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Visualization of lipid rafts in an artificial monolayer membrane by using slit-scanning Raman microscopy

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1. Introduction

Raman scattering microscopy identifies molecular species in a sample by optically detecting molecular vibration, and provides spatial distribution of molecules in a specimen. Our developed slit-scanning Raman microscopy improved imaging speed more than 100 times faster than that of conventional point-scanning Raman microscopy, by providing both diffraction-limited high spatial resolution and chemical information. It allows us to observe dynamic behavior of biomolecules in a living cell during various biological processes, such as cytokinesis, mitosis, and apoptosis [1,2].

2. Raman imaging of lipid rafts in an artificial monolayer membrane using diyne-tagged sphingomyelin

Lipid raft in an artificial monolayer membrane

Here we utilized slit-scanning Raman microscopy to observe lipid rafts in an artificial monolayer membrane. Lipid raft is a specific micro-domain in a membrane, enriched in sphingomyelin (SM) and cholesterol (chol). It has been thought to have important biological functions in a cell, such as membrane signaling and protein trafficking. As a model of lipid raft, artificial ternary membrane of SM/phosphatidylcholine(PC)/chol has been widely investigated. Phase separation of this membrane has been visualized by fluorescently labeled lipid, by discriminating raft-like ordered domain and disordered domain. However, these probes are often excluded from ordered domain due to the steric effect of the bulky fluorophore on lipid packing. Analytical method to observe spatial distribution of lipids, in particular SM, inside of the ordered domain was missing.

Raman imaging of diyne-tagged SM

We focused on Raman scattering microscopy to directly observe SM in a membrane. To specifically observe SM, we synthesized the analogue of SM modified with diyne moiety (Fig. A) [3]. Diyne-tag exhibits distinct peak at Raman silent region of lipid molecules (Fig. B) [3,4]. Since it has small chemical structure, it also maintains the original property of SM to be incorporated into ordered domains. We then prepared diyne-SM/DOPC/chol ternary monolayer on a quartz substrate, and performed imaging by using slit-scanning Raman microscopy. Raman intensity distribution, reconstructed by the peak height of diyne, visualized heterogeneous distribution of diyne-SM, forming micrometer-sized domains (Fig. C) [5]. High spatial resolution of our system allows us to analyze lipid distribution inside of the domain, where the dinye-SM was enriched in the central area of the domain rather than the peripheral area.

3. Conclusions

We succeeded in observing lipid rafts in an artificial membrane, by using slit-scanning Raman microscopy with single lipid-layer sensitivity and hyperspectral imaging capability. Raman microscopy together with alkyne-tag will further contribute to lipid membrane research.

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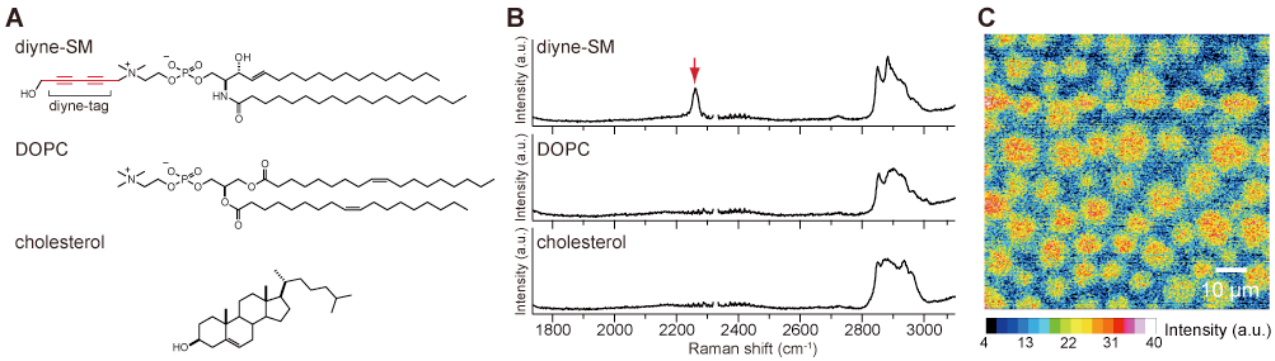

Figure (**A**) Chemical structures and (**B**) Raman spectra of diyne-SM, DOPC and cholesterol**.** (**C**) Raman image of diyne-SM/DOPC/cholesterol ternary monolayer on a quartz substrate, reconstructed by peak intensity of diyne at 2263 cm^{-1}. 412x400 pix.