論 文 内 容 の 要 旨

博士論文題目

Protein assembly fabrication and evaluation utilizing laser micro-manipulation
(レーザー微細操作を駆使したタンパク質集合体の作製と評価)

氏　　名 Yi Po-Wei

(論文内容の要旨)

Protein assembly, including protein crystal, is an indispensable material in bio-process engineering. Processes and methodologies for fabricating and evaluating the protein assembly are widely investigated and discussed. In this study, micro-scale control and evaluation of the protein assembly were newly realized by laser micro-manipulation techniques, which are optical trapping for its fabrication and pulsed-laser-activated impulse response encoder (PLAIRE) for its evaluation. In the optical trapping experiment, formation of a single submillimeter-sized lysozyme assembly was demonstrated and dynamics for its structuring was clarified by adding dye-labeled lysozyme and polystyrene microparticles. In the experiment by PLAIRE, Young's modulus on the surface of a single micro-crystal of lysozyme was evaluated. After the introduction in the chapter 1, experimental methods for the optical trapping and PLAIRE for Hen egg white lysozyme are described in the chapter 2.

In the chapter 3, optical trapping for fabricating a single lysozyme assembly was described. When an infrared continuous-wave laser (1064 nm, 1 W) is focused on a high-concentration lysozyme solution (375 mg/mL) through a 60x objective lens (NA 0.9), a large lysozyme assembly was formed at and around the laser focal point. In addition, when polystyrene microparticles (Φ 3 μm) were added in the solution, a linear assembly of the microparticles associated with the lysozyme assembly was observed. A three-dimensional linear structure composed of the microparticles is prepared along the bottom edge of the lysozyme assembly, reflecting the shape of lysozyme growing from the solution surface during the irradiation. Their morphology was reconstructed by shifting the imaging plane immediately after the laser was turned off. Fluorescence imaging visualized expansion of the lysozyme assembling. No denaturation of the assembly was confirmed by Raman micro-spectroscopy.

In the chapter 4, this new phenomenon found in the optical trapping found was examined by simultaneous transmission and fluorescence imaging. Firstly, lysozyme assembly was formed along the solution surface, where lysozyme association and

orientation were controlled by the lysozyme at the surface. Secondly, the pumped-up lysozyme clusters invaded to the adsorption layer and pushed the layer to the side, expanding to a few tens micrometer. This started when the lysozyme concentration at the focus reached about 300 mg/mL. Association and orientation of the clusters were determined by intense laser irradiation at the focus and kept during the transport to the peripheral region. This would be ascribed to long-range electrostatic interaction between clusters. This mechanism was explained as two-stage optical trapping.

In the chapter 5, a femtosecond laser pulse (800 nm, 100 fs, 3 μJ/pulse) was focused on a lysozyme crystal as a model for evaluating the mechanical property of protein assembly. The single-shot pulse irradiation induced a localized explosion of the lysozyme crystal. The vibration due to the explosion propagated as the surface elastic wave on the crystal. Crystal vibration was detected as the response of an atomic force microscope (AFM) cantilever attached on the crystal. Young's modulus of the crystal was obtained from the surface elastic wave velocity, which was calculated from the arrival-time of the wave as a function of the distance between the laser focus and the contact point of the cantilever. The modulus was estimated to be 50 MPa, which is ~10 times smaller than that (500 MPa) evaluated by the AFM indentation test. The difference would reflect in and out-of-plane planes of the crystal intrinsic property.

The Chapter 6 summarized that methodologies developed by integrating laser and microscope could newly achieve manipulation and evaluation of micro protein assembly. The submillimeter-sized lysozyme assembly was fabricated using optical trapping. The mechanism of its dynamical behavior was considered in molecular level. Moreover, a new method to evaluate mechanical properties was developed for probing characteristic of the surface for a protein micro-crystal. These methodologies opened new ways for controlling and evaluating the protein assembly.

氏　名	Yi Po-Wei

（論文審査結果の要旨）

近年微小な生体材料を対象とするバイオ工学において、タンパク質の凝集状態の制御は重要な技術になろうとしている。本研究では、レーザーと光学顕微鏡を組み合わせた光ピンセットによる微小材料の操作技術を駆使して、新奇なタンパク質凝集状態の制御の可能性を示した。さらに、高出力フェムトレーザーと原子間力顕微鏡（AFM）を組み合わせた微細材料の力学特性の検出技術をタンパク質マイクロ結晶に適用し、タンパク質凝集状態の力学特性を調べる新しい手法を提案するに至った。

光ピンセットによる実験は、倒立顕微鏡上で、高濃度のニワトリ卵白リゾチームの溶液の液滴の表面に、高強度の近赤外レーザーを集光することによりおこなわれた。蛍光標識された高濃度のリゾチーム液滴にレーザーを集光すると、レーザー集光領域に凝集するリゾチームが蛍光像として観察された。さらに、光ピンセットのためのレーザー集光用のラマン散乱励起用レーザーを導入し、凝集状態のラマンスペクトルを測定し、凝集状態について分子レベルでの議論を可能とした。マイクロサイズのポリスチレン微小球を添加したリゾチーム溶液を用いた実験では、集められたポリスチレン微小球が配列されて集光点から伸びていく新奇現象が発見され、その配列メカニズムについて考察された。

マイクロサイズのリゾチームの結晶にフェムト秒レーザーを集光し、その集光点近傍にAFM探針に接触させ、レーザー照射により誘導される結晶表面を伝搬する振動波を検出することに成功した。この表面伝搬波のAFM探針とレーザー集光点の距離依存性を調べることにより、結晶表面の弾性率を検出することができた。通常、AFMによる弾性率計測は、AFM探針を結晶に押し付け、その凹みを評価することによりおこなわれる。この方法では、結晶内部の弾性率が評価されるが、本研究で構築された方法では、反応場となりえる結晶表面の弾性率を特異的に検出することができる新規手法である。

上記のとおり、本論文では、レーザーによる微細操作技術を駆使することにより、タンパク質の集合状態を多様に制御できることが示され、さらには従来法では難しかったタンパク質の集合状態を評価する新手法が提案している。さらに本論文では、手法の提案にとどまらず、その原理となる分子メカニズムについても考察されている。これらの成果は工学としてのみならず、理学的な観点からも学理が深められており、審査委員一同は、本論文が博士（工学）の学位論文として価値あるものと認めた。