrometry for A β identification. Moreover, the multi-kinetic preconcentration approach allowed 5 A β family members to be detected simultaneously via laser induced fluorescence in a single run minimizing the assay time. Finally, excellent agreement (r^2 value > 0.993) between M-PA-LVSEP and current gold standard ELISA methods for A β_{1-42} measurements in CSF was demonstrated. It could be argued that this technique has the potential to identify the complete spectrum of available A β family members in CSF including highly neurotoxic pyroglutamate-A β as well.

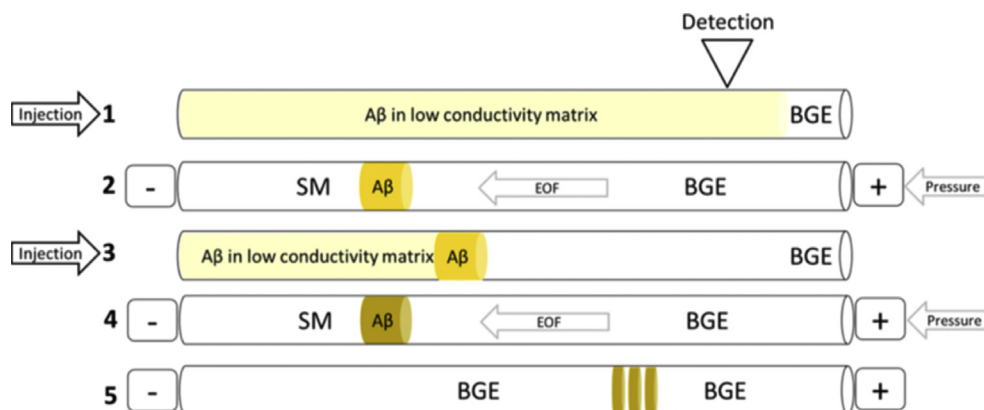


Fig. 5. Principle of the developed M-PA-LVSEP-CZE. (1) First hydrodynamic injection of the sample. (2) Exploitation of conductivity differences between sample and the separation buffer by LVSEP. (3) Second hydrodynamic injection. (4) Repeating LVSEP process (5) Separation and detection of the peptides. Steps 3 and 4 can be repeated several times (cycles) for further preconcentration of analytes newly injected at each cycle. Modified with permission from Refs. [89] American Chemical Society.

Another common analytical technique used to elucidate A β secondary structures in the laboratory is Raman Spectroscopy (RS). RS is a label free, non-invasive spectroscopic technique that provides information about the biochemical structure of analyzed specimens [93]. Within the last decade, numerous hand-held RS spectrometers have been used in the field of forensics making them well-suited to POC settings [94]. RS is categorized depending on the interrogation wavelength. The longer wavelengths of RS (interrogation wavelength, 415 nm) are often used to study the structural organization of fibrils [95] whereas the shorter wavelengths of DUVRR (interrogation wavelength 195–206 nm) are suited to the monitoring of smaller oligomeric species. The oligomeric sensitivity of DUVRR lies in its ability to differentiate between globular and fibrillar β -sheet conformations, and between parallel and antiparallel β -sheets. DUVRR potential was demonstrated by Breydo et al. [96], in which micro-molar concentrations of prefibrillar A β_{40} oligomers (PFO's) from fibrillar oligomers (FO's) were identified. The authors concluded FO's were more ordered than PFO's due to the higher proportion of β -sheets. However, oligomeric concentrations encountered in clinical samples often require a level of A β sensitivity beyond that of DUVRR. In such circumstances a highly sensitive variation of RS known as surface enhanced raman spectroscopy (SERS) can be employed. Recent work by La Rosa's group utilized SERS in combination with nanosilver metal colloids to catalogue different A β_{40} oligomers in buffer doped with human Islet Amyloid Polypeptide (hIAPP) protein [97]. The authors concluded that SERS was capable of discerning different A β_{1-40} oligomeric configurations in protein concentrations as low as 10 nM.

As well as RS, there are many other spectrophotometric techniques such as Fourier transform infrared (FTIR) and near infrared (NIR) that can be used to differentiate secondary structures in AD patient and control samples [98–100]. In the laboratory setting, FTIR in combination with 13 C-labeling was used to evaluate the influence of A β_{40} and A β_{42} oligomers on each other during aggregation. Work by Baldassarre et al. showed that neither one of these oligomers demonstrated structural predominance over the other, with a mixed oligomer solution exhibiting a random distribution of A β_{40} and A β_{42} strands in their β -sheets [98]. In the clinical setting, FTIR spectroscopy can be employed to classify the severity of AD using peripheral blood from control and AD patients [99]. Each assay involved every patient donating 3 mL aliquots of blood which were then centrifuged into white blood cells (WBCs) and serum. One μ L of plasma and WBCs were mounted onto separate ZeSe

crystals and then air dried. FTIR measurements were recorded for each sample. Multivariate analysis was performed using principal component analysis (PCA) followed by linear discriminant analysis (LDA) of infrared spectra from plasma and lymphocyte samples. Major spectral differences between AD and control samples occurred in the lower to mid infrared range (900–1480 cm^{-1}). However, accuracies of 90 and 83% were only achieved when plasma and WBC spectra samples from moderate and severe patients were included in the analysis.

Approximately 500 mL of CSF is absorbed into the blood daily. Characterization of these modified CSF markers has led to a flurry of cheap and non-invasive AD tests well suited to POC. A good example is the recent work by Martin et al. [100], in which a low-cost blood based NIR technique was used in the detection of AD. In brief, blood samples from 111 AD patients and 173 healthy controls (HC) were taken, centrifuged and supernatants extracted. Fifty μ L aliquots of plasma were then mounted on everyday e-glass substrates and exposed to a range of NIR wavelengths. NIR spectroscopy in the transmission mode demonstrated exceptionally high diagnostics accuracies. AD individuals were correctly identified with 92.8% accuracy, 96.1% specificity, and 87.5% sensitivity. However, the authors did note that the patient cohort in this study were already diagnosed with AD and the potential of the technique for presymptomatic detection remains to be further explored.

3. Emerging A β O and A β biosensors

According to the International Union of Pure and Applied Chemistry, a biosensor can be defined as an integrated receptor-transducer device capable of providing specific quantitative or semi quantitative analytical information via a biological recognition element. The diversity of these devices has permitted their application in many different fields of science such as environmental [101], safety [102,103] and remote healthcare [104–106]. In the medical arena, biosensors used at the point of care by an individual or careworker are generally referred to as POC devices. Currently, there is a swath of highly accurate POC biosensors (glucose, triglycerides, lactate, etc) available on-line at minimal cost, providing great relief to the growing number of elderly people who would not been able to access them less than a decade ago. In addition, this has allowed many people to employ healthier lifestyles, thereby reducing their risk of developing serious conditions such as diabetes, cardiovascular diseases and cancer. The simplest biosensors used

with purified media are generally composed of two fundamental layers: 1) the affinity layer, composed of a biological material including antibodies, antigens, DNA, RNA or enzymes that specifically interact with a desired analyte, which in turn changes the physical properties of the biomaterial 2) the transducer, which is able to convert 1 or more of those changes into a measurable signal (Fig. 6).

The complexity of the device is generally determined by the complexity of the fluid or medium to be analyzed. Biosensor design for complex medium such as blood, CSF or to a lesser extent plasma usually involves 3 or more membranes tailored both in porosity and surface chemistry to minimize potential interferents reaching the biological material. Thus developing an A β let alone A β O blood sensor is particularly challenging given the numerous interferents such as, serum albumin, fibrinogen, thrombin, IgG, von Willebrand factor, collagen IV, RNA, hemin, glycosaminoglycan, collagen, zinc and copper ions, and reactive oxygen species [107]. A retro glance at that list suggests copper could be a major problem when developing an A β O sensor as well as the usually monomeric amyloid species. Interestingly recent work by Giau et al., using specific monoclonal and polyclonal antibodies identified the A β _{5–25} region within A β as the most likely area to cross react with fibrinogen [108–110]. Removal of these species can occur via the employment of additional separation (labeled magnetic nanoparticles) and chromatographic techniques as mentioned previously. Alternatively, one can for go these steps, by combining conductive (embedded nanoparticles) selective membranes and pulse micro electric fields thereby minimizing signal interference and the long analyte diffusion times associated with multilayered devices.

At first glance the growing requirements for an A β O sensor can appear insurmountable. Even so, many researchers have attempted to meet these challenges with the development of new optical and electrochemical sensors, many of which we shall discuss in the following sections [111].

3.1. Optical sensors

In the last two decades a variety of optical sensors based on established analytical techniques such as refractive index (RI) surface enhanced Raman spectroscopy (SERS), absorption, infrared, electrochemical luminescence, and near/far field fluorescence has been used to measure and characterize A β O and fibril species in clinical samples and artificial media. The ability to distinguish A β O from fibrils and monomers using a dual molecular beacon assay was recently employed by Zhu et al. [112]. In brief, two stem loop DNA aptamers designated molecular beacons 1 & 2 (MB1 and MB2) both having abasic (AP) sites were used to trap (pseudo base pairing with cytosine opposite the AP site) and quench the fluorescent ligand 2-amino-5,6,7-trimethyl-1,8-naphyridine (ATMND). Upon binding with target DNA, the loop within MB1 unfolds, liberating the fluorescent ligand and allowing unfolded MB1 to interact with MB2 causing additional release of ATMND and the target sequence which is then free to bind to another molecule of MB1. This form of target recycling eliminates the need for enzyme amplification. A DNA aptamer specific for A β O was tested using the target recycling process and was shown to prevent ATMND release, whereas monomer and fibrils did not. Assay sensitivity was lower than that of covalently labeled and enzyme-assisted methods which may have been due to the low binding affinity of the ligand for the AP sites on the MB.

Several other fluorescent based techniques have been used to characterize and distinguish different A β species using DNA specific aptamers. Of note is the work by the Liu et al., in which carboxy-fluorescein (FAM) labeled DNA aptamers specific to A β O's were adsorbed onto the surface of polydopamine nanospheres (PDANs) [113]. In the unbound state, the aptamer's fluorescence was quenched by PDANs. Upon binding with an oligomer, the labeled aptamer adopted a hairpin structure leading to its partial desorption from the PDAN surface, resulting in the restoration of its fluorescence. The limit of detection for A β O was 12.5 nM using said

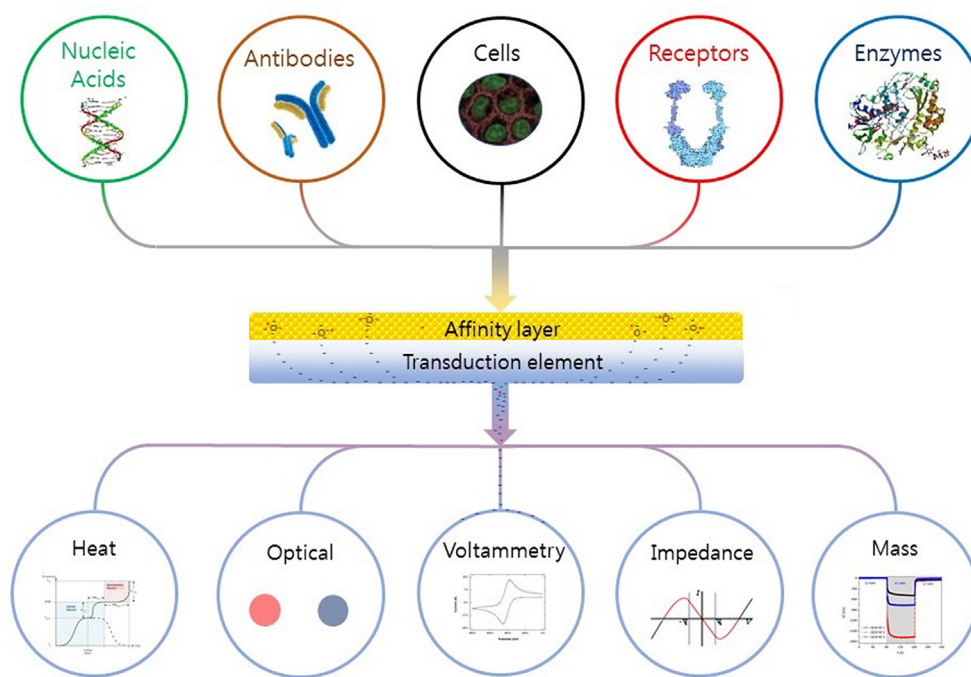


Fig. 6. Schematic displaying the fundamental parts of a biosensor. Reading from the top down; Cellular products used in the tailoring of an affinity layer specific to an analyte or analytes. Binding of an analyte to the layer changes its physical properties (electrochemical, optical, heat etc) some of which are converted into a measurable signal by the transduction element, these are then relayed to and recorded by an electronic meter, or visually (coloration) identified by the subject.

assay. Fluorescence values in the presence of other proteins such as BSA, IgG, lysozyme and myoglobin were almost as low as the background signal, implying that the aptamer was highly A β O—specific. A similar approach employing fluorescent quenching and A β O was also reported by the same group [114]. However, this time the binding event involved A β O and a fluorescently labeled portion of a prion peptide known as PrP-95-110 absorbed on graphene oxide (GO) nanoparticles. As before, binding with A β O induced the desorption of the peptide from the GO surface resulting in an increase of the aptamers fluorescent signal. Applicability of this approach as a diagnostic tool for AD showed promise when PrP-95-110 incubated with GO in artificial CSF (aCSF) demonstrated an LOD of 1 nM for A β O. More recently, Wang et al. employed a fluorescently labeled dual aptamer-conjugated molecular motor to monitor zinc ion levels and their role in A β aggregation [115]. The motor consisted of a Texas Red labeled zinc specific aptamer (Zn6m2)-conjugated to the peptide TGN, adjointed to complementary DNA labeled with the signal quencher (BHQ-2) and the A β -specific aptamer β -55. The proximity of the two labels led to fluorescence quenching via Förster resonance energy transfer (FRET). Upon binding with Zn²⁺, Zn6m2 underwent a conformational change resulting in the release of the complementary DNA and restoration of the motor's fluorescent signal. The assay LOD for zinc was 4.9 μ M.

It is quite evident the LOD of the aforementioned devices do not meet the requirements for presymptomatic AD diagnosis and are better suited to monitoring the later stages of the disease. The low abundance of oligomers and the excessive amounts of monomers encountered in CSF of AD patients has prompted many research groups to focus on the development of devices ultra-sensitive to monomeric A β . A good example is the work by Lee et al., in which a ZnO fluorescent polypeptide amplification platform was reported [116]. In short, the platform configuration consisted of a polyvalent directed peptide polymer (PDPP) constructed from 2 A β ₄₂ specific polypeptides which increased assay sensitivity by 4 orders of magnitude compared to a FITC labeled single peptide. The researchers further improved the binding sensitivity of PDPP by combining it with a nanoporous ZnO film increasing the LOD for A β ₄₂ from the low fg/mL range to 12 ag/mL in CSF. A schematic of the PDPP system used by Lee's group is shown in Fig. 7.

Unlike fluorescent assays, surface plasmon resonance can directly quantify unlabeled A β fibrils from monomeric species in real time. A novel SPR biosensor distinguishing A β O from fibrils was recently reported [117]. Two different antibodies, one specific for A β O and the other for fibrils, were immobilized in carboxymethylated (CM)-dextran-covered SPR chips via amine coupling. These antibodies captured their respective oligomeric and fibrillar targets as the solution flowed through the channels producing a detectable signal. Addition of curcumin interfered with the aggregation process, reducing the amount of oligomeric and fibrillar species as demonstrated by the reduction in SPR signal. In contrast, Cu²⁺ accelerated aggregation within a shorter incubation period while methylene blue only enhanced the fibrillar pathway but inhibited the oligomeric pathway. Similarly, a high-throughput SPRi sensor for monitoring A β fibril elongation on different immobilized “seed” conditions was also reported [118]. Various incubation conditions gave insight to the effect of chelating epigallocatechin-3-gallate (EGCG) and various polyvalent metals (Fe³⁺, Cu²⁺, and Zn²⁺) on the growth of A β fibrils. The results showed that monomer elongation of A β fibril seeds were heightened by the presence of metals compared to A β alone and hindered by chelating polyphenols like EGCG. In addition, EGCG was found to promote the formation of off-pathway highly stable unstructured oligomers.

3.2. Electrochemical sensors

Electrochemical sensors can be classified into amperometric, conductometric, impedimetric, and potentiometric. Construction and modification of these sensors is inexpensive making them highly compatible with POC facilities. Some of the cheapest electrochemical sensors are those constructed from screen printed carbon electrodes (SPCE's). However, unmodified SPCE's are not sufficiently sensitive to detect presymptomatic levels of A β in clinical media. To improve the electrodes limited sensitivity, the surface is often coated with a layer of gold nanoparticles. These gold coated SPCEs or AuNP-SPCE's were recently used by Costa-García et al. [119], as the transduction element in a competitive A β immunoassay. In brief, the surface of an AuNP-SPCE was initially modified with a streptavidin-biotin-A β ₁₋₄₂ solution. Forty μ L of a solution of A β ₁₋₄₂ and antibody anti-A β ₁₋₄₂ (0.5 mg/mL) were then added followed by 40 μ L of an anti-IgG-AP (1:15,000) solution and 3-indoxyl phosphate/silver (3-IP-Ag) solution respectively. AP hydrolyzes 3-IP resulting in an indoxyl intermediate, which in turn reduces the silver ions leading to their deposition on the electrode surface. The analytical signal used by García's group was based on the anodic stripping of enzymatically generated silver by cyclic voltammetry. The immunosensor demonstrated an LOD of 0.1 ng/mL and a wide linear range 0.5–500 ng/mL for A β .

Electrical impedance spectrometry (EIS) is another technique that is readily compatible with SPCE's based sensing devices. For example, Moreira et al. [120] utilized gold coated SPCE's coated with electropolymerized polyaniline (PANI) and a molecularly imprinted poly(α -cyclodextrin), (α -MCD) film to detect A β ₄₂ in PBS. EIS calibration curves of the molecularly imprinted film showed a proportional increase in charge transfer resistance (R_{ct}) values with increasing A β ₄₂ concentrations. The MCD/PANI/Au-SPE devices displayed a linear response down to 0.25 ng/mL and 0.20 ng/mL for A β ₄₂ and A β ₄₀. Shortly after, the same group reported on a simpler version of the sensor using a mediator-free device in which the electroactive element was integrated into the working electrode [121]. The sensor consisted of carbon nanotubes modified with copper nanoparticles (CNT-CuO) layered on a SPCE substrate. An electropolymerized biofilm containing A β ₄₂ imprinted polyaniline was fabricated on top of the copper nanoparticles. Testing of the imprinted film demonstrated an inverse relationship between A β ₄₂ concentration and redox current responses. Calculated LOD was 0.4 ng/mL and recovery rates of diluted serum samples spiked with A β ₄₂ ranged from 88% to 93%.

Inexpensive SPCE's and EIS have also been used in the detection of A β O species. For example, Rushworth et al. constructed a label free impedimetric A β O sensor in which a biotinylated segment of prion protein (PrP(95–110)) was conjugated to the surface of a conducting polymer layered on top of an Au-SPCE substrate. The sensor gave a linear response down to 0.5 pM for synthetically derived A β O [122]. Other researchers have also demonstrated the advantages of using gold electrodes for A β ₄₂ sensing by combining them with prion bioreceptors and conducting polymers. Recent work by Qin et al. [123] utilized a gold electrode functionalized with polypyrrole and PrPc in the detection of synthetic and naturally derived A β O. The multilayer electrode exhibited an electron-transfer resistance (R_{et}) of 89 Ω cm⁻², exhibiting an LOD of 10⁻⁴ pM for synthetic A β O. Further testing using control and AD mouse brain homogenates detected 1.679 $\times$ 10⁻⁷ nM A β O in the control and 1.45 $\times$ 10⁻⁶ nM A β O in the AD homogenate respectively. In 2018, the same group also used EIS and a robust poly (curcumin-Ni) electrode to detect presymptomatic levels of A β O. The sensor surface was easily fabricated using a simple one-step electro polymerization method. The impedance response of the curcumin-based sensor showed a proportional response to synthetic

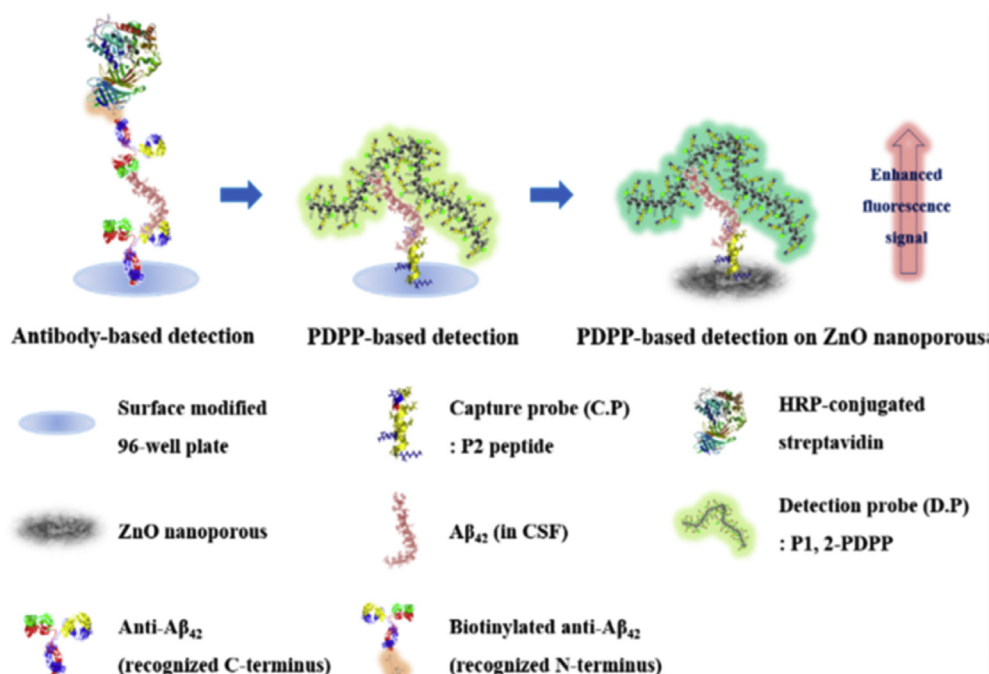


Fig. 7. An Aβ₄₂ detection system utilizing a ZnO nanoporous platform with immobilized peptide (P2) as the capture probe, and multivalent peptide (P1, 2-PDPP) as the detection probe, compared with a traditional sandwich ELISA. Reproduced with permission from American Chemical Society [116].

concentrations of AβO from 0.001 to 5 nM in PBS buffer, with the authors attributing said values to the analytes ability to bind strongly to the polycurcumin-Ni complex and slow electron transfer to the underlying nickel foam. Fig. 8 demonstrates the simple construction of the device, the enhanced conductivity of the curcumin complex and shift in the Nyquist plot when polycurcumin binds to AβO (see insert) [124]. Although it should be noted, in artificial cerebral spinal fluid, the poly (curcumin-Ni) AβO sensor demonstrated a detectable range of 0.1–5 nM for AβO and a moderate RSD value of 8.4. In addition, the authors did not compare the AβO specificity of their curcumin analogue with other hydrophobic curcumin analogues such as CRANAD 102 (52) which may have further improved the sensing performance of their device.

More recently, EIS was used in combination with square wave voltammetry (SWV) in the detection of tAβ₄₀ and pAβ₄₀ respectively [125]. The binding and chelation of pAβ₄₀ resulted in the electrochemical oxidation of cadmium and a subsequent increase in the sensitivity of the device. Concentrations as low as 1 fM for pAβ₄₀ were detected in artificial media. Further testing using CSF samples from healthy and AD patients showed that the concentration ratios of Aβ₄₀ and pAβ₄₀ in AD patients was significantly higher than those donated by healthy individuals.

Electrochemical luminescence (ECL) is an attractive Aβ sensing technique due to its excellent sensitivity, simple instrumentation, and wide linear range. Numerous ECL emitters for bioanalytical application have been utilized in the last decade such as metal nanoclusters, carbon nanotubes, ruthenium (II) and luminol. The application of electrochemiluminescence resonance energy transfer (ECL-RET) via the electron donor-acceptor pair Ru(bpy)₃²⁺ and gold nanorods was recently employed in the detection of Aβ species [126]. The electrochemiluminescent donor consisted of mesoporous carbon nanospheres (MOCs) complexed through a Nafion linker with Ru(bpy)₃²⁺. Gold nanorods (GNRs) bound to the DNA aptamer acted as the electron acceptor. Aβ measurements were performed using a 2-stage ELISA format. First, fluorescent MOC-bound antibodies were used to capture and collect Aβ in solution.

Then, a GNR-Aβ specific aptamer was added resulting in the binding and quenching of the MOC's fluorescence. A control assay was conducted in PBS and diluted plasma containing interferences like AFP, HCG, CEA, and CA19-9 but no signal was detected with any of them. An Aβ LOD of 4.2 fg/mL was demonstrated. Another fluorescent quenching AβO assay involving ruthenium was recently reported by Zhao et al. [127]. In this approach, the authors employed a dual-quenching electrochemiluminescence (ECL) strategy based on tris(2,2'-bipyridyl)-ruthenium(II) [Ru(bpy)₃²⁺] as chromophores caged in three-dimensional (3D) zinc oxalate metal-organic frameworks [Ru(bpy)₃²⁺/zinc oxalate MOFs] and Au@NiFe MOFs as the dual quencher were used in the ultrasensitive detection of Aβ. The assay demonstrated a 100 fg/mL-to 50 ng/mL range and an LOD of 13.8 fg/mL for Aβ.

As well as ruthenium, terbium atoms have been used as an ECL emitters in Aβ assays. In 2019, metal organic coordination polymers (MOCs) comprised of terbium (Tb³⁺) atoms as luminescence centers connected with 1,3,5-benzenetricarboxylate (BTC) were reported. One in every 8 Tb atoms was substituted with Cu²⁺ with the resultant Cu-BTC/Tb MOC complex remaining non-emissive at room temperature. Upon addition of Aβ₄₀ solution, Cu²⁺ vacated the luminescent center permitting ECL emission. A higher selectivity for Aβ₄₀ was observed over Aβ aggregates, though slight interference from L-glutathione (GSH) and L-cysteine (Cys) was reported [128]. Despite this, the measured LOD was 0.3 nM for Aβ₄₀ with the device exhibiting good reproducibility when tested on plasma samples. Another type of ECL Aβ aptamer assay employing copper ions and the EL emitter luminol was also able to detect full length Aβ₄₀ not only in phosphate buffer solution (PBS) solution but also in human serum [129]. In addition, the ECL aptasensor exhibited a favorable selectivity for Aβ₁₆ monomer with a linear range of 1.0×10^{-13} mol/L - 1.0×10^{-8} mol/L and a LOD of 3.5×10^{-14} mol/L (S/N = 3).

Luminol's limited number of primary amino groups hinders its successful conjugation with MOC and metal organic frameworks (MOFs). However, an analogue of luminol namely N-(aminobutyl)-

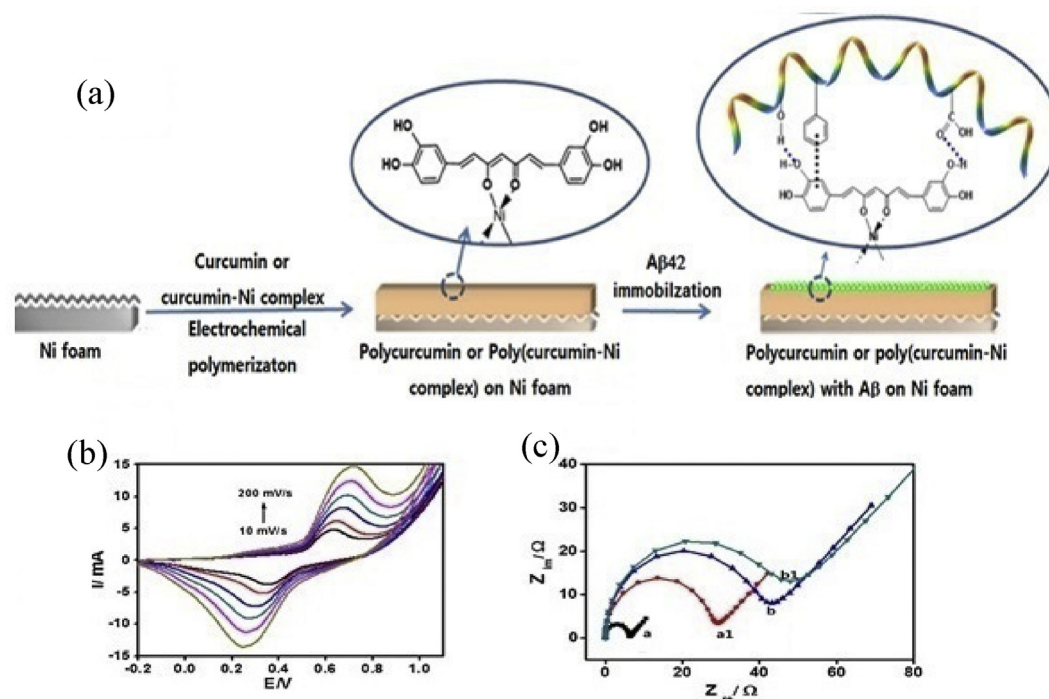


Fig. 8. (a) Schematic showing the complexation of polycurcumin and poly(curcumin-Ni) on Ni foam substrate and subsequent capture of Aβ. (b) CVs of poly(curcumin-Ni) (90th cycles) at different scan rate from 10 to 200 mV s⁻¹ with 0.1 M NaOH solution (c) Nyquist plots, poly(curcumin-Ni) a-a1 binding with AβO (1 nM solution); b-b1. polycurcumin binding with AβO (1 nM solution). Image modified with permission from Elsevier [124].

N-(ethylisoluminol) (ABEI) is well suited to conjugation with a MOF. Recently, Wang et al. [130], synthesized and then employed an ABEI cobalt based MOFs in the immunological detection of Aβ₄₂ in phosphate buffer solution (PBS). A linear correlation efficient of 0.99904 between the ECL intensity and the logarithm of Aβ₄₂ was achieved with the immunoassay exhibiting an LOD of 3 fg/mL. After 20 days' storage, no significant change in the detectable Aβ range was observed. Further testing with mixed protein solutions containing the interferences, carcino-embryonic antigen (CEA), prostate specific antigen (PSA), carbohydrate antigen 125, and Aβ₄₀ generated a negligible response from the ECL sensor.

In addition to EIS and ECL, other techniques such as differential pulse voltammetry (DPV) have been used in the detection of Aβ species. Of note [131] is the simple "aptasensor" reported by Negahdari and Heli for the quantification of Aβ₄₂. The aptasensor surface consisted of a gold electrode patterned with a fern-leaf-like gold structure upon which an RNA-Aβ₁₋₄₂ aptamer was immobilized. Binding of Aβ restricted the access of the redox marker to the aptamer surface resulting in a decrease in the DPV peak current. DPV curves were recorded before and after Aβ binding following immersion in a ferro/ferricyanide redox marker solution. The aptamer sensor exhibited an LOD of 0.4 pg/mL for synthetically derived Aβ₄₂ in purified media. Further testing using CSF and serum samples showed a weak response from protein interferences. Notably though, some overlap in the differential pulse voltammetry (DPV) curves for Aβ₄₂, human serum albumin (HSA), and bilirubin were detected.

Yu et al. employed DPV in conjunction with a nanocomposite sensor in the simultaneous measurement of Cu²⁺ and Aβ₁₋₄₂. The working electrode was coated with 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) and poly(-diallyldimethylammonium chloride), resulting in the formation of (PDDA)-bi functionalized single-walled carbon nanotubes or ABTS-PDDA/CNTs, to which copper selective neurokinin B (NKB) was

immobilized [132]. The single device demonstrated an LOD of 0.04 μM for Cu²⁺ and 0.5 ng/mL for Aβ₁₋₄₂ respectively.

In addition to modified SPCE's, Aβ species have been detected using photo sensitive nano-composite electrodes. In 2018 Ju et al., employed a Bi₂WO₆/CdS patterned ITO electrode coated with anti-Aβ₄₂ antibodies in a competitive Aβ₄₂ immunoassay [133]. The assay solution consisted of a 50:50 (v/v) mixture of MnCdSe-Aβ₄₂ and free Aβ₄₂ in PBS (pH 7.4) electrically enriched with 0.1 mol/L of ascorbic acid. Increasing the concentration of free amyloid in the mixture resulted in a proportional decrease in the photocurrent. The immunosensor demonstrated a linear range of 0.2 pg/mL-50 ng/mL with an ultralow LOD of 0.068 pg/mL for Aβ₄₂. Additional measurements using higher (2 orders of magnitude) concentrations of protein interferences such as PSA, insulin, and CEA resulted in a negligible response from the device. The authors attributed the ultra-high sensitivity of the assay to the high energy conversion efficiencies between CdS and CdSe.

As well as CdS and CdSe, other semiconducting materials such as SnO₂ and SnS₂ with similar transfer efficiencies have been employed in the photoelectrochemical (PEC) detection of Aβ₄₂. A good example is the recent work by Wei's group in which an ITO-SnO₂/SnS₂/Ag₂S layered electrode coated with anti-Aβ₄₂ was used in a simple immunoassay. The specific binding of anti-Aβ with Aβ hindered the electron transfer between ascorbic acid and the SnO₂/SnS₂/Ag₂S working electrode resulting in a decrease in the photocurrent. The standardization conditions were identical to those previously reported [133]. The immunosensor demonstrated a linear range of 0.5 pg/mL-100 ng/mL with an LOD of 0.56 pg/mL for Aβ₄₂. Although lower in sensitivity compared to similar PEC sensors [134], a higher degree of selectivity and larger detectable range for Aβ₄₂ was demonstrated. Moreover, the medical applicability of the device using human blood samples spiked with Aβ₄₂ was evaluated by standard addition methods, with the relative standard deviation (RSD) in the range of 1.5–3.6%. Furthermore, the results obtained

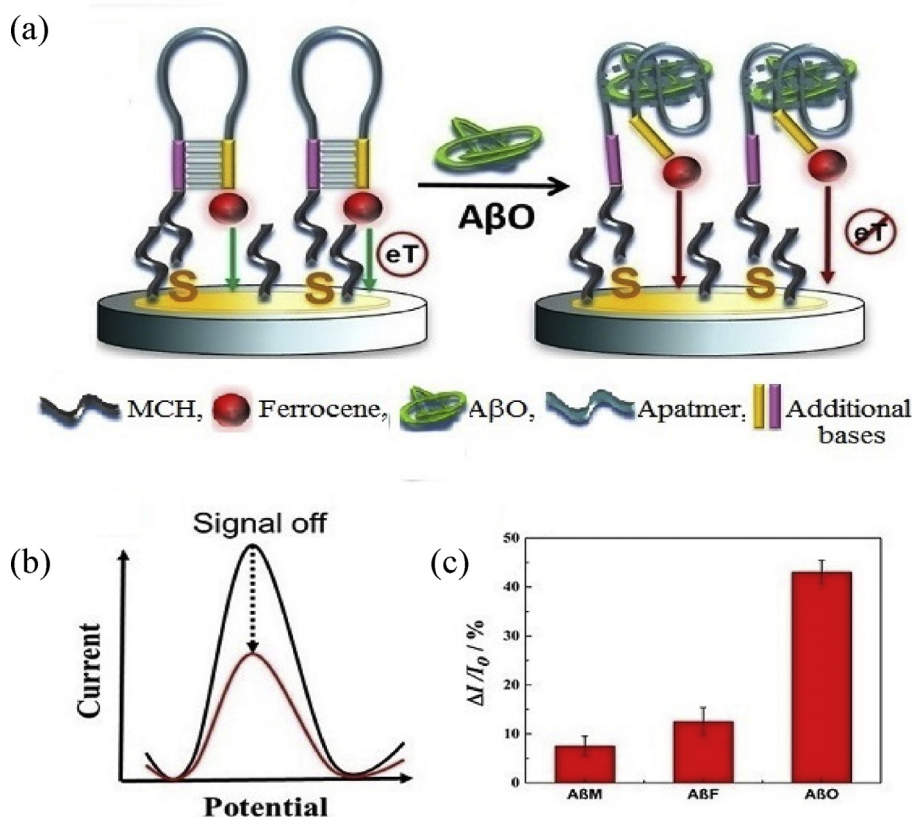


Fig. 9. (a) Schematic depicting the binding of AβO to the immobilized aptamer and ferrocene distancing from the electrode surface, resulting in reduced electron transference (eT) (b) corresponding reduction in current with binding (c) the selectivity of the probe for AβO, monomeric Aβ (mAb) and fibrillar Aβ (AβM) in PBS. MCH is 6-mercapto-1-hexanol. Image modified with permission from American Chemical Society [138].

Table 1

Recent methods used in the detection of AβO and various Aβ species.

Method	LOD	Medium	Aβ species	AD Application	Assay Time (min)	Reference & (Year)
ELISA	10 pg/mL	CSF	AβO	Early	60	69, (2018)
ELISA	200aM-1.7 fM	PBS, CSF	ADDL	Early	>120	70, (2005)
ELISA	0.09 pg/ml	CSF	AβO	Monitoring	>180	73, (2014)
MDS-OAβ	3 pg/mL	Blood	AβO	Presymptomatic	60 min	78, (2015)
SIMOA	0.22 nM	PBS	AβO	Monitoring	>360min	83, (2019)
MPA-LVSEP	0.05 nM	CSF	Aβ ₁₋₄₂ , 2-40, 2-42	Early	<15 min	92, (2018)
SERS	10 nM	Protein solution	AβO	Monitoring	>120min	97, (2018)
Fluorescence	12.5 nM	Protein solution	AβO	Monitoring	>40min	113, (2017)
Fluorescence	1 nM	Artificial CSF	AβO	Monitoring	>5min	114, (2015)
Fluorescence	12 ag/mL	CSF	Aβ ₄₂	Presymptomatic	>120min	116, (2019)
EIS	0.5 pM	Buffer	AβO	Presymptomatic	>45min	122, (2014)
EIS	10 ⁻⁴ pM	Buffer	AβO	Presymptomatic	>60min	123, (2018)

from human samples using the PEC device compared favorably with those obtained with a commercial Aβ ELISA. It is worth noting many of the electrochemical sensor configurations are applicable in the detection of other AD markers such as chlorine ions, blood prion proteins, acetylcholine and Aβ₁₋₄₂ metabolites [135–137]. It is debatable whether either monomeric Aβ₁₋₄₂ or its oligomerized counterpart is the most suitable marker for AD. As previously mentioned, the concentration of AβO is very low comparable to other monomeric species found in CSF, thus ultralow limits of detection are needed to support an accurate diagnosis. In attempt to meet the demand for a simple, ultrasensitive electrochemical AβO sensor suitable for point of care settings Mayer et al. recently reported an amperometric ferrocene based aptasensor, with an exceeding large detection range spanning six orders of magnitude,

with high selectivity and sub-picomolar sensitivity. The sensor consisted of a classic 3 wire electrode configuration upon which an optimized stem loop structure was immobilized via a thiol group at the proximal end, with ferrocene (redox label) attached to the distal end in close proximity to the electrode surface. Binding of AβO to the optimized loop resulted in its opening and subsequent distancing of ferrocene from the electrode surface which in turn reduced the charge transfer current. A schematic depicting sensor operation and its response characteristics to different Aβ species in artificial CSF containing HSA are shown in Fig. 9. However, there are several disadvantages to be considered such as the use of expensive crystal gold electrodes, the long immobilization and assay times needed to obtain the level of sensitivity demonstrated herein. Perhaps the same level of sensitivity could have been achieved, via

a combination of less expensive electrode materials (graphene) and Fourier transformed alternating current voltammetry instead [138]. An up to date summary of the different sensing formats used in the detection of A β O are shown in Table 1.

4. Conclusion

Mounting evidence suggest that A β O is one of the major neurotoxic agents in AD capable of inducing the oligomerization of numerous proteins along the ACT pathway. Currently there are various commercial diagnostic platforms and analytical techniques (MDS-OA β , SIMoA, sFIDA, MPA-LVSEP) capable of providing the sensitivity needed to detect ultra-low A β O levels in the CSF and blood from patients in the pre-symptomatic and prodromal phases of AD. sFIDA is unique in that it uses silica nanoparticles for oligomer standardization and signal enhancement, thus acquiring ultrasensitivity and regulatory alignment at cost. It is also worth noting that M-PA-LVSEP has the potential to generate a complete spectrum of A β species from a single CSF sample including highly neurotoxic agents such as pyroglutamate-A β and other proteoforms of A β that constitute 25% of all A β species. Furthermore, it appears that this technique is scalable and well suited to microcapillary array technologies potentially putting an end to the debate regarding the cost of targeting multiple neurotoxic species in AD diagnosis. Encouragingly, recent attempts to integrate some of the well-known analytical techniques and PET imaging probes such as ACV and CRANAD into handheld biosensing devices have met with some success, although these devices still remain in the early stages of development.

Currently there are no AD sensing devices that are used routinely outside the clinic by untrained individuals. Patient presentation, physician assessment and post-mortem identification are still required for a positive diagnosis of AD making the differential diagnosis of the pre-symptomatic stages of the disease extremely challenging. As AD is generally considered a multifactorial disease it would seem intuitive that a multiplex platform is needed. Until recently there was only one platform capable achieving of this, the Pronucleon platform from Adlyfe, [139]. However, a novel kit that uses labeled fluorescent nanoparticles for early diagnosis of AD was recently reported by Park et al. in which 22 (miR-106b, miR-146b, miR-181a, miR200a, miR-34a, miR-124b, miR-153, miR-155, A β ₁₋₄₂ monomer (mA β), A β ₁₋₄₂ oligomer (oA β), UCHL1, NLRP3, Tau, STAT3, SORL1, Clusterin, APOE3, APOE4, Nogo-A, IL-13, and Visfatin) AD associated biomarkers including AB₁₋₄₂ oligomer were tested on plasma from AD patients and used to identify different stages of the disease [140]. According to the results, miR-106b and miR-153 showed significant potential as early stage diagnostic markers for AD. The advantage of this kit is that it is minimally invasive, uses blood, and unlike other approaches is convenient, inexpensive and fast. Yet the instrumentation used with the kit is very similar to that employed in SIMoA and sFIDA and exceedingly expensive; on the upside once further investigations have completed and groups of biomarkers identified for early stage diagnosis it should be possible to test a smaller battery of biomarkers using a simple biosensor.

Underpinning all these advances in early stage diagnostics and biosensing devices are tailored nanostructured materials. Tailoring of these materials regarding size, physical properties and multi-layered complexity has led to unparallel improvements in diagnostics and device performance (sensitivity and range) as well reductions in sample processing costs. Furthermore, with the advent of paper microfluidics, graphene technologies, smart nanomaterials and micro spectrometers [141] the application of ultrasensitive amyloid beta devices in POC settings is a very real possibility in the near future.

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References

- [1] Alzheimer's Association, Alzheimer's disease facts and figures, *Alzheimer's Dementia* 14 (2018) 367–429.
- [2] L.E. Hebert, J. Weuve, P.A. Scherr, D.A. Evans, Alzheimer disease in the United States (2010–2050) estimated using the 2010 Census, *Neurology* 80 (2013) 1778–1783.
- [3] World Alzheimer Report, Alzheimer's Disease International, 2018. www.alz.co.uk/adi.
- [4] M. Ruthirakuhana, K.L. Lancot, M. Di Scipio, M. Ahmed, N. Herrmann, Biomarkers of agitation and aggression in Alzheimer's disease: a systematic review, *Alzheimer's Dementia* 14 (2018) 1344–1376. <https://doi.org/10.1016/j.jalz.2018.04.013>.
- [5] C. Osorio, T. Kanukuntla, E. Diaz, N. Jafri, M. Cummings, A. Sfera, The post amyloid era in Alzheimer's disease: trust your gut Feeling, *Front. Aging Neurosci.* 11 (2019) 143. <https://doi.org/10.3389/fnagi.2019.00143>.
- [6] E.N. Cline, M.A. Bicca, K.L. Viola, W.L. Klein, The amyloid- β oligomer hypothesis: beginning of the third decade, *J. Alzheim. Dis.* 64 (2018) S567–S610.
- [7] S.C. Neu, J. Pa, W. Kukull, D. Beekly, A. Kuzma, P. Gangadharan, et al., Apolipoprotein E genotype and sex risk factors for alzheimer disease: a meta-analysis, *JAMA Neurol* 74 (2017) 1178–1189. <https://doi.org/10.1001/jamaneurol.2017.2188>.
- [8] A. Aliyan, N.P. Cook, A.A. Martí, Interrogating amyloid aggregates using fluorescent probes, *Chem. Rev.* 119 (2019) 11819–11856. <https://doi.org/10.1021/acs.chemrev.9b00404>.
- [9] N. Naruse, H. Satooka, K. Todo, A. Nakanishi, H. Taguchi, Y. Mera, Oligomers imaging of amyloid- β 1-42 by scanning tunneling microscopy, *Jpn. J. Appl. Phys.* 58 (2019) S11B30.
- [10] L. Gremer, D. Schölzel, C. Schenk, E. Reinartz, J. Labahn, R. Ravelli, G.F. Schröder, Fibril structure of amyloid- β (1-42) by cryo-electron microscopy, *Science* 358 (2017) 116–119. <https://doi.org/10.1126/science.aao2825>.
- [11] C.L. Satizabal, A.S. Beiser, V. Chouraki, G. Chene, C. Dufouil, S. Seshadri, Incidence of dementia over three decades in the framingham heart study, *N. Engl. J. Med.* 374 (2016) 523–532. <https://doi.org/10.1056/NEJMoa1504327>.
- [12] C.R. Jack, D.A. Bennett, K. Blennow, M.C. Carrilod, B. Dunn, et al., NIA-AA Research Framework: toward a biological definition of Alzheimer's disease, *Alzheimer's Dementia* 14 (2018) 535–562. <https://doi.org/10.1016/j.jalz.2018.02.018>.
- [13] A. Maass, S. Landau, S.L. Baker, A. Horng, S.N. Lockhart, R. La Joie, et al., Comparison of multiple tau-PET measures as biomarkers in aging and Alzheimer's disease, *Neuroimage* 157 (2017) 448–463.
- [14] H. Zetterberg, T. Skillback, N. Mattsson, J.Q. Trojanowski, E. Portelius, L.M. Shaw, et al., Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression, *JAMA Neurol* 73 (2016) 60–67. <https://doi.org/10.1016/j.jneuroim.2017.05.058>.
- [15] R.P. Friedland, M.R. Chapman, The role of microbial amyloid in neurodegeneration, *PLoS Pathog.* 13 (2017) e1006654. <https://doi.org/10.1371/journal.ppat.1006654>.
- [16] S.S.A. An, B.S. Lee, Ji S. Yu, K. Lim, G. Je Kim, R. Lee, et al., Dynamic changes of oligomeric amyloid β levels in plasma induced by spiked synthetic A β 42, *Alzheimers Res. Ther.* 9 (2017). <https://doi.org/10.1186/s13195-017-0310-6>.
- [17] M. Novotny, B. Klimova, M. Valis, Nitrendipine and dementia: forgotten positive facts? *Front. Aging Neurosci.* 10 (2018) 418. <https://doi.org/10.3389/fnagi.2018.00418>.
- [18] C.W. Zhu, H. Grossman, J. Neugroschl, S. Parker, A. Burden, X. Luo, M. Sano, A randomized, double-blind, placebo-controlled trial of resveratrol with glucose and malate (RGM) to slow the progression of Alzheimer's disease: a pilot study, *Alzheimer's Dementia (New York, N. Y.)* 4 (2018) 609–616. <https://doi.org/10.1016/j.trci.2018.09.009>.
- [19] H. Li, C.C. Liu, H. Zheng, T.Y. Huang, Amyloid, tau, pathogen infection and antimicrobial protection in Alzheimer's disease-conformist, nonconformist, and realistic prospects for AD pathogenesis, *Transl. Neurodegener.* 7 (2018). <https://doi.org/10.1186/s40035-018-0139-3>.
- [20] J. Cummings, G. Lee, A. Ritter, K. Zhong, Alzheimer's Disease Drug Development Pipeline, *Alzheimers Dement*, NY, 2018, pp. 42018195–42018214. <https://doi.org/10.1016/j.trci.2018.03.009>.
- [21] Biogen and Eisai to Discontinue Phase 3 Engage and Emerge Trials of Aducanumab in Alzheimer's Disease, Press release, 2019 March 21. Retrieved from: <http://investors.biogen.com/news-releases/news-release-details/biogen-and-eisai-discontinue-phase-3-engage-and-emerge-trials>.

- [22] A. Kaushik, R.D. Jayant, S. Tiwari, A. Vashist, M. Nair, Nano-biosensors to detect beta-amyloid for Alzheimer's disease management, *Biosens. Bioelectron.* 80 (2016) 273–287. <https://doi.org/10.1016/j.bios.2016.01.065>.
- [23] J. Knopman, D.S. Weigand, S.D. Wiste, H.J. Vemuri, P. Lowe, et al., An operational approach to National Institute on Aging–Alzheimer's Association criteria for preclinical Alzheimer disease, *Ann. Neurol.* 71 (2012) 765–775. <https://doi.org/10.1002/ana.22628>.
- [24] Y. Lin, P.Y. Shan, W.J. Jiang, C. Sheng, L. Ma, Subjective cognitive decline: preclinical manifestation of Alzheimer's disease, *Neurol. Sci.* 40 (2019) 41–49. <https://doi.org/10.1007/s10072-018-3620-y>.
- [25] T. Kawagoe, K. Onoda, S. Yamaguchi, Subjective memory complaints are associated with altered resting-state functional connectivity but not structural atrophy, *Neuroimage Clin* (2019) 101675. <https://doi.org/10.1016/j.nicl.2019.101675>.
- [26] P.S.J. Weston, J.M. Nicholas, S.M.D. Henley, Y. Liang, K. Macpherson, E. Donnachie, et al., Accelerated long-term forgetting in presymptomatic autosomal dominant Alzheimer's disease: a cross-sectional study, *Lancet Neurol.* 17 (2018) 123–132. [https://doi.org/10.1016/S1474-4422\(17\)30434-9](https://doi.org/10.1016/S1474-4422(17)30434-9).
- [27] I. Dal Prà, A. Chiarini, U. Armato, Antagonizing amyloid- β /calcium-sensing receptor signaling in human astrocytes and neurons: a key to halt Alzheimer's disease progression? *Neural Regen. Res.* 10 (2015) 213–218. <https://doi.org/10.4103/1673-5374.152373>.
- [28] Y. Xie, Y. Tan, Y. Zheng, X. Du, Q. Liu, Ebselen ameliorates β -amyloid pathology, tau pathology, and cognitive impairment in triple-transgenic Alzheimer's disease mice, *J. Biol. Inorg. Chem.* 22 (2017) 851–865. <https://doi.org/10.1007/s00775-017-1463-2>.
- [29] F. Martini, S.G. Rosa, I.P. Klann, B.C.W. Fulco, F.B. Carvalho, F.L. Rahmeier, et al., A multifunctional compound ebselen reverses memory impairment, apoptosis and oxidative stress in a mouse model of sporadic Alzheimer's disease, *J. Psychiatr. Res.* 109 (2019) 107–117. <https://doi.org/10.1016/j.jpsychires.2018.11.021>.
- [30] S. Sanz-Blasco, J.C. Piña-Crespo, X. Zhang, S.R. McKercher, S.A. Lipton, Levetiracetam inhibits oligomeric A β -induced glutamate release from human astrocytes, *Neuroreport* 27 (2016) 705–709. <https://doi.org/10.1097/WNR.0000000000000601>.
- [31] R.D. Moira, R. Lathe, R.E. Tanzi, The antimicrobial protection hypothesis of Alzheimer's disease, *Alzheimer's Dementia* 14 (2018) 1602–1614. <https://doi.org/10.1016/j.jalz.2018.06.3040>.
- [32] K. Bourgade, A. Le Page, C. Bocti, J.M. Witkowski, G. Dupuis, E.H. Frost, et al., Protective effect of amyloid- β peptides against herpes simplex virus-1 infection in a neuronal cell culture model, *J. Alzheimers Dis.* 50 (2016) 1227–1241. <https://doi.org/10.3233/JAD-150652>.
- [33] Y. Wu, S. Du, J.L. Johnson, H.-Y. Tung, C.T. Landers, Y. Liu, et al., Microglia and amyloid precursor protein coordinate control of transient Candida cerebritis with memory deficits, *Nat. Commun.* 10 (2019). <https://doi.org/10.1038/s41467-018-07991-4>.
- [34] Y. Xie, Y. Tan, Y. Zheng, X. Du, Q. Liu, in: *Journal of biological inorganic chemistry JBIC: a publication of the Society of Biological Inorganic Chemistry*, vol. 22, 2017, pp. 851–865.
- [35] P. Carneiro, Simone Morais, M.C. Pereira, Nanomaterials towards biosensing of Alzheimer's disease biomarkers, *Nanomaterials* 9 (2019) 1663. <https://doi.org/10.3390/nano9121663>.
- [36] M. Sochocka, K. Donskow-Lysoniewska, B. Satler Diniz, D. Kurpas, E. Brzozowska, J. Leszek, The gut microbiome alterations and inflammation-driven pathogenesis of Alzheimer's disease—a critical review, *Mol. Neurobiol.* 56 (2019) 1841–1851. <https://doi.org/10.1007/s12035-018-1188-4>.
- [37] S.E. Garfinkle, Matter over mind: liquid phase separation and neurodegeneration, *J. Biol. Chem.* 294 (2019) 7160–7168. <https://doi.org/10.1074/jbc.REV118.001188>.
- [38] E. Chatani, N. Yamamoto, Recent progress on understanding the mechanisms of amyloid nucleation, *Biophys. Rev.* 10 (2018) 527–534. <https://doi.org/10.1007/s12551-017-0353-8>.
- [39] Y.J. Choi, S. Chae, J.H. Kim, K.F. Barald, J.Y. Park, S.H. Lee, Neurotoxic amyloid beta oligomeric assemblies recreated in microfluidic platform with interstitial level of slow flow, *Sci. Rep.* 3 (2013) 1921. <https://doi.org/10.1038/srep01921>.
- [40] J. Park, B.K. Lee, G.S. Jeong, J.K. Hyun, C.J. Lee, S.H. Lee, Three-dimensional brain-on-a-chip with an interstitial level of flow and its application as an *in vitro* model of Alzheimer's disease, *Lab Chip* 15 (2015) 141–150. <https://doi.org/10.1039/c4lc00962b>.
- [41] T. Osaki, Y. Shin, V. Sivathanu, M. Campisi, R.D. Kamm, *In vitro* microfluidic models for neurodegenerative disorders, *Adv. Healthcare Mater.* 7 (2018) 1700489.
- [42] G.F. Chen, T.H. Xu, Y. Yan, Y.R. Zhou, Y. Jiang, K. Melcher, H.E. Xu, Amyloid beta: structure, biology and structure-based therapeutic development, *Acta Pharmacol. Sin.* 38 (2017) 1205–1235. <https://doi.org/10.1038/aps.2017.28>.
- [43] K.A. Ganzinger, P. Narayan, S.S. Qamar, L. Weimann, R.T. Ranasinghe, A. Aguzzi, C.M. Dobson, et al., Single-molecule imaging reveals that small amyloid-beta(1–42) oligomers interact with the cellular prion protein (PrP^C), *Chembiochem* 15 (2014) 2515–2521.
- [44] C. Peters, M.P. Espinoza, S. Gallegos, C. Opazo, L.G. Aguayo, Alzheimer's A β interacts with cellular prion protein inducing neuronal membrane damage and synaptotoxicity, *Neurobiol. Aging* 36 (2015) 1369–1377.
- [45] E.S. Song, D.W. Rodgers, L.B. Hersh, Insulin-degrading enzyme is not secreted from cultured cells, *Sci. Rep.* 8 (2018) 2335. <https://doi.org/10.1038/s41598-018-20597-6>.
- [46] F.A. Caetano, F.H. Beraldo, G.N. Hajj, A.L. Guimaraes, S. Jurgensen, A.P. Wasilewska-Sampaio, et al., Amyloid beta oligomers increase the localization of prion protein at the cell surface, *J. Neurochem.* 117 (2011) 538–553.
- [47] H.B. Nygaard, C.H. van Dyck, S.M. Strittmatter, Fyn kinase inhibition as a novel therapy for Alzheimer's disease, *Alzheimer's Res. Ther.* 6 (2014). <https://doi.org/10.1186/alzrt238>.
- [48] X. Nie, M.F. Falangola, R. Ward, E.T. McKinnon, J.A. Helpner, P.J. Nietert, et al., Diffusion MRI detects longitudinal white matter changes in the 3xTg-AD mouse model of Alzheimer's disease, *Magn. Reson. Imaging* 57 (2019) 235–242. <https://doi.org/10.1016/j.mri.2018.12.003>.
- [49] C. Anckaerts, I. Blockx, P. Summer, J. Michael, J. Hamaide, C. Kreutzer, et al., Early functional connectivity deficits and progressive microstructural alterations in the TgF344-AD rat model of Alzheimer's Disease: a longitudinal MRI study, *Neurobiol. Dis.* 124 (2019) 93–107. <https://doi.org/10.1016/j.nbd.2018.11.010>.
- [50] Y. Guo, X.Y. Li, M. Zhang, S. Wu, J. Lei, Z. Wang, et al., Age-and brain region-associated alterations of cerebral blood flow in early Alzheimer's disease assessed in A β PPSWE/PS1 Δ E9 transgenic mice using arterial spin labeling, *Mol. Med. Rep.* 19 (2019) 3045–3052, 2019.
- [51] C. Marcus, E. Mena, R.M. Subramaniam, Brain PET in the diagnosis of Alzheimer's disease, *Clin. Nucl. Med.* 39 (2014) e413–e422. quiz e423–6.
- [52] L. Mosconi, Glucose metabolism in normal aging and Alzheimer's disease: methodological and physiological considerations for PET studies, *Clin Transl Imaging* 1 (2013). <https://doi.org/10.1007/s40336-013-0026-y>.
- [53] D.F. Wong, P.B. Rosenberg, Y. Zhou, A. Kumar, V. Raymont, H.T. Ravert, et al., *Vivo* imaging of amyloid deposition in Alzheimer disease using the radioligand 18F-AV-45, *J. Nucl. Med.* 51 (2010) 913–920.
- [54] C.M. Clark, J.A. Schneider, B.J. Bedell, T.G. Beach, W.B. Bilker, M.A. Mintun, et al., Use of florbetapir-PET for imaging beta-amyloid pathology, *J. Am. Med. Assoc.* 305 (2011) 275–283.
- [55] V.L. Villemagne, K. Ong, R.S. Mulligan, G. Holl, S. Pejoska, G. Jones, et al., Amyloid imaging with (18)Fflorbetaben in Alzheimer disease and other dementias, *J. Nucl. Med.* 52 (2011) 1210–1217.
- [56] M. Mizoi, M. Yoshida, R. Saiki, M. Waragai, K. Uemura, H. Akatsu, et al., Distinction between mild cognitive impairment and Alzheimer's disease by CSF amyloid beta40 and beta42, and protein-conjugated acrolein, *Clin. Chim. Acta* 430 (2014) 150–155.
- [57] J.T. O'Brien, K. Herholz, Amyloid imaging for dementia in clinical practice, *BMC Med.* 13 (2015) 163.
- [58] H. Tana, K. Zhou, J. Yana, H. Suna, M. Pistozozia, M. Cuic, et al., Dual-functional red-emitting fluorescent probes for imaging beta-amyloid plaques and viscosity, *Sens. Actuators, B* 298 (2019) 126903. <https://doi.org/10.1016/j.snb.2019.126903>.
- [59] X. Zhang, Y. Tian, Z. Li, H. Sun, H. Liu, A. Moore, et al., Design and synthesis of curcumin analogues for *in vivo* fluorescence imaging and inhibiting copper-induced cross-linking of amyloid beta species in Alzheimer's disease, *J. Am. Chem. Soc.* 135 (2013) 16397–16409. <https://doi.org/10.1021/ja405239v>.
- [60] H. Fu, P. Tu, L. Zhao, Amyloid- β deposits target efficient near-infrared fluorescent probes: synthesis, *in vitro* evaluation, and *in vivo* imaging, *Anal. Chem.* 88 (2016) 1944–1950. <https://doi.org/10.1021/acs.analchem.5b04441>.
- [61] Y. Li, J. Yang, H. Liu, J. Yang, L. Du, H. Feng, et al., Tuning the stereo-hindrance of a curcumin scaffold for the selective imaging of the soluble forms of amyloid beta species, *Chem. Sci.* 8 (2017) 7710–7717. <https://doi.org/10.1039/C7SC02050C>.
- [62] C.L. Teoh, D.D. Su, S. Sahu, S.-W. Yun, E. Drummond, F. Prelli, et al., Chemical fluorescent probe for detection of A β oligomers, *J. Am. Chem. Soc.* 137 (2015) 13503–13509. <https://doi.org/10.1021/jacs.5b06190>.
- [63] G. Lv, A. Sun, P. Wei, N. Zhang, H. Lana, T. Yi, A spiropyran-based fluorescent probe for the specific detection of β -amyloid peptide oligomers in Alzheimer's disease, *Chem. Commun.* 52 (2016) 8865–8868. <https://doi.org/10.1039/C6CC02741E>.
- [64] Y. Li, D. Xu, H.N. Chan, C.Y. Poon, S.L. Ho, H.W. Li, et al., Dual-modal NIR-fluorophore conjugated magnetic nanoparticle for imaging amyloid- β species *in vivo*, *Small* 14 (2018) 1800901. <https://doi.org/10.1002/smll.201800901>.
- [65] C. Peng, X. Wang, Y. Li, H.-W. Li, M.S. Wong, Versatile fluorescent probes for near-infrared imaging of amyloid- β species in Alzheimer's disease mouse model, *J. Mater. Chem. B* 7 (2019) 1986–1995. <https://doi.org/10.1039/c9tb00161a>.
- [66] S. Strickland, J. Blood will out: vascular contributions to Alzheimer's disease, *Clin. Invest.* 128 (2018) 556–563. <https://doi.org/10.1172/JCI97509>.
- [67] S. Kumar, P.H. Reddy, MicroRNA-455-3p as a potential biomarker for Alzheimer's disease: an update, *Front. Aging Neurosci.* 10 (2018) 41. <https://doi.org/10.3389/fnagi.2018.00041>.
- [68] U. Smailovic, T. Koenig, I. Kåreholt, T. Andersson, M.G. Kramberger, B. Winblad, et al., Quantitative EEG power and synchronization correlate with Alzheimer's disease CSF biomarkers, *Neurobiol. Aging* 63 (2018) 88–95. <https://doi.org/10.1016/j.neurobiolaging.2017.11.005>.

- [69] T.-Y. Wei, Yun Fu, K.H. Chang, K.J. Lin, Y.J. Lu, C.M. Cheng, Point-of-Care devices using disease biomarkers to diagnose neurodegenerative disorders, *Trends Biotechnol.* 36 (2018). <https://doi.org/10.1016/j.tibtech.2017.11.004>.
- [70] D.G. Georganopoulou, L. Chang, J.M. Nam, C.S. Thaxton, E.J. Mufson, W.L. Klein, et al., Nanoparticle based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease, *Proc. Natl. Acad. Sci. U.S.A.* 102 (2005) 2273–2276.
- [71] M. Fandrich, Oligomeric intermediates in amyloid formation: structure determination and mechanisms of toxicity, *J. Mol. Biol.* 421 (2012) 427–440.
- [72] M. Holttä, O. Hansson, U. Andreasson, J. Hertz, L. Minthon, K. Nagga, N. Andreasen, H. Zetterberg, K. Blennow, Evaluating amyloid-beta oligomers in cerebrospinal fluid as a biomarker for Alzheimer's disease, *PLoS One* 8 (2013), e66381.
- [73] M.J. Savage, J. Kalinina, A. Wolfe, K. Tugusheva, R. Korn, T. Cash-Mason, et al., A sensitive beta oligomer assay discriminates Alzheimer's and aged control cerebrospinal fluid, *J. Neurosci.* 34 (2014) 2884–2897.
- [74] T. Yang, S. Hong, T. O'Malley, R.A. Sperling, D.M. Walsh, D.J. Selkoe, New ELISAs with high specificity for soluble oligomers of amyloid beta protein detect natural A-beta oligomers in human brain but not CSF, *Alzheimers Dement* 9 (2013) 99–112.
- [75] S.S. An, K. Lim, B.S. Lee, G.J. Kim, M. Kang, S. Kang, et al., Detecting amyloid-beta oligomers in Alzheimer patient's blood using multimer detection system, *Alzheimer's Dementia* 6 (2010) S518–S519.
- [76] M.J. Wang, S.H. Yi, J.Y. Han, S.Y. Park, J.W. Jang, I.K. Chun, et al., Alzheimer's Res. Ther. 9 (2017) 98. <https://doi.org/10.1186/s13195-017-0324-0>.
- [77] Y.C. Youn, S. Kang, J. Suh, Y.H. Park, M.J. Kang, J.M. Pyun, et al., Blood amyloid- β oligomerization associated with neurodegeneration of Alzheimer's disease, *Alzheimer's Res. Ther.* 11 (2019) 40. <https://doi.org/10.1186/s13195-019-0499-7>.
- [78] M.C. Park, M. Kim, G.T. Lim, S.M. Kang, S.S. An, T.S. Kim, et al., Droplet-based magnetic bead immunoassay using microchannel-connected multiwell plates (μ CHAMPS) for the detection of amyloid beta oligomers, *Lab Chip* 16 (2016) 2245–2253. <https://doi.org/10.1039/c6lc00013d>.
- [79] L.G. Ceballos, A. Asanov, L. Vaca, Single-channel single-molecule detection (SC-SMD) system, *Methods Mol. Biol.* 1843 (2018) 189–201. https://doi.org/10.1007/978-1-4939-8704-7_16.
- [80] T. Weber, *J. Lab. Med.* 42 (2018) 121–130.
- [81] L. Song, D.R. Lachno, D. Hanlon, A. Shepro, A. Jeromin, D. Gemani, et al., A digital enzyme-linked immunosorbent assay for ultrasensitive measurement of amyloid- β 1–42 peptide in human plasma with utility for studies of Alzheimer's disease therapeutics, *Alzheimer's Res. Ther.* 8 (2016) 58. <https://doi.org/10.1186/s13195-016-0225-7>.
- [82] S. Janelidze, E. Stomrud, S. Palmqvist, H. Zetterberg, D. van Westen, A. Jeromin, et al., Plasma β -amyloid in Alzheimer's disease and vascular disease, *Sci. Rep.* 6 (2016) 26801.
- [83] S.S. Hwan, H. Chan, M. Sorci, J. Van Deventer, D. Wittrup, G. Belfort, D. Walt, et al., Detection of amyloid β oligomers toward early diagnosis of Alzheimer's disease, *Anal. Biochem.* 566 (2019) 40–45. <https://doi.org/10.1016/j.ab.2018.09.011>.
- [84] J.K. Adams, V. Boominathan, B.W. Avants, D.G. Vercosa, F. Ye, R.G. Baraniuk, et al., Single-frame 3D fluorescence microscopy with ultraminiature lensless FlatScope, *Science Advances* 3 (2017) e1701548. <https://doi.org/10.1126/sciadv.1701548>.
- [85] S.K. Fischer, A. Joyce, M. Spengler, T.Y. Yang, Y. Zhuang, M.S. Fjording, et al., Emerging technologies to increase ligand binding assay sensitivity, *AAPS J.* 17 (2014) 93–101. <https://doi.org/10.1208/s12248-014-9682-8>.
- [86] J. Kalinina, J. Brunner, L. Yao, N.G. Hatcher, P.R. Gehrmann, M.A. Kling, et al., A reciprocal relationship between sleep and ALZHEIMER'S disease? *Sleep* 40 (2017) 0280. <https://doi.org/10.1093/sleep/zsx050.279>.
- [87] M. Hulsemann, C. Zafiu, K. Kuhbach, N. Luhmann, Y. Herrmann, L. Peters, et al., Biofunctionalized silica nanoparticles: standards in amyloid-beta oligomer-based diagnosis of Alzheimer's disease, *J. Alzheimers Dis* 54 (2016) 79–88.
- [88] Y. Herrmann, T. Bujnicki, C. Zafiu, A. Kulawik, K. Kuhbach, L. Peters, et al., Nanoparticle standards for immuno-based quantitation of alpha-synuclein oligomers in diagnostics of Parkinson's disease and other synucleinopathies, *Clin. Chim. Acta* 466 (2017) 152–159.
- [89] L. Wang-Dietrich, S.A. Funke, K. Kuhbach, K. Wang, A. Besmehn, S. Willbold, al Cinar, The amyloid-beta oligomer count in cerebrospinal fluid is a biomarker for Alzheimer's disease, *J. Alzheimers. Dis.* 34 (2013) 985–994.
- [90] T.D. Mai, I. Pereiro, M. Hirau, J.-L. Viovy, S. Descroix, M. Taverna, et al., Magneto-immunocapture with on-bead fluorescent labeling of amyloid- β peptides: towards a microfluidized-bed-based operation, *Analyst* 140 (2015) 5891–5900. <https://doi.org/10.1039/c5an01179e>.
- [91] T.D. Mai, D. Ferraro, N. Aboud, R. Renault, M. Serra, N.T. Tran, et al., Single-step immunoassays and microfluidic droplet operation: towards a versatile approach for detection of amyloid- β peptide-based biomarkers of Alzheimer's disease, *Sens. Actuators, B* 255 (2018) 2126–2135. <https://doi.org/10.1016/j.snb.2017.09.003>.
- [92] C.C. Lassichère, T.D. Mai, M. Otto, M. Taverna, Online preconcentration in capillaries by multiple large-volume sample stacking: an alternative to immunoassays for quantification of amyloid beta peptides biomarkers in cerebrospinal fluid, *Anal. Chem.* 90 (2018) 2555–2563. <https://doi.org/10.1021/acs.analchem.7b03843>.
- [93] D. Kuroski, R.P. Van Duyne, I.K. Lednev, Exploring the structure and formation mechanism of amyloid fibrils by Raman spectroscopy: a review, *Analyst* 140 (2015) 4967–4980. <https://doi.org/10.1039/c5an00342c>.
- [94] G. Devitt, K. Howard, A. Mudher, S. Mahajan, Raman spectroscopy: an emerging tool in neurodegenerative disease research and diagnosis, *ACS Chem. Neurosci.* 9 (2018) 404–420.
- [95] D. Shea, C.C. Hsu, T.M. Bi, N. Paranjaype, M.C. Childers, J. Cochran, et al., α -Sheet secondary structure in amyloid β -peptide drives aggregation and toxicity in Alzheimer's disease, *Proc. Natl. Acad. Sci. U. S. A.* 116 (2019) 8895–8900. <https://doi.org/10.1073/pnas.1820585116>.
- [96] L. Breydo, D. Kuroski, S. Rasool, S. Milton, J.W. Wu, V.N. Uversky, et al., 5 Structural differences between amyloid beta oligomers, *Biochem. Biophys. Res. Commun.* 477 (2016) 700–705. <https://doi.org/10.1016/j.bbrc.2016.06.122>.
- [97] L. D'Urso, M. Condorelli, O. Puglisi, C. Tempra, F. Lolicato, G. Compagninia, et al., Detection and characterization at nM concentration of oligomers formed by hA β , A β (1–40) and their equimolar mixture using SERS and MD simulations, *Phys. Chem. Chem. Phys.* 20 (2018) 20588–20596.
- [98] M. Baldassarre, C.M. Baronio, L.A. Morozova-Roche, A. Barth, Amyloid β -peptides 1–40 and 1–42 form oligomers with mixed β -sheets, *Chem. Sci.* 8 (2017) 8247–8254.
- [99] S. Mordechai, E. Shufan, B.S.P. Katz, A. Salman, Early diagnosis of Alzheimer's disease using infrared spectroscopy of isolated blood samples followed by multivariate analyses, *Analyst* 142 (2017) 1276–1284. <https://doi.org/10.1039/c6an01580h>.
- [100] M. Paraskevaidi, C.L. M. Morais, D.L. D. Freitas, K.M.G. Lima, D.M.A. Mann, D. Allsop, et al., Blood-based near-infrared spectroscopy for the rapid low-cost detection of Alzheimer's disease, *Analyst* 24 (2018) 5959–5964. <https://doi.org/10.1039/c8an01205a>.
- [101] T. Thi Vu Nu, T. Hoa, E. Nam, T. Nguyen, W. Jung Yoon, S. Cho, et al., Blood-based immunoassay of tau proteins for early diagnosis of Alzheimer's disease using surface plasmon resonance fiber sensors, *RSC Adv.* 8 (2018) 7855–7862. <https://doi.org/10.1039/C7RA11637C>.
- [102] S. Kim, A.W. Wark, H.J. Lee, *Anal. Chem.* 88 (2016) 7793–7799.
- [103] S. Lisi, S. Scarano, S. Fedeli, E. Pascale, S. Cicchi, C. Ravelet, et al., Toward sensitive immuno-based detection of tau protein by surface plasmon resonance coupled to carbon nanostructures as signal amplifiers, *Biosens. Bioelectron.* 93 (2016) 289–292.
- [104] L. Dujuan, S. Scarano, S. Lisi, P. Palladino, M. Minunni, Real-time tau protein detection by sandwich-based piezoelectric biosensing: exploring tubulin as a mass enhancer, *Sensors* 18 (2018) 946. <https://doi.org/10.3390/s18040946>.
- [105] L. Rivas, A. de la Escosura-Muñiz, J. Pons, A. Merkoçi, Alzheimer disease biomarker detection through electrocatalytic water oxidation induced by iridium oxide nanoparticles, *Electroanalysis* 26 (2014) 1287–1294.
- [106] L. Wu, H. Ji, H. Sun, C. Ding, J. Ren, X. Qu, Label-free ratiometric electrochemical detection of the mutated apolipoprotein e gene associated with Alzheimer's disease, *Chem. Commun.* 52 (2016) 12080–12083.
- [107] H.M. Brothers, M.L. Gosztyla, S.R. Robinson, The physiological roles of amyloid- β peptide hint at new ways to treat Alzheimer's disease, *Front. Aging Neurosci.* 9 (2018). <https://doi.org/10.3389/fnagi.2018.00118>.
- [108] V.V. Giau, S.S.A. An, Epitope mapping immunoassay analysis of the interaction between β -amyloid and fibrinogen, *Int. J. Mol. Sci.* 20 (2019) 496. <https://doi.org/10.3390/ijms20030496>.
- [109] K.G. Malmosa, L.M. Blancas-Mejia, B. Weber, J. Buchner, M. Ramirez-Alvarado, H. Naikie, et al., ThT 101: a primer on the use of thioflavin T to investigate amyloid formation, *Amyloid* 24 (2017) 1–16.
- [110] R. Alonso, D. Pisa, A.M. Fernandez-Fernandez, L. Carrasco, Infection of fungi and bacteria in brain tissue from elderly person and patients with Alzheimer's disease, *Front. Aging Neurosci.* 10 (2018) 159. <https://doi.org/10.3389/fnagi.2018.00159>.
- [111] F. Rahimi, Aptamers selected for recognizing amyloid β -protein-A case for cautious optimism, *Int. J. Mol. Sci.* 19 (2018) 668. <https://doi.org/10.3390/ijms19030668>.
- [112] L.-L. Zhu, J.-Y. Zhang, F.-Y. Wang, Y. Wang, L. Lu, C. Feng, et al., Selective amyloid β oligomer assay based on a basic site-containing molecular beacon and enzyme-free amplification, *Biosens. Bioelectron.* 78 (2016) 206–212. <https://doi.org/10.1016/j.bios.2015.11.048>.
- [113] L. Liu, Y. Chang, J. Yu, M. Jiang, N. Xia, Two-in-one polydopamine nanospheres for fluorescent determination of beta-amyloid oligomers and inhibition of beta-amyloid aggregation, *Sens. Actuators B* 251 (2017) 359–365. <https://doi.org/10.1016/j.snb.2017.05.106>.
- [114] L. Liu, N. Xia, J.-B. Zhang, W. Mao, Y. Wu, X. Ge, A graphene oxide-based fluorescent platform for selective detection of amyloid-A β oligomers, *Anal. Methods* 7 (2015) 8727–8732. <https://doi.org/10.1039/c5ay02018b>.
- [115] J. Wang, Y. Wang, X. Hu, C. Zhu, Q. Ma, L. Liang, Z. Li, Q. Yuan, Dual-aptamer-conjugated molecular modulator for detecting bioactive metal ions and inhibiting metal-mediated protein aggregation, *Anal. Chem.* 91 (2019) 823–829. <https://doi.org/10.1021/acs.analchem.8b03007>.
- [116] S.-C. Lee, H.H. Park, S.-H. Kim, S.-H. Koh, S.-H. Han, M.-Y. Yoon, Ultrasensitive fluorescence detection of Alzheimer's disease based on polyvalent directed peptide polymer coupled to a nanoporous ZnO nanoplateform, *Anal. Chem.* 91 (2019) 5573–5581.
- [117] X. Yi, C. Feng, S. Hu, H. Lib, J. Wang, Surface plasmon resonance biosensors for simultaneous monitoring of amyloid-beta oligomers and fibrils and

- screening of select modulators, *Analyst* 141 (2016) 331–336. <https://doi.org/10.1039/c5an01864a>.
- [118] X.R. Cheng, B.Y.H. Hau, A.J. Veloso, S. Martic, H.B. Kraatz, K. Kerman, et al., Surface plasmon resonance imaging of amyloid- β aggregation kinetics in the presence of epigallocatechin gallate and metals, *Anal. Chem.* 85 (2013) 2049–2055. <https://doi.org/10.1021/ac303181q>.
- [119] E.C. Rama, M. Begoña, G. García, A.C. García, Competitive electrochemical immunosensor for amyloid-beta 1–42 detection based on gold nanostructured Screen-Printed Carbon Electrode, *Sens. Actuators, B* 201 (2014) 567–571. <https://doi.org/10.1016/j.snb.2014.05.044>.
- [120] F.T.C. Moreira, M.G.F. Sales, Smart naturally plastic antibody based on poly (α -cyclodextrin) polymer for β -amyloid-42 soluble oligomer detection, *Sens. Actuators, B* 240 (2017) 229–238. <https://doi.org/10.1016/j.snb.2016.08.150>.
- [121] F.T.C. Moreira, B.A.G. Rodriguez, R.A.F. Dutra, M.G.F. Sales, et al., Redox probe-free readings of a β -amyloid-42 plastic antibody sensory material assembled on copper@carbon nanotubes, *Sens. Actuators, B* 264 (2018) 1–9. <https://doi.org/10.1016/j.snb.2018.02.166>.
- [122] J.V. Rushworth, A. Ahmed, H.H. Griffiths, N.M. Pollock, N.M. Hooper, P.A. Millner Pa, A label-free electrical impedimetric biosensor for the specific detection of Alzheimer's amyloid-beta oligomers, *Biosens. Bioelectron.* 56 (2014) 83–90. <https://doi.org/10.1016/j.bios.2013.12.036>.
- [123] J.L. Qin, D.G. Jo, M. Cho, Y.K. Lee, Monitoring of early diagnosis of Alzheimer's disease using the cellular prion protein and poly (pyrrole-2-carboxylic acid) modified electrode, *Biosens. Bioelectron.* 113 (2018) 82–87.
- [124] J.L. Qin, J.S. Park, D.G. Jo, M. Cho, Y.K. Lee, Curcumin-based electrochemical sensor of amyloid- β oligomer for the early detection of Alzheimer's disease, *Sens. Actuators, B* 273 (2018) 1593–1599. <https://doi.org/10.1016/j.snb.2018.07.078>.
- [125] Z. Yin, S. Wang, B. Shen, C. Deng, Q. Tu, Y. Jin, et al., Co-immunocapture and electrochemical quantitation of total and phosphorylated amyloid- β 40 monomers, *Anal. Chem.* 91 (2019) 3539–3545.
- [126] H. Ke, H. Sha, Y.F. Wang, W. Guo, X. Zhang, Z. Wang, et al., Electrochemiluminescence resonance energy transfer system between GNRs and Ru(bpy)₃2⁺: application in magnetic aptasensor for β -amyloid, *Biosens. Bioelectron.* 100 (2018) 266–273. <https://doi.org/10.1016/j.bios.2017.09.017>.
- [127] G. Zhao, Y. Wang, X.J. Li, Q. Yue, X. Dong, B. Du, et al., Dual-quenching electrochemiluminescence strategy based on three-dimensional Metal–Organic frameworks for ultrasensitive detection of amyloid- β , *Anal. Chem.* 91 (2019) 1989–1996.
- [128] B. Liu, H.-S. Shen, Y.-Q. Hao, X. Zhu, S. Li, Y. Huang, et al., Lanthanide functionalized metal-organic coordination polymer: toward novel turn-on fluorescent sensing of amyloid β -peptide, *Anal. Chem.* 90 (2018) 12449–12455. <https://doi.org/10.1021/acs.analchem.8b01546>.
- [129] H. Qin, X. Gao, X. Yang, W. Cao, S. Liu, A label-free and signal-on electrochemiluminescence strategy for sensitive amyloid-beta assay, *Biosens. Bioelectron.* 141 (2019) 111438. <https://doi.org/10.1016/j.bios.2019.111438>.
- [130] C. Wang, N. Zhang, Y. Lia, L. Yang, D. Wei, T. Yan, et al., Cobalt-based metal-organic frameworks as co-reaction accelerator for enhancing electrochemiluminescence behavior of N-(aminobutyl)-N-(ethylisoluminol) and ultrasensitive immunosensing of amyloid- β protein, *Sens. Actuators, B* 291 (2019) 319–328. <https://doi.org/10.1016/j.snb.2019.04.097>.
- [131] M. Negahdary, H. Heli, An ultrasensitive electrochemical aptasensor for early diagnosis of Alzheimer's disease, using a fern leaves-like gold nanostructure, *Talanta* 198 (2019) 510–517. <https://doi.org/10.1016/j.talanta.2019.01.109>.
- [132] Y. Yu, P. Wang, X. Zhu, Q. Peng, Y. Zhou, T. Yin, et al., Combined determination of copper ions and β -amyloid peptide by a single ratiometric electrochemical biosensor, *Analyst* 143 (2018) 323–331. <https://doi.org/10.1039/c7an01683b>.
- [133] R. Xu, D. Wei, B. Du, W. Cao, D. Fan, Y. Zhang, et al., A photoelectrochemical sensor for highly sensitive detection of amyloid beta based on sensitization of MnCdSe to Bi₂WO₆/CdS, *Biosens. Bioelectron.* 122 (2018) 37–42. <https://doi.org/10.1016/j.bios.2018.09.030>.
- [134] Y. Wang, D. Fan, G. Zhao, J. Feng, D. Wei, N. Zhang, et al., Ultrasensitive photoelectrochemical immunosensor for the detection of amyloid β -protein based on SnO₂/SnS₂/Ag₂S nanocomposites, *Biosens. Bioelectron.* 120 (2018) 1–7. <https://doi.org/10.1016/j.bios.2018.08.026>.
- [135] A.J. Veloso, A.M. Chow, H.V.S. Ganesh, N. Li, D. Dhar, D.C. Wu, et al., Electrochemical immunosensors for effective evaluation of amyloid beta modulators on oligomeric and fibrillar aggregation processes, *Anal. Chem.* 86 (2014) 4901–4909.
- [136] H. Dong, L. Zhang, W. Liu, Y. Tian, Label-free electrochemical biosensor for monitoring of chloride ion in an animal model of Alzheimer's disease, *ACS Chem. Neurosci.* 8 (2017) 339–346. <https://doi.org/10.1021/acscchemneuro.6b00296>.
- [137] F.T.C. Moreira, M.G.F. Sale, M. Di Lorenzo, Towards timely Alzheimer diagnosis: a self-powered amperometric biosensor for the neuro-transmitter acetylcholine, *Biosens. Bioelectron.* 87 (2017) 607–614. <https://doi.org/10.1016/j.bios.2016.08.104>.
- [138] Z. Ying, A.N. Simonov, Z. Jie, A.M. Bond, Fourier transformed alternating current voltammetry in electromaterials research: direct visualisation of important underlying electron transfer processes, *Curr Opin Electrochem* 10 (2018) 72–81.
- [139] E.G. Matveeva, J.R. Moll, M.M. Khan, R.B. Thompson, R.O. Cliff, Surface assay for specific detection of soluble amyloid oligomers utilizing Pronucleon peptides instead of antibodies, *ACS Chem. Neurosci.* 8 (2017) 1213–1221. <https://doi.org/10.1021/acscchemneuro.6b00381>.
- [140] J.S. Park, S.T. Kim, S.Y. Kim, M.G. Jo, M.J. Choi, M. O Kim, A novel kit for early diagnosis of Alzheimer's disease using a fluorescent nanoparticle imaging, *Sci. Rep.* 9 (2019) 13184. <https://doi.org/10.1038/s41598-019-49711-y>.
- [141] Z. Yang, T. Albrow-Owen, H. Cui, J. Alexander-Webber, F. Gu, X. Wang, et al., Single-nanowire spectrometers, *Science* 365 (2019) 1017–1020. <https://doi.org/10.1126/science.aax8814>.
- [142] An, et al., Differentiating blood samples from scrapie infected and non-infected hamsters by detecting disease-associated prion proteins using Multimer Detection System, *Biophys. Res. Commun.* 392 (2010) 505–509. In this issue.
- [143] Youn, et al., Blood Amyloid- β Oligomerization as a Biomarker of Alzheimer's Disease: A Blinded Validation Study, *J. Alzheimers. Dis.* 75 (2020) 493–499.
- [144] Meng, et al., Association between increased levels of amyloid-beta oligomers in plasma and episodic memory loss in Alzheimer's disease, *Alzheimers. Res. Ther.* 11 (2019). <https://doi.org/10.1186/s13195-019-0535-7>.