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Rapid and high-throughput colorimetric screening for anti-aggregation reagents of protein conformational diseases by using gold nanoplasmonic particles

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Abstract

Cellular deposition of destabilized proteins and their aggregates is considered one of the most indisputable factors implicated in protein conformational diseases. Here, we report an innovative high-throughput screening method for discovering anti-aggregation reagents out of numerous potential candidates by using gold nanoplasmonic particles. In our method, nanoparticles act as catalytic activators to accelerate protein aggregation and simultaneously exhibit a colorimetric response according to their embedded shape on the protein aggregates. Using this principle, we observed the colorimetric response to the anti-aggregation effect of amyloid β (Aβ) with the naked eye within a few minutes. Investigation of the anti-aggregation effects of select candidates under three different protein aggregation stages showed that the anti-aggregation efficiency could relate to disease progression. Finally, results obtained with spiked samples in cerebrospinal fluid as well as under various denaturation conditions and different Aβ compositions show the feasibility of future personalized medicine considering individual patient's disease progression.

Key words: Protein conformational disease; Anti-aggregation reagent; Gold nanoplasmonic particle; High-throughput screening; Colorimetric sensor

Conformational denaturation of certain proteins enables to cause spontaneous aggregation. It has been generally considered that the deposition of these protein aggregates results in cellular malfunction implicated in protein conformational diseases (also known as "proteopathy") such as neurodegenerative diseases[1–3] and alcoholic liver disease.[3] Above all, protein conformational diseases, including Alzheimer's disease, Lou Gehrig's disease and Parkinson's disease, are prevalent worldwide. According to the 2015 World Alzheimer Report,[4] it was reported that over 46 million people worldwide have Alzheimer's disease, an important social issue nowadays. Also, approximately 1000 per 100,000 people over the age of 65 years worldwide are living with Parkinson's disease based on the 2014 report,[5] and 1700 per 100,000 people were identified to have Lou Gehrig's disease in the United States.[6] Despite the high number of patients around the world, these protein conformational diseases remain incurable diseases. Therefore, finding effective drugs to efficiently mitigate and inhibit the formation of protein aggregates is an important task to conquer such diseases. Until now, many researchers have consistently reported that certain molecules can act as anti-aggregation reagents with direct or indirect effects on the formation of protein aggregates.[7–22] Many studies, at the protein and cellular levels, on the inhibition of protein aggregation have utilized spectroscopy and microscopy for screening effective anti-aggregation reagents. Fluorescence spectroscopy and circular dichroism (CD) spectroscopy are generally used for the quantitative detection of protein aggregates during the screening process.[7,8,11,17] Fluorescent

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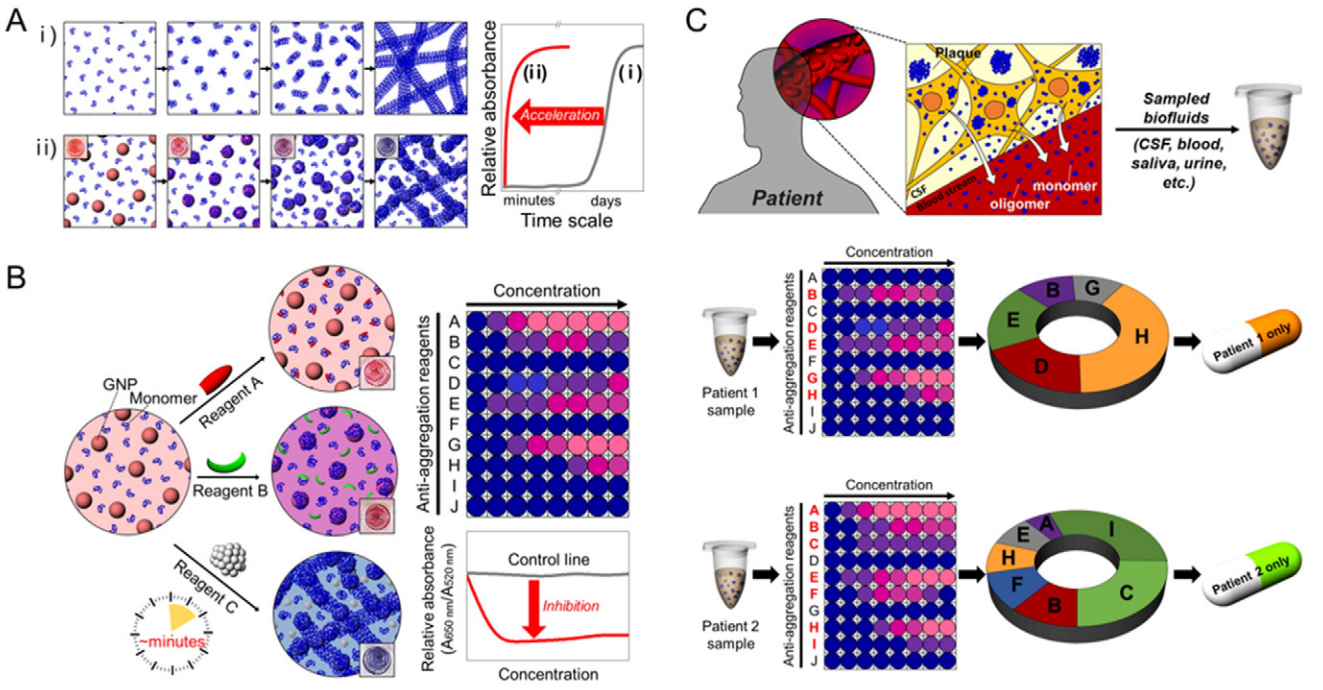

Figure 1. Schematic illustration of a rapid and high-throughput screening method of anti-aggregation reagents for protein conformational diseases. **(A)** Comparison of the physiological aggregation process with the gold nanoparticle-assisted rapid aggregation process. **(B)** Illustration of the principle of rapid colorimetric screening method, exemplary colorimetric responses for multiple anti-aggregation reagents, and concomitant spectral responses. **(C)** Schematic demonstration of application to develop a personalized medicine considering individual patient's disease progression.

optical microscopy, transmission electron microscopy (TEM), and atomic force microscopy (AFM) are used for visualizing the morphology of protein aggregates.[7,8,11,12,16,17] Biological assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay and reactive oxygen species (ROS) assay are also utilized for studying cytotoxicity of protein aggregates.[8,11,16,17] These methodologies are quite useful for studying protein aggregation, however, they have limitations with respect to high-throughput screening of a number of anti-aggregation reagents at once. Many of these techniques usually work under *ex-situ* and/or non-real time conditions and require longer incubation time, complex pre-treatment steps, and high cost of analysis. Therefore, development of a faster, simpler, and easier method for screening efficient anti-aggregation reagents would be useful in developing medicines for protein conformational diseases.

Recently, gold nanoplasmonic particles (GNPs) have drawn great attention for studying biomolecular assemblies owing to their biocompatibility, large surface area, and excellent optical property.[23–25] In our previous reports,[24,25] we suggested that GNPs can be utilized as catalytic activators and optical reporters for rapidly tracking aggregation kinetics (*i.e.*, promotion or inhibition). During the aggregation process, GNPs are embedded along the protein aggregates and their assembly affects light scattering. Therefore, the scattering color generated from the GNPs reflects protein morphology, which enables us to correlate the observed colors with the extent of aggregation. Based on these findings, we envisioned a new colorimetric screening method for discovering anti-aggregation reagents at each stage of structural evolution under various destabilizing conditions in order to develop effective medicine for protein conformational diseases.

Herein, we suggest an innovative screening method for anti-aggregation reagents based on rapid optical transition depending on the morphology of GNPs embedded in protein aggregates. In general, *in vitro* protein aggregation under physiological conditions takes a long time, several days or months, and nucleation of destabilized proteins is considered as a slow, rate-limiting step.[26–28] Whereas, in case of nanoparticle-assisted protein aggregation, gold nanoparticles working as nucleation cores can accelerate aggregation and simultaneously report aggregation levels by color.[24] As described in Figure 1, *A*, the nanoparticles embedded on the protein aggregates exhibit distinct colorimetric responses according to their assembled morphology during aggregation. In our present study, simultaneous color changes accompanied with aggregates formation was applied to rapidly screen the anti-aggregation reagents of protein conformational diseases with the naked eye (without any instrumentation). As illustrated in Figure 1, *B*, it is expected that protein aggregation would be inhibited by treatment with effective binding reagents, because their selective binding to target proteins lowers the level of free monomers and oligomers at an initial growth stage of protein aggregation. As a proof-of-concept demonstration, we exploit the proposed method to screen possible anti-aggregation reagents for amyloid β (1–40) and amyloid β (1–42) peptides implicated in Alzheimer's disease, one of protein conformational diseases. Through a series of screening tests under different aggregation stages, denaturation conditions, and peptide compositions, we further suggest that our method can facilitate finding effective