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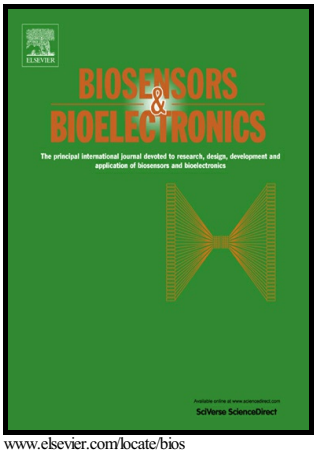

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Monitoring of early diagnosis of Alzheimer's disease using the cellular prion protein and poly(pyrrole-2-carboxylic acid) modified electrode

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Abstract

Amyloid-beta oligomers (AβO) are considered to be reliable biomarkers for the diagnosis of Alzheimer' disease (AD), and the cellular prion protein (PrP^C) is identified as a receptor for AβO. We demonstrated a label and antibody-free electrochemical biosensor for the selective detection of AβO using an electrically conductive poly(pyrrole-2-carboxylic acid) (PPyCOOH) linking agent and PrP^C receptor. In the fabrication of the biosensor, each step was characterized by electrochemical impedance spectroscopy and cyclic voltammetry. The developed PrPc/PPyCOOH biosensor exhibited extremely low detection limit of 10^{-4} pM with high sensitivity which is desirable for the early diagnosis of AD. The sensing performance was further confirmed by the mice infected with AD. The proposed sensor emerged as a promising tool for the early detection of AβO.

Keywords: Cellular prion protein (PrP^C), Amyloid beta oligomer, Alzheimer's disease, Impedance

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the presence of extracellular deposits of amyloid proteins and plaques in the brain, composed primarily of toxic aggregates of amyloid beta (Aβ) peptide (Hardy and Selkoe 2002; Kasai et al. 2013). Sensing of Aβ levels in human cerebrospinal fluid (CSF) has been widely validated as a robust biomarker for the diagnosis of AD, including in its earliest clinical stages (Fagan et al. 2009; Shaw et al. 2009). Various techniques and attempts have been made in the past to develop new assay methods for screening Aβ.

The enzyme-linked immunosorbent assay (ELISA) is widely used for measurements of Aβ peptides, the main constituents of senile plaques in AD brain (Barghorn et al. 2005). The assay is highly dependent on the conformation specificity of the antibodies used in the ELISA. Classical immunoassays including ELISA, Western blot, dot-blot, etc., are time-consuming and require indirect readouts, with long incubation steps and washing procedures that could affect the recognition of transient and unstable species (Dohler et al. 2014; Kang et al. 2013). Recently, an electrochemical sensing methodology has been adopted for probing of Aβ aggregation and its interaction with biomolecules (Kaushik et al. 2016; Zhou et al. 2016), drugs (Veloso et al. 2011; Xia et al. 2017), and metals (Geng et al. 2008) due to advantage that included ease of operation, rapid detention, low-cost, and high sensitivity. The introduction of the nanosized electrode (Wu et al. 2014; Xia et al. 2016), sensing materials (Rushworth et al. 2014), and sensing transducers (Rama et al. 2014) into electrochemical sensor fabrication has been shown to improve the sensing performance. Prabhulkar et. al developed antibody captured carbon fiber electrode for AβO detection (Prabhulkar et al. 2012). Mikula et.al prepared His-tagged protein on a redox active thiol derivative and a Cu layer for Aβ detection (Mikuła et al. 2013). Li and his colleagues utilized a ferrocene-conjugated peptide as a bioreceptor and have demonstrated the AβO detection at nanomolar levels (Li et al. 2012). Suprun and coworkers investigated that the electrochemical oxidation of AβO was determined by the oxidation of the single Tyr-10 residue, which is interacted with Zn(II) ions (Suprun et al. 2016). The AβO selectivity inherent in the methods was not examined in metal binding with Aβ (Zhang et al. 2013). The development of reliable

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tools and sensors to selectively detect the AβO at very low concentration level still represents a major need in AD research.

Several studies have identified that the cellular prion protein (PrP^C) as residues 95-110 (amino acid sequence THSQWNKPSKPKTNMK) can selectively bind AβO within the unstructured N-terminal region of the protein (Guillot-Sestier et al. 2012; Lauren et al. 2009). Since PrP^C was identified as an AβO receptor, many studies have critically explored the role of PrP^C in mediating the effects of AβO (Fluharty et al. 2013; Younan et al. 2013). Rushworth and his groups reported AβO biosensor based on biotinylated PrP^C and POPA copolymer coated electrode using electrochemical impedance spectroscopy (EIS) where the detection range was 0.5 pM to 1000 nM (Rushworth et al. 2014). Liu and his groups reported a sandwich format of cysteine based oligo-peptide containing PrP^C and alkaline phosphatase conjugated PrP^C for AβO detection. For the signal amplified detection of AβO, ferrocene methanol was used as the redox mediator where the detection rage was 3 pM to 2 nM (Liu et al. 2015). Xia and his coworker prepared Ag nanoparticles (AgNPs) based network as the redox reporter, in which adamantine labeled PrP^C induced the aggregation and color change of AgNPs. The introduction of AβO prevented the PrP^C-triggered AgNPs aggregation and allowed the detection range from 20 pM to 100 nM (Xia et al. 2016).

As shown above, the redox mediator and labeling of the peptide substrate to amplify an electrical signal maybe caused the complicated fabrication and limitation of the detection range. For the immobilization of PrP^C on the substrate, the linking agents are relative big molecules and act as considerable resistance. Thus, the change in the impedance by EIS determined charge transfer resistance may not be detected when PrP^C is reacted with AβO at a low level. Hitherto, the AβO detection at the sub-pM level to diagnose AD at early stages is not demonstrated well. To overcome the problems, herein electrically conductive polypyrrole (PPy) containing a carboxyl group was proposed for a linking agent between PrP^C and a gold substrate. PrP^C was modified its end to have an amine group for the covalent binding with the carboxyl group of PPy. Poly(pyrrole-2-carboxylic acid) (PPyCOOH) was electrochemically deposited on a gold substrate and used to immobilize PrP^C. PPyCOOH has proved to be an

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electroactive material as a bioreceptor through a covalent bonding using carboxylic group (Foschini et al. 2013; Kausaite-Minkstimiene et al. 2015). We expected that PPyCOOH allows good adhesion with gold substrate and facile electron transfer in electrochemical analysis. In the preparation of biosensor, the optimal content of PrP^C on the sensing performance in terms of detection range and limit of detection was carefully examined. In order to confirm the effect of the conductive linking agent, a non-conductive self-assembled monolayer (SAM) of 3-mercaptopropionic acid (MPA) on gold substrate was also employed to immobilize to NH_2-terminated PrP^C. It has been known that any change in electron transfer through SAM on an electrode surface can be reflected through the change of sensor response (Chun et al. 2013; Su et al. 2012). The biosensor had sensitively detected Aβ oligomer and showed insignificant response to Aβ monomer and fibrils. The sensing performance was carefully studied depending on the various fabrication conditions. Finally, the biosensor was estimated AβO levels in CSF of mice with AD, and the results demonstrated that this work was to accurately and selectively detect AβO at sub-pM level.

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2. Experimental

2.1 Materials

3-Mercaptopropionic acid (MPA), morpholino ethanesulfonic acid (MES), N-hydroxy-succinimide (NHS), N-(3-dimethyl aminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), $K_3Fe(CN)_6$, $K_4Fe(CN)_6$ and pyrrole-2-carboxilic acid (Py-COOH) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). PrP^C (THSQWNKPSKPKTNMK) was purchased from Peptron Ltd. (Daejeon Korea) and modified its end to have an amine group. The $A\beta_{1-42}$ from AnaSpec, Inc. (Fremont, USA), the tissue protein extraction reagent (T-PER, cat no: 78510) and BCA assay kit (cat no: 23225) from Thermo Fisher Scientific (Korea Ltd.), the protease inhibitor cocktail (cat no: K272-1) and phosphatase inhibitor cocktail (cat no: K276-1) from Biovision, Inc. (San Francisco Bay Area) were used as received, respectively. Phosphate-buffered saline (0.01 M PBS, pH=7.4) was prepared from NaCl, KCl, Na_2HPO_4 and KH_2PO_4. All chemicals were used without further purification.

2.2. Modification of gold electrode with PPyCOOH and Immobilization of PrP^C

The gold disc (2 mm in diameter) was polished according to the literature (Chun et al. 2013; Yang et al. 2014). Subsequently, the gold electrode was electrochemically activated in 0.5 M H_2SO_4 aqueous solution by CV from 0 to 1.5 V at a scan rate of 100 mV s^{-1} until the curves reach a steady state. PPyCOOH films were prepared on the polished gold disc (2 mm in diameter) by cyclic voltammetry from -0.3 to 1.5 V at a scan rate of 50 mV s^{-1} in 0.1 M Py-COOH acetonitrile solution containing 0.1 mol L^{-1} of $LiClO_4$ (Foschini et al. 2013; Lin et al. 2010). NH_2-PrP^C was immobilized on the activated PPyCOOH electrode by dipping in PrP^C /PBS (0.01 M, pH 7.4) solution for 1 h.

2.3. Modification of gold electrode with MPA SAM and Immobilization of PrP^C

The polished electrode was immersed in 2.5 mM MPA in water/ethanol (3/1, v/v) at room temperature (RT) as a function of time, followed by rinsing with ethanol to remove the

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residual MPA molecules. The coverage of MPA on gold electrode was confirmed by cyclic voltammetry (CV) and EIS (Limbut et al. 2006, see supporting information Figure S1). Then the MPA-modified electrode was soaked in 100 mM MES containing 20 mM EDC and 20 mM NHS to activate the carboxyl-terminated group of MPA for another 15 min (Su et al. 2012). Finally, NH_2-PrP^C was immobilized on the activated MPA SAM electrode by dipping in PrP^C /PBS (0.01 M, pH 7.4) solution for 1 h.

2.4 Treatment of Aβ solution (Stine et al. 2003).

To obtain Aβ monomers, the lyophilized peptide was dissolved in hexafluoroisopropanol (HFIP) and incubated overnight at room temperature. HFIP was evaporated, and the Aβ was redissolved in dimethylsulfoxide and stored at -20℃ as a stock solution. The Aβ oligomers and fibrils were obtained by the incubation of the Aβ monomer in the dark 37℃ for 24 h and 72 h, respectively. The successful preparation of AβO was confirmed via Western blot (see Figure S2).

2.5 Characterization

All electrochemical experiments were performed using VSP Potentiostat (Princeton applied research, USA) and the resulting curves were recorded with VSP EC-Lab software. CV and EIS were carried out in a conventional three-electrode system with a gold disc electrode (CH Instruments Inc., USA; diameter: 2 mm), an Ag/AgCl and a platinum plate as the working, reference and counter electrodes, respectively. In CV measurement, the conditions where at constant as followings: applied potential -0.3 - 0.6V in 20 mM $Fe(CN)_6^{3-/4-}$ /PBS (pH 7.4) at a scan rate of 100 mV/s. In the EIS measurement, a 10 mV amplitude sine wave was applied to a gold electrode in the frequency range of 0.1 Hz to 100 kHz and 20 mM $Fe(CN)_6^{3-/4-}$ /PBS (pH 7.4) was used as the electrolyte. The ZSimpWin EIS DATA analysis software (Perkin-Elmer, Version 2.00) was used to analyze the obtained EIS data and to fit an equivalent circuit.

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2.6 Real sample analysis

All animals in these experiments were purchased from the Jackson Laboratory (Maine, USA) and then kept and raised in the School of Pharmacy at Sungkyunkwan University. Hemizygous 5xFAD Alzheimer diseased (AD) mice (APPSwFlLon/ PSEN1*M146L*L286V) were maintained on a C57BL/6J background. All mice were raised in a constant environment, RT (22 ±2℃), and humidity (55±15%), and maintained dark-light cycle were kept on a 12 h light (lights on at AM 08:00) and 12 h dark schedule with free access to feed and water. AD mice were raised for seven months with wild type littermate. All efforts were made to minimize suffering and the number of animals used. At 7 months of age, wild type mice (WT mice) and AD mice were sacrificed. Their brains were collected and stored at -70℃. The collected brain tissues were homogenized to obtain protein extracts using T-PER with protease inhibitor cocktail and phosphatase inhibitor cocktail. The homogenized brain tissues were incubated on ice for 30 min and vortexed at maximum speed every minute. After that, these samples were centrifuged at 13000 rpm for 30 min. The supernatants were collected and calculated protein concentration using BCA assay kit and protein extracts were stored at -70℃ for later use. The trace of Aβ in the brains was confirmed via Western blot and EIS (in Figure S3).

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3. Results and Discussion

3.1. Fabrication of PrP^C/PPyCOOH sensor and its sensing performance

The stepwise preparation of the biosensor and its AβO detection was represented in Figure 1: 1A for PrP^C/PPyCOOH sensor and 1B for PrP^C/MPA SAM sensor. PPyCOOH film was successfully deposited by CV on the gold substrate (in Figure S4). The electrochemical behaviors of PPyCOOH were corresponded well with those obtained by others (Foschini et al. 2013;Kausaite-Minkstimiene et al. 2015). The optimal MPA SAM conditions were discussed in the supporting information (in Figure S1). The surface properties of the gold electrode at each modification step were characterized by EIS, which was conducted and shown in Figure 1B and D. In the inset, R_s, W, R_{et}, and C represent the solution resistance, the Warburg diffusion resistance, the electron-transfer resistance, and the double layer capacitance, respectively (Kim et al. 2016). EIS provided detailed information on surface changes of electrodes during modification processes. Various models of circuit were tested for matching experimental results with the model fitting (Su et al. 2012; Yang et al. 2014). The proposed circuit was well matched to the both of the electrode, PPyCOOH and MPA SAM and EIS response. EIS data were fitted using the selected equivalent circuit. The sequential increase in the electron-transfer resistance R_{et}, indicative of a successful stepwise modification of the gold electrode, arose from each layer added to the electrode surface at the corresponding modification stage (Kim et al. 2017). The bare gold corresponded to an almost straight line in the Nyquist plot of impedance spectroscopy, which was characteristic of a diffusion-controlled process over the whole frequency range. After the modification of PPyCOOH (or MPA SAM), a semicircle up to the mid-frequency region revealed a charge transfer-controlled process. The EIS data indicated that the successful formation of PPyCOOH (or MPA SAM) hindered slightly the electron transfer to the electrochemical probe. After the amine terminated PrP^C peptide was immobilized on the PPyCOOH layer (or MPA SAM), the semicircle of the EIS curve further increased indicating that PrP^C prevents $Fe(CN)_6^{3-/4-}$ ion transference moreover. After binding of PrP^C with AβO, the resistance increased dramatically, because the linked AβO could block the charge exchange and mass

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transfer, further insulating the conductive support and significantly hindering the access of redox probe towards the electrode surface effectively. These results confirmed that the PrP^C/PPyCOOH/Au electrode could be successfully prepared and suitable for AβO detection.

To further confirm the modification step more precisely, the electrochemical behavior gold electrode at each modification step was characterized by CV and discussed in supporting information (Figure S5). The increase in the impedance was proportional to the modification, which results were quite consistent with the results from the CV experiments.

The EIS response indicated the successful formation of PrP^C/MPA on gold (in Figure 1D). The sensing performance of PPyCOOH modified electrode was evaluated with that of conventional mercaptopropanoic acid (MPA) SAM modified electrode.

In order to optimize the PrP^C content for the AβO sensing performances, various amount of PrP^C were incorporated in the sensor and incubated in a different concentration of AβO solution. The EIS response of the various electrodes was recorded in Figures 2. As expected, Figure 2A showed the R_{et} increments according to the amount of PrP^C due to the prevention of $Fe(CN)_6^{3-/4-}$ ions from reaching the electrode surface. The sensing performance was depended on the amount of immobilized PrP^C. The relationship between R_{et} value change as a function of PrP^C amount and the detection range of AβO was plotted in Figure 2B. The R_{et} values were summarized in Table S1. As expected, the sensitivity of the PPyCOOH electrode was gradually increased with the increase of the amount of PrP^C (Limbut et al. 2006; Zhou et al. 2016). However, in the case of the excess amount PrP^C (7 mg/mL), the entry barriers of AβO might be getting higher to yield lowering the upper limit of detection range.

The detailed AβO detection information of the 1 mg/mL PrP^C immobilized PPyCOOH electrode was presented in Figure 3A and B. The PPyCOOH electrode exhibited a R_{et} value of 89 Ω cm^{-2}. There was an original semicircle domain on the prepared PrP^C sensor, implying an inherent electron transfer resistance of about 270 Ω cm^{-2}. When PrP^C bound with AβO on the electrode surface, the semicircle of the EIS curve increased progressively from 296 Ω

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cm^{-2} at 10^{-4} pM to 904 Ω cm^{-2} at 10 nM. In the case of 20 nM AβO, the R_{et} value was not increased any more as shwon in Figure 3A and Figure S6 (with high amplification). The increase of R_{et} was proportional to the logarithmic concentration of AβO, and can be expressed in a linear fashion, as in $y = ax + b$, where a is the slope of the calibration curve in terms of sensitivity, and R^2 is the coefficient of the determination. The linear regression equation was determined to be ΔR_{et} (Ω)=74.5 log $C_{A\beta}$ (nM)+562 in the broad range of 10^{-7} to 10 nM with a detection limit of 10^{-7} nM, an R^2 value of 0.9928 and the RSD of 1.01 % (Figure 3B). The low detection limit and the wide detection range with excellent linear relationship suggested that the prepared electrode could be used in practical application.

The MPA on gold electrode exhibited a R_{et} value of 980 Ω cm^{-2} which is relatively higher than that (89 Ω cm^{-2}) of PPyCOOH layer. For the immobilization of PrP^C(1 mg/mL), the R_{et} value was further increased to 1217 Ω cm^{-2}. After the binding with AβO, the value was increased to 1835 Ω cm^{-2} at 10^{-2} nM in Figure 3C. The limit of detection was much higher than that of PPyCOOH linker which might be due to the relatively high initial R_{et} value limiting the detection of small variation of resistance. The linear regression equation (in Figure 3D) was determined to be ΔR_{et} (Ω)=219.9 log $C_{A\beta}$ (nM)+1109, R^2=0.996. The sensing trends were similar to those obtained by the PPyCOOH electrode. The optimum detection range of PrP^C/MPA was from 10^{-5} to 10^{-2} nM.

The overall performance of PPyCOOH based sensor was superior to that of MPA-based sensor in terms of sensitivity and limit of detection. There was a great discrepancy of initial resistance of the two electrodes: 89 Ω cm^{-2} for PPyCOOH/gold and 980 Ω cm^{-2} for MPA SAM/gold. The variation of R_{et} after the immobilization of PrP^C (1 mg/mL) was much higher for the MPA electrode (Δ R_{et}: 237) than that for the PPyCOOH electrode (Δ R_{et}: 181) as shown in Table S1. The variation was further increased after the binding with AβO (0.001 nM); Δ R_{et}: 590 for the MPA electrode and Δ R_{et}: 377 for the PPyCOOH electrode. The initial high R_{et} value may cause trouble for detecting low concentration of AβO in the impedance sensors. Meanwhile, the electrically conducting PPyCOOH-based electrode yielded much lower R_{et} value, which allowed much more sensitive detection up to10^{-4} pM of AβO.

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The sensing performance of the PrP^C based AβO sensor was compared with sensors reported by others in Table 1. The developed sensors have attached neither a conductive binder nor label compared with others in which PrP^C/PPyCOOH based sensor exhibits exceptionally low detection limit of 10^{-4} pM.

3.2. Real sample test

For the real sample analysis, the scarified brain samples (400 mg) were first dissolved in small quantities of T-PER (1 mL) and then diluted with PBS to the final concentrations. The procedure for the sampling and analysis was represented in Figure S3. In the 0.05 mg/mL normal mice (WT), 1.679 x 10^{-7} nM AβO was found to be used by the PrP^C/PPyCOOH electrode with 3.1 % RSD. In the 0.05 mg/mL AD mice, 1.45 x 10^{-6} nM AβO was found with 2.1 % RSD. Table 2 provides an analysis of AD mice on the both of and PrP^C/PPyCOOH and PrP^C/MPA SAM based electrode. The recoveries of AβO from different concentrations of AD mice brain samples were found to be 98-116% with RSD of less than 5%. These results indicated that the PrP^C based AβO sensor is promising for AβO detection with satisfactory reliability in actual implementation.

3.3. Regeneration and stability and selectivity to Aβ oligomer

The regeneration of biosensors was important for its practical application. Generally, highly acidic or alkaline surroundings would damage the immobilized peptide/ aptamer/protein or affect the interaction between peptide/aptamer/protein and their targets (Chun et al. 2013). The regeneration efficiency of the AβO sensor was evaluated by comparing R_{et} values of EIS responses obtained before and after incubating the sensor in $Fe(CN)_6^{3-/4-}$/PBS solution containing 0.001 nM AβO repeatedly. Following each measurement cycle, the sensor was incubated in pH 4 PBS for 10 min to render desorption of PrP^c from peptide molecules and washed thoroughly with pH 7.4 PBS to restore the conformation of the sensor to its native structure prior to proceeding to the next cycle of measurement. As shown in Figure S7, R_{et} values of EIS responses from AβO-decoupled (A) and AβO-coupled (B) electrodes are

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compared for each measurement cycle. R_{et} values recorded from the fresh (■), first (●), second (▲), third (▼), and fourth (◂) cycle of measurement time regenerated electrodes were almost equal to one another before and after AβO detection with a relative standard deviation (RSD) of only 5 %, indicating that the regenerated sensor worked as efficiently as the freshly fabricated sensor.

For the stability study, the PrP^C based electrodes were stored at 4℃ and the current response was measured every seven days. No significant change was observed after one month, indicating that the sensor possesses adequate stability for amyloid-beta sensing applications.

The selectivity of the PrP^C based electrode was tested by measuring the response to the Aβ monomer, oligomer and fibril (see in Figure S8). Compared with the sensitivity toward Aβ monomer and fibril, the detection of oligomer showed high sensitivity.

4. Conclusion

We developed the AβO sensor based on PPyCOOH and PrP^C without any label and redox mediator. A electrically conductive polymer, PPyCOOH, was designed as an active electrode material and also a linking agent for the first time. The direct electrochemical analysis of AβO using EIS technique and the simple component of only PPyCOOH and PrP^C have allowed detecting at a 10^{-4} pM level. The proposed electrically conductive linking agent emerges as a promising tool for the detection at a low level, and its immobilization strategy will be useful for the development of other biosensor platforms. The sensing performance of the AβO sensor was also evaluated by using the mice infected with AD. The biosensor can provide a simple, sensitive, and selective detection to specifically measure the Aβ oligomer and monitor the early diagnosis of the AD.

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Figure 1. The stepwise preparation of the biosensors and their AβO detection and EIS analysis: Preparation steps (A) and Nyquist plots (B) for PrP^C/PPyCOOH sensor; Preparation steps (C) and Nyquist plots (D) for PrP^C/MPA SAM sensor

Figure 2. Nyquist plots of PPyCOOH electrode and PrP^C immobilized electrode as a function of PrP^C concentration (A); Linear relationship between ΔR_{et} value and logarithmic AβO concentration as a function of PrP^C amount (B) The value in the bracket is a sensitivity.

Figure 3. Nyquist plots of PrP^C (1 mg/mL) immobilized PPyCOOH electrode as a function of AβO concentration (A); Linear relationship between ΔR_{et} value and logarithmic AβO concentration (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 1, 10, and 20 nM) from A (B) Nyquist plots of PrP^C (1 mg/mL) immobilized MPA SAM electrode as a function of AβO concentration (C); Linear relationship between ΔR_{et} value and logarithmic AβO concentration (10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2}) from C (D)

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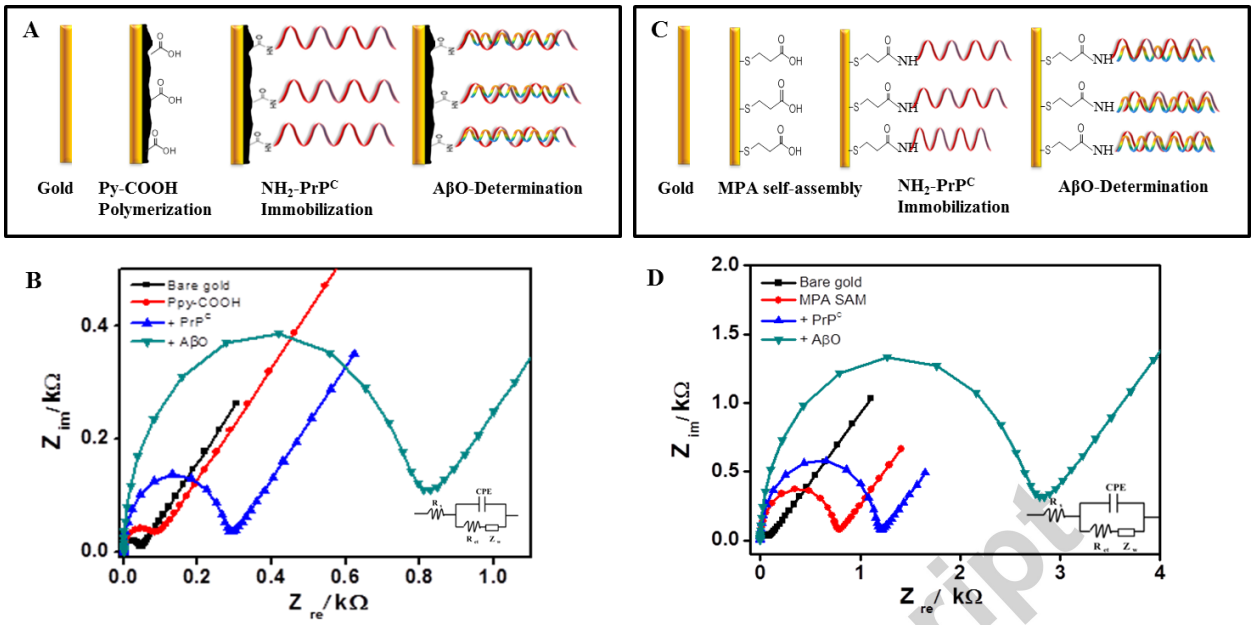

Figure 1

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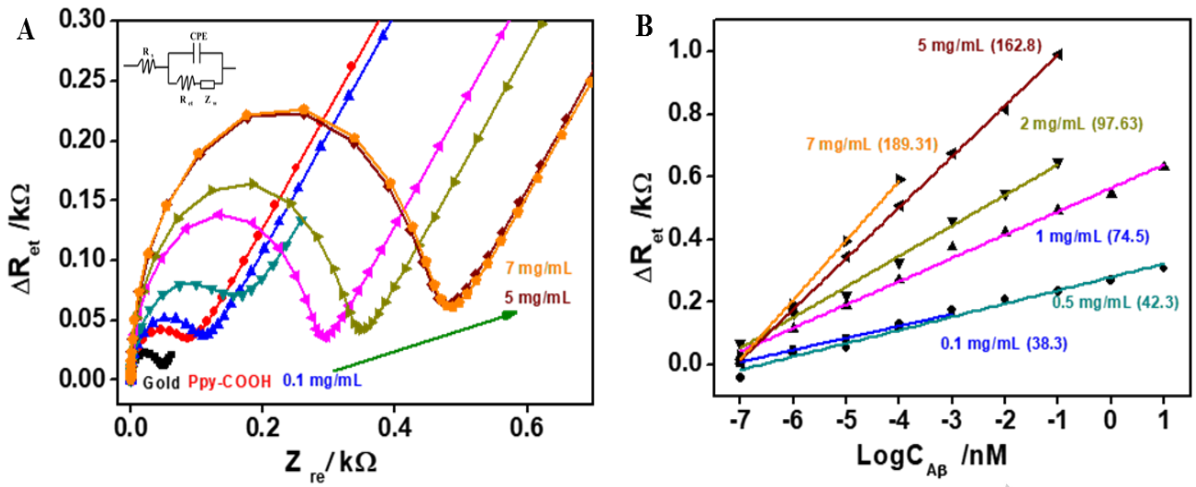

Figure 2

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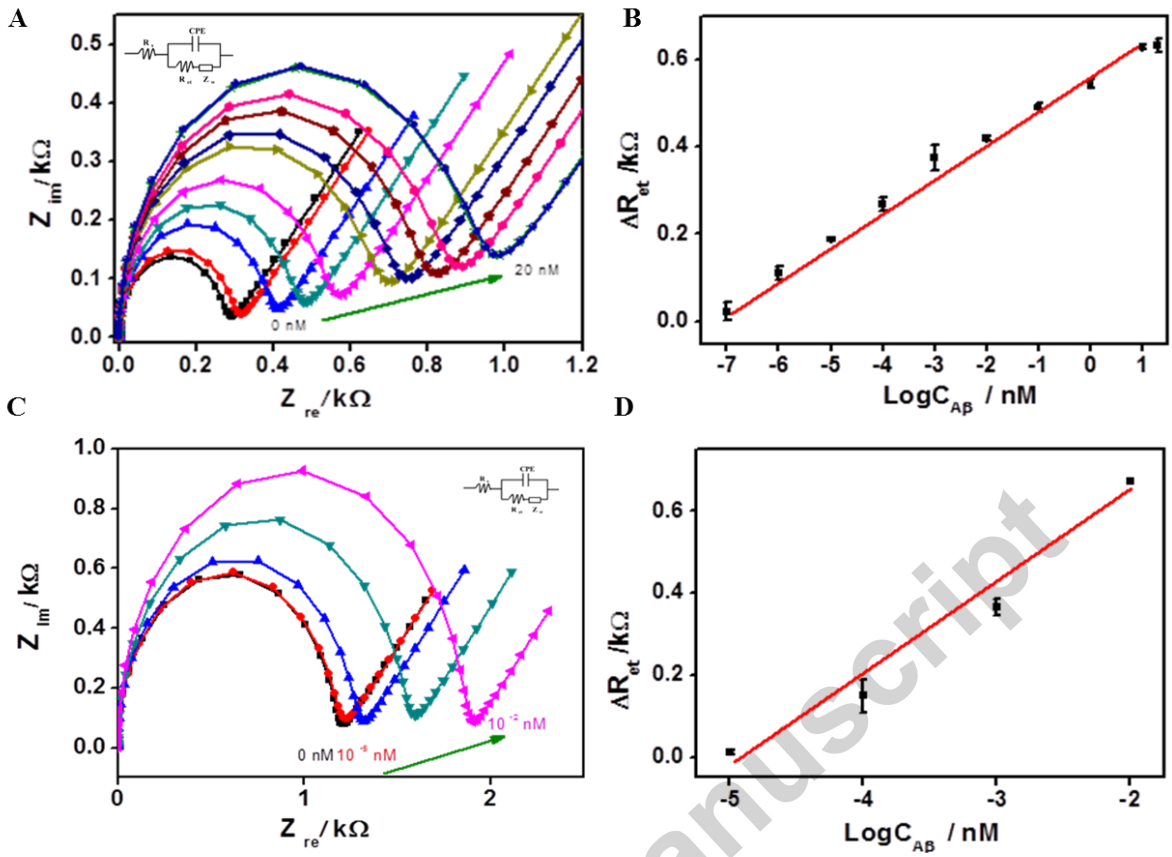

Figure 3

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Table 1. Summary of sensitivity performance of electrochemical amyloid-β sensors

Electrode	Linear range (nM)	Analysis	Limit of detection (nM)	Reference
Ad-PrP^C/MCH/ β-CD/Au [a]	$2x10^{-2}$-10^2	LSV	0.008	(Xia et al. 2016)
PrP^C/POPA/Au [b]	10^{-3}-10^3	EIS	0.0005	(Rushworth et al. 2014)
PrP^C/Cysteine/Au [c]	$5x10^{-3}$-2	CV	0.003	(Liu et al. 2015)
Antibody/AuNP/AAO [d]	$2x10^{-6}$-2.22	EIS	0.00022	(Wu et al. 2014)
An βA Abs/Au [e]	10^{-2}-10^2	EIS	0.01	(Kaushik et al. 2016)
Peptide/MUA/Au [f]	0.48-12	SWV	0.24	(Li et al. 2012)
Antibody-aptamer [g]	0.5-30	DPV	0.1	(Zhou et al. 2016)
PrP^C/PPyCOOH/Au	10^{-7} -10	EIS	10^{-7}	This work

[a] Ad-PrP^C: adamantine labeled PrP^C; [b] PrP^C/POPA: PrP^C/ copolymer of polytyramine and 3-(4-hydroxyphenyl) propionic acid; [c] Cysteine-containing PrP^C /alkaline phosphatase-conjugated PrP^C; [d] Aβ antibody/gold nanoparticle decorated AAO; [e] An βA Abs: specific anti-beta-amyloid antibodies; [f] Peptide: I10 peptide; [g] Antibody-aptamer/graphene/Au

Table 2. Detection of AβO in brain sample of normal mice and Alzheimer diseased mice

Sample	Spiked (nM)	After spiking (nM)	RSD (%)	Recovery (%)
PrP^C /PPyCOOH based electrode				
In 0.05 mg/mL WT, 1.679 $x10^{-7}$ nM (RSD 3.1 %) AβO				
In 0.005 mg/mL AD, 1.45 $x10^{-6}$ nM (RSD 2.1 %) AβO	0.01	$9.86x10^{-3}$	3.5	98.6
In 0.05 mg/mL AD, 1.3 $x10^{-5}$ nM (RSD 1.4 %) AβO	0.01	0.0105	2.3	104.8
In 0.5 mg/mL AD, 1.13 $x10^{-4}$ nM (RSD 1.4 %) AβO	0.01	0.0117	2.9	115.9

WT: wild type, normal mice, AD: Alzheimer's disease mice, RSD: relative standard deviation for three independent measurements, Recovery: (after spiking–before spiking)/(spiked)x100%.

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Research Highlights

►Ultra-sensitive amyloid β oligomer (AβO) sensor based on PPyCOOH and PrP^C

►Label-free and one-step preparation and direct detection of PrP^C to AβO

►Excellent performance in AβO detection with 10^{-4} pM and real sample test in AD mice

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