別紙４－１（課程博士（英文））

Date of Submission（month day, year）： January 10th, 2020

Department	Student ID Number	
Applied Chemistry and Life Science	Student ID Number	D133411
Applicant's name	Yasuhiro Kakimoto	

Supervisors	Ryugo Tero Rika Numano

Abstract（Doctor）

Title of Thesis	Development and application of observation methods for functional lipid domains and assembled structures in supported lipid bilayers

Approx. 800 words

In all living things, the outer and inner worlds of cells are separated by cell membranes. Since they are the outermost of the cells, they provide reaction fields to recognize the ambient environment, and to transport information and materials into and out of cells. These play important roles in the function of cells, such as immunity, infection, and metabolism. Cell membranes have a bimolecular membrane comprising of amphiphilic lipids, which serve as the fundamental skeleton, and contain membrane proteins and glycolipids in it. Localization of these cell membrane components is key factors regulating the recognition and transportation reactions. Artificial cell membrane systems have been studied to elucidate the functions of cell membranes. Supported lipid bilayers (SLBs) are a kind of artificial lipid membrane situated at solid-liquid interfaces. Fluid and fragile lipid membranes are stabilized in the SLB system, and that highly sensitive analytical methods in the field of interfacial science measurements are available. Especially SLBs have been used to investigate the fundamental physicochemical properties and molecular distribution of lipid bilayer membranes.

In this Ph.D. thesis, I aimed to investigate the functional domains containing glycolipids. Two-dimensional localization, e.g. clustering and domain formations, of glycolipids and membrane proteins exert their appropriate functions in the cell membrane reactions of living organisms. Although extensive researches have been conducted on the diffusion and aggregation of lipids and proteins in cell membranes, glycochain dynamics and effects on membrane properties are not well understood. Elucidation of the behavior of cell membranes in consideration of glycochains on cell membranes is expected to be useful for establishing more accurate cell membrane models and for developing new biodevices. For this purpose, I developed observation methods for domains and assembled structures of glycolipid, and sample preparation methods for bacterial model membranes. Additionally, I found out a new application of the glycolipid localization to the domain control in a freestanding bilayer lipid membrane on a microwell-fabricated Si chip.

First, I developed methodologies for glycolipid-containing SLB preparation and observation. I investigated the morphology and properties of domains induced by the hydrophilic-polymer-modified lipid applying poly-ethylene glycol (PEG)-modified lipid. I formed SLBs using a 0–10 mol % range of PEG-modified lipid concentration (C_{PEG}). I studied its morphology and fluidity by fluorescence microscopy, the fluorescence recovery after photobleaching (FRAP) method, and atomic force microscopy (AFM). Fluorescence images and AFM topographies showed that clusters of the PEG-modified lipid and the PEG-lipid-rich domains appeared depending on C_{PEG}. Both localized structures were induced by the hydrophilic PEG chains, but gave different contrast in the AFM topographies.

Frequency-modulation AFM revealed a force-dependent appearance of the PEG-lipid-rich domain. This study demonstrated the hydrophilic-polymer-induced lipid localization, and its physical property that is critical when the domain is evaluated with AFM.

Next, I made bacterial model SLBs, and evaluated their formation mechanisms for utilizing these models to further glycolipid and functional lipid observation. As a bacterial model cell membrane system, reconstituted *Escherichia coli* (*E. coli)* cell membrane has been studied. I formed SLBs of *E. coli* extracted lipids on mica and thermally oxidized SiO_2/Si substrates, and investigated their morphology and physical property with AFM and fluorescence-microscope-based methods. I found substrate-dependent SLB formation, and two- and three-dimensional morphologies depending on Ca^{2+} concentration. I also formed SLBs of phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CA), which are dominant components of *E. coli* extracted phospholipids. The SLBs showed various morphologies depending on the composition of PE, PG and CA. PG- and CA- dependent holes, and PE dependent multiple layers were observed. In this study, I established fabrication method of *E. coli* extracted lipids SLB, and discovered two- and three-dimensional lipid assemblies depending on lipid species, substrate materials, and Ca^{2+} concentration.

Then, I included diacylglycerol (DAG) and membrane protein integrase (MPIase) to the developed SLBs of *E. coli* in order to establish membrane protein insertion model membrane. Membrane proteins are synthesized by ribosomes and inserted into cell membranes, and then perform their functions. Membrane protein insertion is thought to proceed by a universal molecular mechanism in all organisms. Correct insertion of membrane proteins into membranes is important not only in living organisms but also in biotechnology. DAG present in *E. coli* blocks the inadequate spontaneous insertion of membrane proteins. On the other hand, MPIase present in *E. coli* is known to promote the insertion of membrane proteins. The creation and control of various artificial cell membrane systems, including MPIase and DAG, will contribute to the development of understanding and utilization of cell membranes. I formed and investigated SLB of *E. coli* lipids containing DAG or MPIase.

Finally, I discovered the glycolipid localization affects membrane domain formation on a microwell-fabricated substrate. Freestanding bilayer lipid membrane suspended by SLB is an advanced SLB system, which is obtained by placing a lipid bilayer on a substrate with micrometer order holes. This system is designed to place indicators or sensors in the holes for detecting the activities of the freestanding membrane. In this freestanding region, specific lipid localizations are observed because of the competitive interaction between the supported region and the freestanding region. Recent study showed that liquid disordered phase preferentially exists in the freestanding region. I attempted to change the properties of a freestanding membrane by using the interaction between the hydrophilic polymer chains of the glycolipid and the substrate. The bilayer membranes containing various concentrations of ganglioside GM1 were prepared, and phase separation and domain formation on the freestanding membrane were observed and evaluated using a fluorescence microscope. The time course of the phase separation state depended on GM1 concentration, and the formation of functional domains on the freestanding membrane was successfully controlled. This study applied glycolipid localization effect to engineering an artificial lipid membrane system.

This thesis concerns functional lipid assemblies relating to bacterial membranes, experimental methodologies, and application for membrane engineering. I expect that these research works lead to break further veils of roles and mechanism of functional lipids in biomembrane reactions, and to contribute to progress in the field of interface chemistry, biochemistry, and biodevices.