Less is More – Characterization of Protein Structures

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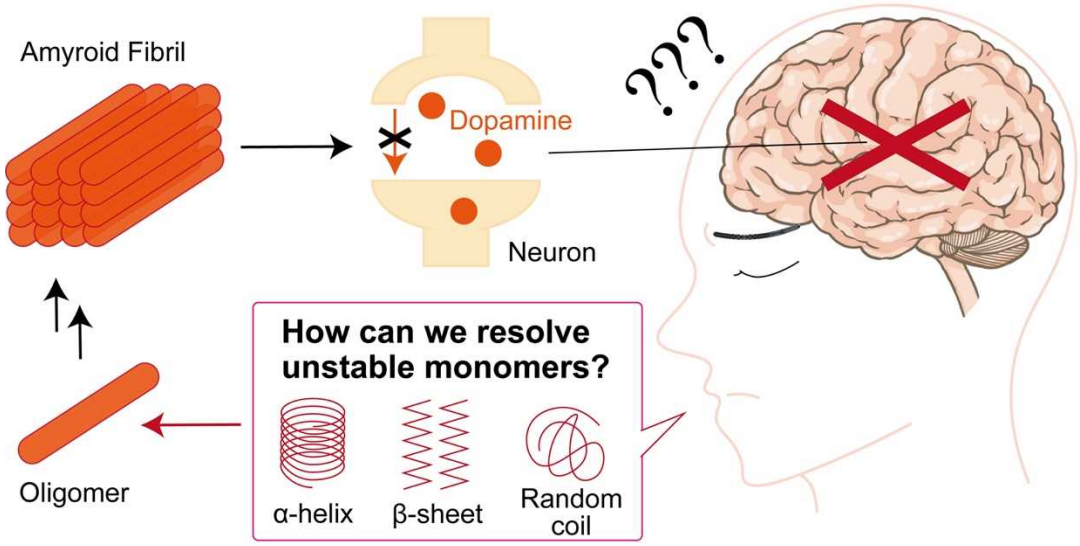

Today's Japan is facing the serious problem of super-aging society. The number of people aged 65 and over is 28% of the total population in Japan, and it is still growing. This seems positively that life expectancy increased with advanced medical care. However, it does not imply that people can live the rest of their lives in good health. One issue is increasing the number of patients with neurodegenerative disease, such as Alzheimer's disease and Parkinson's disease. For instance, Parkinson's disease is associated with age-related decrease of dopaminergic neurons in the brain. Since its main motor symptoms are tremor and postural retention disorder, many of its patients require nursing assistance. Particularly in a super-aging society with a small population of young people who can nurse them, the pathology of this disease will need to be soon clarified for its early detection and treatment.

In recent years, as a cause of Parkinson's disease, pathologists have paid attention to the aggregation of alpha-synuclein that is one of intrinsically disordered proteins (IDPs)[1]. Since IDPs as monomer do not have stable secondary and tertiary

structures (α-helix, β-sheet, and random coil) in aqueous solution, they are more likely to form misfolded proteins. Sometimes, they self-assemble, and in more severe cases, grow into amyloid fibrils with β-sheet structure that damage cells. Thus, in the early pathological stage of Parkinson's disease, the conversion of alpha-synuclein from monomers to oligomers needs to investigate. However, the low concentration of oligomers in pathological environment makes it challenging to characterize their transient structures. To resolve the complex biological systems such as IDP transient structures, here, I will introduce a sensitive, reproducible measurement platform developed in 2021 by using optical tweezer-assisted surface enhanced Raman scattering (SERS) spectroscopic system[2].

Raman spectroscopy is a optical measurement method that enables label-free, non-invasive measurement. This technique is based on an inelastic light scattering called "Raman scattering" in which the incident light to the sample is modulated by the vibrational frequency of the target molecules in the sample, providing Raman spectra with vibrational features specific to the molecules. In biomedical study, it is utilized for identifying various proteins. If they cause a symptom, they are called biomarkers that are quantitative indicators of biological change in an organism. Thus, tracking the amount of those biomarkers in the blood or secretions by Raman spectroscopy will give important information on health conditions; for example, which disease we have and how serious it is. Despite its high availability, Raman spectroscopy is not sensitive enough to detect molecules at physiological concentrations, typically 1 μmol/L (=μM) because of the small scattering cross section of the molecule. Among many types of Raman spectroscopic techniques, SERS is a powerful tool to boost the sensitivity of Raman spectroscopy and enable single molecule detection via huge enhancement of the

signal intensity by $x10^{10}$ - 10^{14}. This enhancement rises from the interaction between the collective motion of metal atoms on the surface of the SERS substrate, called "plasmons" which occurs when the metal surface is irradiated with light, and the scattering light from the target molecules adsorbed on the metal surface.

SERS is expected to be useful for characterization of biomarkers in aqueous solution at low concentration. However, there are practical difficulties in the conventional SERS measurement to probe proteins in diluted solution, which is typically based on measurements in aqueous solution with dispersed metal nanoparticles (NPs) as a SERS substrate; for example, poor efficiency and reproducibility, which need to be improved to satisfy the objective "stable, sensitive detection of the transient structures of alpha-synuclein at its physiological concentrations". The high SERS enhancement comes from the strong electric field induced by the light irradiation on the metal surface. In the NP-based SERS measurements, it is localized at the junction between the NPs, called "hotspot". If the NPs moving in the solution can be manipulated precisely to create a stable hotspot by optical tweezers for trapping the particles using the radiation pressure generated when laser light is irradiated on an object, it is desirable for SERS detection of low concentration proteins.

The researchers in Hong Kong University of Science and Technology developed an optical tweezer-coupled Raman spectroscopic system that enables precise position control of two AgNP-coated beads with optical tweezers in conjunction with SERS detection of passing-by proteins in microfluidic flow channel to reduce the ensemble averaging of the protein measurement. They utilized this platform to investigate the transient structures of alpha-synuclein at its physiological concentration of 1 μM. As a result, the ratio of the secondary structures of 1-μM alpha-synuclein

obtained in their 200 parallel SERS measurements was consistent with that derived from the spectral deconvolution of a spontaneous Raman spectrum of 2-mM alpha-synuclein, indicating that the ensemble averaging of the system is reduced and that this platform enables to resolve detail information of the transient species of alpha-synuclein. Therefore, owing to the high sensitivity and stability of the measurements, this SERS platform holds promise to open new avenues to characterize structures of proteins at their physiological concentrations that was hidden by the ensemble averaging.

In amyloid aggregation, since the amyloid fibrils include β-sheet structure, we can employ this SERS method to directly characterize β-sheet containing oligomers for further investigation in the pathological development of Parkinson's disease. Also, since it can be applied to monitoring other protein structures, it can reveal protein dynamics associated with various neurodegenerative diseases. If the molecular mechanisms in the development of symptoms can be elucidated by using this SERS platform, it will help create treatments for them. In current Japan, where the population is aging, early detection and treatment of diseases in elderly, who are at high risk of serious illness, using such measurement technology may not only improve their healthy life expectancy, but also healthcare system that is overwhelmed to deal with serious ill patients.

[References]

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2. Dai, X., Fu, W., Chi, H., Mesias, V.S.D., Zhu, H., Leung, C.W., Liu, W., and Huang,

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