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A Nanoplasmonic Biosensor for Ultrasensitive Detection of Alzheimer's Disease Biomarker Using a Chaotropic Agent

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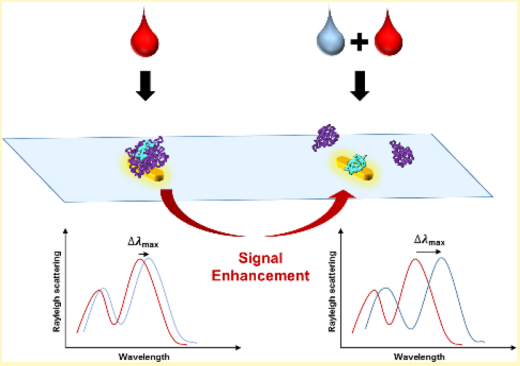
Supporting Information

ABSTRACT: Blood-based diagnosis (hemodiagnosis) of Alzheimer's disease (AD) is emerging as a promising alternative to cerebrospinal-fluid-based methods because blood contains various kinds of AD biomarkers, including amyloid beta 1−40, 1−42, and τ (tau) protein. However, with current technology, the accuracy of the blood-plasma-based methods is relatively low compared to the traditional methods because the concentration of AD biomarkers in blood plasma is incredibly low, and diverse interference is present in blood plasma, which hinders precise detection. Here, we suggest a nanoplasmonic biosensor using gold nanorods with a chaotropic agent for precise ultrasensitive detecting of Alzheimer's disease biomarkers in human plasma. This nanoplasmonic biosensor is based on the localized surface plasmon resonance (LSPR), which is extremely sensitive to the point where it can respond to an insignificant change of the refractive index around the gold nanoparticles. Also, using guanidine hydrochloride as a chaotropic agent, we can overcome the obstacles of blood-based AD diagnostics. In more detail, this agent interrupts the network between water molecules and weakens the hydrophobic interactions between proteins, remarkably improving detection capabilities to target τ protein. By reducing the overlapping ranges between protein levels in an age-matched control and AD patients' plasma, this system can accurately diagnose AD patients. This platform also can analyze disease from mild cognitive impairment using standardized blood biomarker tau protein, which is related to Alzheimer's disease. As a result, our platform can be applied to clinical trials, and thus it has excellent potential in the medical field.



KEYWORDS: *Alzheimer's disease (AD), chaotropic agent, τ (tau) protein, gold nanorods (AuNRs), plasmonic immunoassay, localized surface plasmon resonance (LSPR)*

As the world is rapidly becoming an aging society, dementia has been noted as a crucial social malaise.[1] In 2010, one set of projections revealed that 30 million patients in the world suffer from Alzheimer's disease (AD). This disease is regarded as a familiar form of dementia, and its prevalence is estimated to increase to 106.2 million by 2050.[1−3] AD is also designated a "tauopathy" due to the neurofibrillary tangles (NFTs) that form from pathological lesions.[4] The structural unit of NFTs is the tau (τ) protein, which is normally a microtubule-associated protein.[5] The extension of microtubules is controlled by numerous phosphorylation sites on expressed τ proteins.[6] Although both dephosphorylated and phosphorylated τ protein remain in equilibrium under physiological conditions, forms that are hyperphosphorylated at serine and threonine amino acid forms accumulate rapidly in the AD brain.[5] When the τ protein affinity for microtubules is decreased, soluble protein is released into cerebrospinal fluid (CSF), and phosphorylated τ (p-τ) protein separates from the axonal microtubules and aggregates into paired helical filaments that assemble into NFTs.[6,7] The level of total τ (t-τ) protein as well as p-τ protein was thus suggested to correlate with post-mortem neurofibrillary tangle load, and load-bearing neurons might contribute to the CSF level of t-τ protein.[8,9] CSF t-τ protein levels mirror the severity and intensity of neuronal degeneration and damage.[5] Clinical studies indicate that AD patients have approximately 3-fold more CSF t-τ protein than healthy elderly individuals.[10] Many studies have focused on CSF for the development of methods to diagnose AD early and accurately[11] because these pathological changes in the brain can be detected after the starts of serious stages, which occur at every step that generates irreversible neurofibrillary tangles.[12] Since CSF moves throughout the brain and spinal cord, it contains various proteins produced by the brain, including τ protein.[9] However, the performance of AD diagnostics based on CSF biomarkers is far from optimal. First, many clinically diagnosed cases have revealed a relatively large percentage of misdiagnoses based on the use of CSF biomarkers to discriminate AD patients from the elderly.[13,14] Whether differences in CSF biomarker profiles can be the standard for distinguishing AD patients from the elderly lacks

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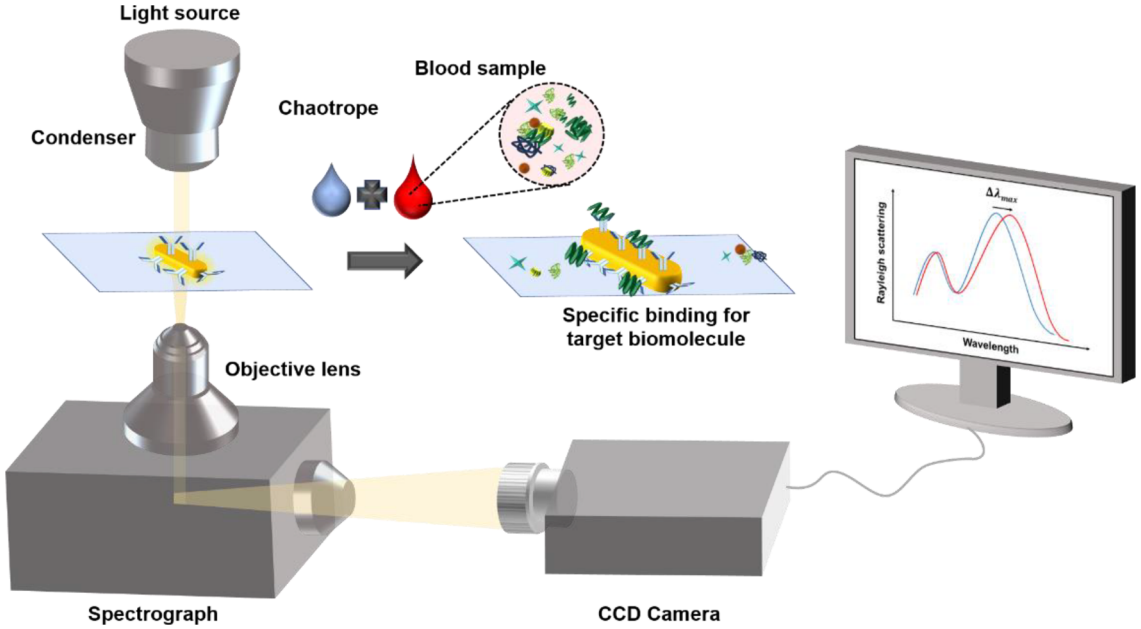

Figure 1. Schematic illustration of nanoplasmonic biosensor system for delicate hemodiagnosis of Alzheimer's disease.

consensus.[15,16] Second, even though CSF directly represents the condition of the central nervous system, the typical process of taking CSF from patients (e.g., lumbar puncture) triggers physical discomfort and poses several serious drawbacks such as risks of infection at the puncture site and damage to the brain and spinal cord.[17]

For the above-mentioned reasons, hemodiagnosis of AD has recently attracted considerable interest. Blood plasma is a great fluid to use as a sample candidate for the diagnosis of AD because it contains many AD biomarkers (i.e., τ protein, Aβ 1−40, and Aβ 1−42). Plasma τ protein levels positively correlate with CSF τ protein levels in AD patients, and the concentrations of τ protein in both the blood and brain of AD patients are 3-fold greater than those of age-matched patients.[18,19] In addition, the liquid biopsy step for plasma is relatively facile and safe compared to CSF.[20] Very few studies measure τ protein levels in plasma, moreover they only use technologies such as enzyme-linked immunosorbent assays (ELISAs), a Simoa HD-1 analyzer (SIMOA), and immunomagnetic reduction (IMR) assays.[15,21−26] However, conventional ELISA assays for plasma τ protein have demonstrated inconsistent results with disease-associated differences due to execrable sensitivity, use of different antibodies among the ELISAs, and wide overlap between groups.[21−23] Recent studies that applied SIMOA and IMR assays have improved the mean detectable value of the protein in plasma over the values detected in previous methods and have discriminated AD patients from the elderly with high accuracy, but these assays could not overcome the existing problems, including low detection limits or overlap ranges between AD and control patients.[15,24−26] This most common issue for the detection of small molecules in protein-rich media (e.g., plasma and serum) is primarily caused by the insufficient sensitivity of the detection methods[27] that can cope with the inherent challenges posed by hemodiagnosis. These challenges include the following: (1) the blood plasma sample has an infinitesimal amount of AD biomarker, making the precise detection of the analyte difficult by traditional detection tools,[28] and (2) miscellaneous interference and matrix effects are present in blood plasma and plasma, which renders the accurate detection of target proteins impossible.[29,30] Although the changes in τ protein concentration represent the collapsing of the microtubules and neuronal cell death and enable the diagnosis of AD, they are the rarest detectable AD blood plasma biomarkers and the most disrupted by inherent problems in blood.[31] Reliable AD hemodiagnosis, i.e., a system established to enable the precise detection of the τ protein in blood plasma, is urgently needed.

In this study, we suggest a highly sensitive biosensor using a chaotropic agent for the detection of τ protein in human plasma as an AD biomarker (Figure 1). The immunogold is shaped as a long rod (aspect ratio 3.67) that binds to a monoclonal antibody (mAb) that specifically reacts with an AD biomarker. The aspect-ratio-3.67 gold nanorod is known as the most sensitive surface plasmon oscillator among the various gold nanoparticles.[32] Nanometer-sized gold particles are sequestered under the electromagnetic field of incoming light, and their free electrons undergo a collective coherent oscillation caused by the incident photon frequency.[33−35] Changes in frequency result from the nanoplasmonic biosensor and cause Rayleigh light scattering, which is also called localized surface plasmon resonance (LSPR).[33−35] This phenomenon is extremely sensitive and responds to an infinitesimal change of the refractive index surrounding the gold nanoparticles. The mAb leads the distinct changes around the surface of the gold nanorod by having a high binding affinity that specifically targets biomarkers in a complex medium and a lower cross-reactivity than polyclonal antibodies.[36] The presence of biomolecular analytes bound to the immunogold surface thus enables their detection via the corresponding refractometric sensing.[33,37]

To improve the sensitivity of the system, we treated the samples with guanidine hydrochloride (Gua-HCl), as a chaotropic agent, which reveals the hindrance from other proteins concealing the epitope of the τ protein in blood plasma.[38] Plasma includes over 3700 identified proteins that are known to be stabilized by hydrogen bond networks between solvent molecules.[32,39] It has been suggested that the

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greatest challenge in the search for actionable AD blood plasma markers is that many proteins are tightly bound to core proteins in this fluid.[30] This type of sequestration may interfere with the accurate detection of blood plasma biomarkers.[30] Gua-HCl can overcome this obstacle in blood-plasma-based AD diagnostics through disordering water molecule networks and weakening the hydrophobic interactions between proteins.[38] This combination of nanoplasmonic biosensor with the use of a chaotropic agent offers greatly enhanced sensitivity to maximize the number of target biomolecules captured (Figure 1). This platform allows the τ protein at the femtomolar levels in the blood plasma to be identified to aid the diagnosis of mild cognitive impairment or the early stages of AD.

RESULTS AND DISCUSSION

Confirmation of Chaotropic Effects in Plasma. To apply the chaotropic agent to the nanoplasmonic biosensor for increasing capturable τ protein, ELISA initially confirmed that chaotropic agents affect the measurement of AD core proteins in blood. The τ protein biomarker was quantitated by a τ protein-specific ELISA, and the apparent level of this biomarker changed as the optical density at 450 nm changed due to chaotropic agents in the sample. Both full-length and fragments of τ protein form high-molecular-weight aggregates in blood, while reducing conditions, such as the presence of chaotropic agents, are known to partly disintegrate aggregated forms.[40] To find the optimal chaotropic agent and the concentration of detecting tau protein in a nanoplasmonic biosensor system, we have identified changes in the concentration of detecting τ protein using three well-known chaotropic agents (guanidine hydrochloride, guanidine thiocyanate, and potassium thiocyanate) under various concentration conditions (Figure S1). Among them, 6 M guanidine hydrochloride most increased the concentration of detected τ protein in repeated experiments. The concentration of τ protein that was detected increased by 67.59% with 6 M Gua-HCL. This result indicated that this treatment ensures the release of the peptide from multimeric complexes or blood proteins and consequentially reveals a higher number of the buried epitopes of the AD pathway-related protein, τ protein.[38,40] We also demonstrate that by applying 6 M guanidine hydrochloride to the nanoplasmonic biosensor system, it ameliorates the causes of contradictions between many studies that used clinical samples and improves the accuracy of AD diagnosis through increasing the number of detected target peptides in blood.

Construction of Nanoplasmonic Biosensor for τ Protein Detection. To fabricate the nanoplasmonic biosensor platform, we synthesized gold nanorods (GNRs) as follows: we made a prepared solution, a seed solution, and a growth solution and followed that by performing Murray's wet chemical method.[41] The prepared particles were confirmed to have a size and aspect ratio of 28.0 nm × 102.7 nm and 3.67, respectively, via TEM images (Figure S2A). Owing to the morphology of the gold nanoparticles, their UV−vis spectra showed two absorbance peaks, one at 564.5 nm and the other at 730.5 nm, corresponding to the short axis and the long axis of GNRs, respectively (Figure S2B).

To develop the gold nanorod particles as plasmonic sensing factors, we replaced hexadecyltrimethylammonium bromide (CTAB) on the surface of GNRs with heterobifunctional polyethylene glycol (COOH-PEG-SH, M_w: 5000). The polymer also helped the antibody to bind to the gold nanoparticle by facilitating the bonding of functional groups in the GNR-PEG-antibody immune complexes. The successful replacement was confirmed with Fourier transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (^{1}H NMR; Figure S3). The FT-IR spectra show the appearance of the characteristic (C−O−C) stretching vibration at 1100−1200 cm^{-1} resulting from the functionalization of PEG molecules (Figure S3A).[42] The ^{1}H NMR spectra also confirmed the chemical structure of GNR-PEG with a characteristic peak at 3.6 ppm, whereas the peaks of CTAB (3.1, 1.2, and 0.8 ppm) were not detected (Figure S3B). The peaks at 4.8 ppm were negligible since they are indicative of deuterium oxide (D_2O) peaks from the solvent.[43] These results demonstrated that the COOH-PEG-SH was successfully exchanged for the surfactant CTAB.

Next, the PEGylated gold nanorods, immobilized onto an APTES-treated glass substrate, were used for detection of τ proteins in blood.[44] When two gold nanoparticles are brought close together, they become optically coupled due to the dipolar interactions.[45] Therefore, to avoid dipolar interaction between adjacent nanoparticles, only individual nanoparticles with an interparticle spacing larger than apporximately 2.5 times the optimal diameter of nanoparticle were chosen (Figure S4).[46] The PEGylated gold nanorods were conjugated with an antibody with 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide and *N*-hydroxysuccinimide (EDC-NHS) chemistry to convert the carboxyl groups of PEG to NHS esters. The prepared immunogold appeard with a reddish-orange color in dark-field images, and its longtitudinal plasmon band (LPB) shift is 10 times longer than that for the transversal plasmon band (TPB) when a PEGlayted gold nanoparticle became an antibody-bound gold nanoparticle; the shift was determined fitting the spectra with the Lorentzian algorithm in the Origin pro 8.0 software (Figure S5). The LPB of immunogold is known to be highly sensitive to the change of dielectric constant of the surrounding medium and refractive index.[47] Furthermore, the LSPR is sensitive to a change of the refractive index in the local dielectric environment. Therefore, the prepared immunogold extended the plasmon oscillation and generated a more sentivie reaction on the LPB band, which was used as reference for the LSPR peak shift in this study.[48] Figure 2 demonstrates typical Rayleigh scattering spectra of individual gold nanorods collected after every chemical binding procedure. The LSPR wavelength peak shifts were approximately 16 nm for the adsorption of τ protein antibody (50 μg mL^{-1}), which captures all the isoforms of the τ protein because the antibody is specific for a mid-domain epitope, amino acids 159−163,[49] and 26 nm for the τ protein (50 ng mL^{-1}) compared to the LSPR spectrum of PEGylated gold nanorods. Binding of chemical molecules such as antibody and τ protein on the nanoparticle surface changes its surface plasmon resonance properties and refractive index on the nanorod surface, which causes the red-shift spectra of the surface plasmon resonance, contributing to the basis for surface plasmon resonance biosensing.[50,51]

Selectivity and Sensitivity of the Nanoplasmonic Biosensor Platform. To verify the observed LSPR $\Delta\lambda_{max}$ from the specific interaction between immunogold and τ protein, nonspecific absorptions were investigated by incubating the solutions containing the other AD core biomarkers such as Aβ 1−40 and Aβ 1−42 at concentrations which were 100 times larger than the τ protein concentration. Figure 3

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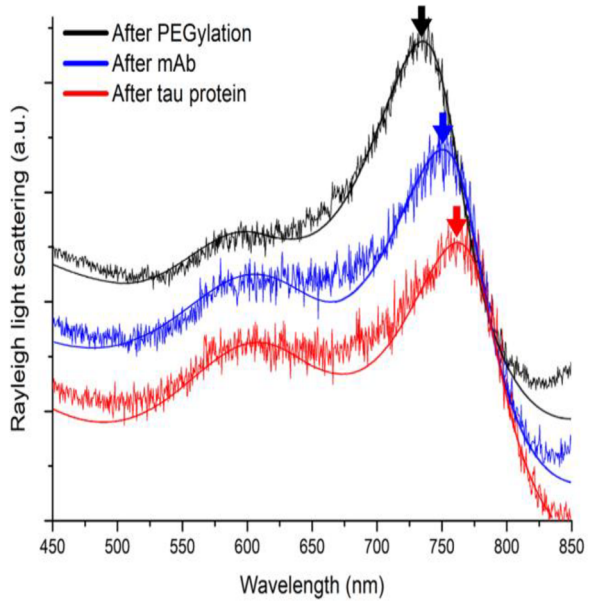

Figure 2. Typical Rayleigh scattering spectra of individual gold nanorods (aspect ratio 3.67), fitted to a Lorentzian algorithm. LSPR wavelength peaks are red-shifted after every chemical binding step.

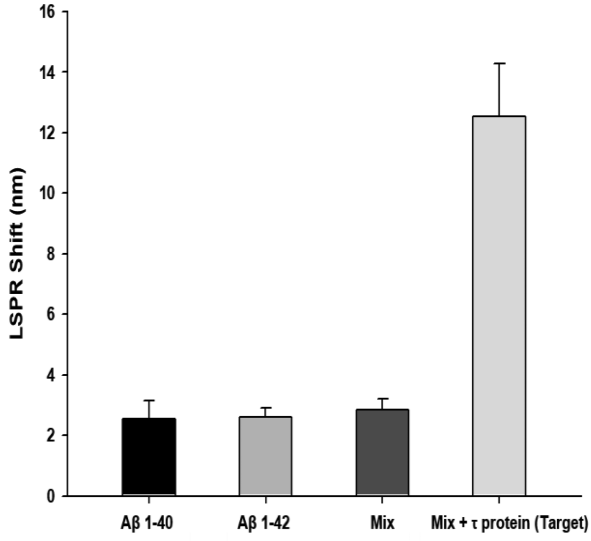

Figure 3. Confirmation for non-cross reactivity of the nanoplasmonic biosensor to Aβ 1−40 and Aβ 1−42. LSPR $\Delta\lambda_{max}$ shifts: 2.55, 2.6, and 2.84 nm for 100 nM controls such as Aβ 1−40, Aβ 1−42, and a mixture solution, as well as 12.55 nm for an about 1 nM mixture solution with τ protein. Error bars correspond to standard errors measured from 20 nanorods.

demonstrated the nonspecific absorptions of Aβ 1−40 and Aβ 1−42 compared to the specific binding between the τ protein and nanoplasmonic biosensor. After the undesired proteins (Aβ 1−40, Aβ 1−42, and mixture solutions) were exposed onto the nanoplasmonic biosensor, no obvious $\Delta\lambda_{max}$ was measured. On the other hand, the nanoplasmonic biosensor produces a significant LSPR peak shift in the mixture solution with τ protein. As a consequence, these results indicate that nonspecific binding by the nanoplasmonic biosensor was negligible even at high concentrations of nontarget proteins. Therefore, we confirmed that this proposed biosensor can be detected selectively at low concentrations in the presence of high concentrations of other AD core biomarkers. Furthermore, we confirmed that the nanoplasmonic biosensor detected femtomolar amounts of τ protein from a fluid resembling blood and determined the detection limit of this sensor with and without the chaotropic agent (Figure 4). The τ

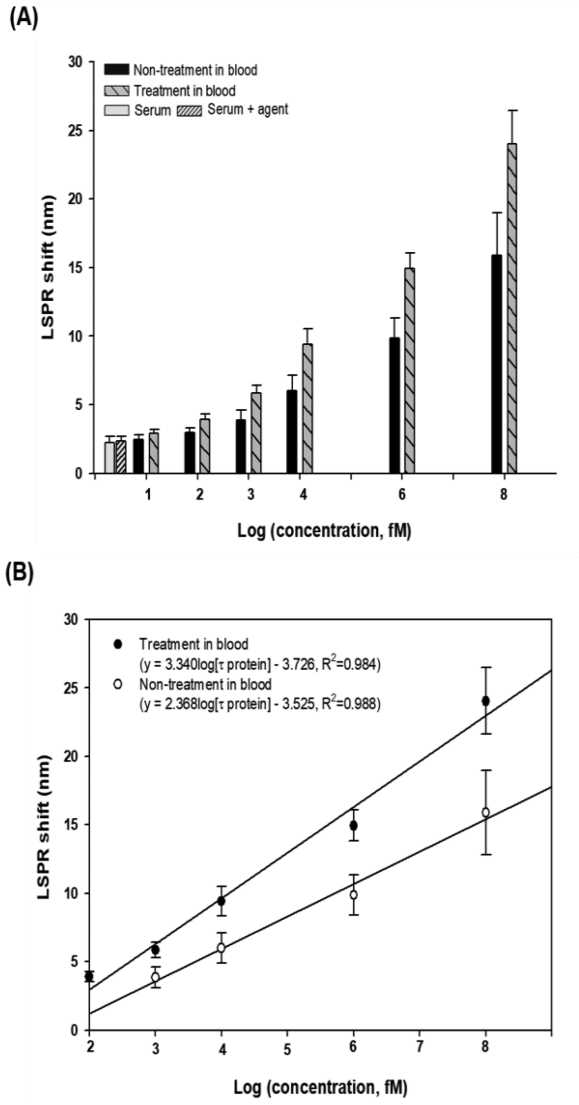

Figure 4. (A) Detection of control (Aβ 1−40, Aβ 1−42, and pure plasma) and τ protein at concentrations ranging from 10^1 fM to 10^8 fM in plasma. (B) Linear regression of the calibration curve describing the relationship between the LSPR wavelengths peak shifts and τ protein concentrations. Error bars correspond to standard errors measured from 20 nanorods.

protein is present in minute amounts in peripheral blood, accompanied by large amounts of other proteins and biomolecules.[17,23,28] To verify our platform, we monitored the LSPR shift for τ protein concentrations from 1×10^1 to 1×10^8 fM in plasma both with and without 6 M Gua-HCl. The values increased when reactions occurred with τ protein in nontreated blood at concentrations from 10^3 to 10^8 fM (black bars in Figure 4A). However, no change was detected in the signal for concentrations below 10^3 fM, indicating that the strong linearity of the signal response to the logarithm of the concentration only occurred within the range from 10^3 to 10^8 fM (white circles in Figure 4B). In addition, the limit of

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detection (LOD, 3 × δ/slope, where δ is the standard deviation of the blank and slope is the slope of calibration curve)[52] was 1.56−1.66 pM, or approximately 1 pM τ protein in the absence of treatment. The known levels of τ protein in the blood of a patient with AD were approximately 239.13 fM to 1.49 pM,[18,23,28] and the minimal detectable values were 815.2 fM to 2.72 pM based on existing blood-based practical and clinical assays such as double-sandwich ELISAs and IMR assays.[15,28] In accordance with the above-mentioned results and studies, this LSPR plasmonic biosensor without the chaotropic agent was lacking in its detection of τ protein for the diagnosis of AD and lagged behind existing methods. To enhance the sensitivity for the detection of τ protein, we combined the LSPR plasmonic biosensor with the use of 6 M Gua-HCl as a chaotropic agent. This combination extended the linear dynamic range to reach from 10^2 to 10^8 fM with determination coefficients (R^2) of approximately 0.984 (gray bars with hashes in Figure 4A, black circles in Figure 4B), and its minimum detectable concentration was 153.17−179.85 fM or approximately 100 fM τ protein (black circles in Figure 4B). These results indicate that a greater amount of τ protein is consequently present and can bind to the immunogold, leading to greater refractive index changes around the gold nanoparticles since Gua-HCl hinders τ proteins from forming aggregates with themselves or plasma proteins.[38] As a result, the sensitivity of the τ protein specific plasmonic biosensor was enhanced from the picomolar to the femtomolar scale, a 10-fold improvement. To perform the recovery tests, the known amounts of τ protein were added into the human serum (Sigma-Aldrich). As shown in Table 1, recovery values were all

Table 1. Recovery Test of τ Proteins in the Human Serum

added (ng mL^{-1})	recovered (ng mL^{-1})	RSD (%)	recovery (%)
0.05	0.0495	1.98	98.97
0.5	0.4877	2.91	97.55
5	4.7215	3.39	94.43
50	51.1346	3.60	102.27

between 90 and 110%. The mean recovery data for the samples spiked at 0.05, 0.5, 5, and 50 ng mL^{-1} were 98.97, 97.55, 94.43, and 102.27%, respectively. The RSDs for the recovery tests were all below 3.6%, demonstrating sufficient accuracy. These results indicate that proposed biosensors have a strong potential in clinical tests. In addition, our proposed assay utilizing the assistance of a chaotropic agent enables AD core protein levels to be used as clinical evidence, detecting the target proteins with high sensitivity and quantifying them in a dynamic range of complex human biological fluids.

Confirmation of Nanoplasmonic Biosensor in AD Patient Samples. Furthermore, we confirmed the medical usefulness of this nanoplasmonic biosensor that was assisted by a chaotropic agent for the diagnosis of AD from the blood samples of patients (Figure 5). We also proved that this highly sensitive platform distinguishes between normal control and AD patients. The plasma samples were obtained from pooled normal human plasma and AD human plasma (Innovative Research Inc., Novi, MI). The patients had been clinically diagnosed as AD, and this status continues (mean age: 72.6 ± 6.3; 3 women, 2 men). We measured the Rayleigh scattering shifts of τ protein-specific immunogold before/after the injection of cognitive normal control plasma and the patients' samples (patients no. 1−5), with or without 6 M Gua-HCl. In

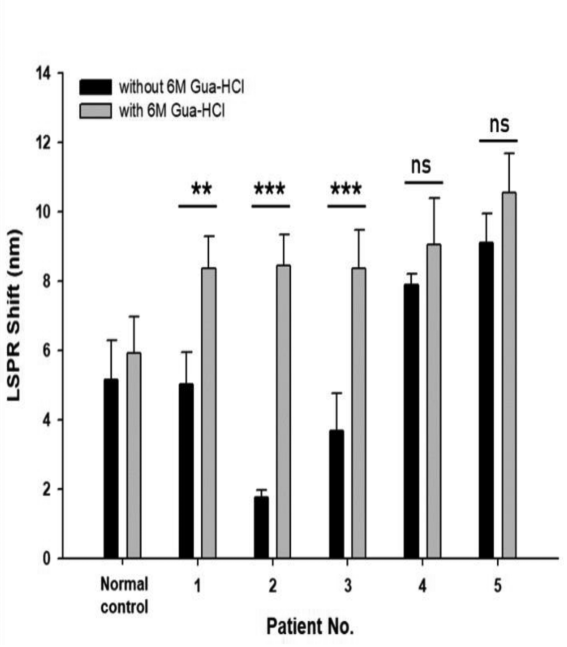

Figure 5. LSPR shifts of nanoplasmonic biosensor for detecting τ protein in cognitive normal control and AD patients' blood samples (patients no. 1−5) combined with 6 M Gua-HCl. Data are means ± SD from three independent experiments (n = 20 single gold nanorods examined for each experiment). *P < 0.01, **P < 0.001, ***P < 0.0001 (Student's t test). Error bars correspond to standard errors measured from 20 nanorods.

general, it is well-known that the concentration of τ protein in blood is high in the case of Alzheimer's disease patients.[10,18,19] However, tau protein in the blood exists in various forms like abnormal or aggregated form.[53−55] Thus, the detection of τ protein in the blood is challenging. In cases 1, 2, and 3, the LSPR shift values were lower than that of the control when the chaotropic agent was not used (black bars in Figure 5). The assay without the chaotropic agent could not discriminate between AD and control patients. In contrast, the combination of the nanoplasmonic biosensor and the chaotropic agent resulted in the assays of patients having a higher LSPR shift than those of controls with the chaotropic agent (gray bars in Figure 5). The chaotropic agent partly disassembled aggregated τ proteins in blood[40] and uncovered the τ protein epitope from blood proteins (Figure 5). This finding was supported by studies that showed that τ protein concentrations in plasma samples increased in the order from subjects with normal control and mild cognitive impairment (MCI) to AD and early stage AD patients.[15,18,23] On the other hand, the two Aβ isoforms did not differentiate between AD and control patients unlike the tau protein case (Figure S6). Recent studies have also shown that plasma Aβ 40 and 42 levels are similar in AD and control groups.[56] These findings sustain the idea that only plasma τ protein can provide insights regarding this pathological process in the brain. Our proposed system is an optimized clinical assay for protein in plasma and reduces the overlapping range between concentrations of the protein in an age-matched control and AD patients' blood that presented a problem in prior studies.[12,31,57,58] This combined use of a nanoplasmonic biosensor and a chaotropic agent provides an accurate medical investigation method to determine the core pathophysiology of AD in blood and to distinguish AD patients with high accuracy.

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CONCLUSIONS

We developed a novel nanoplasmonic biosensor for the ultrasensitive detection of Alzheimer's disease core protein (τ protein) in undiluted human plasma using a chaotropic agent. The platform can detect an τ protein at femtomolar levels; this biomarker can barely be captured in plasma because it is a 10- to 100-fold lower concentration in plasma than CSF;[15] however, this new approach can differentiate AD patients from normal age-matched controls through the highly sensitive and specific detection of an existing biomarker in blood. This combined use of an LSPR biosensor and a chaotropic agent is a progressive approach, compared with current methods, for discovering the core pathology of AD and the detection of τ protein in blood. Its sensitivity may lead to diagnosing the MCI stage, which has not previously been reported in the literature. The improvement in this work indicates that plasma τ protein is a meaningful biomarker to differentiate a normal patient from a cognitively impaired individual or a patient with AD. Highly reliable results from blood might support medical evidence regarding AD. Additionally, this nanoplasmonic biosensor will help to standardize various biomarkers for the diagnosis of AD and establish the hemodiagnosis of other tauopathies.

EXPERIMENTAL SECTION

Reagents and Materials. N-ethyl-N(diethylaminopropyl)-carbodiimide (EDC), gold(III) chloride trihydrate (≥99.0%), sodium borohydride ($NaBH_4$, 99%), N-hydroxysuccinimide (NHS), recombinant τ protein, hexadecyltrimethylammonium bromide (CTAB, >98.0%), and hydrochloric acid (HCl, 37 wt % in water) were purchased from Sigma-Aldrich (Korea). Sodium oleate (NaOL, >97.0%) was purchased from TCI America. COOH-PEG-SH (M_w 2000, 3400, and 5000) was purchased from Laysan Bio, Inc. Anti-τ protein antibody was purchased from Invitrogen. Normal control plasma and AD patient plasma were purchased from Innovative Research (USA). Coverslip slides (22 × 40 × 0.1 mm) were purchased from Deckglaser (Germany). Ultrapure water (18.2 mΩ cm^{-1}) was used to prepare all solutions.

Preparation of Single Gold Nanoparticle Biosensor. Gold nanoparticles (AuNPs) were synthesized by the seed-mediated growth method of gold nanorods and followed synthesis methods reported in the previous study.[32] To enhance the biocompatibility of gold nanoparticles and conjugated antibodies, we replaced CTAB molecules with heterofunctionalized polyethylene glycol (PEG), which functionalized the surface of gold nanoparticles as a new ligand.

A total of 2 mL of gold nanorod solution (300 μg Au/mL) was mixed with 40 mg of PEG (M_w 5000) under gentle vortexing. The resulting solution was centrifuged at 5000 rpm for 4 min, after which the mixture was orbitally swirled for 4 days at 200 rpm. The pellet was redispersed in deionized water and stored at 4 °C for stability.

Fabrication of Nanoplasmonic Platform. A cover glass slide (22 × 40 × 0.1 mm) was first cleaned in acetone with a sonicator for 30 min and then rinsed thoroughly with absolute ethanol three times. The glass was cleaned in piranha solution (3:1 = H_2SO_4/H_2O_2) after incubation for 1 day and then rinsed thoroughly with distilled water and absolute ethanol. The cleaned slide glass was subsequently dried with nitrogen gas, and the surface was treated with 10% (v/v) (3-aminopropyl)triethoxysilane (APTES) in 99.9% absolute ethanol for 20 min. Finally, the slide was sonicated in ultrapure water three times and then dried at 10 °C for 10 h.

For nanoplasmonic biosensor fabrication, PEGylated AuNP solution was diluted to OD ≈ 0.05 at the peak wavelength of its UV−vis spectrum, and 10 μL of it was drop-coated on the APTES-coated glass for 10 min. The glass slide was mounted to an RC-30HV closed-bath imaging chamber (Warner Instruments, USA) to visualize the AuNPs after every chemical step. The imaging chamber was subsequently inserted into the holder of the dark-field microscope. The surface of the glass slide was washed with ultrapure water for 30 min to expunge all contaminants and unbound immuno-gold nanoparticles.

To conjugate the antibody to the surface of carboxylated gold nanoparticles, 200 μL of 0.4 M EDC/0.1 M NHS solution was injected into the chamber to convert the carboxyl groups of PEG to NHS esters to form bonds with the amine groups of antibodies for 10 min. After the washing step was repeated, 100 μL of target protein monoclonal antibody (mAb) solution (50 μg/mL) was added to the ester-activated gold nanoparticles. The solutions were incubated for 2 h. The imaging chamber was washed at 100 μL/min via a connection to a syringe pump (Harvard Apparatus) to flow away unreacted antibody molecules.

A total of 1 mL of 0.2 M ethanolamine solution was injected into the chamber and incubated for 30 min to prevent nonspecific binding to the unreactive surface of gold nanoparticles. Various concentrations of each Alzheimer's disease biomarker, ranging from 1×10^1 to 1×10^8 fM, were subsequently diluted in phosphate-buffered saline (PBS) and mixed with human plasma samples. These antigen−plasma complexes were flowed onto the surface of the antibody-bound AuNPs and incubated for 1 h. The single nanoplasmonic biosensors on the glass slides were cleaned through ultrapure water in a flow cell for 5 min after each chemical binding step, and the distinct Rayleigh light scattering induced by analyte binding to the sensor was measured by a dark field, a spectrograph, and a CCD camera. To determine plasmon shift ($\Delta\lambda_{max}$) of the localized surface plasmon resonance (LSPR) that had occurred on the sensor, the wavelength shift $\Delta\lambda_{max}$ was analyzed with the following formula: λ_{max} (after reaction) − λ_{max} (before reaction) after the spectra had been fit with the Lorentzian algorithm in OriginPro 8 and GraphPad Prism 5 software.

Preparation of Plasma Sample Mixed with Chaotropic Agent. To improve the sensor sensitivity, we added 6 M guanidine hydrochloride to the AD patient plasma sample. The guanidine hydrochloride solution was prepared in 25 mM ammonium bicarbonate solution to a concentration of 20 M. The solution was diluted to 6 M in plasma samples.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.8b01242.

HR-TEM image of gold nanorod, UV−vis spectrum of gold nanorod, FT-IR of PEGylated gold nanorods, ^{1}H NMR of PEGylated gold nanorods, dark field images of the immunogold nanoparticle, Rayleigh light scattering spectra of individual gold nanorods, LSPR peak shifts of the nanoplasmonic biosensor for detecting AD core proteins (Aβ 1−40 and Aβ 1−42) in cognitive normal control and AD patients' blood samples (patient No. 1−5) with and without 6 M Gua-HCl (PDF)

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Notes

The authors declare no competing financial interest.

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