Biosensors and Bioelectronics 101 (2018) 96–102

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# A shape-code nanoplasmonic biosensor for multiplex detection of Alzheimer's disease biomarkers

Hanbi Kim, Jong Uk Lee, Sojin Song, Soohyun Kim, Sang Jun Sim*

*Department of Chemical and Biological Engineering, Korea University, Seoul 02841, South Korea*

ARTICLE INFO

*Keywords:*
Shape-code
Multiplex detection
Alzheimer's disease (AD)
Gold nanoparticles (AuNPs)
Plasmonic biosensors
Localized surface plasmon resonance (LSPR)

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease associated with the loss of nerve cells in the brain. The disease is affected by multifactorial pathways and leads to changes in related biomolecular levels as AD progresses. Therefore, AD should be diagnosed with combined detection of several lesions to improve accuracy. Amyloid beta 1–40, 1–42 and τ (tau) protein are milestones in AD pathology and can be used as main screening and diagnostic target markers. Here, we suggest a highly selective biosensor for detection of AD core biomarkers on one platform through distinct localized surface plasmon resonance (LSPR) depending on gold nanoparticles shapes, called a shape-code biosensor. This plasmonic sensor consists of only gold nanoparticles and antibody, but does not need additory methods for precise separation from multifarious samples and identification of markers. Under physiological condition, we determined a detection limit of 34.9 fM for amyloid beta (Aβ) 1–40, 26 fM for Aβ 1–42 and 23.6 fM for τ protein corresponding to the ~ 1, ~ 2.23 and ~ 3.12 nm of Rayleigh scattering peak shift on shape-code plasmon system for each biomarker in mimicked blood. This is the first highly sensitive shape-code biosensor to detect AD biomarkers which can be used to diagnose AD easily in the future.

## 1. Introduction

Alzheimer's disease (AD) is an irreversible neurodegenerative disease causing severe cognitive decline and is the most frequent form of dementia in the elderly (Liu et al., 2013).

The disease is generated by complex pathological ways and several molecules, such as formation of amyloid load, tauopathy, inflammation and oxidation damage (Gupta et al., 2013). The formation of amyloid senile plaques and neurofibrillary tangles in the brain is a hallmark of AD (Citron et al., 1995). Amyloid plaques mainly contain overexpressed amyloid beta (Aβ) 1–40 and Aβ 1–42 derived from the abnormal processing of amyloid precursor protein (APP) (Citron et al., 1995). Extracellular deposits of proteins and resulting plaques generate neurotoxicity in the brain by interrupting signal transfers between neurons (Kanekiyo et al., 2014; Liao et al., 2012). There is growing evidence that simultaneous or perhaps even earlier neurofibrillary tangles are composed of intraneuronal aggregates of abnormally hyper-phosphorylated τ proteins disengaged from microtubules (Frank et al., 2003; Martin et al., 2011). Dissociation of τ protein leads to collapse of microtubules and impairs the neuron system (Martin et al., 2011).

Some pathological processes are similar with senility in the aged, for example, the concentration of both amyloid beta (Aβ) 1–40 and Aβ 1–42 increases with age older than 65 and increments are also known as a beginning milestone of amyloid aggregation in the brain of AD patients. Yet, as the lesion progresses, plasma Aβ 1–42 level diminishes at an average rate of 12% per year in people with AD and concentration of plasma Aβ 1–40 rises constantly regardless of having the disease (Van Oijen et al., 2006). In addition, a growing body of literature has revealed that Aβ amyloid deposition in the brain correlates inversely with concentration of Aβ 1–42, while τ protein levels reflect the stage of neuronal degeneration and brain damage (Kang et al., 2012). Consequently, Aβ1–40, Aβ1–42 and τ protein, the constituents of the amyloid load and neurofibrillary tangles, respectively, are considered as reliable and complementary biomarkers for AD diagnosis based on combined detection (Frank et al., 2003; Schneider et al., 2009). AD patients cannot be accurately diagnosed by individual physiological variation due to the heterogeneity of dementia pathology. It is imperative to deviate from the traditional approach of investigation standardized on single biomarker candidates and develop diagnosis methodology of multiplex biomarker detection to reflect the complexity of AD pathogenesis. Simultaneous detection of two or more markers achieves higher sensitivity than one marker to distinguish Alzheimer's disease patients and others, indicating that each marker may be complementary and not redundant with the other for accurate diagnosis (Abdi et al.,

* Corresponding author.
*E-mail address:* simsj@korea.ac.kr (S.J. Sim).

http://dx.doi.org/10.1016/j.bios.2017.10.018
Received 8 September 2017; Accepted 9 October 2017
Available online 11 October 2017
0956-5663/ 

2006). For instance, the combination of AD core biomarkers (e.g. Aβ and τ protein) revealed balanced value of prediction factor for distinguishing of AD from healthy control as 85.7% of sensitivity and 84.6% of specificity with ratio τ protein/Aβ1–42 in brain, as compared with single biomarker based methodology (tau with 69.6% and 92.3% of sensitivity and specificity; Aβ1–42 with 96.4% and 76.9% of sensitivity and specificity, respectively) (Shaw et al., 2009).

To this end, many studies have formulated groundbreaking methods to discriminate between age-matched controls and AD patients and evaluated different combinations of protein chips to yield high sensitivity and specificity (Hye et al., 2006), but thus far, existing methods are predominantly required two steps that are same as cognate with conventional protein identification and measurement: separation of proteins biomarkers from sample and peptide ionization for quantification of targeted markers. The first separation process is accomplished by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) or high-performance liquid chromatography (HPLC) (Butterfield et al., 2003); surface chromatography absorbing proteins to activated surfaces (Carrette et al., 2003) and then mass spectroscopy (MS) is used for peptide ionization procedures from gels or protein chips as quantitative measurement of the target molecules. While these technologies are typically accessible to researchers, they are incapable of application for low molecular weighted, hydrophobic peptides and heterogeneous compounds as well as measurement of dynamic quantitative range. Surface enhanced laser desorption/ionization-time of flight MS has been newly introduced with more thorough quantification and capture of native proteins, notwithstanding complex procedure is required for post-processing and reproduction (Gupta et al., 2013). Other universal studies have been based on enzyme-linked immunosorbent assay (ELISA) methodology such as xMAP-Luminex multiplex platform using Innogenetics AlzBio3 immunoassay reagents to detect concentration of Aβ 1–42, total τ protein and phosphorylated τ protein at 181 (Kang et al., 2012). It improved dynamic range and decreased required sample volume to approximately half for analyses of three biomarkers as compared to conventional ELISA, but requires 10 handling steps and 19 h to analyze three biomarkers, totally.

In this study, we suggest a highly selective non-labeling platform for analyzing three predominant AD biomarkers including Aβ 1–40, Aβ 1–42 and τ protein using an ultra-sensitive 'shape-code' nanoplasmonic sensor. We simultaneously exploit three different kinds of immuno-AuNPs, such as spheres (diameter of 50 nm), short rods (aspect ratio of 1.6) and long rods (aspect ratio of 3.6) for detecting Aβ 1–40, Aβ 1–42 and τ protein, respectively, showing individual properties on optical spectrometers as if the bar-code is scanned. Nanoparticles consisted of 'shape-code' plasmonic biosensor based on localized surface plasmon resonance (LSPR). The plasmonic biosensor under the electromagnetic field of incoming light enables detection of incident photon frequency produced by collective coherent oscillation of free electrons that hem in nanometer-sized metallic particles. The result is Rayleigh light scattering, called LSPR (McFarland and Duyne, 2003; Anker et al., 2008; Lee et al., 2015). The plasmon resonance wavelength on the surface of the nanoparticle (LSPR) is dependent on shape, size, and local refractive index surrounding AuNPs (Lee et al., 2015). On this account, varied shapes and sizes easily tune optical properties of gold nanoparticles including surface plasmon resonance wavelength without any dyes (Link et al., 2000). Since the $\lambda_{max}$ position directly relates to the color of AuNPs in dark-field, recognition of each immuno-particle can easily be conducted without labeling steps or scattering measurements (Gish et al., 2007; Rosi and Mirkin, 2005; Truong et al., 2012). Also, this nanoplasmonic biosensor sensitively detects molecular interaction (e.g. antibody-protein interactions and DNA hybridization) occurring on the surface of gold nanoparticles, since surface-bound events arise from changes in the dielectric environment surrounding the particle having significant effect on the extinction peak ($\lambda_{max}$) of nanomaterials (Hall et al., 2011). Based on that, our 'shape-code' plasmonic biosensor detects individual antibody-protein interaction on distinct immuno-gold nanoparticles (AuNPs) and the colorimetric feature of it enables multiplex detection of biomarkers from single sample without additional steps for protein identification. It also reduces analytical time via detection of three biomarkers in one analytical run and increases detected throughput. Such highly sensitive and selective immunoassays in multiplexed fashion are essential methods for disease diagnostics, therapeutics and biodefense applications (Stoeva et al., 2006). As a consequence, we successfully developed a novel methodology for detecting AD-related biomarkers with considerably high selectivity, sensitivity and close approach to AD complex pathway promoting realization of diagnosis of AD.

## 2. Materials and methods

### 2.1. Materials

NHydroxysuccinimide (NHS), sodium citrate, absolute ethanol, 3-aminopropyltriethoxysilane (APTES), sodium citrate, L-ascorbic acid, silver nitrate ($AgNO_3$, > 99%), NethylN(diethylaminopropyl)carbodiimide (EDC), sodium borohydride, gold(III) chloride trihydrate (≥ 99.0%), dimethyl sulfoxide (DMSO), Sodium borohydride ($NaBH_4$, 99%), hydrochloric acid (HCl, 37 wt% in water), Hexadecyltrimethylammonium bromide (CTAB, > 98.0%) were purchased from Sigma Aldrich (Korea). Sodium oleate (NaOL, > 97.0%) was purchased from TCI America. COOH-PEG-SH (Mw 2000, 3400 and 5000) was purchased from Laysan Bio, Inc. Anti-Aβ 1–40 antibody and recombinant Aβ 1–42 were purchased from Abcam. Anti- Aβ 1–42 antibody was purchased from Millipore. Recombinant Aβ 1–40 was purchased from Anaspec. Coverslip slides (22 × 40 × 0.1 mm) were purchased from Deckglaser (Germany). Ultra–pure water (18.2 mΩ $cm^{-1}$) was used to prepare all solutions.

### 2.2. Preparation of gold nanoparticles for shape-code biosensor

Gold nanoparticles (AuNPs) were synthesized citrate reduction method for gold nanospheres and seed-mediated growth method of gold nanorods followed synthesized methods in previous studies (Jv et al., 2010; Ye et al., 2013). To connect with antibody and render gold nanoparticle stable in physiological conditions, heterofunctionalized polyethylene glycol (PEG) was used as a ligand to functionalize the surface on gold nanoparticles.

For functionalization of 50 nm gold nanospheres, 20 μL of 1 mM PEG (Mw 2000) was added in 1 mL of synthesized AuNSs solution and mixed with full turn for 24 h. The mixture was centrifuged at 7000 rpm for 15 min to discard excess PEG molecules and unreactive AuNPs, and re-suspend in 100 μL of deionized water. Conversely, gold nanorods were functionalized with longer polyethylene glycol. 40 mg of Mw 3400 or 5000 PEG were proportionally mixed to 2 mL AuNRs solution (300 μg Au/mL). Resulting solution was centrifuged at 5000 rpm for four minutes after which the mixture was swirled vigorously for four days.

On subsequent step to conjugate antibody to carboxylated gold nanoparticles, 3 μL of 0.7 M EDC/NHS was mixed with 300 μL of functionalized gold nanoparticles solution to convert carboxyl groups of PEG to NHS esters for connecting with amine group of antibodies after 15 min, 150 μL of each target protein monoclonal antibody (mAb) (50 μg/mL) was added to ester activated AuNPs solution. The solutions were mixed overall and incubated at room temperature. After four hours, the immune-gold nanoparticles were centrifuged at 5000 rpm for 10 min or 3000 rpm for four minutes to separate unreactive particles and molecules. The pellet was re-dispersed in deionized water and stored at 4 °C for stabilization.