Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-019-02350-8

RESEARCH PAPER

A dip-and-read optical aptasensor for detection of tau protein

Iva Ziu[1] · Erving T. Laryea[1] · Fayza Alashkar[1] · Colin G. Wu[1] · Sanela Martic[2]

Received: 9 September 2019 / Revised: 3 December 2019 / Accepted: 11 December 2019

Abstract

Neurodegeneration currently remains without a differential diagnosis or cure. Tau protein is one of the biomarkers of neurodegenerative diseases commonly known as tauopathies. Tau protein plays an integral role in stabilizing microtubules and cell structure; however, due to post-translational modifications, tau protein undergoes self-assembly into cytotoxic structures and is co-localized intra- and extracellularly. Hence, tau protein is a viable biomarker associated with protein pathogenesis and neurodegeneration. The novel optical biosensor for tau441 protein is based on the aptamer recognition probe and the biolayer interferometry (BLI) method for detection. The current biotin-aptasensor in combination with the streptavidin surface provides real-time monitoring of tau441 protein in the nanomolar range, with the limit of detection at 6.7 nM in vitro. The tau441 detection is achieved with high selectivity over other neurodegeneration biomarkers which include amyloid-β and α-synuclein. The aptasensor also allows for tau441 protein detection in a complex matrix such as fetal bovine serum, indicating its utility in other biological fluids for diagnostic applications. The optical method is simple, rapid and highly selective for point-of-care application which is critical for achieving the early and differential diagnosis of neurodegenerative diseases and identifying their treatments.

Keywords Aptamer · Biolayer interferometry · Optical spectroscopy · Neurodegeneration · Tau protein

Introduction

Tau is a highly soluble and natively unfolded protein found primarily in neuronal cells. Its main function is to bind and stabilize the microtubules (MT), facilitate neuronal transport and maintain cell integrity and viability [1]. Post-translational modifications of tau, such as phosphorylation by protein kinases, are thought to be responsible for tau aggregation and its detachment from microtubules, leading to cell death and cytotoxicity [2, 3]. Additionally, aggregates of tau can spread from cell to cell, further compromising cell functions and contributing to neurodegeneration [4, 5]. Since tau protein is related to normal and diseased states, it is a viable biomarker for disease detection, diagnosis, and therapeutics development.

In vitro detection of tau protein has been achieved by using several optical and electrochemical methods, including ELISA, electrochemical impedance spectroscopy, surface plasmon resonance (SPR), and capillary electrophoresis, among others [6–11]. A single-molecule ELISA digital array technology has been used for the detection of tau in blood at the femtomolar level [12]. The SPR method was used to quantify the interaction between tau protein and antibodies, as well as tau protein and DNA [13–15]. The tau protein was also detected by using nanoparticle-localized SPR, surface-enhanced Raman scattering, and multiphoton scattering [16–18]. The integration of nucleic acid aptamer and antibody with SPR was also demonstrated for the detection of tau protein [8].

Aptamers are functional single-stranded deoxyribonucleic or deoxyribonucleic oligonucleotides selected from combinatorial libraries of random sequences by in vitro selection, using a technique called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) [19]. Aptasensors can bind a variety of targets such as proteins, small molecules, tissues, and cells, and offer a variety of advantages over antibodies, such as high stability, low cost, and enhanced reproducibility [20–23]. It has been demonstrated that tau protein can bind to a single-stranded DNA and double-stranded DNA [14, 24]. In addition, the aptamer-antibody pairing for tau

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00216-019-02350-8) contains supplementary material, which is available to authorized users.

✉ Sanela Martic
sanelamartic@trentu.ca

[1] Department of Chemistry, Oakland University, Rochester, MI 48309, USA

[2] Department of Forensic Science, Environmental and Life Sciences, Trent University, Peterborough, ON K9L 0G2, Canada

detection has been achieved by using SPR [8]. The aptamer was attached to the SPR sensor by using amide coupling chemistry and the subsequent binding of tau protein to the aptamer was followed by the sandwich-like antibody binding for the generation of a highly sensitive sensor.

Biolayer interferometry (BLI) has undergone rapid advancement as a label-free and real-time optical method to study biomolecular interactions without the need for labeled reagents. It is a powerful technique that can be used for the detection of targets such as small molecules, proteins, and cells, among others [25–27]. The BLI is based on a Dip-and-Read™ format in which biosensors are dipped into microplate wells containing purified or complex samples, providing a highly parallel, user-friendly technique to study molecular interactions. The interference pattern of white light reflected on the disposable fiber optic–based biosensor can be analyzed. Changes in the number of molecules bound to the biosensor tip cause a shift in the interference pattern that can be measured in real time [28, 29]. BLI has been previously used for the detection of proteins and antibodies but has never been utilized for the detection of neurodegeneration biomarkers or tau protein [30–32].

Herein, we report a portable, label-free, and real-time optical BLI biosensor for the detection of tau protein by using a biotinylated-aptamer with streptavidin interface as a specific capture probe for the first time. The biosensor's selectivity was evaluated with additional neurodegeneration biomarkers, including amyloid-β and α-synuclein. The biosensor performance was also tested in a complex medium such as fetal bovine serum.

Experimental

Materials and reagents

A construct to express recombinant tau441 protein in bacteria was purchased from Vectorbuilder (USA). Biotinylated DNA aptamers with the following sequences were synthesized by Integrated DNA Technologies (USA): 5′-Biotin-GCGGAGCGTGGCAGG-3′ and 5′-Biotin-TTTTTTTT TTTTTTT. Streptavidin-coated (SA) biosensors were obtained from Pall Fortebio (USA). Amyloid β_{40} and α-synuclein were purchased from AnaSpec Inc. (USA) as lyophilized powders. Bovine serum albumin (BSA) was procured from Amresco (USA). A stock solution of MES buffer, pH 6.8, was prepared by using 0.5 mM ethylene glycol-O, O′-bis (2-aminoethyl)-N, N, N′, N′- tetraacetic acid (EGTA) (Alfa Aesar (England)), 50 mM 2-(N-5-morpholino) ethanesulfonic acid (MES), and 100 mM sodium chloride (NaCl) (Fisher Scientific (USA). The pH was adjusted using sodium hydroxide (NaOH) obtained from Fisher Scientific (USA). Phosphate buffer saline (PBS) at pH 7.4 was purchased from Fortebio (USA). The lysogeny broth (LB), Terrific broth (TB), and glycerol were purchased from Fischer Scientific (USA). Imidazole, sodium chloride, nonidet P40 (NP40), and NaPi (NaH_2PO_4 and Na_2HPO_4) were obtained from VWR (USA). Nickel-NTA beads were purchased from ThermoScientific (USA). Isopropyl β-d-thiogalactoside (IPTG) and DTT were procured from Gold Biotechnology (USA). The Proteostat fluorescence aggregation assay kit was purchased from Enzo Life Sciences (USA). D8 antibody specific to N-terminal of tau441 was obtained from Santa Cruz Biotechnology Inc. Secondary antibody IRGAM680 was purchased from Biorad (USA). SfGFP-TEV protease construct was obtained from VectorBuilder (USA) and expressed as previously reported [33]. Fetal bovine serum (FBS) was obtained from Hyclone GE Healthcare Life Science.

Expression and purification of tau441 protein

Full-length tau441 (2N4R) protein was expressed and purified based on previous procedures [34]. First, the tau441 construct, which possesses a C-terminal polyhistidine tag, was transformed into *Escherichia coli* NiCo21 competent cells. Transformed cells were plated on LB-Agar supplemented with 50 μg/mL ampicillin at 37 °C. A single colony was selected for an overnight starter culture in LB. This was used to inoculate terrific broth (TB) the next day using a 1:50 dilution. The culture was left at 37 °C until $O.D._{.675} = 0.4–0.6$ is reached. Tau441 protein expression was induced with 1 mM isopropyl β-d- thiogalactoside (IPTG). The cell growth was allowed to continue overnight at 16 °C, after which cells were harvested by centrifugation (4000 rpm, 4 °C, 90 min). The cell pellet was immediately resuspended in a lysis binding buffer (20 mM NaPi pH 7.5, 300 mM NaCl, 30 mM Imidazole, 1 mM DTT, 5% glycerol) and 1% NP40 in a ratio of 1 g of cells to 10 mL of solution. The supernatant from the resuspended cells was sonicated at 45% amplitude for 15 min (15 s on, 45 s off pulses) to lyse the cells. The cell lysate was heated (70 °C, 1 h) to precipitate out the endogenous *E. coli* proteins. The crude lysate was clarified by centrifugation (18,000 rpm, 4 °C, 90 min) and the supernatant was filtered through a 0.22-μm filter before NiNTA chromatography.

The filtered supernatant was mixed with NiNTA beads for 15 min to allow batch binding and the mobile phase was collected as the flow-through (FT) fraction. The NiNTA media was washed with wash 1 buffer (20 mM NaPi pH 7.5, 300 mM NaCl, 30 mM Imidazole, 1 mM DTT, 5% glycerol) and the first of three washes was collected as Wash 1 (W1). The first of three washes using with wash 2 buffer (20 mM NaPi pH 7.5, 30 mM NaCl, 30 mM Imidazole, 1 mM DTT, 5% glycerol) was collected as Wash 2 (W2). His-tagged tau441 protein was eluted off the NiNTA column by elution buffer (20 mM NaPi pH 7.5, 300 mM NaCl, 2 M Imidazole, 1 mM DTT, 5% glycerol) was collected as Elute (EL). The

polyhistidine tag on tau441 was cleaved off the C-terminus by incubation with TEV protease at room temperature for 72 h in the ratio of 1 mol of TEV protease to 10,000 mol of tau441. Following cleavage, a second Nickel affinity purification was done to remove the cleaved His_6-tag and the TEV protease. SDS–PAGE and western blot analyses (D8 primary antibody to tau (1:1000 dilution in TBST) and IRGAM680 secondary antibody (1:5000 dilution in TBST) were performed to assess the purity of the protein (see Electronic Supplementary Material (ESM) Fig. S10).

Biolayer interferometry

BLI experiments were carried out using a Blitz instrument which was obtained from Fortebio (USA). All BLI measurements were performed at 25 °C with shaking at 2200 rpm in 0.5-mL black microtubes (Argos Technologies, USA), containing 200 μL of each solution. For method definition and execution, the Data Acquisition software Blitz pro 1.2.1.5 from ForteBio was used. Triplicate measurements were performed for each sample to ensure statistical significance.

Biotin-aptamer biosensor fabrication

Streptavidin (SA)-biosensor tips were activated by hydrating in PBS, pH 7.4 buffer (250 μL). Next, SA-tips were used in order to immobilize biotin-aptamer (5′-Biotin-GCGGAGCG TGGCAGG-3′) which was diluted in PBS, pH 7.4. The biosensor fabrication was carried out and monitored in real-time by BLI as follows: (1) 200 μL PBS, pH 7.4, baseline (1 min); (2) 200 μL of 1 μM biotin-aptamer (5 min); (3) 200 μL PBS, pH 7.4, baseline (1 min). The biotin-aptamer loading time was also varied from 5 to 15 min, but a similar BLI signal was obtained and a 5-min loading time was selected for further measurements. In order to optimize the biotin-aptamer surface coverage, various biotin-aptamer concentrations were tested: 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 5, and 10 μM.

Detection of tau441 protein

For the tau441 protein concentration–dependent studies, following BLI experiments were carried out: (1) 200 μL PBS, pH 7.4 buffer baseline (1 min): (2) biotin-aptamer loading (5 min, 200 μL of 1 μM in PBS, pH 7.4); (3) 200 μL PBS, pH 7.4, baseline (1 min); (4) 200 μL MES buffer, pH 6.8, baseline (1 min); (5) tau441 (5 min, 200 μL of tau protein at various concentrations in MES buffer, pH 6.8); (6) dissociation (1 min, 200 μL of MES buffer, pH 6.8). Tau441 protein dilutions were prepared using MES buffer, pH 6.8, at the following concentrations of the protein: 2, 5, 11, 16, 21, 32, 43, 55, and 64 nM.

Control experiments with amyloid-β_{40}, α-synuclein, BSA, and MES buffer, pH 6.8

BLI experiments were carried out with Amyloid-β_{40}, α-synuclein, and BSA proteins (21 nM in MES buffer, pH 6.8) instead of tau441. Additional control measurement was also conducted with a protein-free solution with MES buffer, pH 6.8, alone.

Tau441 detection in spiked fetal bovine serum

For fetal bovine serum (FBS) studies, FBS was diluted by 100× in PBS, pH 7.4 buffer to minimize nonspecific binding to the biosensor. The following BLI experiments were carried out: (1) 200 μL PBS, pH 7.4 buffer baseline (1 min): (2) biotin-aptamer loading (5 min, 200 μL of 1 μM in PBS, pH 7.4); (3) 200 μL PBS, pH 7.4, baseline (1 min); (4) protein association (5 min, 200 μL FBS 100x diluted in PBS buffer, pH 7.4, or spiked with 210 nM tau441 protein); (5) dissociation (10 min, 200 μL of PBS buffer, pH 7.4).

Fluorescence aggregation assay

The Proteostat fluorescence aggregation assay kit (EnzoSciences, USA) was used for the detection of protein aggregation. Fluorescence was acquired using the H1 synergy microplate reader from Biotek (USA) at an excitation of 550 nm and emission of 600 nm. The sample solutions were loaded (3 μL onto each well) onto a Take3 micro-volume plate (Biotek, USA). Solutions of tau441, Amyloid-β_{40}, and α-synuclein (21 nM in MES buffer, pH 6.8) were prepared and a Proteostat detection reagent added using the sample-to-detection reagent ratio of 10:1% v/v. After incubation at room temperature for 15 min, the fluorescence measurements were performed. The positive and negative controls (aggregated and native lysozyme, respectively) were used as per the Proteostat aggregation protocol. Triplicate measurements were carried out for each sample to ensure statistical significance.

Results and discussion

Confirmation of biosensor assembly by BLI

An overview of the BLI sensor for tau protein detection is provided in Fig. 1. The BLI sensor tip functionalized with streptavidin was first modified with biotin-aptamer (5′-Biotin-GCGGAGCGTGGCAGG-3′). This particular sequence was previously reported to bind to tau381 and tau410 protein isoforms [8, 24]. For optimization purposes,

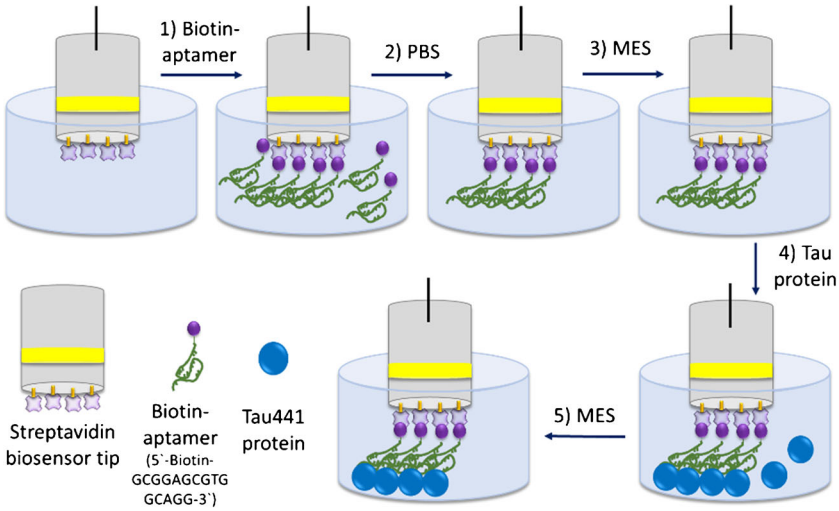

Fig. 1 Schematic overview of sensing strategy for tau protein detection. Streptavidin BLI tip was used for immobilization of biotin-aptamer, followed by extensive rinsing with buffers. Specific adsorption of tau441 protein was monitored in buffer ((1) biotin-aptamer loading (5′-Biotin-GCGGAGCGTGGCAGG-3′); (2) PBS, pH 7.4 (rinsing); (3) MES buffer, pH 6.8 (rinsing); (4) tau441 protein binding (in MES buffer, pH 6.8); (5) MES buffer, pH 6.8 (rinsing))

the biotin-aptamer loading time was tested (5–15 min) but the BLI signal did not improve with longer loading time, hence, 5 min was chosen as the loading time for biotin-aptamer step. In order to determine the optimal biotin-aptamer surface coverage, the change in BLI signal was monitored by using a series of biotin-aptamer concentrations in PBS, pH 7.4: 10 μM, 5 μM, 1 μM, 0.75 μM, 0.5 μM, 0.25 μM, 100 nM, 75 nM, 50 nM, 25 nM, 10 nM, 5 nM, 2.5 nM, and 1 nM. The BLI signal as a function of concentration was monitored for each sensor tip and is plotted in Fig. 2a. The BLI signal change, at 360 s, was also plotted versus biotin-aptamer concentration (Fig. 2b). The BLI response was linear from 0.005–1 μM, indicating that the optimal biotin-aptamer coverage was obtained at 1 μM. Hence, the 1 μM biotin-aptamer concentration was selected for subsequent measurements (see ESM Fig. S2).

Detection of tau441 protein by the BLI biosensor

The corresponding BLI spectrum for real-time monitoring of biotin-aptamer immobilization (1) and subsequent tau441 protein binding to aptamer (4) is depicted in Fig. 3a. Prior to tau441 protein binding, the biosensor was rinsed with PBS, pH 7.4 (2), and MES, pH 6.8 (3), buffers. In addition, after the tau441 binding event, the biosensor was rinsed with MES, pH 6.8 buffer (5).

Next, the biotin-aptamer biosensor was exposed to tau441 solution in MES buffer, pH 6.8, at various tau441 concentrations (0, 2, 5, 11, 16, 21, 32, 43, 55, 64 nM) as depicted in Fig. 1 and BLI signal monitored as a function of time (Fig. 3b). An increased amount of tau441 caused an increase in the BLI response. The BLI data for tau441 binding were fitted with a 1:1 isotherm model to obtain dissociation constant (K_D) value of $6.20\ (\pm 0.001) \times 10^{-9}$ M. This confirmed the high affinity of the aptamer for tau441 which was comparable to its affinity forthe tau381 protein isoform ($K_D = 1.60\ (\pm 0.1) \times 10^{-9}$ M) [8]. The K_D values for other protein-aptamer systems were also at $\sim 10^{-9}$ M [35, 36]. From the BLI binding curves (Fig. 3b), the signal intensity (at 780 s) was plotted as a function of tau441 concentrations in Fig. 3c. The BLI biosensor exhibited a good linear detection range from 2 to 55 nM of tau441. From the linear data fit, the equation: $y = 0.0212x + 0.014$ was obtained with a correlation coefficient of $R^2 = 0.9724$. The standard deviation for the MES buffer, pH 6.8, was found to be 0.0657 nm. Hence, based on the IUPAC (International Union of Pure and Applied Chemistry), the limit of detection (LOD) is defined as the mean of the blank measures (− 0.0679 nm), plus triple of its standard deviation (3 x (0.0657) = 0.1973) for higher than 99% confidence level [37]. The LOD for tau441 was calculated to be (− 0.0679) + (0.1973) = 0.1294 nm. From the linear fitting equation from the standard curve in Fig. 3c, the LOD for tau441 using the BLI aptasensor was ≥ 6.7 nM. The coefficient of variation was determined by dividing the standard deviation with the mean followed by multiplication (× 100%) to obtain a percent value which indicates the precision and repeatability of the assay. The CV values in 5–25% range were obtained across tau protein concentrations from 3 to 0.25 μg/mL. Next, biosensor was rinsed with PBS buffer, pH 7.4, followed by MES buffer, pH 6.8. At this point, the biotin-aptamer was immobilized on the surface by binding to the streptavidin-coated sensor tips and was ready for tau specific adsorption measurements.

Notably, no significant BLI signal (0.03 ± 0.04 nm) was observed when the biotin-aptamer-free biosensor was used (PBS, pH 7.4 solution exclusively) for detection of 20 nM

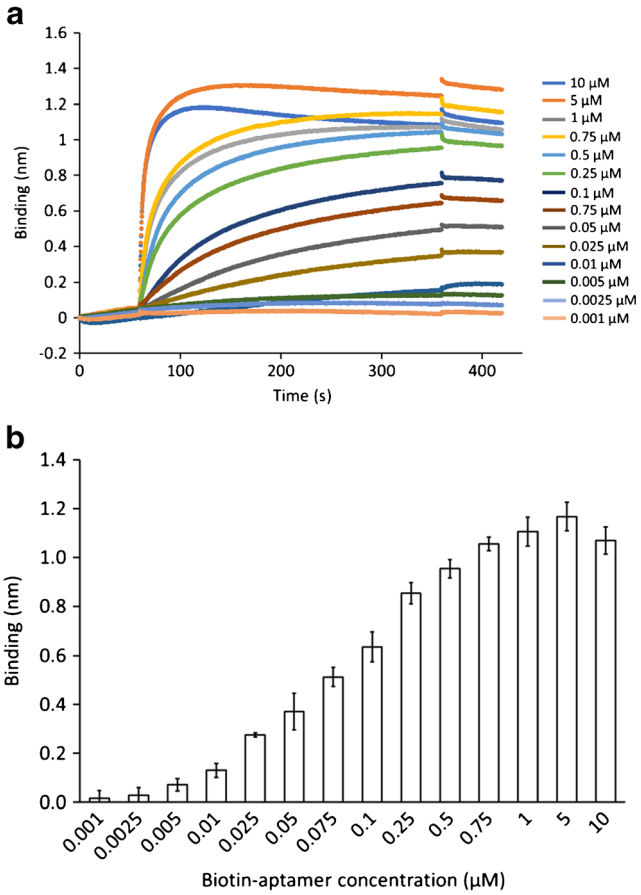

Fig. 2 **a** BLI signal intensities for biotin-aptamer binding to streptavidin-coated biosensor and **b** plot of BLI signal change, at 360 s, as a function of biotin-aptamer concentrations (in PBS buffer, pH 7.4).The error bars represent the standard deviation of three independent measurements

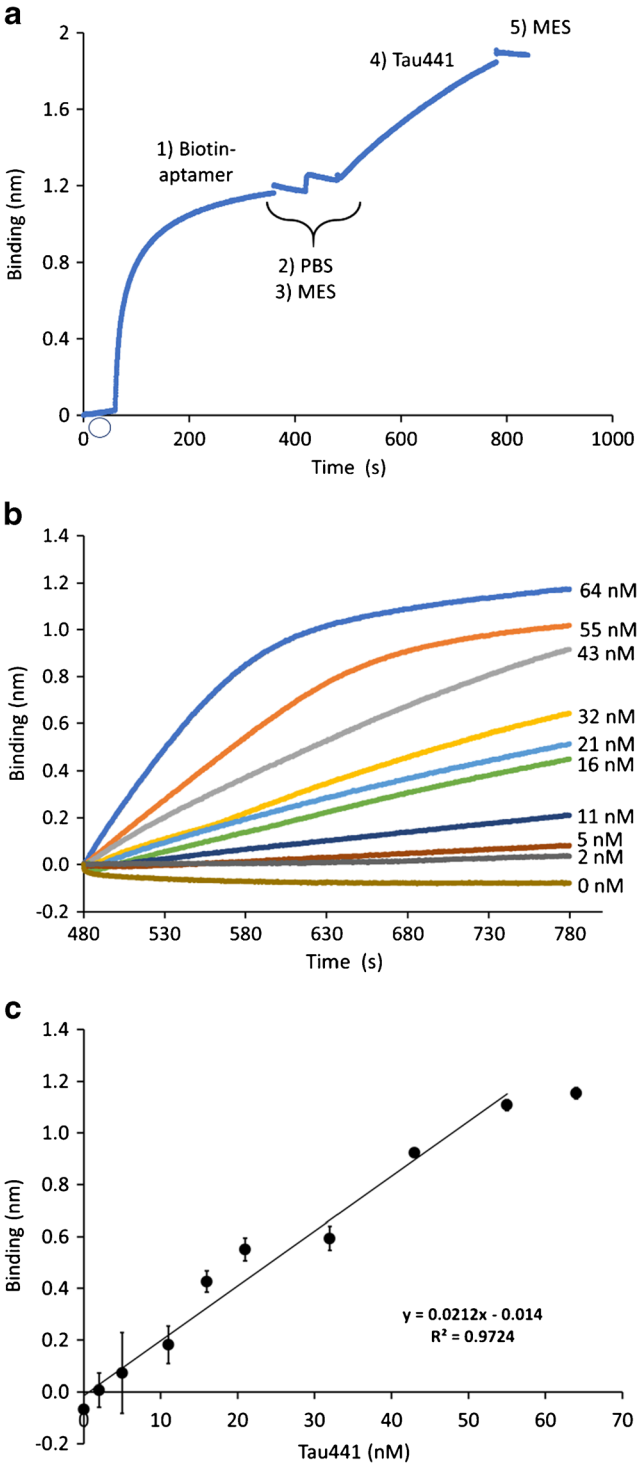

Fig. 3 **a** Representative BLI spectrum for tau binding to biotin-aptamer-SA biosensor: (1) biotin-aptamer (5 min); (2) PBS buffer, pH 7.4 (1 min); (3) MES buffer, pH 6.8 (1 min); (4) tau441 protein (5 min), and (5) MES buffer, pH 6.8 (1 min). **b** Partial BLI spectra of tau441 binding to biotin-aptamer biosensor as a function of tau441 concentrations (0–64 nM). **c** The plot of BLI signal for binding of tau441 as a function of protein concentration as determined from the plot (**b**) ($t = 780$ s; error bars represent the standard deviation of three independent sets of measurements; data are fitted using linear fit)

tau441 protein, indicating minimal non-specific adsorption of tau441 on the sensor surface. The tau441 non-specific binding was also evaluated by replacing the tau-aptamer by T15 aptamer (5′-Biotin-TTTTTTTTTTTTTTT) which was not specific for tau441. The small BLI signal change (0.19 ± 0.03 nm) was observed upon incubation of tau441 using the biotin-T15 aptamer sensor, which is significantly lower than when tau-aptamer was used. Notably, when the biotin-T15 aptamer was exposed to MES buffer, pH 6.8 (free of tau441), a similar BLI signal change was observed (0.18 ± 0.03 nm). The background BLI signal change may be ascribed to the aptamer reorganization due to the MES buffer. This data indicate that the tau441 selectively and specifically bound the tau-aptamer sequence allowing for its detection in the BLI format.

Selectivity of BLI aptasensor

The selectivity of the tau441 biosensor was investigated in the presence of bovine serum albumin (BSA) at 21 nM in MES buffer, pH 6.8. The biosensor was unresponsive to BSA protein (Fig. 4) which was similar to that of MES buffer. Hence,

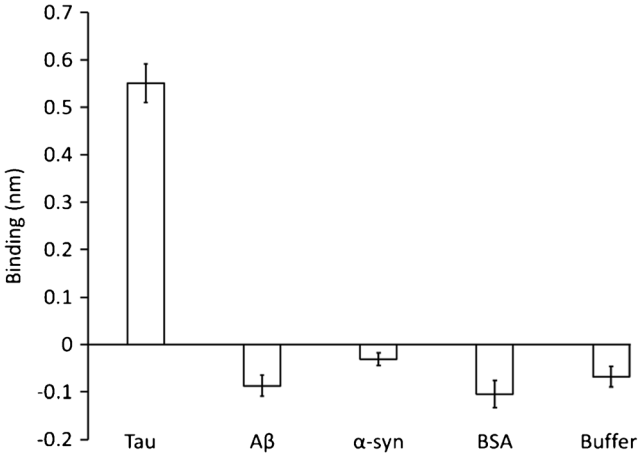

Fig. 4 Selectivity of BLI aptasensor for tau441 and other proteins: Amyloid-β_{40}, α-synuclein, and BSA (21 nM), and MES buffer, pH 6.8 (biotin-aptamer loading at 1 μM; the average of three independent trials with standard deviation errors)

the aptamer-based biosensor was specific to tau441 and exhibited minimal non-specific binding to BSA. Additional biosensor selectivity was tested in the presence of other neurodegeneration biomarkers, such as Amyloid-β_{40} and α-synuclein (see ESM Fig. S5-6). Hence, demonstrating the sensor selectivity for tau441 biomarkers against other neuronal proteins is critical for the development of a functional biosensor for tauopathies. Figure 4 shows high selectivity for tau441 (~ 0.55 nm) compared with the negligible signals obtained for Amyloid-β_{40} (~ − 0.07 nm) and α-synuclein (~ − 0.03 nm), both of which were similar to the signal in the MES buffer. The aggregation of tau441, Amyloid-β_{40}, and α-synuclein at 1 μg/mL was also evaluated by using the fluorescence aggregation assay in order to determine if these proteins and peptides were present in the aggregated or non-aggregated forms during the BLI measurements. The Proteostat aggregation assay was used to monitor aggregation via β-sheet formation, wherein the increase in fluorescence intensity was associated with the aggregation. The (non)aggregated lysozyme was used as the positive and negative control in the fluorescence assay. None of the proteins exhibited aggregation, indicating that all proteins were likely in the monomeric or dimeric states during BLI measurements.

Validation of the BLI aptasensor using the fetal bovine serum (FBS) spiked with tau441

The ability of the BLI aptasensor to detect tau441 in FBS was evaluated next, since the FBS is a complex solution, similar to cerebrospinal fluid or blood, which mimics the biological matrix. FBS contains proteins such as BSA, as well as small molecules including amino acids, sugars, lipids, and hormones, and represents a suitable matrix to mimic the biological setting for the proof-of-concept design and development of the biosensor. As a result, it is important to find out whether the aptamer BLI platform can be used to detect tau441, neurodegeneration biomarker, in the presence of any such protein. The BLI signal observed in the presence of diluted FBS (100× dilution) was 0.344 ± 0.056 nm indicating significant non-specific adsorption of proteins in FBS solution onto the biotin-aptasensor surface. However, when FBS (100× diluted) was spiked with 21 nM tau441, the BLI signal was at 1.13 ± 0.02 nm, which was significantly higher than in the absence of tau441. More importantly, the BLI signal for 21 nM tau441 in the MES buffer, pH 6.8, was at 1.26 ± 0.11 nm, which is very similar to the biosensor response in the presence of FBS (100× diluted). Hence, ~ 90% signal recovery for tau441 was observed even in the presence of a complex mixture such as an FBS solution. Despite the high recovery, the non-specific adsorption on the surface observed may be further reduced by blocking the surface with diluted BSA solution prior to measuring tau441 protein in FBS. In this approach, only specific interactions between the aptamer and tau441 protein would be observed resulting in a signal, with minimal adsorption from proteins in FBS. Our experimental data with FBS clearly demonstrate that out BLI assay is highly sensitive to tau441 protein and it can distinguish it from proteins and small molecules in FBS mixture, which is analogous to CSF or blood. Other studies have shown that a biosensor maintained their specificity for tau in artificial CSF and diluted FBS, hence the results obtained in the current study in diluted FBS are beneficial [38].

Comparison of the current method to literature reports for tau protein detection

The characteristics of the literature-reported techniques, including the type of target, matrix tested, LOD value, and sensor platform used, are provided in Table 1. The parameters associated with the current method are also included.

While the usage of commercially available ELISA kits is common, the results may vary based on different manufacturers [6]. For example, CV values were seen to be above 20% due to usage of different materials for reagents or procedures in antibody preparation, purification, and plate coating [6]. In addition, ELISA kits from the same vendors may have lot-to-lot variability and cause a discrepancy in results. ELISA protocols also require extensive incubation time and plate washing between steps, as well as large sample volumes (~ 200 μL) depending on the plates. Recently, SIMOA (an improvement to ELISA) platform was developed with reported LOD at 0.3 pg/mL for tau441 protein [39]. Alternative methodologies to ELISA have been reported to utilize UV-vis spectroscopic signature from gold nanoparticles for the detection of tau in a colorimetric format, or detection of tau protein by using fluorescence spectroscopy in combination with magnetic iron oxide nanoparticles or quantum dots or detection of tau by SERS [16, 38, 42, 44, 46]. Both spectroscopic assays rely

Table 1 The LOD values reported for the detection of the specific tau biomarker in a matrix by using various sensor platforms and techniques

Biomarker	Matrix	Sensor platform	Technique[a]	LOD[b]	Reference no.
Total tau protein	Cerebrospinal fluid	Immunoassay	ELISA	1 ng/mL (22 pM)	[6]
Tau441 isoform	Buffer	Immunoassay	EIS	10 μg/mL (217 nM)	[7]
Tau441 isoform	CSF	Immunoassay	ELISA/SIMOA	0.3 pg/mL (6.5 fM)	[39]
Tau441 isoform	Buffer and diluted human serum	Immunoassay	EIS	30 fM	[10]
Tau441 isoform	Spiked CSF	Immunoassay	EIS	0.15 nM	[9]
Tau381 isoform	Serum	Immunoassay/aptamer	DPV	0.42 pM	[40]
Tau381 isoform	Plasma	Immunoassay/aptamer	SPR	10 fM	[8]
Tau441 isoform	buffer or spiked CFS	Immunoassay	QCM	50 nM	[41]
Tau protein	Buffer	Immunoassay	SERS	100 fg/mL (2.2 fM)	[42]
Tau441 isoform	Buffer or spiked CSF	Immunoassay/MWCNT	SPR	500 pM	[43]
Tau protein	Buffer	Immunoassay/Au NP	Au NP calorimetric UV-vis spectroscopy	1 pg/mL (22 fM)	[44]
Tau protein	Buffer	Immunoassay	LSPR UV-vis spectroscopy	10 pg/mL (0.22 pM)	[16]
Phosphorylated tau protein	Serum	Immunoassay	SPR	2.4 pg/mL (52 fM)	[45]
Tau441 isoform	Buffer	Magnetic iron oxide NP/immunoassay	Fluorescence spectroscopy	0.1 μg/mL (2.2 nM)	[38]
Tau381 isoform	Buffer human serum	Immunoassay/quantum dots/aptamer	Fluorescence spectroscopy	10 pM	[46]
Tau441 isoform	Buffer and FBS	Aptamer	BLI	6.7 nM	This study

[a] Biolayer interferometry (*BLI*); cerebrospinal fluid (*CSF*); differential pulse voltammetry (*DPV*); electrochemical impedance spectroscopy (*EIS*); enzyme-linked immunosorbent assay (*ELISA*); quartz-crystal microbalance (*QCM*); single molecule array (SIMOA); surface-enhanced Raman spectroscopy (*SERS*); surface plasmon resonance (*SPR*). [b] Conversion from reported mass/volume to molarity was carried out by assuming the longest isoform of tau (tau441) (45,900 Da), unless otherwise stated

on using an immunosensor as a recognition probe for the analyte, mainly tau protein. The optical biosensors were also developed based on immunoassay platform and surface plasmon resonance (SPR) in combination with aptamer for the detection of tau [8, 43, 45]. Unlike ELISA, SPR provides the ability to monitor analyte binding in real time and determine binding parameters. In general, the optical methods are largely benchtop and their uses in the point-of-care applications are challenging. Hence, designing and developing new analytical platforms which allow for rapid and sensitive detection of analytes by using small sample volumes in a portable point-of-care format are of interest. Towards this goal, tau protein detection has been demonstrated by using electrochemical rather than optical methods. Electrochemical methods provide several advantages compared with traditional optical methods such as ELISA and include tailored and specific surface modification rather than adsorption; reduced number of washing steps, if any; comparable sensitivity; and portability for point-of-care applications, which are not commonly seen with optical methods. Among electrochemical methods, voltammetry, impedance, and piezoelectrics have been used for immunodetection of tau protein in buffer, spiked CSF, and serum [9, 10, 40, 41]. The current study reports on an optical BLI method to be used for detection of neurodegeneration biomarker for the first time. BLI combines advantages associated with SPR as well as electrochemical methods in that it offers (1) real-time measurements of analyte binding, (2) small sample volume, (3) aptamer surface immobilization for targeted detection, (4) rapid analyte measurements (1–5 min), and (5) portability for point-of-care applications. However, unlike ELISA, the current BLI method does not require antibodies, labeled-antibodies, or washing steps. Our experiments indicate this bioassay is rapid and takes less than 15 min from aptamer binding to tau441 detection and analysis, which is much faster than ELISA-type experiments which may take hours or days to conduct. While the reported LOD with the present BLI method for tau protein may not be as low as those reported by using SPR, the advantages listed for BLI use provide additional attractive features when designing and developing a biosensor for point of care application. Unlike SPR which is a flow-through method and requires washing steps, the BLI is a dip-and-read platform which does not require special pumps and large sample volumes for flow-through applications. Several optimizations may be made to

the current BLI method, in order to improve LOD (towards the picomolar or fentomolar range), and include the selection of new aptamers, or use of antibodies with aptamers, as was reported in the SPR method [8].

Conclusions

In summary, a novel optical aptamer biosensor has been prepared for tau441 protein detection. The BLI sensor was based on the biotin-aptamer specific to tau and the streptavidin interface. The BLI aptasensor possesses a linear dynamic range over 2 orders of magnitude with an LOD of 6.7 nM. While the LOD reported is at the higher end of the biologically relevant tau protein concentrations (typically within a wide picomolar range), this is the proof-of-concept optical sensor for tau441 protein which is the biomarker of neurodegeneration. In addition, the tau441 binding to biotin-aptamer on the surface is selective and specific to tau441 protein compared with other biomarkers such as Amyloid-β of α-synuclein. Notably, the detection of tau441 was also achieved in the complex mixture such as fetal bovine serum indicating that the device may find utility in the biomarker detection in the other biological fluids. To the best of our knowledge, this is the first trial of a reproducible, selective, and specific BLI aptasensor for neurodegeneration biomarker, tau441 protein. Furthermore, the proposed biosensing strategy demonstrates great potential for differential disease diagnosis based on the type of closely related biomarkers present in the biological fluids. This platforms may also offer an alternative for the inhibitor screening related to tau protein aggregation or phosphorylation. A further improvement of the sensitivity of the sensor may be achieved by optimizing the aptamer sequence as well as minimizing the non-specific surface adsorption.

Acknowledgments The authors thank the Department of Chemistry at Oakland University for support.

Author contributions All authors contributed equally to this work.

Funding information This project was funded in part by the NIH NIGMS R15 to S.M. (2016-2018) and C.W. (2019-2020).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Wingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. Proc Natl Acad Sci U S A. 1991;72:1858–62.
2. Alonso AD, Clerico JD, Li B, Corbo CP, Alaniz ME, Grundke-Iqbal I, et al. Phosphorylation of tau at Thr212, Thr231, and Ser262 combined causes neurodegeneration. J Biol Chem. 2010;285:30851–60.
3. Lee VM, Goedert M, Trojanowsk JQ. Neurodegenerative tauopathies. Annu Rev Neurosci. 2001;24:1121–59.
4. De Calignon A, Polydoro M, Suarez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, et al. Propagation of tau pathology in a model of early Alzheimer's disease. Neuron. 2012;73:685–97.
5. Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. Trans-cellular propagation of tau aggregation by fibrillar species. J Biol Chem. 2012;287:19440–51.
6. Babić M, Vogrinc Ž, Diana A, Klepac N, Borovečki F, Hof P, et al. Comparison of two commercial enzyme-linked immunosorbent assays for cerebrospinal fluid measurement of amyloid β1-42 and total tau. Transl Neurosci. 2013;4:234–40.
7. Carlin N, Martic-Milne S. Anti-tau antibodies based electrochemical sensor for detection of tau protein biomarkers. J Electrochem Soc. 2018;165:G3018–25.
8. Kim S, Wark AW, Lee HJ. Femtomolar detection of tau proteins in undiluted plasma using surface plasmon resonance. Anal Chem. 2016;88:7793–9.
9. Derkus B, Bozkurt PA, Tulu M, Emergul KC, Yucesan C. Simultaneous quantification of myelin basic protein and tau proteins in cerebrospinal fluid and serum of multiple sclerosis patients using nanoimmunosensor. Biosens Bioelectron. 2017;89:781–8.
10. Wang SX, Acha D, Shah AJ, Hills F, Roitt I, Demosthenous A, et al. Detection of the tau protein in human serum by a sensitive four-electrode electrochemical biosensor. Biosens Bioelectron. 2017;92:482–8.
11. Nehmé H, Chantepie S, Defert J, Morin P, Papy-Garcia D, Nehmé R. New methods based on capillary electrophoresis for in vitro evaluation of protein tau phosphorylation by glycogen synthase kinase 3-β. Anal Bioanal Chem. 2015;407:2821–8.
12. Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol. 2010;28:595–600.
13. Kontsekova E, Zilka N, Kovacech B, Skrabana R, Novak M. Identification of structural determinants on tau protein essential for its pathological function: novel therapeutic target for tau immunotherapy in Alzheimer's disease. Alz Res Ther. 2014;6:45.
14. Camero S, Benitez MJ, Cuadros R, Hernandez F, Avila J, Jimenez JS. Thermodynamics of the interaction between Alzheimer's disease related tau protein and DNA. PLoS One. 2014;9:e104690.
15. Yanamandra K, Kfoury N, Jiang H, Mahan TE, Ma S, Maloney SE, et al. Anti-tau antibodies that block tau aggregate seeding in vitro markedly decrease pathology and improve cognition in vivo. Neuron. 2013;80:402–14.
16. Vestergaard M, Kerman K, Kim D, Hip HM, Tamiya E. Detection of Alzheimer's tau protein using localised surface plasmon resonance-based immunochip. Talanta. 2008;74:1038–42.
17. Neely A, Perry C, Varisli B, Singh AK, Arbneshi T, Senapati D, et al. C. Ultrasensitive and highly selective detection of Alzheimer's disease biomarker using two-photon Rayleigh scattering properties of gold nanoparticle. ACS Nano. 2009;3:2834–40.
18. Zengin A, Tamer U, Caykara T. A SERS-based sandwich assay for ultrasensitive and selective detection of Alzheimer's tau protein. Biomacromolecules. 2013;14:3001–9.
19. Jayasena SD. Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clin Chem. 1999;45:1628–50.
20. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990;346:818–22.
21. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249:505–10.

22. Gao S, Zheng X, Jiao B, Wang L. Post-SELEX optimization of aptamers. Anal Bioanal Chem. 2016;408:4567–73.
23. Tan W, Donovan MJ, Jiang J. Aptamers from cell-based selection for bioanalytical applications. Chem Rev. 2013;113:2842–62.
24. Krylova SM, Musheev M, Nutiu R, Li Y, Lee G, Krylov S. N. tau protein binds single-stranded DNA sequence specifically–the proof obtained in vitro with non-equilibrium capillary electrophoresis of equilibrium mixtures. FEBS Lett. 2005;579:1371–5.
25. Mechaly A, Cohen H, Cohen O, Mazor O. A biolayer interferometry-based assay for rapid and highly sensitive detection of biowarfare agents. Anal Biochem. 2016;506:22–7.
26. Verzijl D, Riedl T, Parren P, Gerritsen A. A novel label-free cell-based assay technology using biolayer interferometry. Biosens Bioelectron. 2017;87:388–95.
27. Zhang M, Jiang X, Le H, Wan P, Ye B. Dip-and-read method for label-free renewable sensing enhanced using complex DNA structures. ACS Appl Mater Interfaces. 2013;5:473–8.
28. Do T, Ho F, Heidecker B, Witte K, Chang L, Lerner L. A rapid method for determining dynamic binding capacity of resins for the purification of proteins. Prot Expr Purif. 2008;60:147–50.
29. Ciesielski GL, Hytöne VP, Kaguni L. S. Biolayer interferometry: a novel method to elucidate protein-protein and protein-DNA interactions in the mitochondrial DNA Replisome. Meth. Mol. Biol. 2016;1351:223–31.
30. Auer S, Koho T, Uusi-Kerttula H, Vesikari T, Blazevic V, Hytonen VP. Rapid and sensitive detection of norovirus antibodies in human serum with a biolayer interferometry biosensor. Sens. Act. B Chem. 2015;221:507–14.
31. Gao S, Zheng X, Wu J. A biolayer interferometry-based competitive biosensor for rapid and sensitive detection of saxitoxin. Sens Act B Chem. 2017;246:169–74.
32. Gao S, Zheng X, Wu J. A biolayer interferometry-based enzyme-linked aptamer sorbent assay for real-time and highly sensitive detection of PDGF-BB. Biosens Bioelectron. 2018;102:57–62.
33. Wu X, Wu D, Lu Z, Chen W, Hu X, Ding YJ. A novel method for high-level production of TEV protease by superfolder GFP tag. Biomed Biotech. 2009;2009:108.
34. Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, et al. Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. J Neurochem. 2005;95:834–47.
35. Kim S, Lee HJ. Direct detection of α-1 antitrypsin in serum samples using surface Plasmon resonance with a new Aptamer-antibody Sandwich assay. Anal Chem. 2015;87:7235–40.
36. Kim S, Lee J, Lee SJ, Lee HJ. Ultra-sensitive detection of IgE using biofunctionalized nanoparticle-enhanced SPR. Talanta. 2010;81:1755–9.
37. Mocak J, Bond AM, Mitchell S, Scollary G. A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: application to voltammetric and stripping techniques. Pure Appl Chem. 1997;69:297–328.
38. Li Y, Lim E, Fields T, Wu H, Xu Y, Wang A, et al. Improving sensitivity and specificity of amyloid-β peptides and tau protein detection with antibiofouling magnetic nanoparticles for liquid biopsy of alzheimer's disease. ACS Biomater Sci Eng. 2019;5:3595–605.
39. Chen Z, Mengela D, Keshavanb A, Rissman RA, Billinton A, Perkinton M, et al. Learnings about the complexity of extracellular tau aid development of a blood-based screen for Alzheimer's disease. Alzheimers Dement. 2019;15:487–96.
40. Shui B, Tao D, Cheng J, Mei Y, Jaffrezic-Renault N, Guo Z. A novel electrochemical aptamer–antibody sandwich assay for the detection of tau-381 in human serum. Analyst. 2018;143:3549–54.
41. Li D, Scarano S, Lisi S, Palladino P, Minunni M. Real-time tau protein detection by sandwich-based piezoelectric biosensing: exploring tubulin as a mass enhancer. Sensors. 2018;18:946–56.
42. Demeritte T, Nellore BPV, Kanchanapally R, Sinha SS, Pramanik A, Chavva SR, et al. Hybrid graphene oxide based plasmonic-magnetic multifunctional nanoplatform for selective separation and label-free identification of Alzheimer's disease biomarkers. ACS Appl Mater Interfaces. 2015;7:13693–700.
43. Lisi S, Scarano S, Fedeli S, Pascale E, Cicchi S, Ravelet C, et al. Toward sensitive immuno-based detection of tau protein by surface plasmon resonance coupled to carbon nanostructures as signal amplifiers. Biosens Bioelectron. 2017;93:289–92.
44. Neely A, Perry C, Varisli B, Singh AK, Arbneshi T, Senapati D, et al. Ultrasensitive and highly selective detection of Alzheimer's disease biomarker using two-photon rayleigh scattering properties of gold nanoparticle. ACS Nano. 2009;3:2834–40.
45. Nu TTV, Tran NHT, Nam E, Nguyen TT, Yoon WJ, Cho S, et al. Blood-based immunoassay of tau proteins for early diagnosis of Alzheimer's disease using surface plasmon resonance fiber sensors. RSC Adv. 2018;8:7855–62.
46. Chen L, Lin J, Yi J, Weng Q, Zhou Y, Han Z, et al. A tyrosinase-induced fluorescence immunoassay for detection of tau protein using dopamine-functionalized CuInS2/ZnS quantum dots. Anal Bioanal Chem. 2019;411:5277–85.