Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Gel Filter Trapping Approach for Ultra-sensitive Detection of Biopolymers
by Surface-enhanced Raman Scattering
（表面増強ラマン散乱による高感度検出のためのゲル網目構造を利用した生体高分子捕捉法）

Sensitive detection of biomarkers, such as proteins, is essential for the early diagnosis of diseases and point-of-care testing systems. As a powerful tool in molecular detection, surface-enhanced Raman scattering (SERS) provides molecular fingerprint information with high sensitivity by utilizing the noble metal nanostructured substrates. With laser excitation, free electrons in metals oscillate as the localized surface plasmon resonance (LSPR) effect especially at the nanogap and tip featured structure, forming intense local electromagnetic fields (so called hotspots), which can be used to amplify the Raman signals of analytes in it. While high sensitivity, the application of SERS is still limited in the detection of biopolymers. To realize sensitive detection of trace biopolymers, highly intensity and localized hotspots in nanogaps are necessary. However, their large molecular volume hinders their approach to nanogaps, always absorb out of hotspots.

In this study, I fabricated an actively gap-distance tunable plasmonic device by attaching a Au nanoTriangle plate (AuNT) arrangement on a thermo-responsive hydrogel surface. As a particle assembly, 2D hexagonal close-packed (hcp) superlattice prepared by colloidal AuNTs show extremely strong hotspot area in its central gap. With the utilization of hydrogel, the Au nanoTriangle plate Array on Gels (AuTAGs) as a SERS substrate could support protein insertion into the hotspot with a widened gap and higher signal enhancement with a closed gap. Further, I designed a Gel Filter Trapping (GFT) approach as an active protein delivery strategy based on the characteristics of hydrogels, which can absorb water and separate biopolymers through their crosslinked polymer networks. The GFT approach was applied on a gel-based AuTAG substrate for sensitive protein SERS detection.

In Chapter 1, the capability of gold nanostructure, especially AuNT array in the generation of enhancement fields for Raman signals, and the underlying logics to realize ultra-sensitive molecular SERS detection are introduced. To achieve the sensitivity in biopolymer detection, hydrogels potential in this field are mentioned as a novel material in SERS, including their capability in molecular trapping.

In Chapter 2, I prepared AuTAG substrate by attaching AuNT monolayers onto surfaces of hydrogel poly (*N*,*N*-diethyl acrylamide) (pDEAA) by a nano-transfer printing technique (**Figure 1a**). AuNTs were synthesized in colloid and modified with charged molecules, which allowed AuNTs could be assembled as a highly ordered (**Figure 1b**) superlattice and show charged surfaces in water to prevent the unspecific absorption with proteins. By increasing/decreasing the temperature, the distance between AuNTs on gel could be reduced/increased in the nanoscale level with gel shrinkage/swelling (**Figure 1c**). This result indicates that the optical property in extinction could be reversibly tuned due to the changing of volume of gel, in which the thermo-responsive property of pDEAA. Such nano-transfer printing method also was improved that can be used for various kind of gels.

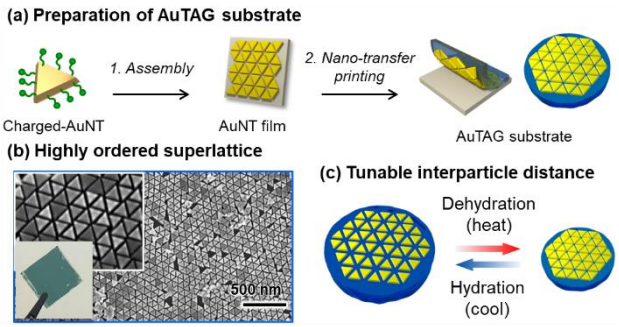

Figure 1. (a) Preparation procedure of AuTAG. (b) surface morphology of AuNT array on ITO. (c) Tunable structure of AuTAG with temperature

In Chapter 3, I discussed the details of the conduction of GFT approach on the surface on AuTAG substrate. By controlling the hydrophobic/hydrophilic properties repeatedly with temperature, pDEAA gel would actively

absorb water in a low temperature while in its dehydration state (**Figure 2a**). Gel swelled with water absorption and resulted that analyte proteins in a droplet were guided to nanogaps and gel surface with water flow. The charged surface of AuNT inhibited protein's adsorption on particle surface, and the large volume made proteins trapped by the polymer network between particle's gap. Inter-particle gaps were closed again by heating for providing significant hotspots in SERS, which were also evaluated in the simulation of electromagnetic fields by changing the gap distance.

In Chapter 4, the versality in the combination of AuTAG substrate and GFT method was evaluated with SERS sensitivity. Here, negative charged protein (hemoglobin and myoglobin) and positive charged protein (lysozyme) were detected with the same chagred AuTAG. Proteins were successfully introduced in nanogaps between AuNTs and give rise to the ultra-sensitivity in SERS by applying GFT method (**Figure 2b**) even in the single-molecular level, which allowed the discovery of single protein with SERS mapping in 2D space.

In Chapter 5, I summarize the results of this dissertation from chapters 2 to 5.

In summary, I have proposed a fabrication procedure of a hydrogel-based SERS substrate by attaching an assembled AuNT monolayer on to a gel surface. Furthermore, based on the property of hydrogel, biopolymer proteins can be guided into the nanogaps for efficiently using hotspots and give rise to an ultra-sensitivity which was rarely reported in past. By combining the nanoparticle with chiral chemistry or optic, the detection can be optimized and more suitable for chiral biomolecules. As a state of art work, the application of hydrogel in SERS is expected to be widely used and commercialized in SERS in future.

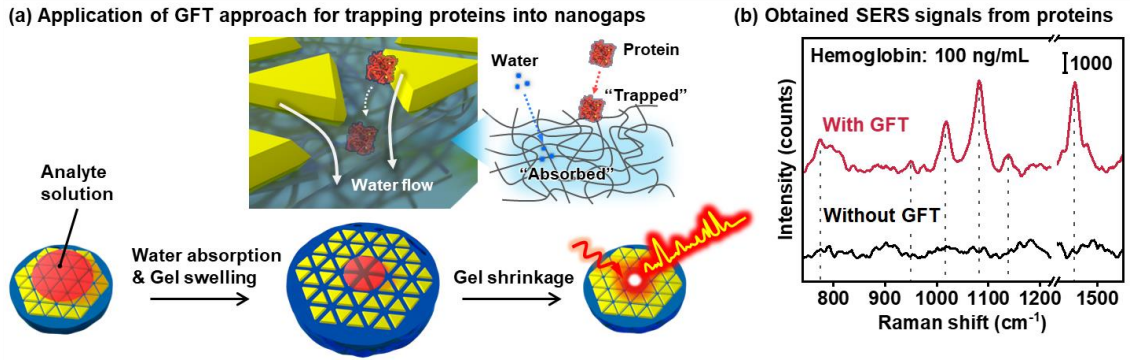

Figure 2. (a) application of GFT approach on AuTAG for protein trapping and sensing. (b) Difference in hemoglobin SERS obtained with (red) and without (black) GFT approach.