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Vibrational Spectroscopic Studies on the Structure of Bio-model Interfaces

（振動分光法による生体モデル界面の構造に関する研究）

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2013

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Chapter 1

Introduction

1-1 General Introduction

Biological membranes are the most common cellular structures in both animals and plants, and are involved in almost all aspects of animal and plant cellular activity, ranging from simple mechanical functions, such as motility, food entrapment, and transport, to highly specific biochemical processes, such as energy transduction, immunological recognition, nerve conduction, and biosynthesis.¹⁻³ Biological membranes are highly complex and varied because they contain at least 50 different kinds of proteins, various glycolipids, steroids, and pure hydrocarbon molecules. Nonetheless, all biological membranes are based on a two-dimensional space made of lipid molecules, which are held together by hydrophobic interactions, and self-assembled as a continuous bilayer, as shown in Figure 1.1.^{4,5}

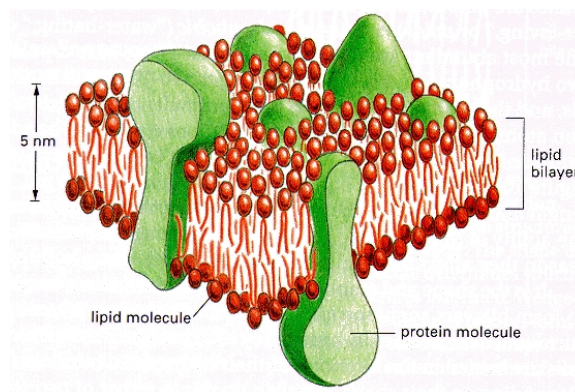


Figure 1.1 Typical view of a cell membrane, which consists of a lipid bilayer and some large protein molecules.

The deposition of model membranes on planar solids, which is expected to be an

effective cell membrane-mimicking model system, is essential for studying cell membrane characteristics.⁶⁻⁸ Although many characterization techniques, such as Brewster angle microscopy (BAM),⁹ fluorescence microscopy,¹⁰ X-ray diffraction,¹¹ atomic force microscopy (AFM),^{12,13} Fourier transform infrared spectroscopy (FTIR),¹¹ and attenuated total reflectance FTIR (ATR-FTIR), have been utilized in studying the membrane structures on the surface, the interfacial structure of adsorbed molecules remains unclear because of the lack of an effective experimental technique for interfacial study in the molecular level.

Sum frequency generation (SFG) spectroscopy, which is an interface-sensitive technique based on a second-order nonlinear optical effect, is used to determine the membrane structures on the surface. In this method, a photon is generated at a frequency that is equal to the sum of the frequencies of the two incident photons.¹⁴⁻¹⁷

In the following sections, the adsorption of lipid monolayer and bilayer is first introduced, followed by a concise review of the stabilization of lipid membrane in dry state. Then, an introduction on soap film and soap bubble is provided. Finally, the operation principles of SFG spectroscopy are presented.

1-2 Lipid Membranes

1-2-1 Intermolecular Interactions

1-2-1-1 Hydrogen Bond

The intramolecular O–H distance is approximately 0.10 nm, which is the expected value for this type of covalent bond. However, the intermolecular O···H distance is only 0.18 nm, which is significantly lower than the sum of two van der Waals radii (0.26 nm), but still greater than the covalent distance of 0.10 nm, as shown in Figure 1.2 (a).¹⁸ These findings suggest that the intermolecular O···H bond initially exhibits covalent characteristics.^{19,20}

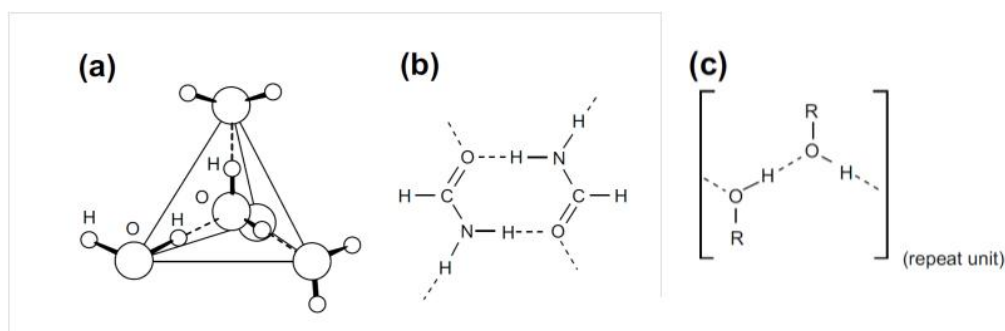


Figure 1.2 Different types of hydrogen bonds and hydrogen-bonded structures. (a) Threedimensional structures (e.g., ice). (b) Two-dimensional (layered) structures (e.g., formamide). (c) One-dimensional (chain and ring) structures (e.g., alcohols, HF).¹⁸

Hydrogen bonds are not unique to water; such bonds exist in varying degrees between electronegative atoms (e.g., O, N, F, and Cl) and H atoms covalently bound to similar electronegative atoms. These bonds only involve H atoms because of the tendency of H atoms to become positively polarized. Moreover, the uniquely small size of H atoms allows them to interact strongly with nearby electronegative atoms, resulting in an effective H-mediated “bond” between two electronegative atoms. The hydrogen bond was previously believed to be a quasi-covalent bond that involved the sharing of an H atom or proton between two electronegative atoms. However, the currently accepted notion is that the hydrogen bond is predominantly an electrostatic interaction. With a few exceptions, the H atom is not shared but remains closer to and covalently bound to its parent atom. The hydrogen bond between two groups XH and Y is usually denoted by $X-H\cdots Y$. Nevertheless, certain characteristics of hydrogen bonds do make them appear like weak covalent bonds.

Hydrogen bonds can occur intermolecularly or intramolecularly, and can exist in a nonpolar environment. Hydrogen bonds are particularly important in macromolecular and biological assemblies, such as in proteins, in which hydrogen bonds link different segments inside the molecules, and in nucleic acids, in which hydrogen bonds are responsible for the DNA molecular structure. The involvement of hydrogen bonds in setting up one-, two-, and three-dimensional macromolecular structures is sometimes referred to as hydrogen-bond polymerization, as illustrated in

Figures 1.2 (a) to (c).

1-2-1-2 Hydrophobic and Hydrophilic Interactions

The concept of “hydrophobicity” was introduced to describe the low solubility and the unexpectedly strong attraction between hydrocarbon molecules in water. This concept was initially applied to small solute molecules only and was believed to be associated with the structure of the water molecules around the solute molecules. Hydrophobic solutes, such as hydrocarbons, fluorocarbons, and vapor bubbles, are invariably nonpolar. Thus, polar, ionic, and hydrogen bonds cannot be formed between hydrophobic solutes.

Hydrophobic interaction refers to the unusually strong attraction between hydrophobic molecules and surfaces in water. This interaction is often stronger than the attraction in free space. For example, the van der Waals interaction energy between two contacting methane molecules in free space is -2.5×10^{-21} J, whereas that in water is -14×10^{-21} J. Similarly, the surface tensions of most saturated hydrocarbons range from 15 mJ m^{-2} to 30 mJ m^{-2} , whereas their interfacial tensions with water range from 40 mJ m^{-2} to 50 mJ m^{-2} . This strong interaction in water was previously believed to be caused by a sort of “hydrophobic bond”. However, a bond-associated entropic phenomenon, which primarily arises from the rearrangement of hydrogen-bond configurations in the overlapping solvation zones when two hydrophobic species approach each other, was not observed. Therefore, this strong interaction had a significantly longer range than any typical bond. Several manifestations of hydrophobicity are illustrated in Figure 1.3.¹⁸

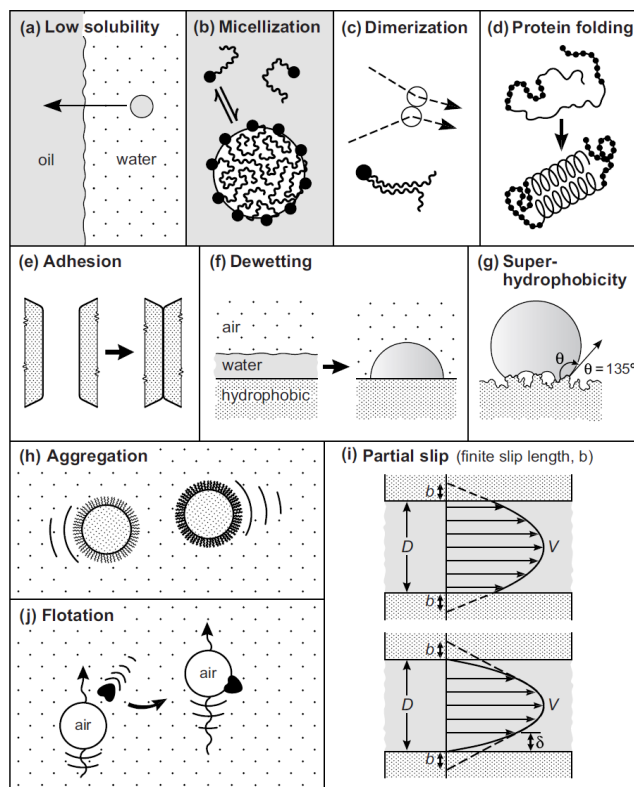


Figure 1.3 Manifestations of hydrophobicity and hydrophobic interactions. (a) Low solubility/immiscibility; (b) micellization; (c) dimerization and association of hydrocarbon chains; (d) protein folding; (e) strong adhesion; (f) nonspreading of water on hydrophobic surfaces; (g) Superhydrophobic surfaces are found in plants and animals and, more recently, as man-made coatings; (h) rapid coagulation of hydrophobic particles or surfactant-coated surfaces; (i) partial slip boundary condition at a solid-liquid interface. Partial slip can arise from true partial slip (top figure) as occurs at a liquid-vapor interface, or zero wall slip (apparent partial slip, bottom figure) due to a reduced liquid density and viscosity within a depletion zone of thickness δ ($\neq b$) at the interface as may be occurring for water flowing past a hydrophobic surface; (j) hydrophobic particle attachment to rising air bubbles (basic mechanism of “froth flotation” used to separate hydrophobic and hydrophilic particles).¹⁸

Although no phenomenon known as a hydrophilic interaction actually exists, such effects can be recognized when certain molecules and groups appear to be water-soluble or strongly repel each other in water. This behavior is in contrast to the strong attraction exhibited by hydrophobic groups. Hydrophilic groups prefer to be in contact with water rather than each other. Strongly hydrated ions and zwitterions are presumably hydrophilic. However, some uncharged and nonpolar molecules can be hydrophilic if they have the right geometry and if they contain atoms capable of

H-bonding with water, such as the O atoms in C=O, OH (alcohol), ethylene-oxide groups, and the N atoms in amines.

Table 1.1 Hydrophilic Groups and Surfaces¹⁸

Molecules and ions			
Alcohols (CH ₃ OH, C ₂ H ₅ OH, glycerol)		Polyelectrolytes (polysaccharides)	
Sugars (glucose, sucrose)		Soluble proteins	
Chaotropes (urea)		Nucleic acids DNA, RNA	
Polyethylene oxide (—CH ₂ CH ₂ O—) _n		Na ⁺ , Li ⁺ , Mg ²⁺	
Molecular groups ^a			
Anionic		Zwitterionic	
Carboxylate	—COO [−]	Phosphatidylcholine (lecithin)	—OPO ₂ [−] OCH ₂ CH ₂ N ⁺ (CH ₃) ₃
Sulfonate	—SO ₃ [−]		
Sulfate	—SO ₄ [−]		
Phosphate ester	—OPO ₂ [−] O—		
Cationic		Polar (nonionic)	
Trimethyl ammonium	—N ⁺ (CH ₃) ₃	Amine	—NH ₂
Dimethyl ammonium	>N ⁺ (CH ₃) ₂	Amine oxide	—NO(CH ₃) ₂
		Sulfoxide	—SOCH ₃
		Phosphine oxide	—PO(CH ₃) ₂
Solid Surfaces			
Hydroxylated silica (below 600°C, surface characterized by silanol Si-OH groups ^b)			
Swelling clays (montmorillonite)			
Chromium			
Gold (when clean)			
Polar groups that are not hydrophilic when attached to a long hydrocarbon (hydrophobic) chain ^a			
Alcohol	—OH	Amide	—CONH ₂
Ether	—OCH ₃	Nitroalkanes	—NO, —NO ₂
Mercaptan	—SH	Aldehyde	—CHO
Amines	—NH(CH ₃), —N(CH ₃) ₂	Ketone	—COCH ₃

^aCompiled from a longer list given by Laughlin (1978, 1981).

^bAbove 600°C, a dehydroxylation reaction takes place where two adjacent Si—OH groups liberate a water molecule (H₂O) and combine to form a hydrophobic siloxane Si—O—Si group. Dehydroxylated silica surfaces are hydrophobic.

Table 1.1 lists several common hydrophilic molecules and molecular groups, as well as some hydrophilic surfaces that are wetted by water or each other in aqueous solutions.¹⁸ This table shows that a polar group is not necessarily hydrophilic, and a nonpolar group is not always hydrophobic.

Figure 1.4 shows the structure of water on a hydrophilic surface that contains a normal van der Waals attraction and a short-range steric repulsion between two hydrophilic surfaces.¹⁸ The strong and directional binding of water or solute molecule to a hydrophilic surface adds an additional steric barrier, which increases the range of the repulsive force or the effective size of the molecule. In addition, the orientation of all water molecules toward a certain direction partially restricts the charge transport

processes, such as proton-hopping.

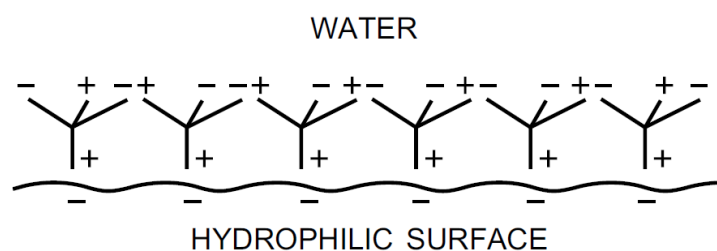


Figure 1.4 Water structure at a hydrophilic solid/water interface. For example, at a negatively charged surface, the water molecules are forced to orient to a certain direction with one of the two positive H^+ atoms pointing toward the surface.¹⁸

1-2-1-3 Hydration Forces

Despite extensive experimental and theoretical studies, the highly unusual properties displayed by the short-range forces between surfaces in water and aqueous salt solutions remain unexplained. These forces can be strongly monotonically repulsive, attractive, oscillatory, or a combination of these characteristics. For instance, certain clays, uncharged surfactants, and lipid bilayers swell spontaneously or repel each other in aqueous solutions, whereas silica dispersions and other colloidal particles sometimes remain stable in very high salt. Therefore, the surfaces or the particles of these systems are expected to remain in adhesive contact or to coagulate in a primary minimum because of the hydration forces.

The concept of hydration forces was first proposed by Langmuir, Derjaguin and Zorin to explain the unexpected stability of uncharged colloidal particles, such as “coacervates” (giant vesicles), in solutions. Subsequent experiments showed that the particles were monotonically repulsive, roughly exponential, and ranged from 10 Å to 30 Å between amphiphilic surfaces. However, the particles were of a longer range between hydrophilic mineral surfaces. The idea that these forces are due to the “water structuring” on the surfaces gained steady popularity, and the frequently observed exponential decay length of approximately 2.5 Å was attributed to some characteristic property of water, such as size. These forces between fluid-like amphiphilic surfaces,

such as surfactants and lipid bilayers, are known as steric hydration forces.

Strong short-range monotonically repulsive forces have been measured across soap films composed of various surfactant monolayers, between uncharged bilayers composed of lipids with uncharged sugar or zwitterionic head-groups, and between biological membranes. The hydrophilicity of such surfaces is due to the presence of strongly hydrophilic groups. The repulsive force between these groups is caused by the confinement of the protruded and thermally excited chains on the surface, as well as the attraction between the head groups.

1-2-2 Lipid Monolayer

The study of the structure of lipid layers is essential understanding biological membranes because lipid layers are key constituents of biological membranes. Lipid molecules, including fatty acids, phospholipids, glycerolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides, consist of a hydrophilic head group and a hydrophobic tail region, as shown in Figure 1.5 (a).

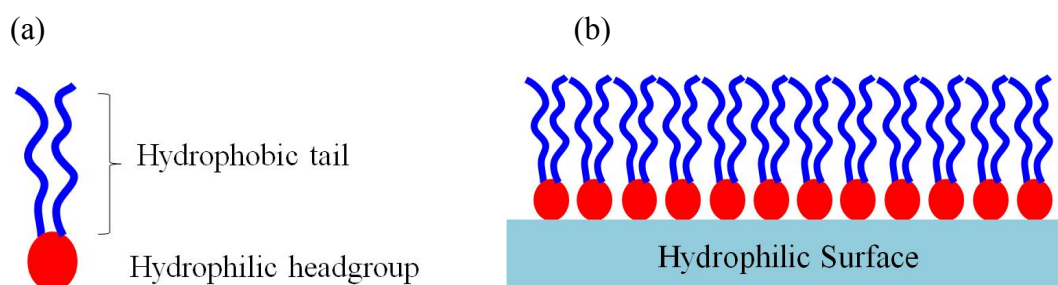


Figure 1.5 Structure of (a) a lipid molecule and (b) a supported planar lipid monolayer.

Monolayers are excellent model systems for membrane biophysics because a biological membrane can be considered as two weakly coupled monolayers.²¹ The substrate-supported lipid monolayer represents an intermediate stage in the fabrication of functionalized model membrane systems and needs to be properly understood.^{22,23} Although many studies have been performed, the interfacial molecular structure of the lipid monolayer remains unclear.

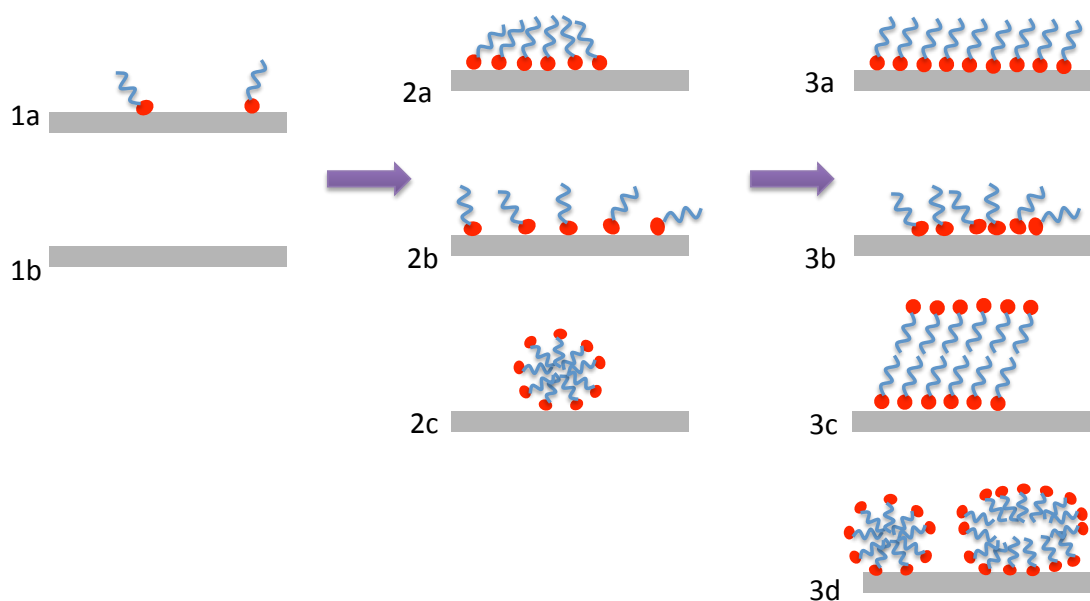


Figure 1.6 Cartoons displaying different structures that may form during the adsorption of lipid molecule to a clean hydrophilic substrate (1b). At very low coverage, few lipid molecules may be randomly adsorbed on the surface (1a). As the coverage increases, the adsorbed molecule layer shows a formation of hemimicelles (2a), a disordered single layer (2b), or admicelles (2c). At high coverage, a range of structures are conceivable: well-ordered monolayer (3a), disordered molecule layer (3b), bilayer (3c), or admicelles (3d).

Figure 1.6 shows different lipid molecule adsorption models that have been previously proposed. At low lipid molecule concentrations, only several molecules adsorb on the surface with a randomly oriented hydrocarbon chain, as shown in Figure 1.6 (1a). As the concentration increases, the interactions between lipid molecules may result in the formation of hemimicelles (2a), the random distribution of the molecules in a single layer (2b), or the adsorption of the admicelles structure (2c). At high lipid molecule concentrations, the interactions between lipid molecules may result in the formation of a well-ordered monolayer on the surface (3a), an adsorbed molecule layer with a disordered structure (3b), a bilayer (3c), or admicelles (3d).

These models are not mutually exclusive but describe systems that are based on various possibilities between two limiting cases. One of the limiting cases corresponds to lipid molecules, particularly when the interaction between the head groups and the surface is weak.²⁴ At a certain concentration, which is known as the

critical surface aggregation concentration, the aggregates adsorb to the surface (2c and 3d). Another limiting case is a self-assembled monolayer. The interaction between the head group and the surface is sufficiently strong to form a monolayer below the critical micelle concentration (2a, 2b, 3a, and 3b). A second layer does not form at these dilute concentrations because the elimination of water-hydrocarbon contact does not compensate for the loss of translational entropy upon adsorption. As the concentration is further increased, the isolated molecules adsorb to the hydrophobic surface of the formed monolayer, and the surface coverage gradually increases until bilayer is approached at the critical micelle concentration.²⁵⁻²⁷

1-2-3 Lipid Bilayer

1-2-3-1 Supported Lipid Bilayer

The deposition of model membranes on solid supports has become highly popular for studying cell membrane characteristics.⁶ The solid-supported lipid bilayer, which has several variations, is a single bilayer deposited onto a hydrophilic solid surface, such as mica or silica, as shown schematically in Figure 1.7. These systems are highly fluid and are achieved by the hydrophilic interactions between the head group of the phospholipids and the surface, as well as the hydrophobic interactions between the hydrocarbon tails of the lipid molecules.⁴ In addition, a thin water layer (approximately 10 Å to 20 Å) often exists between the solid surface and the bilayer. Consequently, the lipids can diffuse laterally, which preserves an important dynamic property of the biological membrane.

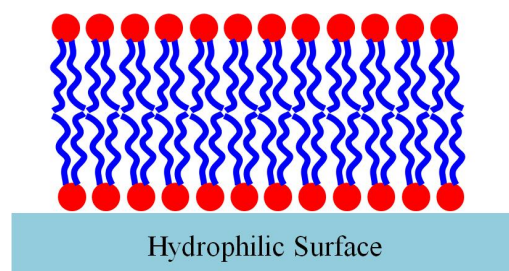


Figure 1.7 Illustration of supported lipid bilayer.

Solid-supported lipid bilayers can be utilized to study membrane characteristics and processes because they act as useful models of biological cell membranes. A great number of physical investigations and biological research have demonstrated the importance of solid-supported lipid bilayers in various applications, such as the study of membrane structure thermodynamics, the determination of immune cell response mechanisms, and the development of biosensors using membrane-based integrin receptors.^{7,28}

In previous studies, supported lipid bilayers were mostly formed through the Langmuir-Blodgett (LB) deposition of two lipid monolayers on hydrophilic substrates.²⁹⁻³² However, McConnell et al. found that small vesicles can undergo rupture and fusion to form an extended, adsorbed, planar bilayer on suitably prepared surfaces.³³ Currently, this spontaneous self-assembly of supported continuous bilayers from vesicles in solutions is commonly used in creating supported lipid bilayers. The vesicle deposition method is preferred over LB deposition because of its experimental simplicity and reproducibility.

The growing interest in introducing lipid membranes on surfaces is motivated by the continued development of highly effective surface-sensitive characterization techniques such as SFG spectroscopy.

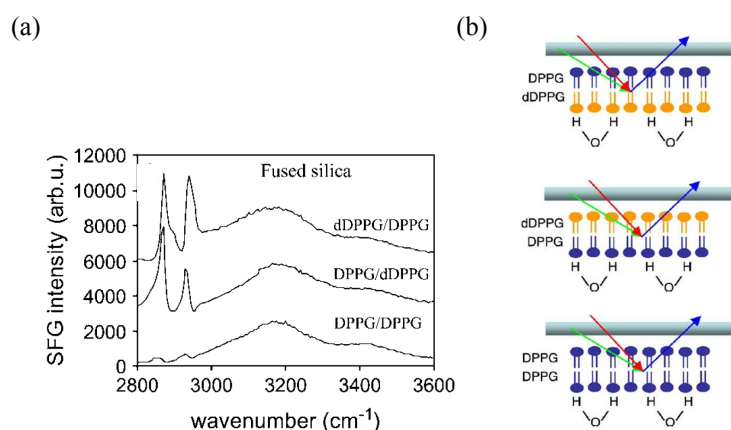


Figure 1.8 (a) SFG spectra from DPPG/dDPPG, dDPPG/DPPG, and DPPG/DPPG bilayers on fused silica³⁴ and (b) schematic of conformation of bilayer and SFG light.³⁵

Figure 1.8 shows a typical structure of a supported bilayer obtained through SFG

spectroscopy. Figure 1.8 (a) shows the SFG spectra of DPPG/dDPPG, dDPPG/DPPG, and DPPG/DPPG bilayers. The spectra for isotopically asymmetric bilayers are dominated by CH₃ symmetric stretching mode (2875 cm⁻¹) and Fermi resonance (2940 cm⁻¹). The strong C-H signals from the dDPPG/DPPG and DPPG/dDPPG bilayers on the fused silica substrate indicate that stable and ordered bilayers formed on the substrate, as illustrated in Figure 1.8 (b). Weak C-H signals from isotopically symmetric bilayers on the substrate indicate that the proximal and distal leaflets had similar structures, and the signals generated from these leaflets effectively cancelled each other.^{34,35} Thus, SFG spectroscopy is highly sensitive to the conformational order of interfacial molecules.

1-2-3-2 Lipid Bilayer Vesicles

Unilamellar lipid bilayer vesicles can provide a free-standing bilayer. These vesicles consist of a spherical shell-shaped bilayer that separates the “intracellular” liquid volume from the “extracellular” space, as shown in Figure 1.9.

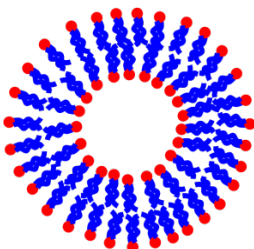


Figure 1.9 Model of a unilamellar lipid bilayer.

The length scale L is used to classify vesicles size. For small unilamellar vesicles (SUVs), L approximately ranges from 1 nm to 50 nm. For large unilamellar vesicles (LUVs), L approximately ranges from 100 nm to 500 nm. For giant unilamellar vesicles (GUVs), L approximately ranges from 1 μ m to 100 μ m. The larger dimension of GUVs, which can be prepared using the electroformation method developed by Angelova and Dimitrov,^{36,37} is similar to that of cells.^{21,22} Thus, GUVs are often used to model cells.³⁸

Vesicle fusion method, which is an effective and widely used method for

forming supported lipid bilayers, was used in the present work. When hydrophilic surfaces, i.e., SiO_2 and quartz, were immersed in a lipid vesicle suspension, the vesicles adhered on the surface, ruptured, spread, and finally transformed into a planar bilayer, as shown in Figure 1.10.

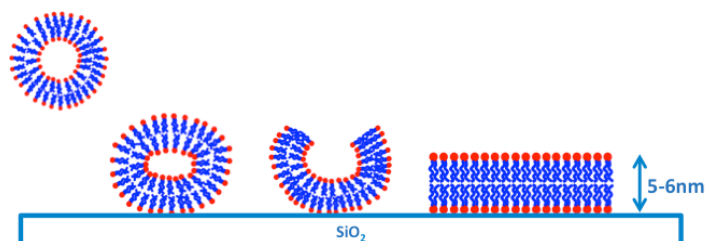


Figure 1.10 Schematic drawings of the planar membrane formation process from vesicles.

1-3 Stabilization of Lipid Membrane in Dry state

1-3-1 Trehalose in Stabilization Lipid Membrane in Dry state³⁹

Trehalose is a non-reducing disaccharide in which two glucose molecules are linked together in a α (1 $\rightarrow$ 1)-glucoside bond, as shown in Figure 1.11.

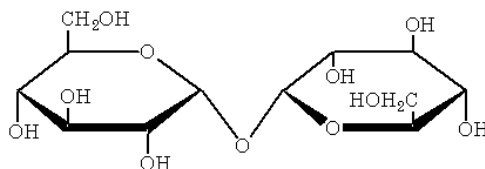


Figure 1.11 Structure of trehalose.

Although water is obviously necessary for life, some organisms can survive in almost complete dehydration or even if 99% of their water content is removed. These organisms include plants, yeast cells, fungal spores, and some microscopic animals, such as nematodes, rotifers, and the cysts of brine shrimp.⁴⁰⁻⁴² Under favorable conditions, some of these dried organisms can remain in this state known as anhydrobiosis for decades until water becomes available. These organisms swell and resume an active state when they come in contact with water. Figure 1.12 shows an

example of the rehydration of a revival plant. In dry state, the fronds of the plant are curled up tightly (a). After rehydration, the fronds spread out and turn green ((b) and (c)).



Figure 1.12 Rehydration of a revival plant. (a) Initial, dry state; (b) 2 h after adding water; and (c) 24 h after adding water.

Many studies on these organisms have demonstrated mechanisms that allow their survival during dehydration. Some of these mechanisms can be used to develop new methods for preserving biological materials that are normally sensitive to drying.⁴³ Anhydrobiotic organisms generally contain high concentrations of trehalose (and sometimes other disaccharides and oligosaccharides). When the nematode *Aphelenchus avenae* was slowly dehydrated, it converted 20% of its dry weight into trehalose.⁴⁴ The ability of anhydrobiotic organisms to survive in the absence of water is strongly correlated with the synthesis of trehalose.^{42,44} Log-phase cultures of yeast have low trehalose concentrations and are quite susceptible to dehydration. However, log-phase yeast cells can enhance their ability to survive during dehydration by entering a stable phase of growth levels as their trehalose increases.⁴⁵ This ability to survive in the presence of trehalose is independent of the growth phase of the cells because log-phase cells subjected to rapid heat-shock trehalose synthesis can also survive dehydration.⁴⁶ These results are also applicable to various other organisms, including the brine shrimp and the resurrection plant.⁴⁷ The use of trehalose to enable cells to survive dehydration may be an ancient form of adaptation given that archaebacteria have been found to accumulate trehalose in response to stress.⁴⁸ The two primary stresses that are proposed to destabilize lipid bilayers during dehydration

are fusion and lipid phase transitions. Studies using laser light scattering demonstrated that trehalose and other sugars inhibit the fusion between the vesicles during dehydration. However, the mere inhibition of fusion alone is not sufficient to preserve the dry vesicles. Trehalose is also necessary in preventing phase transitions. Evidence suggests that trehalose depresses the phase transition temperature of the dry lipids. The phase transition temperature maintains these dry lipids in liquid crystalline phase in the absence of water.⁴⁹

1-3-2 Mechanisms of Trehalose for Stabilizing Lipids during Dehydration^{50,51}

Trehalose has an important function in stabilizing bio-membrane systems, such as lipids. This function can be explained by two mechanisms. The first mechanism is the “direct interaction” of trehalose with the compound, in which stabilization in the dry state is achieved through hydrogen bonding with the phospholipid head group. The second mechanism is the “indirect interaction” of trehalose, that is, the effect of trehalose on the environment surrounding the compound caused by restricted mobility and increased hydration.

1. Direct interaction.

One of the reasons why lipid membranes lose activity during freezing or drying is the structural change in the physical state of membrane lipids caused by the removal of water. During dehydration, the various interaction forces that maintain the lipid membrane structure are disrupted, resulting in structural changes. Trehalose inhibits these changes by forming a hydrogen bond with the phospholipid head group, thereby maintaining lipid membrane conformation and activity. Water replacement hypothesis is generally used to explain this membrane protection phenomenon by trehalose.^{41,52-55} The direct interaction between trehalose and dipalmitoylphosphatidylcholine (DPPC) during dehydration was demonstrated using IR spectroscopy.⁴¹ In the presence of trehalose, the band at 1246 cm^{-1} , which has been assigned to the vibrations of the phosphate head group, became broad, depressed, and shifted to 1240 cm^{-1} . Results indicate that trehalose and DPPC interact by hydrogen bonding between the OH groups in the carbohydrate and the polar head groups of

DPPC. Furthermore, water replacement hypothesis was studied using molecular dynamics (MD) simulations of the interaction between trehalose and the lipid membrane.⁵⁶ The interactions between trehalose and a lipid membrane made of DPPC at 323 K were studied using MD simulations. Results show that trehalose bonded to the phospholipid head groups, with the main axis of trehalose parallel to the membrane normal. Trehalose established hydrogen bonds with the carbonyl and phosphate groups and replaced water molecules from the lipid head group.

In addition to water replacement hypothesis, head group bridging hypothesis was used to explain this mechanism. Phospholipid bilayers were simulated as model cell membranes to understand their dynamic and structural properties, particularly how these properties are affected by the presence of cell-preservation agents, such as trehalose.⁵⁷ The simulations covered a wide range of temperatures and concentrations of trehalose. Various properties, including area per head group, lipid-tail order parameter, density profile, conformation, and trehalose binding, were examined. The results indicate that trehalose specifically interacted with the phospholipid bilayer by binding to the polar head groups (H-bonds). This binding serves as a bridge between the lipids and prevents lipid aggregation.

2. Indirect interaction.

Given the unique physical properties of trehalose, it can induce several “indirect effects” on labile biologicals. During dehydration, trehalose can form an amorphous matrix, which can be used to restrict the mobility of biomolecules.⁵⁸⁻⁶⁰ This matrix impedes the chemical degradation rate and maintains the separation between the neighboring biomolecules, which may aggregate in the absence of trehalose.^{60,61} Trehalose can form a unique glassy structure that is stable at high temperatures, which allows the use of trehalose in a wide range of extreme environmental conditions.⁶² The glassy state maintains the entrapped biomolecules in a form that allows them to return to their native structure and function upon rehydration.^{62,63} However, vitrification alone is insufficient to confer stability.

Hydration force hypothesis states that nonspecific osmotic and volumetric effects are responsible for the observed membrane phase behaviors in the presence of

sugars.⁶⁴⁻⁶⁶ According to hydration force hypothesis, the presence of small solutes between the membranes during dehydration hinders the approach of membrane bilayers. This impediment reduces the hydration force between the surfaces, thereby decreasing the mechanical stresses that arise when the bilayers are in close proximity. The increased osmotic pressure generated by the solutes between the bilayers limits the water removal between the membranes, thereby keeping the bilayers further apart. The volumetric effects indicate that the solutes possess a molecular volume that keeps the membranes separated. Thus, the stresses and strains in the membranes at low hydrations may be diminished by interlamellar solutes, which include sugars, consequently reducing the effects that cause the transition to gel phase. The effect of increasing solute size on phosphatidylcholine (PC) phase behavior at a range of hydrations was studied.⁶⁶ Various molecular weights of saccharide polymer were employed to investigate the size-exclusion limit for polymers between PC bilayers. Solutes with sizes ranging from glucose to dextran 1000 limited the increase in lipid T_m during dehydration, which suggests that the lipids remained between the dehydrated bilayers. At the lowest hydrations, the solutions vitrified, and T_m was further depressed to about 20 °C below the transition temperature for the lipid in excess water, T_o . The depression of T_m below T_o occurred when the interlamellar solution vitrified between fluid phase bilayers. The largest saccharide polymers, namely, dextran 5000 and 12,000, had slight effects on T_m of PCs at any hydration. Moreover, the vitrification of these large polymers had no effect on lipid phase behavior. These findings suggest that large saccharide polymers are excluded from the interlamellar region during dehydration.

1-3-3 Current Research Applications⁵¹

The lipid membrane has been extensively studied as a model cell membrane to understand the effects of dehydration on the stability of the membrane by examining the shift in phase transition temperature T_m ,⁶⁷⁻⁶⁹ and the degree of phase separation.^{70,71} The effects of various disaccharides, such as trehalose, on the structural stability of lipid membrane were examined. The main observations include

the decrease in T_m ,^{68,72,73} the inhibition of aggregation/fusion,^{72,74} and the inhibition of phase separation,^{70,71} all of which contribute to the enhanced stability of lipid membranes. T_m indicates the temperature at which the membrane lipids undergo a transition from gel phase ($T < T_m$) to liquid crystalline phase ($T > T_m$). The dehydration of lipids increases the packing between lipid molecules because of water removal, thereby stabilizing the gel phase and increasing T_m . For DPPC, T_m increases from 42 °C in the hydrated state to over 100 °C upon dehydration.⁴⁹ By adding trehalose before dehydration, T_m can be lowered to approximately 25 °C (below T_m of the hydrated state),^{49,67} which shows that trehalose can preserve the membrane structure during dehydration. In another example, trehalose provided complete protection of freeze-dried lipids, which consisted of palmitoylcholine and phosphatidylserine, based on the retention of an entrapped marker (isocitrate).⁷⁵ Maximal stability was observed at 1 g trehalose/g lipid. However, the preservation of trehalose depends on the presence of sugar on both sides of the lipid structure. For LUVs composed of egg PC, the amount of Na^+ entrapped within the lipids was significantly higher (84%) in the air-dried membrane with trehalose on both sides than that with trehalose on the outside of the membrane structure only (17%).⁷⁶ Furthermore, the addition of trehalose inhibited the fusion of unilamellar vesicles. Dynamic light scattering revealed that the addition of trehalose to lipid at a 5:1 weight ratio resulted in the complete inhibition of fusion. In the absence of the sugar, vesicle size increased from 100 nm to over 3000 nm.⁷³ Both direct (i.e., water replacement hypothesis) and indirect (i.e., hydration force hypothesis) interactions contribute to the lipid-stabilizing mechanisms of trehalose. Among the saccharide polymers, trehalose has the highest ability for hydration, which suggests that trehalose stabilizes the lipid bilayer structure by ordering the water molecules around the membrane.⁷⁷ Numerous reports also examined the effects of trehalose on the stability of lipids in solution.⁷⁸⁻⁸⁰

1-4 Soap Films and Soap Bubbles

1-4-1 History of the Science of Soap Films and Soap Bubbles⁸¹

The scientific study of soap films and soap bubbles dates back to the 17th century when Isaac Newton observed colors and black spots in soap films and bubbles. These colors and black spots were from the interference of light produced by the reflection from the surfaces of the films. During the 19th century, Belgian physicist Joseph Antoine Ferdinand Plateau showed that dipping wire frameworks into a bath of soap solution minimizes the surface area. A soap film was formed in the frame, and the frame was bounded by the edges of the framework with a minimum surface area after withdrawing the framework from the bath. During the latter half of the 19th century, Josiah Willard Gibbs, who is well known for his theoretical contributions to the study of statistical mechanics and thermodynamics, observed the draining and thinning of soap films.

Charles Vernon Boys popularized the properties of soap films and soap bubbles at the turn of this century. His research covered the behavior of surface water of bubbles, bubble pressures, bubbles and electricity, and the differences between inside and outside bubbles in double bubbles. His book entitled “Soap bubbles and the forces which mold them” has been popular with young people since its publication in 1890. Biologist D’Arcy Wentworth Thompson presented the similarities between the shape of soap bubbles and the shapes that occur in living organisms. During the First World War, Sir James Dewar investigated the draining and stability of soap films.

A large chemical industry dependent on the science of surface phenomena has developed. By understanding the surface properties, such as wetting, dyeing, foaming, coalescing, and emulsification, these industries have developed several products. The study of soap films and soap bubbles is an essential part of the science of surface phenomena. Thus, large research programs in this field are being conducted by the industry and universities. Biochemists have been studying biological membranes in animal and plant cells with increasing interest. These cells are composed of lipid molecules that are similar in both structure and behavior to soap molecules. Thus,

studying soap films can provide insight into the function and properties of lipid molecules and lipid membranes.

1-4-2 Molecular Structures of Soap Films

Soap solutions have the remarkable property of forming stable bubbles and films. This property is due to the surface structure of the soap solution and the soap film. The surface of the soap solution and the soap film consists of an amphiphilic monolayer, which has a hydrophilic head group and a hydrophobic tail. The hydrophilic tail prefers to be surrounded by water because of the attraction between the hydrophilic head group and the water molecules. By contrast, the hydrophobic part is averse to the water environment that the hydrocarbon tails points out from the water.

An example of a soap molecule is sodium dodecyl sulfate (SDS), $\text{C}_{12}\text{H}_{25}\text{SO}_4^-\text{Na}^+$, which has a hydrophilic SO_4^- group and a hydrophobic $\text{C}_{12}\text{H}_{25}$ chain. The positive metallic sodium ions are dispersed throughout the bulk fluid in the soap solution or film. Figure 1.13 (a) shows a monolayer that is adsorbed on the surface of the soap solution. The amphipathic ions on the surface of the soap solution can orient themselves so that their heads remain in the water and their tails remain out of the water. A soap film consists of two surfaces of amphipathic ions, as shown in Figure 1.13 (b). These two surfaces are separated by a thin layer of fluid, which has a thickness ranging from $2 \times 10^5 \text{ \AA}$ to $50 \text{ \AA}$ ($10 \text{ \AA} = 1 \text{ nm}$). Each surface is composed of soap molecules that are largely separated by water molecules. The greatest thickness occurs immediately after the formation of a film. Once the film forms, it begins to become thin because the water starts to drain away from the film. The thickness of the film decreases until a final equilibrium thickness is reached, provided that the film does not rupture.

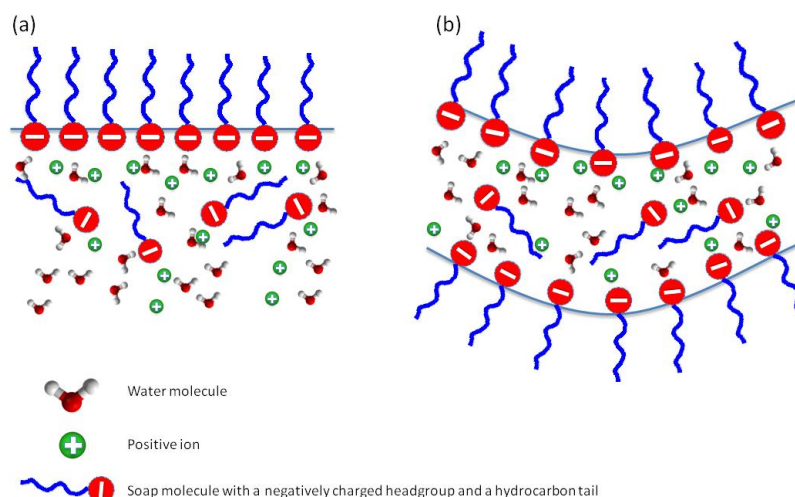


Figure 1.13 Surface structures of (a) a soap solution and (b) a soap film.

Studies on the thinning of vertical soap films have revealed various aspects of time evolution of film drainage, resulting in the discovery of the Newton black film.^{82,83} UV-vis and IR spectroscopy were used to study the time-dependent changes in the thickness of soap films and to obtain information regarding the structure and chemical composition of films.⁸⁴⁻⁸⁶ However, the real-time change in the structure and arrangement of soap and water molecules during bubble thinning remains unclear. The study of these structural changes requires an improved stability of soap membrane.

1-5 Fundamental of Sum Frequency Generation (SFG) Spectroscopy

Spectroscopic studies are essential for real understanding of surfaces and interfaces. Unlike the linear optical spectroscopy, which lacks the intrinsic surface specificity for most surface studies, nonlinear optical spectroscopies can be highly surface specific by symmetry if the nonlinear optical process involved is of even order. Second order nonlinear processes such as second harmonic generation (SHG), sum frequency generation (SFG), and difference frequency generation (DFG) are more attractive due to their simplicity and sensitivity. In the present thesis, SFG spectroscopy is the main experimental technique for investigating molecular structure

at bio-interface. Thus, I focus my discussion on SFG spectroscopy.⁸⁷⁻⁸⁹

1-5-1 Theory of SFG⁸⁷

1-5-1-1 Nonlinear Optics

In the intense electric field, for example, a laser source, the induced dipole moment $\boldsymbol{\mu}$ of a molecule placed in the electric field $\boldsymbol{E}$ is described as

$$\boldsymbol{\mu} = \boldsymbol{\mu}^0 + \vec{\alpha}:\boldsymbol{E} + \vec{\beta}:\boldsymbol{E}\boldsymbol{E} + \vec{\gamma}:\boldsymbol{E}\boldsymbol{E}\boldsymbol{E} + \dots \quad (1-1)$$

Where, $\boldsymbol{\mu}^0$ is a static dipole, and $\vec{\alpha}$, $\vec{\beta}$, $\vec{\gamma}$... are the linear polarizability, the first and second ... hyperpolarizabilities of the molecule, respectively, and are represented by second, third and forth ... rank tensor. These give rise to nonlinear optical effects that are known as second and third order nonlinear effects, respectively.

In condensed phases, it is more convenient to consider the dipole moment per unit volume, or polarization $\boldsymbol{P}$.

$$\begin{aligned} \boldsymbol{P} &= \boldsymbol{P}^{(0)} + \boldsymbol{P}^{(1)} + \boldsymbol{P}^{(2)} + \boldsymbol{P}^{(3)} + \dots \\ &= \boldsymbol{P}^{(0)} + \varepsilon_0(\vec{\chi}^{(1)}\boldsymbol{E} + \vec{\chi}^{(2)}:\boldsymbol{E}\boldsymbol{E} + \vec{\chi}^{(3)}:\boldsymbol{E}\boldsymbol{E}\boldsymbol{E} + \dots) \end{aligned} \quad (1-2)$$

Where, $\vec{\chi}^{(1)}$, $\vec{\chi}^{(2)}$, $\vec{\chi}^{(3)}$ are the linear, second order and third order nonlinear susceptibilities, respectively, and ε_0 is dielectric constant in vacuum. For a simple molecular material, the susceptibility depends on the number of molecules per unit volume, N , multiplied by molecular polarizability averaged over all orientation of molecules in the material.

$$\vec{\chi}^{(1)} = N\langle\vec{\alpha}\rangle/\varepsilon_0, \vec{\chi}^{(2)} = N\langle\vec{\beta}\rangle/\varepsilon_0, \vec{\chi}^{(3)} = N\langle\vec{\gamma}\rangle/\varepsilon_0, \dots \quad (1-3)$$

Where, the brackets $\langle \rangle$ denote the ensemble average of the molecular

orientation. Since few materials have a static polarization, the first term $\mathbf{P}^{(0)}$ can be dropped.

$$\mathbf{P} = \varepsilon_0(\tilde{\chi}^{(1)}:\mathbf{E} + \tilde{\chi}^{(2)}:\mathbf{E}\mathbf{E} + \tilde{\chi}^{(3)}:\mathbf{E}\mathbf{E}\mathbf{E} + \dots) \quad (1-4)$$

One of the nonlinear processes that will be extensively studied in this thesis is SFG. Assuming two laser beams with electric fields of $E_1(\mathbf{r})\cos\omega_1 t$ and $E_2(\mathbf{r})\cos\omega_2 t$ present simultaneously and generate the second order nonlinear polarization $P^{(2)}$ which is given by

$$\mathbf{P}^{(2)} = \varepsilon_0\tilde{\chi}^{(2)}\mathbf{E}_1\mathbf{E}_2 \quad (1-5)$$

$$= \varepsilon_0\tilde{\chi}^{(2)}E_1(\mathbf{r})\cos\omega_1 t \cdot E_2(\mathbf{r})\cos\omega_2 t \quad (1-6)$$

This equation can be further expanded to:

$$\mathbf{P}^{(2)} = 1/2\varepsilon_0\tilde{\chi}^{(2)}E_1(\mathbf{r})E_2(\mathbf{r})\{\cos[(\omega_1 + \omega_2)t] + \cos[(\omega_1 - \omega_2)t]\} \quad (1-7)$$

The oscillating polarization can emit light at frequencies of $2\omega_1$, $2\omega_2$, $\omega_1 + \omega_2$, $\omega_1 - \omega_2$, which give rise to SHG for both input beams, SFG, and DFG, respectively.

However, SFG are prohibited in media with inversion symmetry under the electric dipole approximation. In the bulk of a centrosymmetric medium as shown in Figure 1.14, an inversion operation of Eq. (1-5) gives:

$$-\mathbf{P}^{(2)} = \varepsilon_0\tilde{\chi}^{(2)}(-\mathbf{E}_1)(-\mathbf{E}_2) \quad (1-8)$$

In order to satisfy Eq. (1-5) and Eq. (1-8) at the same time, $\tilde{\chi}^{(2)}$ must be zero. Thus, the bulk with inversion symmetry structure, i.e., bulk liquids, gases, or amorphous and centrosymmetric solid, is SFG inactive. To be emphasizing, SFG is allowed only at the interface between these media where the inversion symmetry is necessarily

broken.

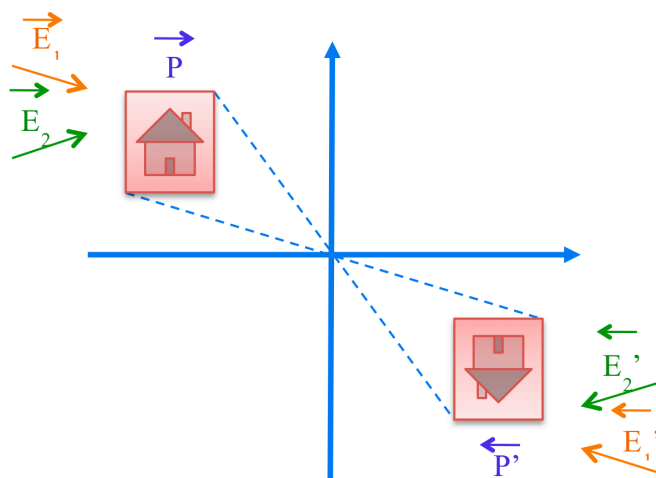


Figure 1.14 Schematic image of the bulk of inversion symmetry structure.

1-5-1-2 SFG Vibration Spectroscopy

In SFG vibration spectroscopy, one of the beams needs to be scanned in the infrared region or spectrally broadened beam in the infrared region can be used, while the other beam is usually fixed at the frequency lower or close to the electronic transitions in the visible region. When the infrared frequencies resonate with vibration transition frequencies of a surface molecule, SFG signal will be enhanced as described in Figure 1.15.

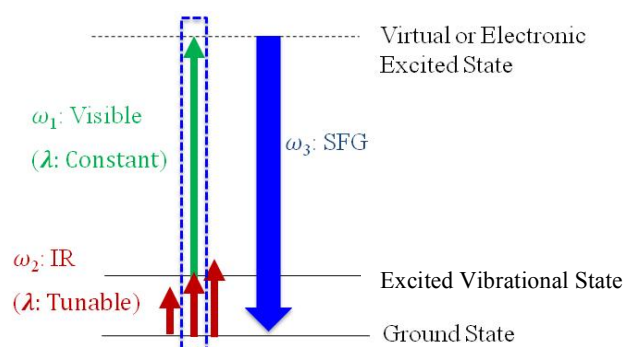


Figure 1.15 Molecular energy schemes of SFG resonant processes.

In the most widely used geometry, the visible laser beam and the tunable infrared laser beam are incident in either counter or collinear propagating geometry, and

reflected SFG light (ω_{SFG}) is detected. Figure 1.16 shows visible and infrared beams are overlapped from the medium 1 to the interface between the medium 1 and 2, and generated SFG light goes to the medium 1.

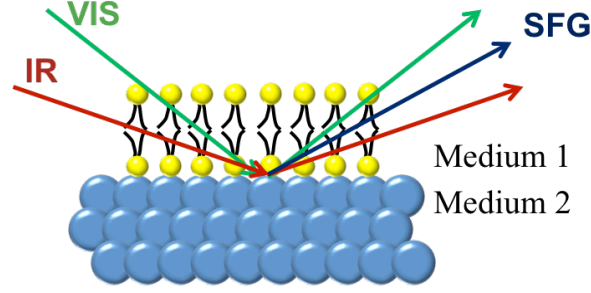


Figure 1.16 Schematic diagram of SFG process in the collinear incidence.

The direction of emission is determined by the conservation of the momentum parallel to the surface,

$$\omega_{SFG} \sin \theta_{SFG} = \omega_{vis} \sin \theta_{vis} + \omega_{IR} \sin \theta_{IR} \quad (1-9)$$

Where θ_{SFG} is the emission angle of the SFG light and θ_{vis} and θ_{IR} are the incident angles of visible and infrared lights, respectively. Since the incident angles θ_{vis} and θ_{IR} and the frequency ω_{vis} is constant in a SFG measurement, θ_{SFG} can be written as a function of ω_{IR} :

$$\theta_{SFG}(\omega_{IR}) = \sin^{-1} \frac{\omega_{vis} \sin \theta_{vis} + \omega_{IR} \sin \theta_{IR}}{\omega_{vis} + \omega_{IR}} \quad (1-10)$$

The intensity of SFG light (I_{SFG}) is described as follows:⁸⁸

$$I_{SFG}(\omega_{IR}) = \frac{8\pi^3 \omega_{SFG}^2 \sec^2 \theta_{SFG}}{c^3 n_1(\omega_{SFG}) n_1(\omega_{vis}) n_1(\omega_{IR})} |\mathbf{F} : \overleftrightarrow{\chi}^{(2)}|^2 I(\omega_{vis}) I(\omega_{IR}) \quad (1-11)$$

Here, $I(\omega_{vis})$ and $I(\omega_{IR})$ are the intensities of the input visible and infrared

lasers, $n_1(\omega_i)$ is the refractive index of medium 1 at frequency ω_i and $\mathbf{F}$ is the Fresnel factor. If the input lasers have a pulsewidth T and an overlapping beam cross section A at the sample, then the SFG output, in terms of photons per pulse can be written as:

$$S = I_{SFG}(\omega_{IR})AT/\hbar\omega_{SFG}$$

$$= \frac{8\pi^3\omega_{SFG}^2\sec^2\theta_{SFG}}{\hbar c^3 n_1(\omega_{SFG})n_1(\omega_{vis})n_1(\omega_{IR})} |\mathbf{F}:\tilde{\chi}^{(2)}|^2 I(\omega_{vis})I(\omega_{IR})AT \quad (1-12)$$

The number of photos which generated by a SFG process can be calculated as follows. For a polar monolayer of molecules at an interface, the resonant $|\tilde{\chi}^{(2)}|$ is typically $10^{-14} \sim 10^{-16}$ esu. If we choose $|\tilde{\chi}^{(2)}| \sim 10^{-15}$ esu, $I(\omega_{vis}) \sim 1$ mJ/pulse and $I(\omega_{IR}) \sim 100$ μ J/pulse, $T \sim 25$ ps, $\omega_{vis}=5.64 \times 10^{14}$ s^{-1} (532 nm), $\omega_{IR}=9.0 \times 10^{13}$ s^{-1} (3333nm), $\omega_{SFG}=6.55 \times 10^{14}$ s^{-1} (458.77 nm), $A \sim 10^{-3}$ cm^2 , $\theta_{SFG} \sim 67^\circ$, according to Eq. (1-12), the signal of 1.5×10^4 photon/pulse can be expected. Such signal can be detected by a photo-multiplier tube (PMT) or CCD detector.

If neither ω_{vis} nor ω_{SFG} is in resonance with the electric dipole transition in the material and only the electric dipole transition is considered, the microscopic hyperpolarizability, β_{ijk} , near a vibrational transition in a molecule is given by:^{87,88,90}

$$\beta_{ijk} = \frac{1}{2\hbar^2} \cdot \sum_s \left(\frac{\langle g|\mu_i|s\rangle\langle s|\mu_j|v\rangle}{\omega_{SFG}-\omega_{sg}} - \frac{\langle g|\mu_j|s\rangle\langle s|\mu_i|v\rangle}{\omega_{vis}+\omega_{sg}} \right) \left(\frac{\langle v|\mu_k|g\rangle}{\omega_{IR}-\omega_{vg}+i\Gamma} \right) + \text{const.} \quad (1-13)$$

Where $|g\rangle$ is the ground state, $|v\rangle$ is the excited vibrational state, $|s\rangle$ is any other state, ω_{sg} is the transition frequency from $|g\rangle$ to $|s\rangle$, Γ is the relaxation time of the excited vibrational state, const. is off-resonant terms which can be considered as a constant and $\mu = er$ is the electric dipole operator. The first bracket is identified as Raman transition dipole moment, $\mathbf{M}_{ij}$, and the term $\langle v|\mu_k|g\rangle$ as the infrared transition dipole moment, μ_n^{vg} . If we substitute ω_{vg} to ω_0 , Eq. (1-13) becomes:

$$\beta_{ijk} = \frac{1}{2\hbar^2} \cdot \frac{a_{ijk}}{\omega_{IR} - \omega_0 - i\Gamma} + \text{const.} \quad (1-14)$$

$$\text{where, } a_{ijk} = \mathbf{M}_{ij} \cdot \boldsymbol{\mu}_n^{vg} \quad (1-15)$$

From the above equations, β_{ijk} takes the maximum value when the infrared frequencies resonate with vibration transition frequencies of a surface molecule, i.e., $\omega_{IR} = \omega_0$, and the molecular vibration is SFG active which is only occurred at both infrared and Raman active. This is an important selection rule for SFG spectroscopy.

If we introduce $\vec{A} = \frac{N}{\varepsilon_0 \langle \vec{\alpha} \rangle} = \frac{N}{\varepsilon_0 \langle \mathbf{M}_{ij} \cdot \boldsymbol{\mu}_n^{vg} \rangle}$, the description of a macroscopic second order susceptibility, $\vec{\chi}_R^{(2)}$, is simplified by:

$$\vec{\chi}_R^{(2)} = \frac{\vec{A}}{\omega_{IR} - \omega_0 - i\Gamma} \quad (1-16)$$

The intensity of emitted SFG light is proportional to a sum of resonant contributions with q th vibrational states, $\vec{\chi}_{R,q}^{(2)}$, and nonresonant contribution, $\vec{\chi}_{NR}^{(2)}$, which are not negligible.

$$\vec{\chi}^{(2)} = \vec{\chi}_{NR}^{(2)} + \sum_q \vec{\chi}_{R,q}^{(2)} = \vec{\chi}_{NR}^{(2)} + \sum_q \frac{\vec{A}_q}{\omega_{IR} - \omega_q - i\Gamma_q} \quad (1-17)$$

Here, $\vec{\chi}_{NR}^{(2)}$ includes three parts of the nonresonant contributions, which is molecules ($\vec{\chi}_{NR,mol}^{(2)}$), substrate ($\vec{\chi}_{NR,sub}^{(2)}$) and the interaction between the molecules and the substrate ($\vec{\chi}_{NR,m-s}^{(2)}$) as shown below:

$$\vec{\chi}_{NR}^{(2)} = \vec{\chi}_{NR,mol}^{(2)} + \vec{\chi}_{NR,sub}^{(2)} + \vec{\chi}_{NR,m-s}^{(2)} \quad (1-18)$$

Combining Eq. (1-11) and (1-17), the SFG intensity can be written by:

$$I_{SFG} \propto \left| \chi_{eff,NR}^{(2)} + \sum_n \frac{\vec{A}_{eff,n}}{\omega_{IR} - \omega_n - i\Gamma_n} \right|^2 \quad (1-19)$$

Where, $\chi_{eff,NR}^{(2)} = \mathbf{F} : \vec{\chi}_{NR}^{(2)}$, $\vec{A}_{eff,n} = \mathbf{F} : \vec{A}_n$. This is the equation which is generally used for analyses an observed SFG spectrum.

1-5-2 Peak Deconvolution and Shape of a SFG Spectrum

Similar to CARS the profile of a resonance can appear in various different forms depending on the interference between a resonance and a nonresonant background as well as resonances. In order to deduce the parameters characterizing the resonance, Eq. (1-19) must be used to fit the observed spectrum. Since both $\vec{\chi}_R^{(2)}$ and $\vec{\chi}_{NR}^{(2)}$ are complex quantities, the overall signal depends on the relative phases of the two susceptibilities, ε and δ , respectively. If we consider a single resonance for simplification, we obtain:

$$\vec{\chi}_R^{(2)} = \frac{\vec{A}}{\sqrt{(\omega - \omega_{IR})^2 + \Gamma^2} \cdot e^{i\delta(\omega)}} = \left| \vec{\chi}_R^{(2)} \right| e^{-i\delta(\omega_{IR})} \quad (1-20)$$

$$\delta(\omega_{IR}) = \tan^{-1} \frac{\Gamma}{\omega - \omega_{IR}} \quad (1-21)$$

$$\vec{\chi}_{NR}^{(2)} = \left[\vec{\chi}_{NR}^{(2)} \right] e^{i\varepsilon} \quad (1-22)$$

When ω_{IR} is scanned from lower to higher frequency around ω , $\delta(\omega_{IR})$ changes its value from 0 to π continuously across $\pi/2$ at $\omega_{IR} = \omega$. ε is the constant which is determined mainly by the substrate. Using Eqs. (1-20) and (1-22), Eq. (1-19) can be written as:

$$I_{SFG} \propto \left[\left| \vec{\chi}_{NR}^{(2)} \right| e^{i\varepsilon} + \left| \vec{\chi}_R^{(2)} \right| e^{i\delta(\omega_{IR})} \right] \times \left[\left| \vec{\chi}_{NR}^{(2)} \right| e^{-i\varepsilon} + \left| \vec{\chi}_R^{(2)} \right| e^{-i\delta(\omega_{IR})} \right]$$

$$= \left| \chi_{NR}^{(2)} \right|^2 + \left| \chi_R^{(2)} \right|^2 + 2 \left| \chi_{NR}^{(2)} \right| \left| \chi_R^{(2)} \right| \cos[\varepsilon - \delta(\omega_{IR})] \quad (1-23)$$

Since $\delta(\omega_{IR})$ in Equ. (1-23) is dependent on ω_{IR} , spectral shape of SFG spectra is not simple.

Figure 1.17 shows a series of simulated curves of $\left| \chi^{(2)} \right|^2$ with different ω when $\left| \chi_{NR}^{(2)} \right| = 0$, the $\left| \chi^{(2)} \right|^2$ spectra are expressed by a Lorentz function centered at ω and independent of ε . Whereas, when $\left| \chi_{NR}^{(2)} \right| = |\bar{A}/\bar{\Gamma}| > 0$, the spectral shape is dependent on ε . the spectral profile gives Lorentz shape only when $\varepsilon \sim \pi/2$. Differential-shape profiles are observed when $\varepsilon \sim 0$ and π and even negative peak profile is observed when $\varepsilon \sim 3\pi/2$. A non-Lorentz shape SFG spectrum indicates that there is measureable non-resonant contribution in the spectrum, but the opposite is not always true (in the case of $\varepsilon = \pi/2$ and $\left| \chi_{NR}^{(2)} \right| > 0$)> Note that the peak frequency in appearance is often different from that in truth, which must be obtained by peak fitting.

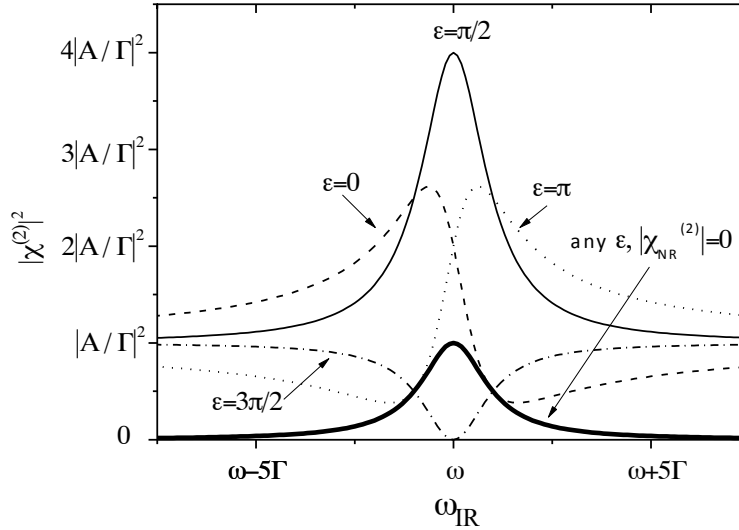


Figure 1.17 The effect of interference between a resonant and nonresonant components on a spectral profile. In all cases, $\left| \chi_R^{(2)} \right| = |A/\Gamma| = \left| \chi_{NR}^{(2)} \right|$. ε for each case is shown in the Figure.

Thick solid line shows a simulation with $\left| \chi_{NR}^{(2)} \right| = 0$, where the spectral shape is independent of ε .

In practical use, it is convenient to separate $\vec{\chi}^{(2)}$ into real and imaginary part, where we introduce phase angle ε between $\vec{\chi}_R^{(2)}$ and $\vec{\chi}_{NR}^{(2)}$. Then Eq. (1-19) is written as:

$$\begin{aligned}
 I_{SFG} &\propto \left| \vec{\chi}_{eff,NR}^{(2)} + \sum_q \frac{\vec{A}_{eff,q} e^{i\varepsilon}}{\omega_q - \omega_{qIR} - i\Gamma_q} \right|^2 \\
 &= \left\{ \sum_q \frac{\vec{A}_{eff,q}(\omega_q - \omega_{qIR})}{(\omega_q - \omega_{qIR})^2 + \Gamma_q^2} + \left| \vec{\chi}_{eff,NR}^{(2)} \right| \cos \varepsilon \right\}^2 + \left\{ \sum_q \frac{\vec{A}_{eff,q} \Gamma_q}{(\omega_q - \omega_{qIR})^2 + \Gamma_q^2} + \left| \vec{\chi}_{eff,NR}^{(2)} \right| \sin \varepsilon \right\}^2
 \end{aligned}
 \tag{1-24}$$

All the SFG spectra were fitted by Eq. (1-24) using Origin 6.1. An example of the fitting function by using Origin is shown as follows.

```

> Parameters=A1,B1,C1,A2,B2,C2,A3,B3,C3,A4,B4,C4,A5,B5,C5,NR,F,s0
[FORMULA]
> M1=A1/((B1-w)^2+C1^2);
> M2=A2/((B2-w)^2+C2^2);
> M3=A3/((B3-w)^2+C3^2);
> M4=A4/((B4-w)^2+C4^2);
> M5=A5/((B5-w)^2+C5^2);
> U=Pi/180*F;
> R=M1*(B1-w)+M2*(B2-w)+M3*(B3-w)+M4*(B4-w)+M5*(B5-w)+NR*cos(U);
> I=M1*C1+M2*C2+M3*C3+M4*C4+M5*C5+NR*sin(U);
> y=(R)^2+(I)^2+s0;
> INDEPENDENT VARIABLES: w
> DEPENDENT VARIABLES: y

```

1-5-3 Fresnel Factor

The Fresnel factor ($\mathbf{F}$) in Eq. (1-11) and (1-12) represents the combination of Fresnel coefficients. In the laboratory fixed (XYZ) axis system, a component of $\mathbf{F}$, F_{IJK} , is defined as^{12,14}:

$$\mathbf{F}_{IJK} = L_I(\omega_{SFG}) \cdot \hat{\mathbf{e}}(\omega_{SFG}) \cdot L_J(\omega_{vis}) \cdot \hat{\mathbf{e}}(\omega_{vis}) \cdot L_K(\omega_{IR}) \cdot \hat{\mathbf{e}}(\omega_{IR}) \quad (1-25)$$

Where, $\hat{\mathbf{e}}(\omega_{SFG})$, $\hat{\mathbf{e}}(\omega_{vis})$ and $\hat{\mathbf{e}}(\omega_{IR})$ are the unit vectors of SFG, visible and IR beams, respectively. $L_I(\omega_{SFG})$, $L_J(\omega_{vis})$ and $L_K(\omega_{IR})$ represent the Fresnel coefficients at the interface for the electric field of SFG, visible and IR beams in the I , J , K direction, respectively. The Fresnel coefficients for the electric fields in the X , Y , Z direction at a frequency ω_i , $L_X(\omega_i)$, $L_Y(\omega_i)$ and $L_Z(\omega_i)$ are given by^{2,14}:

$$L_X(\omega_i) = \frac{2n_1(\omega_i)\cos\varphi}{n_1(\omega_i)\cos\varphi + n_2(\omega_i)\cos\theta} \quad (1-26)$$

$$L_Y(\omega_i) = \frac{2n_1(\omega_i)\cos\theta}{n_1(\omega_i)\cos\theta + n_2(\omega_i)\cos\varphi} \quad (1-27)$$

$$L_Z(\omega_i) = \frac{2n_2(\omega_i)\cos\theta}{n_1(\omega_i)\cos\varphi + n_2(\omega_i)\cos\theta} \left(\frac{n_1(\omega_i)}{n'(\omega_i)} \right)^2 \quad (1-28)$$

Where, ω_i denotes either ω_{SFG} , ω_{vis} or ω_{IR} and $n_1(\omega_i)$, $n_2(\omega_i)$ and $n'(\omega_i)$ are the refraction index of the medium 1, medium 2 and the interface, respectively. θ and φ represent angles of the reflection and refraction, respectively. $\mathbf{F}_{IJK}$ for the polarization combination with at least one non-zero component which are described in the following section 1-5-3 are summarized as follows:

$$\mathbf{F}_{YYZ} = L_Y(\omega_{SFG}) \times L_Y(\omega_{vis}) \times L_Z(\omega_{IR}) \times \sin(\theta_{IR}) \quad (1-29)$$

$$\mathbf{F}_{YZY} = L_Y(\omega_{SFG}) \times L_Z(\omega_{vis}) \times L_Y(\omega_{IR}) \times \sin(\theta_{vis}) \quad (1-30)$$

$$\mathbf{F}_{ZYY} = L_Z(\omega_{SFG}) \times L_Y(\omega_{vis}) \times L_Y(\omega_{IR}) \times \sin(\theta_{SFG}) \quad (1-31)$$

$$\mathbf{F}_{ZZZ} = L_Z(\omega_{SFG}) \times L_Z(\omega_{vis}) \times L_Z(\omega_{IR}) \times \sin(\theta_{IR}) \sin(\theta_{vis}) \sin(\theta_{SFG}) \quad (1-32)$$

$$\mathbf{F}_{XXZ} = (-L_X(\omega_{SFG})) \times L_X(\omega_{vis}) \times L_Z(\omega_{IR}) \times \sin(\theta_{IR}) \sin(\theta_{vis}) \cos(\theta_{SFG}) \quad (1-33)$$

$$\mathbf{F}_{XZX} = (-L_X(\omega_{SFG})) \times L_Z(\omega_{vis}) \times L_X(\omega_{IR}) \times \cos(\theta_{IR}) \sin(\theta_{vis}) \cos(\theta_{SFG}) \quad (1-34)$$

$$\mathbf{F}_{ZXX} = L_Z(\omega_{SFG}) \times L_X(\omega_{vis}) \times L_X(\omega_{IR}) \times \cos(\theta_{IR}) \cos(\theta_{vis}) \sin(\theta_{SFG}) \quad (1-35)$$

$$\mathbf{F}_{XXX} = L_X(\omega_{SFG}) \times L_X(\omega_{vis}) \times L_X(\omega_{IR}) \times \cos(\theta_{IR}) \cos(\theta_{vis}) \cos(\theta_{SFG}) \quad (1-36)$$

In practice, the Fresnel factors were calculated by these equations on Maple V (Maplesoft). An example of the calculation by using Maple V is shown as below:

```
> Fresnel factors for the n1/n2 interface (n1: quartz; n2: water;)  
> anglev:=x: n1v:=1.45: n2v:=1.33;  
> angleI:=50; n1I:=1.41; n2I:=1.31; n_m:=1.44; n_m_IR:=1.44;  
> Fresnel factor  
# angle  
> T1v:=Pi/180*(anglev):  
> T1I:=Pi/180*(angleI):  
> T1s:=arcsin((18797*sin(T1v)+3200*sin(T1I))/(18797+3200));  
> T2I:=(arcsin(n1I*sin(T1I)/n2I)):  
> T2s:=(arcsin(n1v*sin(T1s)/n2v)):  
> T2v:=(arcsin(n1v*sin(T1v)/n2v)):  
# visible  
> Lxxv:= evalf( (2*n1v*cos(T2v) / (n2v*cos(T1v)+n1v*cos(T2v))) );  
> Lyyv:= evalf( (2*n1v*cos(T1v) / (n1v*cos(T1v)+n2v*cos(T2v))) );  
> Lzzv:= evalf( (2*n1v^2*n2v*cos(T1v)/n_m^2 / (n2v*cos(T1v)+n1v*cos(T2v))) );
```

```

# IR
> LxxI:= evalf( (2*n1I*cos(T2I) / (n2I*cos(T1I)+n1I*cos(T2I))) );
> LyyI:= evalf( (2*n1I*cos(T1I) / (n1I*cos(T1I)+n2I*cos(T2I))) );
> LzzI:= evalf( (2*n1I^2*n2I*cos(T1I)/n_m_IR^2 / (n2I*cos(T1I)+n1I*cos(T2I))) );
# SFG
> Lxxs:= evalf( (2*n1v*cos(T2s) / (n2v*cos(T1s)+n1v*cos(T2s))) );
> Lyys:= evalf( (2*n1v*cos(T1s) / (n1v*cos(T1s)+n2v*cos(T2s))) );
> Lzzs:= evalf( (2*n1v^2*n2v*cos(T1s) / n_m^2 / (n2v*cos(T1s)+n1v*cos(T2s))) );

> Xssp:= evalf( (Lyyv * Lyys * LzzI * sin(T1I)));
> Xsps:= evalf( (Lyyv * LyyI * Lzzs * sin(T1v)));
> Xppp:= evalf( (Lzzv * Lzzs * LzzI * sin(T1v)*sin(T1s)*sin(T1I)));
> plot(Xssp,x=0..90);

```

1-5-4 Symmetry Consideration of $\vec{\chi}^{(2)}$ ¹⁵

$\vec{\chi}^{(2)}$ is a third-rank three dimensional tensor with 27 components as given by:

$$\vec{\chi}^{(2)} = \begin{pmatrix} \chi_{xxx}^{(2)} & \chi_{xxy}^{(2)} & \chi_{xxz}^{(2)} & \chi_{xyx}^{(2)} & \chi_{xyy}^{(2)} & \chi_{xyz}^{(2)} & \chi_{xzx}^{(2)} & \chi_{xzy}^{(2)} & \chi_{xzz}^{(2)} \\ \chi_{yxx}^{(2)} & \chi_{yxy}^{(2)} & \chi_{yxz}^{(2)} & \chi_{yyx}^{(2)} & \chi_{yyy}^{(2)} & \chi_{yyz}^{(2)} & \chi_{yzx}^{(2)} & \chi_{yzy}^{(2)} & \chi_{yzz}^{(2)} \\ \chi_{zxx}^{(2)} & \chi_{zxy}^{(2)} & \chi_{zxz}^{(2)} & \chi_{zyx}^{(2)} & \chi_{zyy}^{(2)} & \chi_{zyz}^{(2)} & \chi_{zzx}^{(2)} & \chi_{zzy}^{(2)} & \chi_{zzz}^{(2)} \end{pmatrix} \quad (1-37)$$

The symmetry of the homogeneous media and the interface implies that the surface symmetry is related with above elements vanish. The highest symmetry surfaces, i.e., isotropic surfaces, the non-vanishing elements of $\vec{\chi}^{(2)}$ are only following 7 elements:

$$\vec{\chi}^{(2)} = \begin{pmatrix} 0 & 0 & \chi_{xxz}^{(2)} & 0 & 0 & 0 & \chi_{xzx}^{(2)} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \chi_{yyz}^{(2)} & 0 & \chi_{yzy}^{(2)} & 0 \\ \chi_{zxx}^{(2)} & 0 & 0 & 0 & \chi_{zyy}^{(2)} & 0 & 0 & 0 & \chi_{zzz}^{(2)} \end{pmatrix} \quad (1-38)$$

Here, $\chi_{xzx}^{(2)} = \chi_{yzy}^{(2)}$, $\chi_{zxx}^{(2)} = \chi_{zyy}^{(2)}$, $\chi_{xxz}^{(2)} = \chi_{yyz}^{(2)}$.

For an isotropic surface, if we put the incident plane on the XZ plane, where Z is perpendicular to the surface, s-polarized light corresponds to Y component and p-polarized light includes both X and Z components. As a result, polarization combinations that have more than one non-vanishing element are summarized as follows:

ppp: $\chi_{xzx}^{(2)}$, $\chi_{zxx}^{(2)}$, $\chi_{xxz}^{(2)}$, $\chi_{zzz}^{(2)}$

ssp: $\chi_{yyz}^{(2)}$

sps: $\chi_{zyy}^{(2)}$

pss: $\chi_{zyy}^{(2)}$

Where, ppp denotes a combination of p- p- p- polarized beams of SFG, visible and infrared beams, respectively. The ssp, sps and pss are defined in the same way. The SFG intensities with those polarization combinations are described as follows:

$$I_{ppp} \propto \left| F_{xzx}\chi_{xzx}^{(2)} + F_{zxx}\chi_{zxx}^{(2)} + F_{xxz}\chi_{xxz}^{(2)} + F_{zzz}\chi_{zzz}^{(2)} \right|^2 \quad (1-39)$$

$$I_{ssp} \propto \left| F_{yyz}\chi_{yyz}^{(2)} \right|^2 \quad (1-40)$$

$$I_{sps} \propto \left| F_{zyy}\chi_{zyy}^{(2)} \right|^2 \quad (1-41)$$

$$I_{pss} \propto \left| F_{zyy}\chi_{zyy}^{(2)} \right|^2 \quad (1-42)$$

1-6 Objective and Overview of the Present Thesis

This study aims to study the surface structures of three typical model membrane systems, namely, supported planar monolayer, supported planar bilayer, and

free-standing soap films depending on different environments. This study also aims to clarify the stabilizing mechanism of disaccharide on the lipid bilayer in dry state using SFG, AFM, and FTIR spectroscopy.

In Chapter 2, a brief description of the experimental details, such as the materials, experimental instruments, femtosecond and picosecond SFG, FTIR, and AFM measurements, and the details of the sample preparation are provided.

In Chapter 3, the adsorption behavior of sodium stearate molecules at fused quartz/solution interface was studied by obtaining the SFG spectra in sodium stearate aqueous solution, under Ar gas, in pure water, and under Ar gas flow again. The conformational order of the adsorbed stearic acid monolayer was very high under Ar gas flow. However, this well-ordered monolayer became an entirely disordered structure upon contact with water because no SFG signal was detected. The adsorption of stearic acid molecules on the fused quartz surface was very fast and was completed within 5 s. Furthermore, the ordered monolayer was only formed below the critical micelle concentration (CMC).

In Chapter 4, the effect of trehalose on the stability of the bilayer in dry state was studied using SFG, ATR-FTIR, and AFM. Results show that the stability of the bilayer in dry state increased when trehalose was increased. ATR-FTIR revealed that no hydrogen bond was formed between trehalose and the head group of the lipid molecule, which suggests that the stabilizing effect of hydrogen-bond formation on the bilayer in dry state was small. Thus, the volumetric effect is proposed to elucidate the stabilizing mechanism on the bilayer in dehydration state.

In Chapter 5, the stability of free-standing SDS membrane was studied using FTIR spectroscopy. The results show that the stability of the SDS membrane increased by introducing 1-dodecanol and trehalose. The effect of 1-dodecanol on stabilizing the soap membrane was attributed to the decrease in the evaporation rate of water. The effect of trehalose was caused by the insertion of trehalose into the head group of the soap molecule to reduce the packing defect, thereby stabilizing the soap membrane. The structural changes in both water and soap molecules were also studied. A more ordered water structure was present before the membrane collapsed, which

may support the ordering of the SDS molecule in the membrane.

In Chapter 6, a general conclusion is given, and a prospect for the future study is briefly presented.

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Chapter 2

Experimental

2-1 Materials

Sulfuric acid (ultrapure grade), hydrochloric acid (ultrapure grade), hydrogen peroxide (ultrapure grade), deuterium oxide (99.9%), SDS (95%), trehalose (99.9%), glucose (99%), and maltose (99.9%) were purchased from Wako Pure Chemical. Hydrofluoric acid (46%, semiconductor grade) was purchased from Morita Chemical. Sodium stearate (99%) and 1-dodecanol (>98%) were obtained from Aldrich. Deuterium stearic acid (98%) was obtained from Cambridge Isotope Laboratories. Dimyristoylphosphatidylcholine (DMPC, 99%) was purchased from NOF CORPORATION. Ultrapure water was supplied by a Milli-Q purification system (Millipore).

All chemicals were used as received without further purification.

Ultrapure argon (99.999%) and nitrogen (99.999%) were purchased from Suzuki Shokan.

Double-side polished Si(111) single-crystal wafers (100 mm diameter; 480 μm thickness; 1 Ωcm to 10 Ωcm resistivity; n-type, phosphorous-doped; p-type, boron-doped) were donated by Shin-Etsu Semiconductor.

Fused quartz prisms with hemi-cylindrical shape (IR grade, $d = 25$ mm, $l = 25$ mm) were purchased from Daico MFG.

2-2 Sample Preparations

2-2-1 Hydrophilic Fused Quartz Prism

Fused quartz prisms with hemi-cylindrical shape (IR grade, $d = 25$ mm, $l = 25$ mm, Daico MFG) were used as substrates to improve the sensitivity in SFG

measurements. The fused quartz prism was thoroughly cleaned using sulfuric acid and rinsed several times with Milli-Q water. UV-ozone cleaning treatment was performed for 1.5 h to remove the organic contaminations on the fused quartz surface. The fused quartz surface was hydrophilic after these treatments.

2-2-2 Oxidized Si(111) Single Crystal Substrate^{1,2}

Si(111) single-crystal wafers were cleaned using acetone, followed by UV-ozone cleaning. The substrates were further cleaned through sequential immersion in (1) freshly prepared sulfuric acid/30% hydrogen peroxide aqueous solution (2:1 by volume) at 80 °C for 20 min, (2) aqueous solution of 0.5% hydrofluoric acid at room temperature (RT) for 5 min, and (3) freshly prepared RCA solution (water/hydrogen peroxide/hydrochloric acid, 4:1:1 by volume) at 80 °C for 20 min. The substrates were thoroughly rinsed with Milli-Q water after immersion in each solution. After these treatments, the substrates became hydrophilic. The silicon substrates for AFM were cut into 1 cm × 1 cm pieces. The parallelogram ATR prism, which was prepared by polishing with 45 bevels, was cut into a 32 cm × 32 cm piece.³⁻⁵

2-2-3 Sodium Stearate Monolayers

Sodium stearate was dissolved in Milli-Q water at various concentrations (10, 50, 100, 200, 300, 400, and 500 μM) at RT. Then, sodium stearate and deuterated stearic acid were dissolved in Milli-Q water at 80 and 20 μM, respectively. A mixed solution was prepared by mixing 80 μM sodium stearate and 20 μM deuterated stearic acid.

1. For SFG measurements:

SFG measurements were performed using a flow cell (Figure 2.1) in the following manner: (1) sodium stearate solution was injected into the SFG cell and kept inside for 10 min; (2) the fused quartz surface was dried by flowing Ar gas; (3) the SFG cell was filled with Milli-Q water; and (4) the fused quartz surface was dried by flowing Ar gas again.

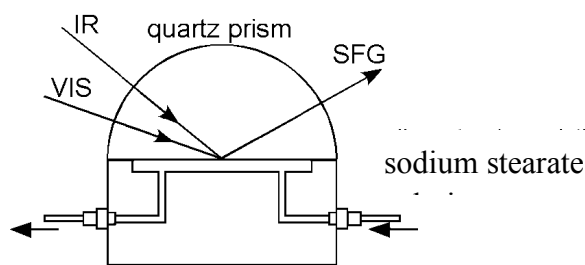


Figure 2.1 Flow cell used in the present study for sum frequency generation (SFG) spectroscopy.

2. For ATR-FTIR measurements:

The oxidized Si(111) surface was immersed in 200 μM sodium stearate solution for 10 min. Then, the surface was dried by N_2 gas flow.

2-2-4 DMPC Bilayers

DMPC vesicles (0.1 mg mL^{-1}) were prepared by sonicating as-delivered DMPC in D_2O , followed by extrusion through a 100 nm polycarbonate filter for over five times to obtain uniform DMPC vesicles. The DMPC suspension was kept above the transition temperature from gel phase to liquid crystal phase (T_c , 24 $^\circ\text{C}$ for DMPC) during the preparation processes.^{6,7}

1. For SFG measurements:

The SFG measurements were performed using a flow cell (Figure 2.1). The prepared DMPC vesicle suspension was injected into the SFG cell, kept inside at above 35 $^\circ\text{C}$ for 60 min, and washed by changing the D_2O solution five times. The DMPC bilayer was formed on the fused quartz surface. All processes were kept in aqueous solution without exposing the sample surface to air.

2. For ATR-FTIR and AFM measurements:

The oxidized Si(111) surface was incubated in 0.1 mg mL^{-1} of 100 nm filtered DMPC vesicle suspension at above 35 $^\circ\text{C}$ for 60 min and washed by changing the D_2O solution five times. The DMPC bilayer was formed on the oxidized Si(111) surface. All processes were kept in aqueous solution without exposing the sample surface to air.

2-2-5 SDS Membranes

The SDS membrane was prepared as follows: (1) 0.65 g (0.11 M) of SDS was dissolved in 20 mL of distilled water and then boiled until the solution was nearly a dry paste; (2) 20 mL of water was introduced to the cooled paste, and the solution was boiled until a dry paste was obtained again; (3) this process was repeated six times; (4) 20 mL of water was introduced, and a colloidal solution was obtained; and (5) 0.22 g (0.04 M) of SDS was introduced to the solution.⁸ The SDS membrane obtained was highly stable. To clarify the reason behind the increased stability of the SDS membrane, the following samples were prepared.

SDS (0.11 M) was dissolved in Milli-Q water and mixed with various concentrations of 1-dodecanol (0.002, 0.004, 0.006, 0.008, 0.01, and 0.015 M).

1. The time from the beginning of membrane formation to its ultimate collapse was measured to examine the stability of the SDS membrane. Setup (a) of this experiment and the preparation procedure of SDS membrane are shown in Figure 2.2. Setup (a) was immersed in the SDS solution, and the SDS membrane was formed after removal from the solution.
2. For transmission FTIR measurement, the first spectrum of each sample was obtained when the SDS membrane was formed, whereas the last spectrum of each sample was obtained when the SDS membrane collapsed. Four or 16 scans were integrated for each spectrum.

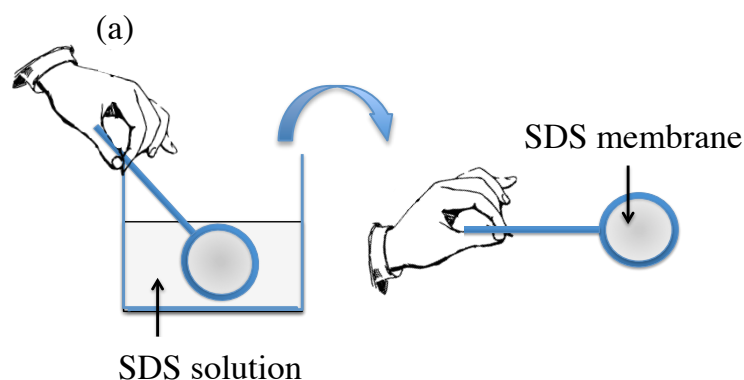


Figure 2.2 Preparation of SDS membrane.

2-3 Ar Gas Cleaning

Given that SFG spectroscopy is a highly surface-sensitive technique, the Ar gas flow used in the SFG experiment required cleaning of any contamination. To ensure that the Ar gas flow was cleaned, SFG measurement of the cleaned fused quartz surface by Ar gas flow was performed, as shown in Figure 2.3.

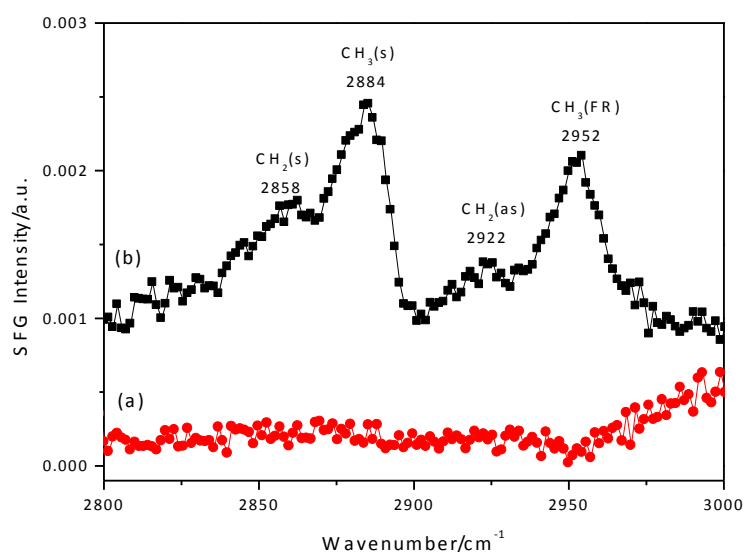


Figure 2.3 SFG spectra of (a) cleaned quartz surface and (b) cleaned quartz surface after Ar gas flow for 1 h. The polarization combination used was s (SFG), s (visible), and p (IR).

No CH peaks were observed in the C–H stretching region, as shown in Figure 2.3 (a), which suggests that the quartz surface was very clean and without any contamination. After Ar gas flowed for 1 h, two strong peaks at 2884 and 2952 cm^{-1} and two weak peaks at 2858 and 2922 cm^{-1} were observed. The two strong peaks were attributed to the symmetric C–H stretching ($\text{CH}_3(\text{s})$) and Fermi resonance ($\text{CH}_3(\text{FR})$) of the methyl group, respectively, whereas the two weak peaks were attributed to the symmetric C–H stretching ($\text{CH}_2(\text{s})$) and asymmetric C–H stretching of the methylene group ($\text{CH}_2(\text{as})$), respectively, as shown in Figure 2.3 (b).^{9,10} Strong SFG peaks were observed in the C–H stretching region at the fused quartz/gas interface because of the flowing Ar gas. This result indicates that the Ar gas was contaminated. Given that the Ar gas contained certain contaminants, it was further

purified by passing through a liquid N₂ trap. Figure 2.4 shows the SFG spectra of the quartz surface cleaned by Ar gas flow, which passed through the liquid N₂ trap.

No CH peaks were detected at the fused quartz/gas interface after Ar gas flowed from 4 min to 60 min, which shows that the contaminants in the Ar gas were removed by the liquid N₂ trap. The further purified Ar gas was used in the following experiments.

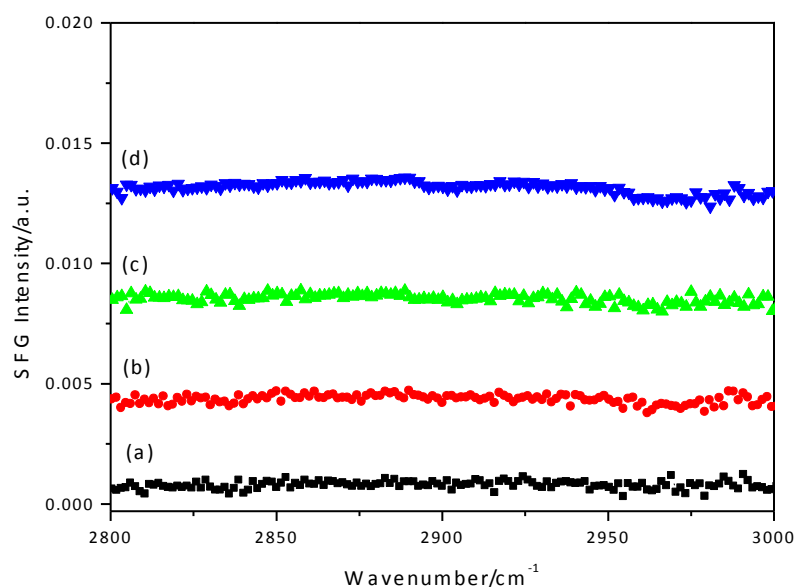


Figure 2.4 SFG spectra of cleaned quartz surface after Ar gas flowed for (a) 4, (b) 10, (c) 20, and (d) 60 min. Ar gas was further purified by passing through a liquid N₂ trap. The polarization combination used was ssp.

2-4 Measurements

2-4-1 SFG Measurement Systems

2-4-1-1 Femtoseconds SFG Measurement System

Figure 2.5 shows a schematic of the femtosecond laser system used in this study. This femtosecond SFG system has been described in detail elsewhere.^{11,12} In brief, a Ti:sapphire oscillator (Spectra-Physics, MaiTai) was used as a seeding laser, which included two diode lasers, an Nd:YVO₄ laser, an LBO crystal, and a Ti:sapphire laser. Finally, a pulse with a duration of 100 fs and centered at 800 nm was created. A regenerative amplifier (Spectra-Physics, Spitfire Pro) pumped by an Nd:YLF laser

(Spectra-Physics, Empower) was used to amplify the seed beams without changing the pulse duration. The typical output energy of the Spitfire Pro was approximately 2.1 mJ per pulse. Half of the output from the amplifier was sent to a homemade spectral shaper to generate a narrowband ps pulse (ca. 10 cm^{-1}) at 800 nm (30 $\mu\text{J}/\text{pulse}$) for improving the spectral resolution. The remaining output was used to pump an optical parametric amplifier/difference frequency generation (OPA/DFG) system for IR beams (Light Conversion, TOPAS). Frequency-tunable IR pulses were generated from $2.5\text{ }\mu\text{m}$ to $10.0\text{ }\mu\text{m}$ (6 $\mu\text{J}/\text{pulse}$ at $3\text{ }\mu\text{m}$) at a spectral width of 200 cm^{-1} . The narrowband ps visible pulses and the broadband fs IR pulses overlapped on the sample surface at incident angles of 70° and 50° , respectively. SFG spectra were obtained using two charge-coupled devices (CCDs) attached to a spectrograph or polychromator (Solar-TII, MS3504). One CCD was for the visible region (Andor Technology, DU420-BV), whereas the other CCD was for the UV region (Andor Technology, DU420A-BU2).

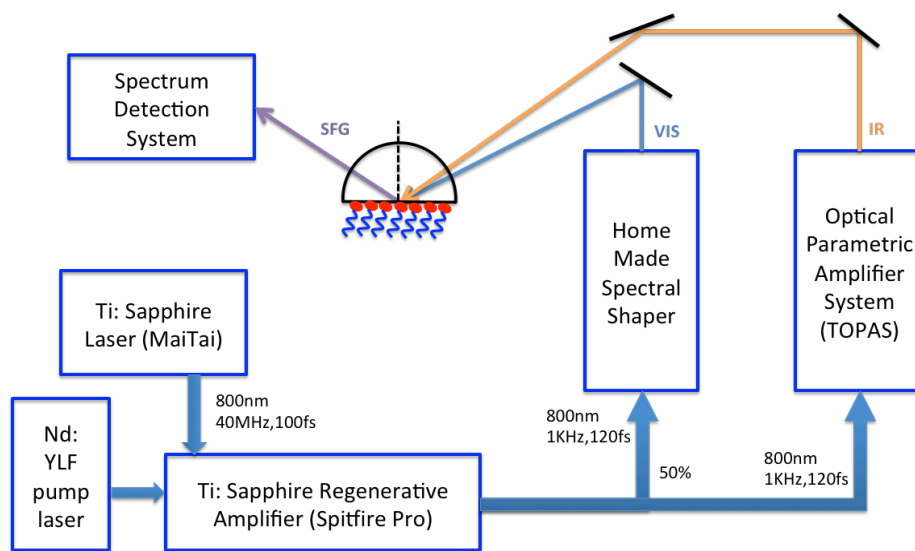


Figure 2.5 Schematic view of femtosecond SFG setup.

SFG measurements were carried out using a flow cell, as shown in Figure 2.1. To enhance sensitivity, hemicylindrical fused quartz prisms were used for SFG measurements. The flat surface of the fused quartz prism was in contact with the

sample solution. The IR and visible beams were incident to the side of the fused quartz prism and were overlapped at the fused quartz/sample solution surface. The sample solution, i.e., sodium stearate, was injected into the SFG cell and was changed by water or Ar gas.

2-4-1-2 Picoseconds SFG Measurement System^{13,14}

Figure 2.6 shows a schematic of the picosecond SFG setup used in this study. A passive mode-locked Nd:YAG laser system (model PL2143B, EKSPLA) generated 30 ps pulses at a repetition rate of 20 Hz. The output of the narrowband ps pulse (ca. 10 cm^{-1}) at 1064 nm (8 mJ/pulse) was sent to the harmonic unit. Half of the output was converted to 532 nm, directed to a sample through a time delay, and used as the incident visible light for SFG measurement. The power of the incident visible light can be adjusted by combining a half-wave plate and a Gran–Taylor prism, and the polarization can be changed by an additional half-wave plate. The residual output was passed through the OPG/OPA system to generate a tunable IR output (ca. 6 cm^{-1}) from $2.3\text{ }\mu\text{m}$ to $8.5\text{ }\mu\text{m}$ by DFG. The power of the incident IR light can be roughly adjusted by tuning the total power of the laser and the ratio of the harmonic generator. The polarization of the IR beam can be changed by exchanging the mirror sets, which contains three and four mirrors.

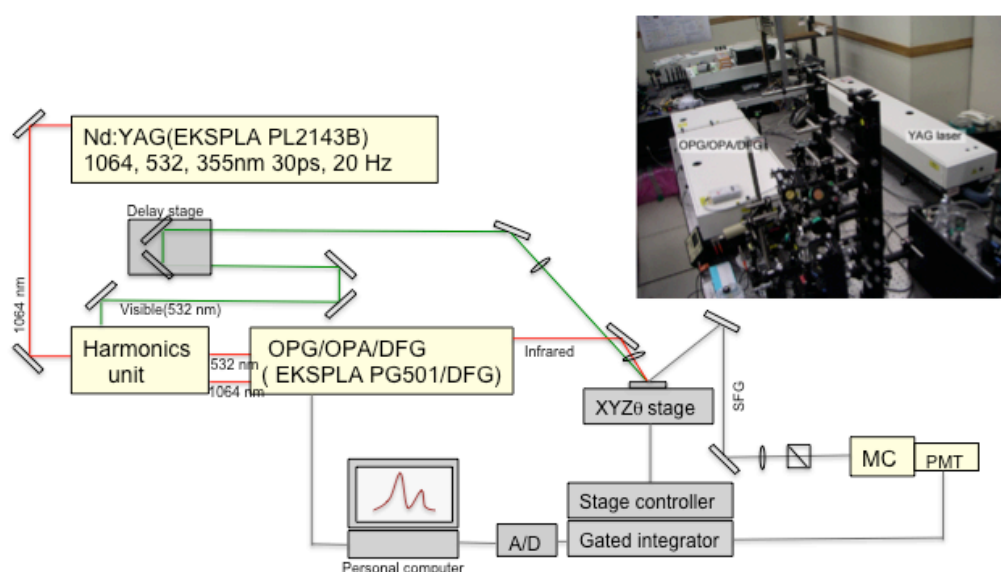


Figure 2.6 Schematic view of picosecond SFG setup.

The visible laser and tunable IR laser were incident and overlapped at the sample surface at 70° and 50°, respectively. A BK7 lens ($f = 600$) and a ZnSe lens ($f = 50$) were used to focus the visible and IR beams, respectively. The position of the ZnSe lens was controlled by an XY stage for spatially overlapping the two incident beams. The beam spot of the visible and IR lights had an elliptical shape with dimensions of 2.5×0.9 and $0.3 \times 0.2 \text{ mm}^2$, respectively. The SFG signal was collected through a monochromator (Oriel instruments, MS-257) by a photomultiplier (Hamamatsu R630-10 or R3896) and a gated electronic system (Stanford Research System). When the 532 nm visible light was used, two holographic Super Notch filters (Kaiser Optical System, INC, HSPF-532-1.0) were placed in front of the monochromator to reduce scattered visible light.

The flow cell (Figure 2.1) was used in this picosecond SFG system.

2-4-2 Fourier Transform Infrared (FTIR) Measurements

2-4-2-1 ATR-FTIR Measurements

ATR-FTIR measurements were carried out using a Bio-RAD FTS-30 spectrometer equipped with an HgCdTe (MCT) detector cooled with liquid nitrogen. Figure 2.7 shows the experimental setup of ATR-FTIR measurements. N_2 gas was continuously provided to the ATR-FTIR chamber. All spectra were obtained in p-polarization by integrating 128 interferograms at a resolution of 2 cm^{-1} . A spectrum of an oxidized Si(111) surface was used as a reference.

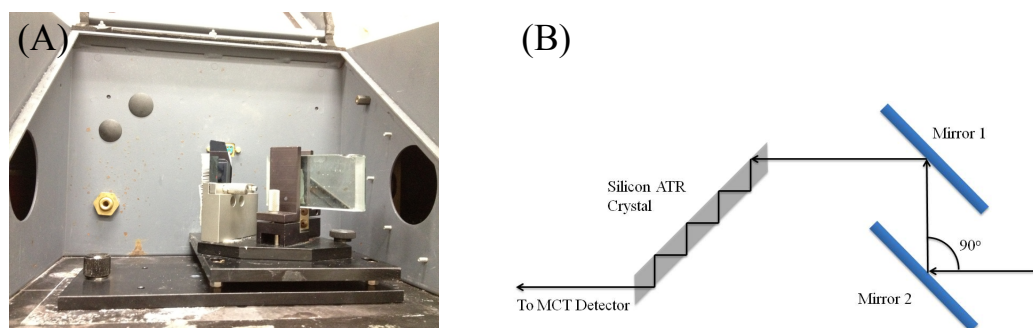


Figure 2.7 (A) Experimental setup and (B) configuration of ATR-FTIR setup.

2-4-2-2 Transmission FTIR Spectroscopy

Transmission FTIR measurements were carried out using a FT/IR-6200 spectrometer (JASCO) equipped with a DLATGS detector. Four or 16 interferograms scanned at a rate of 1 kHz were integrated to obtain a single beam spectrum with a resolution of 4 cm^{-1} . The experimental setup for SDS membrane measurement is shown in Figure 2.8. The electric field of the IR beam is perpendicular to the stage.

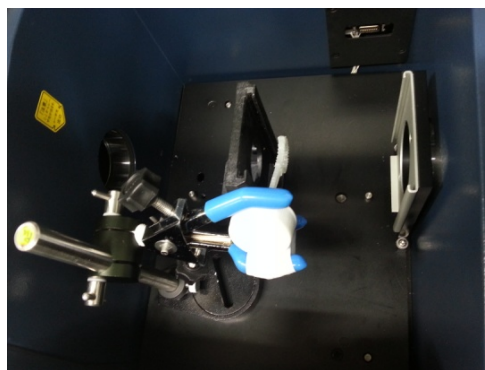


Figure 2.8 Experimental setup of transmission FTIR measurements.

2-4-3 AFM Measurements

AFM (Agilent Technologies 5500, USA) in tapping mode was used for AFM observations. The n^+ -silicon cantilever (NANOSENSORS, PPP-FM-50) had a force constant of 0.5 N/m to 9.5 N/m, a resonance frequency of 45 KHz to 115 KHz, and a tip with a thickness of $3.0 \pm 1\text{ }\mu\text{m}$, a length of $225 \pm 10\text{ }\mu\text{m}$, and a width of $28 \pm 7.5\text{ }\mu\text{m}$. Imaging was conducted at a low force to maintain the integrity of the lipid bilayer. All measurements were carried out at RT ($24\text{ }^\circ\text{C}$).

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Chapter 3

Structure of Adsorbed Stearic Acid on Fused Quartz Surface

3-1 Introduction

The monolayers are excellent model systems for membrane biophysics, since a biological membrane can be considered as two weakly coupled monolayers.¹ The substrate supported lipid monolayer represents an intermediate stage in the fabrication of functionalized model membrane systems and therefore needs to be properly understood.^{2,3} Recently, the adsorption characteristics of wide variety of monolayer at solid/liquid interfaces have been investigated by various techniques such as small angle neutron scattering (SANS),⁴ total internal reflection Raman spectroscopy,⁵ and Fourier transform infrared spectroscopy.⁶ However, the interfacial structure of adsorbed molecule is still not well understood due to a lack of effective experimental probe for interfacial study in molecular level. Many studies has been carried out by using Langmuir-Blodgett (LB) and Sum frequency generation (SFG) vibrational spectroscopy to study a model molecular structure in air and in aqueous solutions.^{7,8} However, it is going to simulate a more realistic molecular adsorption process through in situ SFG measurements in the present work. SFG vibration spectroscopy is proved to be one of the most effective methods to study the molecular structure at solid/liquid interfaces.⁹⁻¹² Furthermore, SFG spectrum of alkyl chain is sensitive to the conformational order. If highly ordered monolayer with all-trans conformation such as octadecyltrichlorosilane (OTS) monolayer is formed, only CH stretching peaks of CH₃ group should be observed as CH₂ group is in centrosymmetry structure and is

SFG inactive.¹² CH stretching peaks of CH₂ group increase if gauche defects are introduced.

Here, the adsorption behavior of sodium stearate, which is one of the most common saturated fatty acid salts at solid/liquid interface were studied by measuring the SFG spectra in sodium stearate aqueous solution, followed by under Ar gas flow, in pure water, and under Ar gas flow again. Dependencies of adsorption time and stearate concentration were also investigated. Importance of the critical micelle concentration (CMC) was found. The structure of sodium stearate was shown in Figure 3.1.

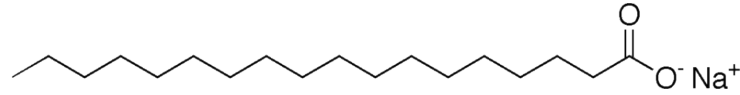


Figure 3.1 Structure of sodium stearate molecule

3-2 Results and Discussion

3-2-1 Environment Dependent Structure of Stearic Acid on a Fused Quartz Surface

SFG measurements were sequentially carried out as follows: (1) in sodium stearate solution, (2) under Ar gas flow, (3) in pure water, and (4) under Ar gas flow. Here, a polarization combination of s-polarized SFG, s-polarized visible, and p-polarized IR (ssp-polarization) was used. SFG spectra were normalized by the SFG spectrum of gold thin film deposited on a fused quartz prism and fitted by using the following equation^{13,14}

$$I_{SFG}(\omega) \propto \left| \sum_q \chi_{R,q}^{(2)} + \chi_{NR}^{(2)} \right|^2 = \left| \sum_q \frac{A_q}{\omega - \omega_q + i\Gamma_q} \exp(i\theta_q) + \chi_{NR}^{(2)} \right|^2 \quad (3-1)$$

Where ω is the infrared frequency, $\chi_{NR}^{(2)}$ is the non-resonant contribution to the surface non-linear susceptibility, and ω_q , A_q , θ_q , and Γ_q are the resonant frequency,

transition amplitude, phase difference between resonant and non-resonant term and homogeneous width of the q th vibrational mode, respectively.

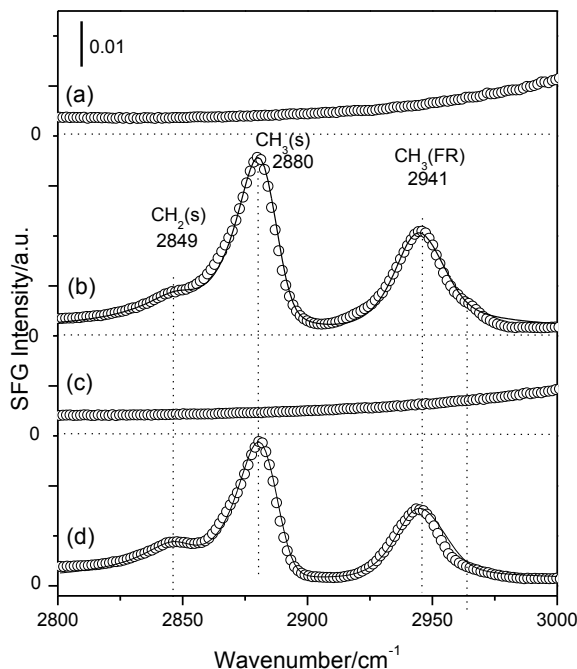


Figure 3.2 SFG spectra (circle symbol) of the fused quartz surface and the fitting results (solid line) in CH stretching region ($2800\text{--}3000\text{ cm}^{-1}$) obtained (a) in $200\text{ }\mu\text{M}$ sodium stearate solution, (b) in Ar gas after (a), (c) in pure water after (b), and (d) in Ar gas after (c).

Figure 3.2 (a) shows SFG spectrum of the fused quartz surface in contact with $200\text{ }\mu\text{M}$ sodium stearate solution. The peak assignment and fitting parameters of Figure 3.2 were listed in Table 3.2. No SFG signal was detected, suggesting no adsorption of stearate molecules or adsorbed molecular layer with centrosymmetric structures such as bilayer¹⁵ and totally disordered structures.

After the fused quartz surface was in contact with sodium stearate solution for 10 min, sodium stearate solution was replaced by Ar gas and SFG measurements were carried out under Ar gas flow. Two strong peaks at 2880 and 2943 cm^{-1} and one weak peak at 2844 cm^{-1} , which can be attributed to the symmetric CH stretching ($\text{CH}_3(\text{s})$) and the Fermi resonance ($\text{CH}_3(\text{FR})$) of methyl group and symmetric CH stretching of methylene group ($\text{CH}_2(\text{s})$), respectively, were observed as shown in Figure 3.2 (b).

Strong signal related to methyl group and weak methylene signal show the formation of highly ordered layer of stearate species with only few gauche defects in the alkyl chain.¹⁶

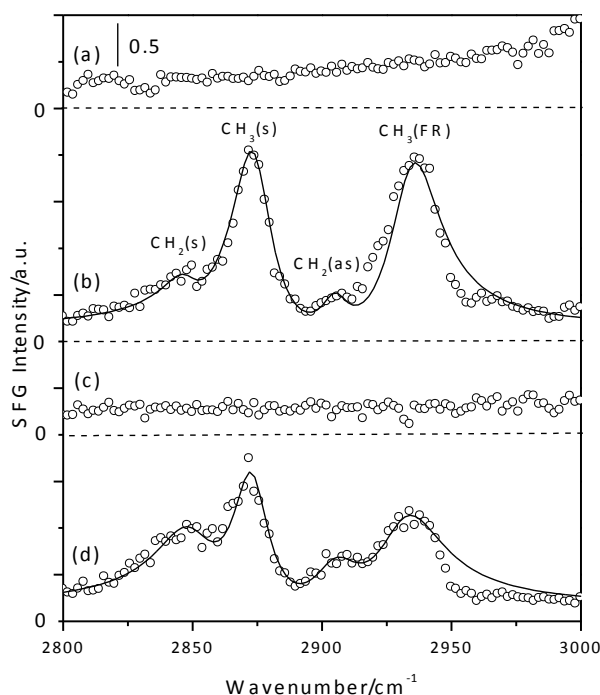


Figure 3.3 SFG spectra of the fused quartz surface in CH stretching region ($2800\text{--}3000\text{ cm}^{-1}$) obtained (a) in $80\text{ }\mu\text{M}$ sodium stearate and $20\text{ }\mu\text{M}$ deuterated stearic acid solution, (b) in Ar gas after (a), (c) in pure water after (b), and (d) in Ar gas after (c).

To see if bilayer or disordered monolayer was present in solution, SFG measurements were carried out utilizing deuterated stearic acid. No SFG peak was observed in a solution containing $80\text{ }\mu\text{M}$ sodium stearate and $20\text{ }\mu\text{M}$ deuterated stearic acid as shown in the Figure 3.3 (a), although the peaks were observed after being dried under N_2 stream (Figure 3.3 (b)). If bilayer was formed, inversion symmetry was broken in this case since the bilayer should be consisting of both H- and D-stearate species and, therefore, SFG peak should be observed in solution. ATR-FTIR measurements of this sample after being dried in N_2 stream confirmed the presence of 80% H- and 20% D- stearate species. Thus, it was concluded that not bilayer but disordered monolayer was present on the surface in the stearate solution.

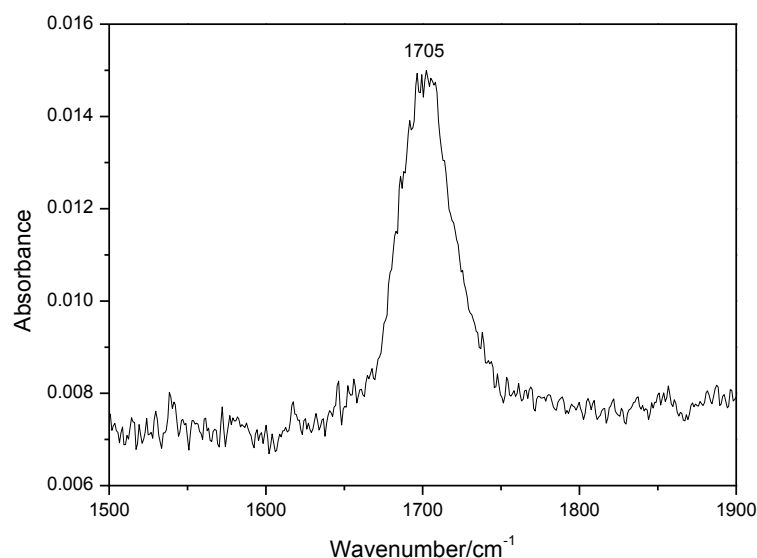


Figure 3.4 ATR-FTIR spectrum in C=O stretching region of oxidized Si(111) obtained in N₂ gas after surface was in contact with 200 μ M sodium stearate solution.

To see which species is adsorbed on the fused quartz surface, ATR-FTIR measurement of oxidized Si(111) surface after it was immersed in 200 μ M sodium stearate solution and then dried by N₂ gas were carried out. Because of strong absorption by SiO₂/Si substrate below 1500 cm⁻¹, reasonable spectrum was not obtained below 1500 cm⁻¹ and only one peak was observed in C=O stretching region above 1500 cm⁻¹ at 1705 cm⁻¹, which is attributed to be due to C=O stretching of COOH group¹⁷ as shown in Figure 3.4, showing the adsorption of stearic acid on the surface. In the IR spectrum obtained in 300 μ M sodium stearate solution, peaks due to COO⁻ and COOH specie were observed at around 1525 cm⁻¹ and 1725 cm⁻¹, respectively. If COO⁻ species were present on the surface, the sign of absorption even above 1500 cm⁻¹ should be able to see, although it cannot obtain a reasonable spectrum below 1500 cm⁻¹. It is also reasonable to think that stearic acid not stearate adsorbs on negatively charged quartz surface. Thus, it was concluded that stearic acid not stearate was adsorbed on the surface.

No SFG signal was detected when the fused quartz surface was made in contact with water as shown in Figure 3.2 (c), showing bilayer formation, totally disordered

structure, or desorption of stearate monolayer, and SFG signal reappeared again but smaller CH_3 related peaks and larger CH_2 related peak compared with the results in Figure 3.2 (b) after water was replaced by Ar as shown in Figure 3.2 (d). These results showed that stearic acid molecule was still present on the surface even in pure water. Figure 3.3 (c) shows SFG spectrum of the mixed layer of H- and D- stearic acid in water after the measurement shown in Figure 3.3 (b). Again no peak was observed, although the peaks were observed after being dried under N_2 stream (Figure 3.3 (d)). These results show the presence of disordered layer not bilayer in water. Although ordered monolayer was formed again in Ar after being in contact with water, more gauche defects were introduced. Disorder of alkyl chain induced by liquid water was also observed in the previous study on alkylated polyvinylpyridine brushes, which showed no CH stretching peaks when alkyl brushes were in contact with liquid water.¹⁰ Thus, it was concluded that the stearic acid monolayer was in totally random structure when they were in contact with sodium stearate solution and liquid water. It is interesting to note that atomic force microscopy (AFM) study showed that highly ordered stearic acid monolayer prepared on SiO surface by Langmuir-Blodgett (LB) technique was nearly free from defects over large surface areas in ambient air and converted to the bilayer in water.¹⁸ The difference may be caused by the difference in the rigidity of the monolayer prepared by LB method and that formed in solution.

A model used to describe the interfacial molecule structure on the fused quartz surface depending on different environment was shown in Figure 3.5.

A disordered molecule layer adsorbed on the fused quartz surface in solution, while the molecule layer became well ordered in Ar gas.

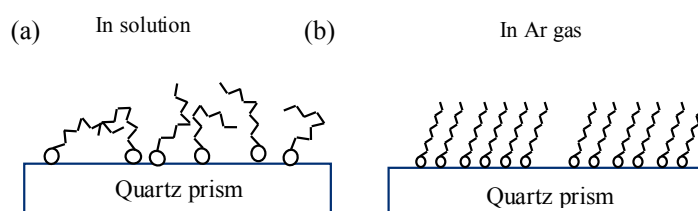


Figure 3.5 The model of molecule structure on the fused quartz surface of (a) in solution and (b) in Ar gas.

3-2-2 Adsorption Time Dependence

Figure 3.6 shows SFG spectra and their fitting results in the CH stretching region (2800-3000 cm^{-1}) of the fused quartz surface obtained under Ar gas flow after the surface was in contact with 200 μM sodium stearate solution for 0, 5, 30, 60, 180, 600 and 1200 s. Two strong peaks related to CH_3 group and one small peak related to CH_2 group were observed in the CH stretching region even at the fused quartz surface which had been in contact with sodium stearate solution only for 5 s.

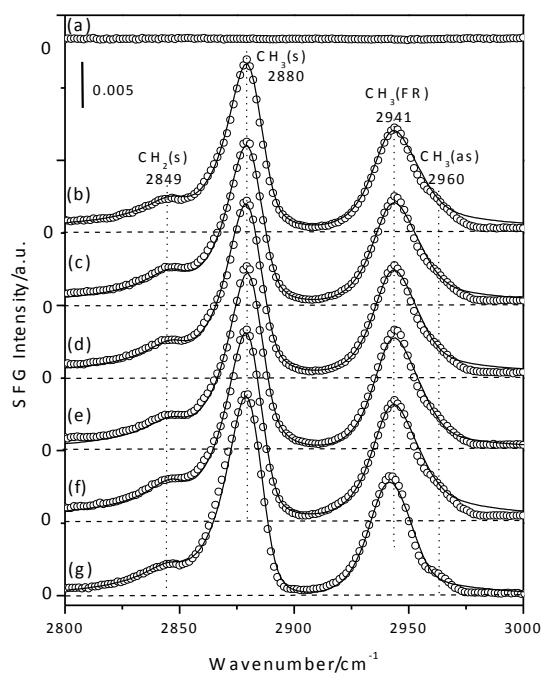


Figure 3.6 SFG spectra (circle symbol) and the fitting results (solid line) of the fused quartz surface obtained in Ar gas after the surface was in contact with 200 μM sodium stearate solution for (a) 0, (b) 5, (c) 30, (d) 60, (e) 180, (f) 600, and (g) 1200 s.

The peak assignment and fitting parameters of Figure 3.6 were listed in Table 3.1. The parameters in Table 3.1 were as follows, amplitude (A), vibrational frequency (ω) and dumping constant (Γ), which were obtained from the fitting of SFG spectra using Eq. (3-1).

Table 3.1 Peak assignment and fitting parameters for the SFG spectra in Figure 3.6.

Immersion time/s		5	30	60	180	600	1200
CH ₂ (s)	<i>A</i>	0.23	0.26	0.23	0.22	0.23	0.2
	ω	2849	2849	2849	2849	2849	2848
	Γ	11.2	11	11	11	10	11
CH ₃ (s)	<i>A</i>	1.68	1.65	1.71	1.75	1.8	1.89
	ω	2880	2880	2880	2880	2880	2880
	Γ	11	11	11	11	11	11
CH ₃ (FR)	<i>A</i>	1.25	1.28	1.28	1.3	1.36	1.38
	ω	2941	2941	2941	2941	2941	2939
	Γ	13	13	13	13	13	13

Figure 3.7 shows time dependencies of the amplitude of the peak at 2880 cm⁻¹ (CH₃(s)) obtained by fitting the spectrum using Eq. (3-1) and the ratio between the amplitudes of the peak at (CH₃(s)) and that at 2849 cm⁻¹ (CH₂(s)). It is suggest that highly ordered stearic acid monolayer was formed very quickly on the fused quartz surface and confirm that the spectrum shown in Figure 3.2 (b) obtained after 10 min contact with the sodium stearate solution is of final structure.

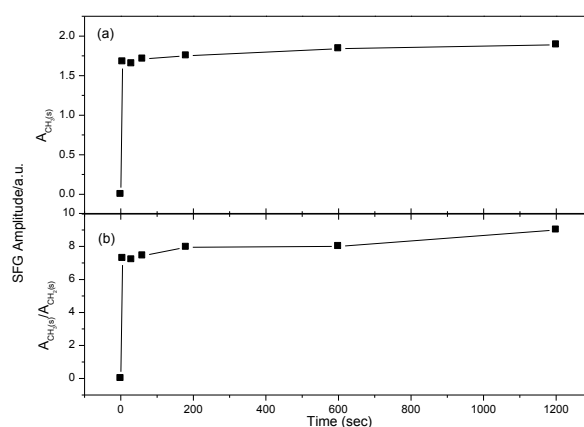


Figure 3.7 The time dependencies of (a) the amplitude of the methyl peak (CH₃(s)) and (b) the amplitude ratio of methyl to methylene peak (CH₃(s)/CH₂(s)).

3-2-3 Concentration Dependence

Concentration dependent behavior of sodium stearate adsorption on the fused quartz surface was studied by measuring SFG spectra under Ar flow after the fused quartz surface was in contact with stearate solutions of various concentrations (10, 50, 100, 200, 300, 400 and 500 μM) for 10 min. It has reported the kinetics of self-assembly process is proportional to the concentration.¹⁹ Since the full coverage was reached within 5 s in 200 μM sodium stearate, it can expect the adsorption in 10 μM solution should complete within 100 s. Thus, 10 min is long enough to achieve final structure even in the solution of lowest concentration employed in the present study.

Figure 3.8 (a) shows the SFG spectra and their fitting results in the CH stretching region (2800-3000 cm^{-1}). No SFG signal was detected under Ar gas flow after immersion in 10 μM solution (curve I), suggesting very low coverage. Two strong peaks at 2880 and 2943 cm^{-1} corresponding to $\text{CH}_3(\text{s})$ and $\text{CH}_3(\text{FR})$ and one small peak at 2844 cm^{-1} corresponding to $\text{CH}_2(\text{s})$ were observed under Ar gas flow after the fused quartz surface was in contact with stearate solutions of 50 - 400 μM (curves II to VI), indicating the presence of well ordered stearic acid monolayer on the fused quartz surface. However, no SFG signal was detected when the concentration was 500 μM (curve VII).

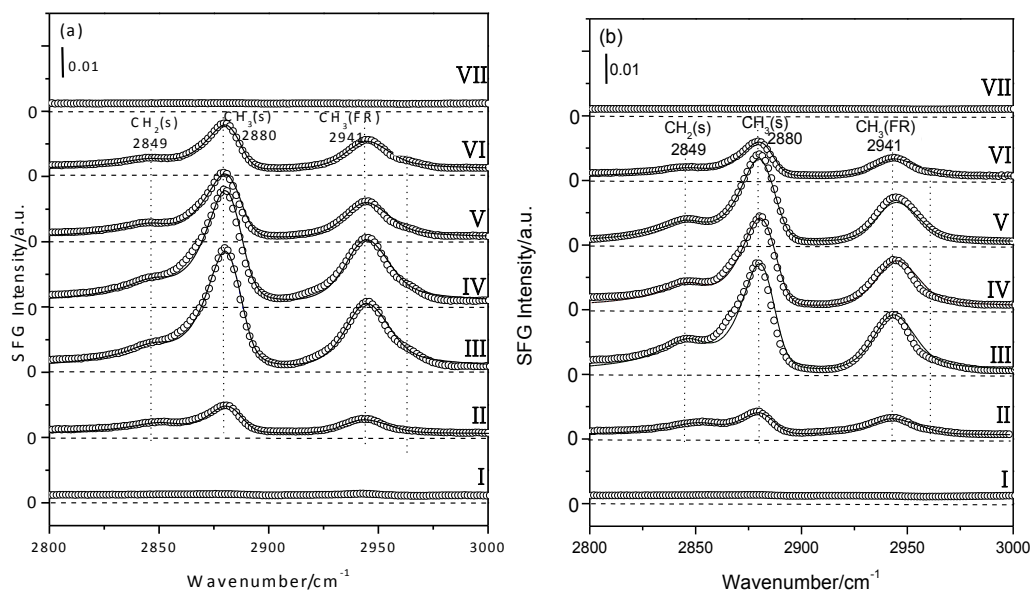


Figure 3.8 SFG spectra (circle symbol) and the fitting results (solid line) in CH stretching region (2800-3000 cm^{-1}) of the fused quartz surface obtained under Ar gas flow (a) after the surface was in contact with sodium stearate solutions of 10 (I), 50 (II), 100 (III), 200 (IV), 300 (V), 400 (VI), and 500 μM (VII) for 10 min and (b) after the surface was in contact with water after (a).

The peak assignment and fitting parameters of Figure 3.8 were listed in Table 3.2.

Table 3.2 Peak assignment and fitting parameters for the SFG spectra in Figure 3.8.

Concentration/ μM		100(a)	100(b)	200(a)	200(b)	300(a)	300(b)	400(a)	400(b)
$\text{CH}_2(\text{s})$	A	0.43	0.79	0.35	0.6	0.4	0.57	0.35	0.37
	ω	2850	2849	2847	2849	2848	2848	2848	2849
	Γ	14	14	15	14	14	14	14	14
$\text{CH}_3(\text{s})$	A	2.49	2.44	1.98	2.4	2.3	2.2	1.86	1.64
	ω	2881	2880	2878	2880	2881	2880	2881	2881
	Γ	12	12	11.4	13	13	13	12	12
$\text{CH}_3(\text{FR})$	A	1.92	1.82	1.46	1.65	1.44	1.5	1.3	0.93
	ω	2942	2940	2939	2942	2942	2941	2942	2940
	Γ	14	14	12	14	14	14	14	13

The parameters in Table 3.2 were as follows, amplitude (A), vibrational frequency (ω) and dumping constant (Γ), which were obtained from the fitting of SFG spectra using Eq. (3-1). (a) and (b) in Table 3.8 were the SFG spectrum measured after the surface was in contact with sodium stearate solutions of 100, 200, 300 and 400 μM for 10 min and after the surface was in contact with water after (a).

The concentration dependencies of the amplitude of the $\text{CH}_3(\text{s})$ peak obtained by fitting to Eq. (3-1) and the ratio between the amplitudes of $\text{CH}_3(\text{s})$ and $\text{CH}_2(\text{s})$ peaks are summarized in Figure 3.8. Both the amplitude of $\text{CH}_3(\text{s})$ peak (open circle in Figure 3.8 (a)) and the $\text{CH}_3(\text{s})$ peak to $\text{CH}_2(\text{s})$ peak ratio (open circle in Figure 3.8 (b)) increased when the concentration of sodium stearate was increased from 50 to 100 μM , suggesting an increased coverage and the conformational order of the alkyl chains. The amplitude of $\text{CH}_3(\text{s})$ peak and the $\text{CH}_3(\text{s})$ peak to $\text{CH}_2(\text{s})$ peak ratio were almost constant when the concentration was higher than 100 μM , indicating the coverage and the conformational order of the monolayer were constant and remained to be high. However, when the concentration of sodium stearate increased further to 400 μM , the amplitude of $\text{CH}_3(\text{s})$ peak and the $\text{CH}_3(\text{s})$ peak to $\text{CH}_2(\text{s})$ peak ratio decreased, showing both the coverage and conformational order decreased. When the concentration of sodium stearate increased further to 500 μM , no SFG peak was detected as mentioned before but the peaks corresponding to C-H and C=O were observed in ATR-FTIR spectrum, although they were very weak, showing the adsorption of fully disordered stearic acid layer. The decrease of the adsorbed amount and conformational order can be explained by considering the presence of micelle in solution above CMC, 400 μM at room temperature.^{20,21} If micelles are present in the solution, they adsorb on the surface competing with monomer. When the surface was dried under Ar, micelles should be ruptured and disordered layer should be formed. At 400 μM , concentration of monomer is high compared to that of micelle and, therefore, the coverage of monomer is high enough to form monolayer with relatively high conformational order, although the intensity decreased slightly. At 500 μM , more micelles in solution and, therefore, the coverage of monomer was lower and totally disordered layer was formed.

Figure 3.8 (b) shows the SFG spectra and their fitting results in the CH stretching region ($2800\text{--}3000\text{ cm}^{-1}$) of the fused quartz surface obtained under Ar gas flow after the surface being contacted with sodium stearate solution of various concentrations followed by the contact with water. Three peaks were still observed at 2844 , 2880 and 2943 cm^{-1} as shown in curves II to VI but slight increase and decrease of the $\text{CH}_2(\text{s})$ peak and the CH_3 peaks, respectively, were observed. These results suggest that although ordered stearic acid monolayer was formed on the fused quartz surface again, more gauche defects were introduced and small amount of stearic acid molecules were dissolved by immersing the surface in water.

Figure 3.9 shows the concentration dependence of the amplitude of the $\text{CH}_3(\text{s})$ peak obtained by fitting the spectrum using Eq. (3-1) (close square in Figure 3.9 (a)) and the ratio between the amplitude of $\text{CH}_3(\text{s})/\text{CH}_2(\text{s})$ (close square in Figure 3.9 (b)) after stearic acid monolayer was contacted with liquid water and dried in Ar gas flow. The amplitude of $\text{CH}_3(\text{s})$ peak slightly decreased after contact with liquid water, suggesting the dissolution of a small amount of stearic acid molecules. The $\text{CH}_3(\text{s})$ peak to $\text{CH}_2(\text{s})$ peak ratio was affected more significantly by the immersion in water, suggesting more gauche defects were introduced in the monolayer.

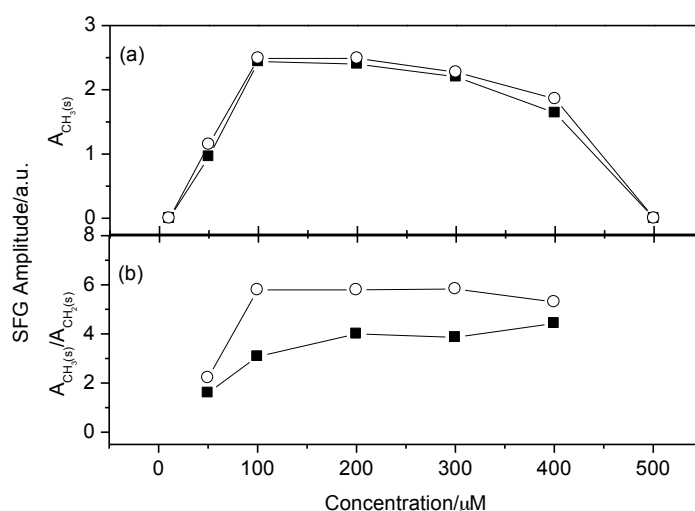


Figure 3.9 The concentration dependencies of (a) the amplitude of the methyl peak ($\text{CH}_3(\text{s})$) and (b) the amplitude ratio of methyl to methylene peak ($\text{CH}_3(\text{s})/\text{CH}_2(\text{s})$). Open circle: data obtained from Figure 7 (a). Close square: data obtained from Figure 7 (b).

The model used to explain the structure of sodium stearate molecule on the fused quartz surface depending on the concentration was shown in Figure 3.10.

When the concentration of sodium stearate was very low (10 μM), disordered molecule layer adsorbed on the surface due to very low coverage. When the concentration of sodium stearate was increase to 100 μM , the well ordered monolayer adsorbed on the surface. The ordered monolayer and small amount of micelle may adsorb on the surface when the concentration was close to CMC of sodium stearate. However, a totally disordered molecule layer adsorbed on the surface when the concentration was higher than CMC.

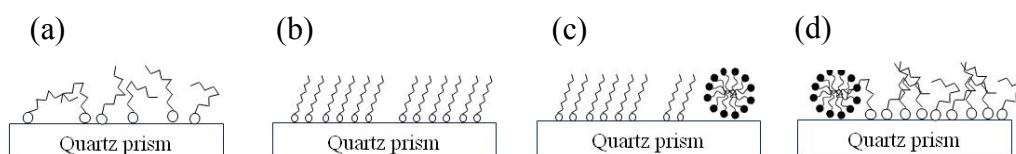


Figure 3.10 The structure of sodium stearate molecule on the fused quartz surface depending on the concentration of (a) 10, (b) 100-300, (c) 400, and (d) 500 μM .

3-4 Conclusions

In conclusion, it has investigated the structure of adsorbed molecular layer on fused quartz surface by SFG spectroscopy sequentially in sodium stearate solution, dry Ar, pure water, and dry Ar. It was revealed that totally disordered layer was formed on the surface in the stearate solution and it was converted to the monolayer of high conformational order after the solution was replaced by Ar. The monolayer became totally disordered in water and the monolayer of high conformational order but with increased gauche defects was formed after being dried by Ar gas flow. Time dependent SFG measurements revealed that the adsorption of stearic acid molecules on the fused quartz surface was very fast and almost completed within 5 s in 100 μM solution. The amount and conformational order of the molecular layer on the fused quartz surface increased as the concentration of stearate in the solution increased and

were almost constant when the concentration was higher than 100 μM . SFG intensity slightly decreased at CMC, 400 μM . No SFG signal was detected when the concentration became higher than CMC because the molecular layer was totally disordered.

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Chapter 4

Effects of Saccharides on the Stability of Lipid Bilayer in Dry State

4-1 Introduction

A biological membrane is an enclosing or separating membrane that acts as a selective barrier within or around a cell. Such a membrane consists of a lipid bilayer with embedded proteins that constitute close to 50% of membrane content.¹ However, this membrane can be damaged by dehydration, which can change the physical state of membrane lipids. Removal of water from the headgroup region of phospholipid bilayers increases headgroup packing and forces acyl chains to group together.^{2,3} Thus, the lipid membranes transform from liquid crystalline phase to gel phase. Upon rehydration, dry membranes in gel phase at RT transform to liquid crystal phase. Several regions have packing defects when the membranes pass through this phase transition, which make the membranes leak.²⁻⁴ However, some organisms can survive in the anhydrobiotic state for many years and can rapidly swell and resume metabolic activity as long as they make contact with water.⁵

Trehalose is a non-reducing disaccharide of glucose that is found in high concentrations in these organisms and can protect them even in extreme life conditions, such as complete dehydration.⁶⁻⁸ Trehalose has an important function in stabilizing biomembrane systems, which can be explained by two hypotheses. In water replacement hypothesis, trehalose replaces water molecules bound to lipid headgroups and maintains them similarly to those in the hydrated state to protect the phospholipid bilayer structure in the dry state.^{2,9-11} In hydration force hypothesis, nonspecific osmotic and volumetric effects are responsible for the observed

membrane phase behavior in the presence of disaccharides.¹²⁻¹⁵

In this chapter, the stability of the DMPC bilayer in complete dry state was verified to be related to the volume of disaccharides. The molecular structure of DMPC, which consists of a hydrophilic headgroup and a hydrophobic tail, is shown in Figure 4.1.

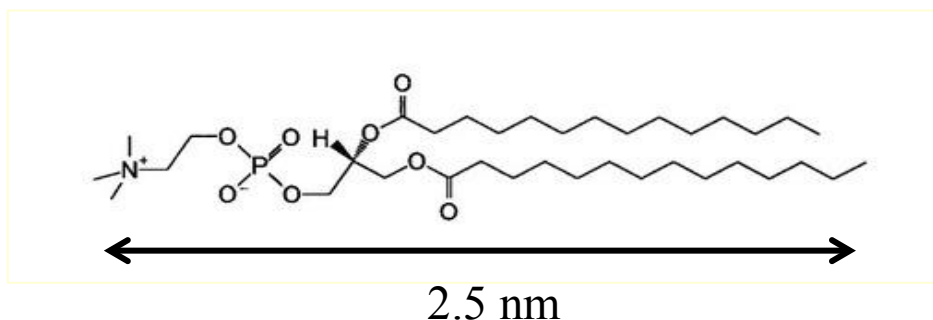


Figure 4.1 Molecule structure of DMPC.

Koster et al. reported that the interaction between disaccharides and membranes is reduced by large disaccharides because of the exclusion limit.¹⁴ The disaccharide with a molecular weight of 342 g/mol remained between bilayers during dehydration, and solutes were excluded at a molecular weight of above 1000 g/mol. The experiments described in this chapter showed that the stability of the bilayer structure in dry state also decreased by reducing the size of disaccharides. The DMPC bilayer was stable in D₂O, but broke and formed an ordered monolayer when D₂O was removed. By contrast, the DMPC bilayer structure was preserved even in dry state when disaccharides were introduced. The size of the DMPC bilayer domain in dry state increased by increasing the concentration of trehalose or maltose (342 g/mol). However, the bilayer domain was small in dry state even when a high glucose concentration (180 g/mol) was introduced.

The molecular structures of (a) trehalose, (b) glucose, and (c) maltose molecules are shown in Figure 4.2. The size of the glucose molecule was half that of the trehalose molecule, whereas the maltose and trehalose molecules had similar sizes.

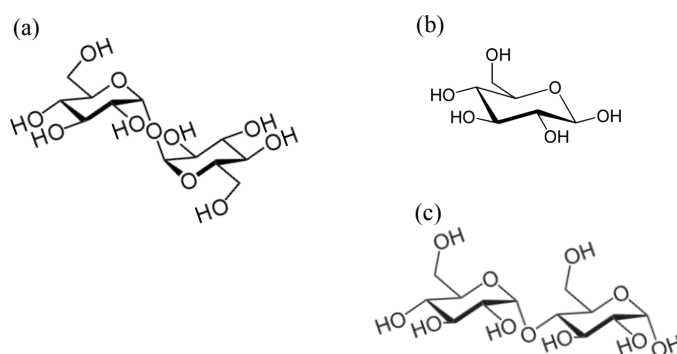


Figure 4.2 Structure of (a) trehalose, (b) glucose, and (c) maltose.

In this chapter, a visual model is demonstrated to clarify the function of trehalose on the stability of the bilayer structure in dry state by in situ SFG measurements, which is one of the most effective methods in studying molecular structures at interfaces to understand the molecular details of the interaction between trehalose and the lipid bilayer.¹⁶⁻¹⁹ The volumetric effect was proposed to have a main function in preserving the bilayer structure in dry state based on SFG, AFM, and ATR-FTIR measurements.

4-2 Results and Discussion

4-2-1 Structure of DMPC Bilayer on Quartz Surface in Water and Under Ar Gas

SFG measurements were carried out as follows: (1) in D_2O solution and (2) under Ar gas flow. D_2O was used instead of H_2O because the OH stretching of trehalose can easily be detected using D_2O . Otherwise, distinguishing the OH signal from trehalose and H_2O molecules is difficult. The use of D_2O can also avoid strong OH signals in the CH region.

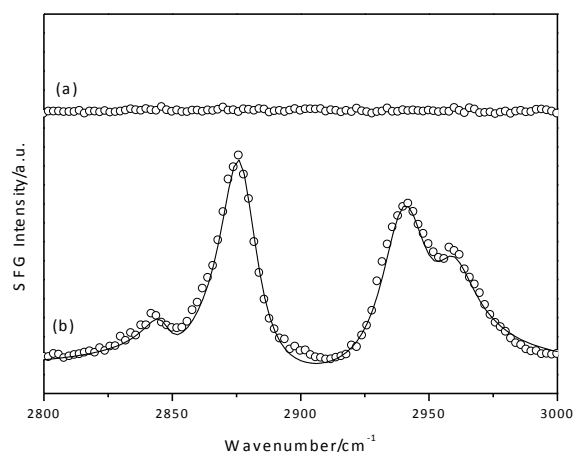


Figure 4.3 SFG spectra of DMPC-covered fused quartz surface in D₂O (a) and Ar gas (b) without contact with trehalose solution. The polarization combination used was s (SFG), s (visible), and p (IR). The SFG spectra were fitted according to Eq. (3-1).

Figure 4.3 (a) shows the SFG spectrum of the fused quartz surface on which the DMPC bilayer was formed by vesicle fusion in D₂O solution. No SFG signal was detected, which suggests three possibilities: (1) no adsorption of DMPC molecules; (2) an adsorbed molecular layer with centrosymmetric structures, such as a bilayer; or (3) completely disordered structures.

To determine the structure of the DMPC bilayer in dry state without contact with trehalose solution, Ar gas flow was used. After the fused quartz surface was dried by Ar gas, SFG measurement was carried out under Ar gas flow. Three strong peaks at 2876, 2941, and 2960 cm⁻¹ and one weak peak at 2848 cm⁻¹ were observed, as shown in Figure 4.3 (b).¹⁹ The three strong peaks were attributed to the symmetric C–H stretching (CH₃(s)), Fermi resonance (CH₃(FR)) of the methyl group, and asymmetric C–H stretching (CH₃(as)), respectively. The weak peak was attributed to the symmetric C–H stretching of the methylene group (CH₂(s)). When a highly ordered monolayer with all-trans conformation, such as an octadecyltrichlorosilane monolayer, formed, only the C–H stretching peaks of the CH₃ group was observed because the CH₂ group is centrosymmetric and SFG-inactive.¹⁸ The C–H stretching peaks of the CH₂ group increase when gauche defects are introduced. Strong signals related to the

methyl group and weak methylene signals indicate the formation of a highly ordered DMPC monolayer with only a few gauche defects in the alkyl chain and not the bilayer, which has a centrosymmetric structure and shows no peak in the C–H stretching region.^{20,21}

To determine the presence of the DMPC bilayer or disordered monolayer in D₂O solution, AFM observation was carried out in D₂O solution. Figure 4.4 (a) shows a bare SiO₂ surface in air with a roughness of less than 0.4 nm. Figure 4.4 (b) shows the SiO₂ surface in D₂O after the surface was incubated in 0.1 mg/mL of 100 nm filtered DMPC vesicle suspension and washed by changed D₂O solution five times. DMPC membranes were observed on the SiO₂ surface. These membranes were assigned to the DMPC single bilayers because the membrane height was 6 nm, which was twice that of a DMPC molecule. This membrane height was consistent with that reported in previous AFM studies (4 nm to 6 nm).^{22,23} Thus, a bilayer, not a disordered monolayer, was present on the SiO₂ surface in D₂O solution.

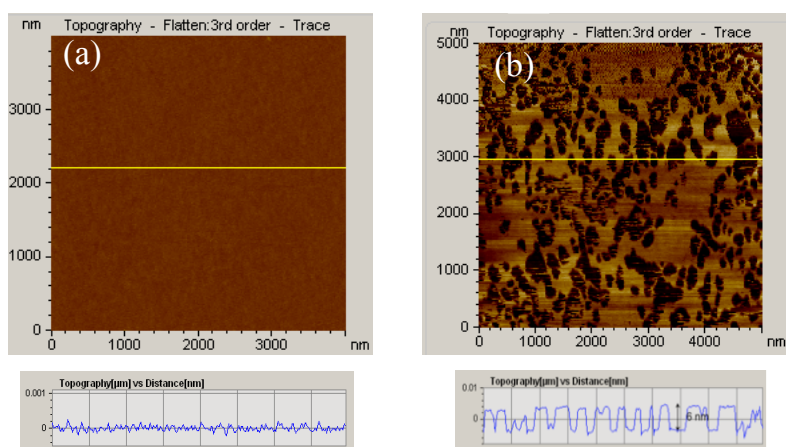


Figure 4.4 AFM images of (a) bare SiO₂ surface and (b) SiO₂ surface after incubation in 0.1 mg mL⁻¹ of 100 nm filtered DMPC vesicle suspension and drying by N₂ gas. (a) was carried out in air, whereas (b) was carried out in D₂O. The surface cross-section profiles of each sample are shown at the bottom of each image.

4-2-2 Effect of Trehalose on the Stability of DMPC Bilayer

Figure 4.5 (a) shows the SFG spectra of the fused quartz surface, on which the DMPC bilayer formed by vesicle fusion in D₂O solution, followed by contact with

0.01 M trehalose solution, and observed in Ar gas. Compared with Figure 4.3 (b), smaller CH_3 peaks and larger CH_2 peaks were observed when the DMPC bilayer was in contact with 0.01 M trehalose solution.

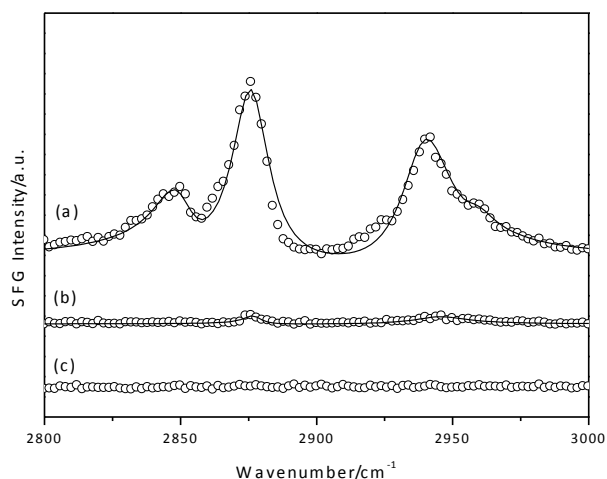


Figure 4.5 SFG spectra of DMPC-covered fused quartz surface in Ar gas after contact with (a) 0.01, (b) 0.1, and (c) 1 M trehalose solutions. The polarization combination used was ssp. The SFG spectra were fitted according to Eq. (3-1).

AFM measurement was carried out to confirm the changes in the SFG spectrum. Figure 4.6 (a) shows the AFM measurement of the SiO_2 surface on which the DMPC bilayer formed, followed by immersion in 0.01 M trehalose solution and drying by N_2 gas. DMPC membranes with a height of 6 nm and a single bilayer thickness were observed on the SiO_2 surface in dry state, which suggests that DMPC single bilayers were present on the SiO_2 surface. Given that AFM measurements in the same condition showed that a bilayer structure was present on the surface in dry state, the intensity of the SFG spectrum was decreased by trehalose because of the partial formation of the DMPC bilayer.

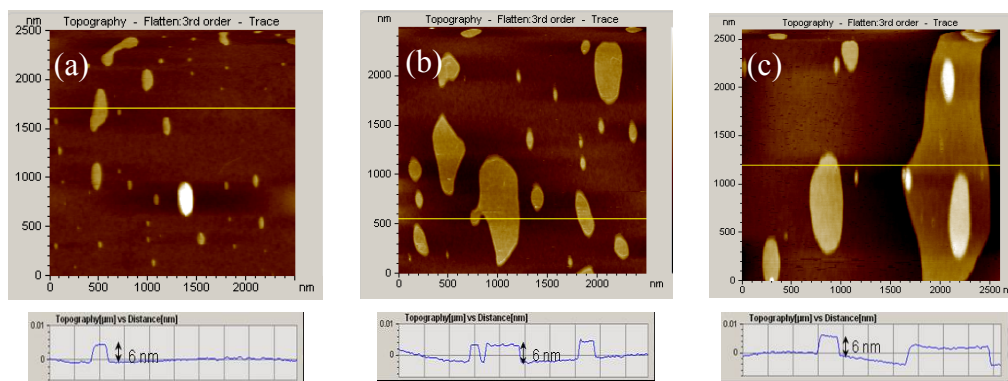


Figure 4.6 AFM images of (a) SiO₂ surfaces after incubation in 0.1 mg mL⁻¹ of 100 nm filtered DMPC vesicle suspension, immersion in (a) 0.01, (b) 0.10, and (c) 1.00 M trehalose solution, and drying by N₂ gas. All AFM images were carried out in air. The surface cross-section profiles of each sample are given at the bottom of each image.

Concentration-dependent experiments were carried out with the same experimental sequences in the following experiment. When the DMPC bilayer was in contact with 0.1 and 1.0 M trehalose solutions and dried by Ar gas, only very weak CH peaks were observed, as shown in Figures 4.5 (b) and 4.5 (c). The intensity of the CH peaks dramatically decreased, which suggests that the adsorbed molecule layer had a bilayer or disordered structure in dry state. To confirm whether a uniform bilayer or a molecule layer with a disordered structure was adsorbed on the surface, AFM measurements were carried out in the same condition. Figures 4.6 (b) and 4.6 (c) show the SiO₂ surfaces on which DMPC bilayers formed, followed by immersion in 0.1 and 1.0 M trehalose solutions and drying by N₂ gas. DMPC membranes with a single bilayer height of 6 nm were present on the SiO₂ surface, and the size of DMPC membranes increased with increasing trehalose concentration. These results suggest that the very weak SFG signals were due to the presence of a uniform DMPC bilayer because the bilayer structure has a centrosymmetric structure and should show no peak in the C–H stretching region.^{20,21} Thus, the stability of the DMPC bilayer increased with increasing trehalose concentration.

To clarify the effect of trehalose on stabilizing the DMPC bilayer in dry state, ATR-FTIR measurements were carried out in the same condition. The ATR-FTIR

spectra of the oxidized Si(111) surface on which the DMPC bilayer formed, followed by immersion in trehalose solution and drying by N₂ gas, are shown in Figure 4.7.

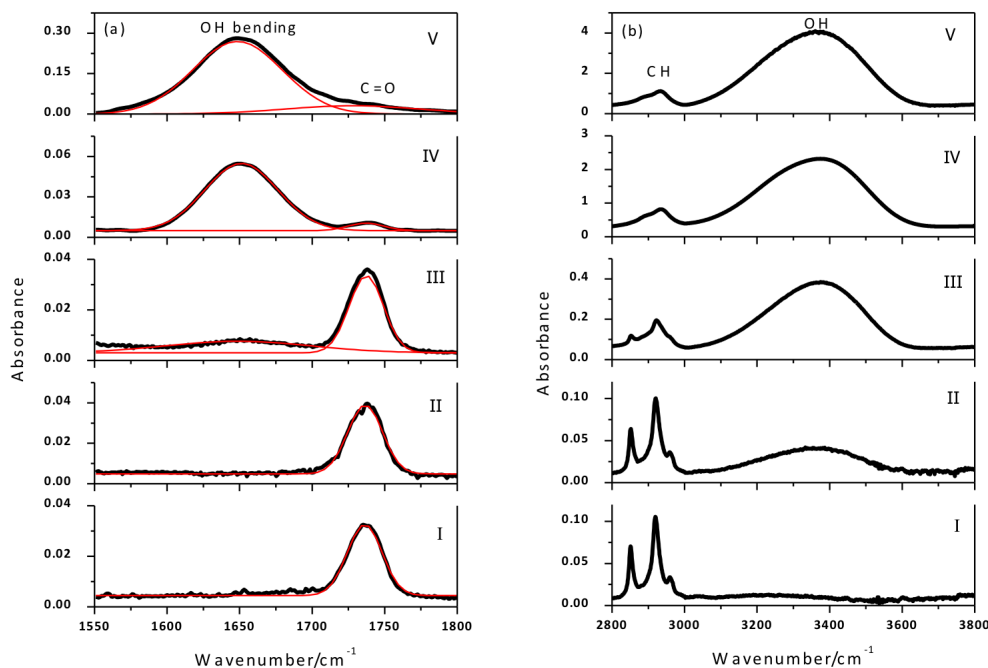


Figure 4.7 ATR-FTIR spectra in (a) C=O stretching vibration region and (b) CH and OH stretching regions of the DMPC bilayer-covered oxidized Si(111) surface obtained in N₂ without contact with trehalose solution (curve I) and after contact with 0.01, 0.10, 0.50, and 1.00 M trehalose solution (curves II to V).

Figure 4.7 (a) shows the ATR-FTIR measurements in the C=O stretching region (1550 cm^{-1} to 1850 cm^{-1}) of the DMPC bilayer without contact with trehalose solution (curve I) and of the DMPC bilayer in contact with 0.01, 0.1, 0.5, and 1 M trehalose solution (curves II to V) in N₂ gas. Only one peak at 1737 cm^{-1} was observed in curve I because of the non-hydrogen-bonded carbonyls of DMPC.²⁴ The peak at 1650 cm^{-1} appeared when the DMPC bilayer made contact with 0.1 M trehalose solution (curve III). The intensity of this peak increased when the trehalose concentration increased (curves III to V). Non-hydrogen-bonded carbonyls shift to a low wavenumber if a hydrogen bond forms.^{25,26} To clarify whether the peak at 1650 cm^{-1} was due to the hydrogen-bonded C=O groups or trehalose itself,

ATR-FTIR measurements were carried out at various trehalose concentrations, as shown in Figure 4.8.

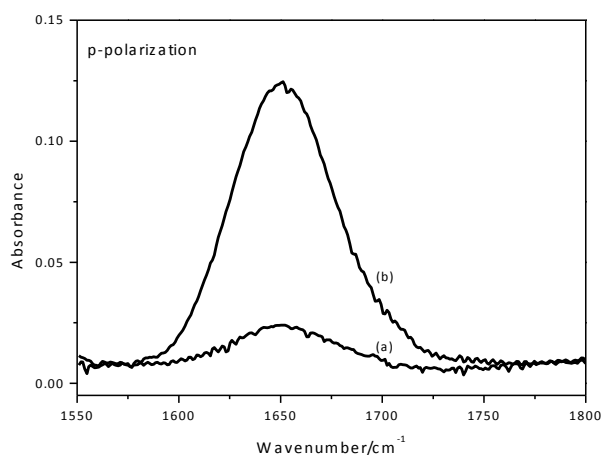


Figure 4.8 ATR-FTIR spectra of (a) 0.1 M and (b) 0.5 M trehalose on oxidized Si(111) surface in N₂.

Figure 4.8 (a) shows the ATR-FTIR measurement in the C=O stretching region (1550 cm⁻¹ to 1850 cm⁻¹) of 0.1 M trehalose in N₂ gas. One peak at 1650 cm⁻¹ was observed, which originated from the OH bending of the trehalose molecule.²⁷ The intensity of this peak increased when the trehalose concentration increased to 0.5 M (Figure 4.8 (b)). Thus, the peak at 1650 cm⁻¹ was due to the OH bending of trehalose itself and not the hydrogen-bonded C=O groups.

Figure 4.7 (b) shows the ATR-FTIR measurements in the CH (2800 cm⁻¹ to 3000 cm⁻¹) and OH stretching regions (3000 cm⁻¹ to 3800 cm⁻¹) of the DMPC bilayer without contact with trehalose solution (curve I) and of the DMPC bilayer in contact with 0.01, 0.1, 0.5, and 1 M trehalose solution (curves II to V). Two strong peaks at 2851 and 2919 cm⁻¹ and one weak peak at 2960 cm⁻¹ were observed in curve I. These peaks were attributed to the symmetric C–H stretching of the methylene group (CH₂(s)), the antisymmetric C–H stretching of the methylene group (CH₂(as)), and the asymmetric C–H stretching (CH₃(as)), respectively.²⁸⁻³⁰ However, no OH peak was observed in the O–H stretching region, which suggests that no interfacial water molecule was absorbed. A small OH peak was observed when 0.01 M trehalose solution was introduced, but not much difference was observed in the C–H stretching

region in curve II. This result indicates that only a few trehalose molecules were adsorbed, and the majority of the DMPC molecule layer was a monolayer because of the low trehalose concentration. The intensity of both CH and OH peaks increased when the trehalose concentration increased, which can be attributed to the adsorption of the trehalose molecules.^{27,31}

These results suggest that trehalose preserved the DMPC bilayer in dry state. However, the formation of a hydrogen bond produced minimal effects. The volumetric effect was proposed to have a main function in stabilizing the bilayer structure in dry state. The presence of trehalose between the headgroups of phospholipids during dehydration hindered the close approach of membrane bilayers and reduced the compressive stress that occurred when the bilayers were in close proximity. Trehalose kept the membrane separated. The stresses and strains in membranes at low hydration may be minimized by interlamellar trehalose, thereby reducing packing defects from phase transition. To clarify whether the volumetric effect stabilizes the DMPC bilayer in dry state, glucose (half the volume of trehalose) and maltose (the same volume of trehalose) were selected in the following experiments. The molecular structures of trehalose, glucose, and maltose are shown in Figure 4.2.

4-2-3 Effect of Glucose on Stability of DMPC Bilayer

To study the volumetric effect of glucose on stabilizing the DMPC bilayer in dry state, SFG spectra were obtained in the same way as mentioned in 4-2-2.

Figure 4.9 (a) shows the SFG spectrum of the fused quartz surface, on which the DMPC bilayer was formed by vesicle fusion in D₂O solution and observed in Ar gas. Three strong peaks at 2875 (CH₃(s)), 2941 (CH₃(FR)), and 2960 cm⁻¹ (CH₃(as)) and one weak peak at 2845 cm⁻¹ (CH₂(s)) were observed. Strong signals related to the methyl group and weak methylene signals indicate the formation of a highly ordered DMPC monolayer with only a few gauche defects in the alkyl chain.

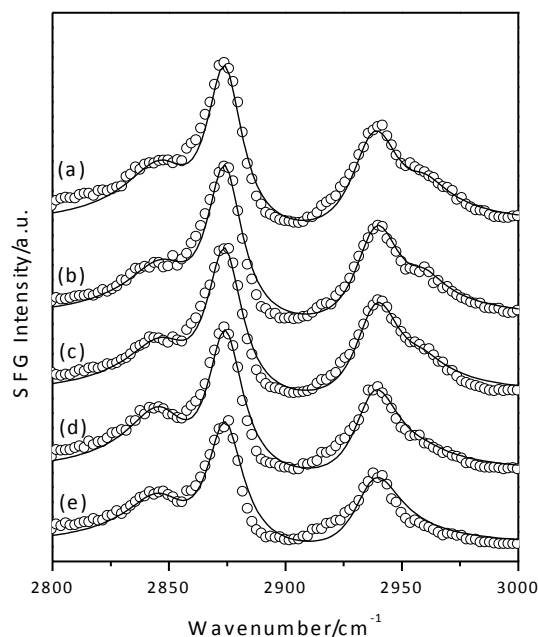


Figure 4.9 SFG spectra of DMPC-covered fused quartz surface in Ar gas (a) without contact and after contact with (b) 0.01, (c) 0.1, (d) 0.5 and (e) 1 M glucose solutions. The polarization combination used was ssp. The SFG spectra were fitted according to Eq. (3-1).

When the DMPC bilayer made contact with 0.01, 0.1, 0.5, and 1 M glucose solution and was dried by Ar gas, the CH₃ peaks only slightly decreased, whereas the CH₂ peak only slightly increased, as shown in Figures 4.9 (b) to 4.9 (e). To confirm these changes in the SFG spectra, AFM measurements were carried out using the same procedure in 4-2-2.

Figures 4.10 (a), 4.10 (b), and 4.10 (c) show the SiO₂ surfaces on which DMPC bilayers formed, followed by immersion in 0.1, 0.5, and 1 M glucose solutions and drying by N₂ gas. DMPC membranes with a single bilayer height of 5 nm were observed on the SiO₂ surface, and the size of DMPC membranes almost never changed with increasing glucose concentration. These results suggest that the small changes observed in the SFG spectra were due to the formation of small bilayer domains. Furthermore, the size of the bilayer did not increase when glucose concentration increased, which suggests that glucose only preserved the small bilayer domains in the dry state.

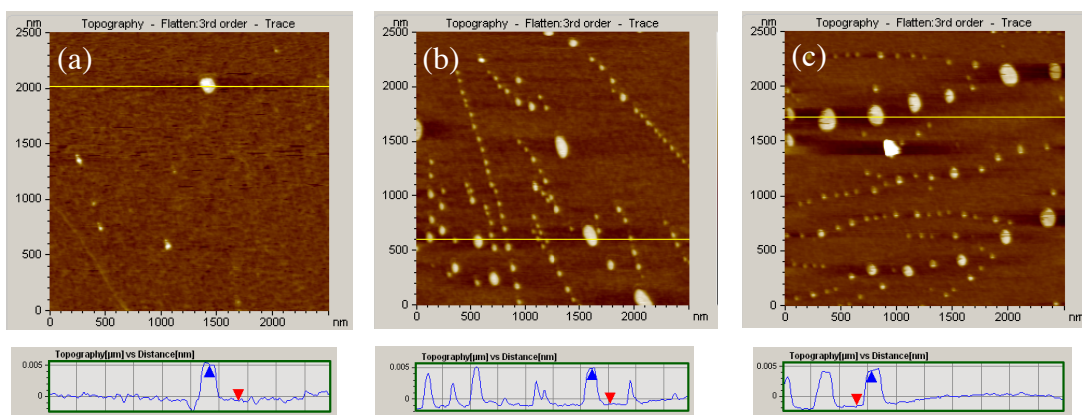


Figure 4.10 AFM images of SiO₂ surfaces after incubation in 0.1 mg mL⁻¹ of 100 nm filtered DMPC vesicle suspension and immersing in (a) 0.1 M, (b) 0.5M, and (c) 1M glucose solution and drying by N₂ gas. The surface cross-section profiles of each sample are shown at the bottom of each image.

To clarify whether a hydrogen bond formed between the headgroup of the DMPC molecule and glucose, ATR-FTIR measurements were carried out in the same conditions. The ATR-FTIR spectra of the oxidized Si(111) surface on which DMPC bilayer formed, followed by immersion in glucose solution and drying by N₂ gas, are shown in Figure 4.11.

Figure 4.11 (a) shows the ATR-FTIR measurements in the C=O stretching region (1550 cm⁻¹ to 1850 cm⁻¹) of the DMPC bilayer without contact with glucose solution (curve I) and the DMPC bilayer in contact with 0.01, 0.1, 0.5, and 1 M glucose solutions (curves II to V) in N₂ gas. Only one peak at 1737 cm⁻¹ was observed in curve I because of the non-hydrogen-bonded carbonyls of DMPC.²⁴ When glucose was introduced, the peak at 1737 cm⁻¹ only slightly changed, which suggests that the hydrogen bond between the glucose molecule and the phospholipid headgroup was small. The peak at 1650 cm⁻¹ appeared when the DMPC bilayer made contact with 0.1 M glucose solution (curve III). The intensity of this peak increased when the glucose concentration increased (curves III to V), which suggests that the peak at 1650 cm⁻¹ was due to the OH bending of the glucose molecule.³²

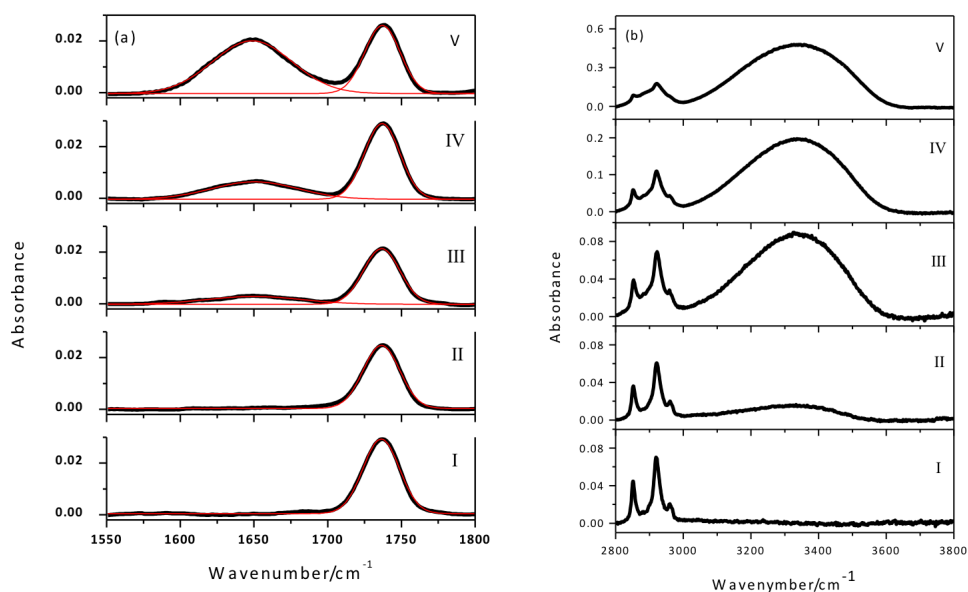


Figure 4.11 ATR-FTIR spectra in (a) C=O stretching vibration region and (b) CH and OH stretching regions of the DMPC bilayer-covered oxidized Si(111) surface obtained in N₂ without contact with glucose solution (curve I) and after contact with 0.01, 0.1, 0.5, and 1 M glucose solution (curves II to V).

Figure 4.11 (b) shows the ATR-FTIR measurements in the CH (2800 cm⁻¹ to 3000 cm⁻¹) and OH stretching regions (3000 cm⁻¹ to 3800 cm⁻¹) of the DMPC bilayer without contact with glucose solution (curve I) and the DMPC bilayer in contact with 0.01, 0.1, 0.5, and 1 M glucose solutions (curves II to V). Two strong peaks at 2851 (CH₂(s)) and 2919 cm⁻¹ (CH₂(as)) and one weak peak at 2960 cm⁻¹ (CH₃(as)) were observed in curve I, which suggests that the DMPC monolayer was adsorbed on the surface. However, no OH peak was observed in the OH stretching region, which suggests that no interfacial water molecule was absorbed. A small OH peak was observed when 0.01 M glucose solution was introduced but this peak was not significantly different from the C–H stretching region in curve II. This result indicates that only a few glucose molecules were adsorbed and the majority of the DMPC molecule layer was a monolayer because of the low glucose concentration. The intensity of both CH and OH peaks increased when the glucose concentration increased, which can be attributed to the adsorption of glucose molecules.

These results show that glucose was ineffective in stabilizing large bilayer domains in dry state, which is consistent with previous reports of monosaccharides being less effective than disaccharides.^{33,34} The volume of glucose was possibly too small to create an adequate space between the headgroups of phospholipids. Thus, significantly reducing the packing defects and effectively stabilizing the bilayer structure in dry state were difficult. However, if the results of the experiments described in 4-2-2 are considered, the size of the bilayer domain dramatically increased when the trehalose concentration increased. These results suggest that the volume of trehalose was suitable for insertion into the headgroup of phospholipids and effectively reduced the packing defects, thereby enhancing the stability of the bilayer domain in dry state.

To confirm whether the volumetric effect is the main reason for the stability of the DMPC bilayer in dry state, maltose was used in the following experiments.

4-2-4 Effect of Maltose on the Stability of DMPC Bilayer

SFG measurements were carried out in the same way as described in 4-2-2 and 4-2-3 to study the volumetric effect of maltose on stabilizing the DMPC bilayer in dry state, as shown in Figure 4.12.

Figure 4.12 (a) shows the SFG spectrum of the fused quartz surface, on which the DMPC bilayer was formed by vesicle fusion in D₂O solution and observed in Ar gas. Three strong peaks at 2875 (CH₃(s)), 2941 (CH₃(FR)), and 2960 cm⁻¹ (CH₃(as)) and one weak peak at 2845 cm⁻¹ (CH₂(s)) were observed. Strong signals related to the methyl group and weak methylene signals show the formation of a highly ordered DMPC monolayer with only a few gauche defects in the alkyl chain. This result is the same as that in Figures 4.3 (b) and 4.9 (a).

When the DMPC bilayer made contact with 0.01 M maltose solution and was dried by Ar gas, the CH₃ peaks decreased, CH₂(s) increased, and a new peak formed at 2928 cm⁻¹, as shown in Figure 4.12 (b).

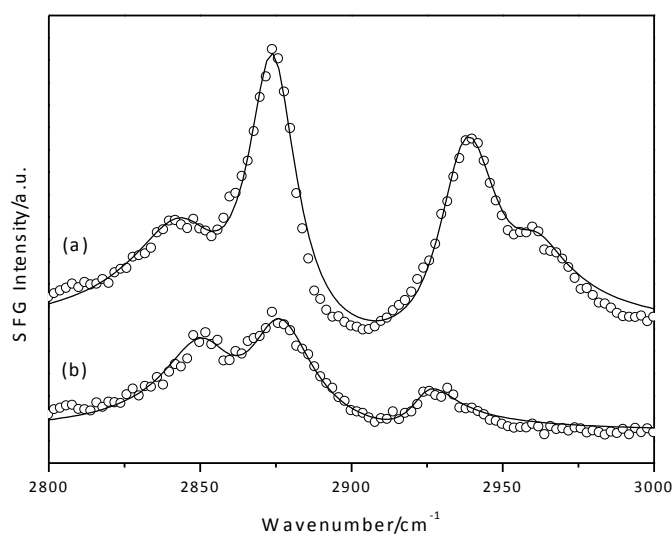


Figure 4.12 SFG spectra of DMPC-covered fused quartz surface in Ar gas (a) without contact and after contact with (b) 0.01 M maltose solutions. The polarization combination used was ssp.

To confirm these changes in SFG spectra, AFM measurement was carried out using the same procedure described in 4-2-2 and 4-2-3.

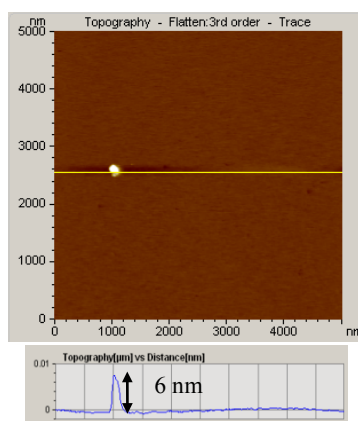


Figure 4.13 AFM images of SiO₂ surfaces after incubation in 0.1 mg mL⁻¹ of 100 nm filtered DMPC vesicle suspension and immersing in 0.01 M maltose solution and drying by N₂ gas. The surface cross-section profile are shown at the bottom of each image.

Figure 4.13 shows the SiO₂ surfaces on which DMPC bilayers formed, followed by immersion in 0.01 M maltose solution and drying by N₂ gas. A DMPC membrane with a single bilayer height of 6 nm was present on the SiO₂ surface, which suggests

that the decrease in CH_3 peaks and increase in $\text{CH}_2(\text{s})$ observed in the SFG spectrum were possibly due to the partial formation of the DMPC bilayer. However, these changes observed in Figure 4.12(b) remain unclear. To clarify the contribution of these peaks, the SFG spectrum of 0.01 M maltose was obtained, as shown in Figure 4.14.

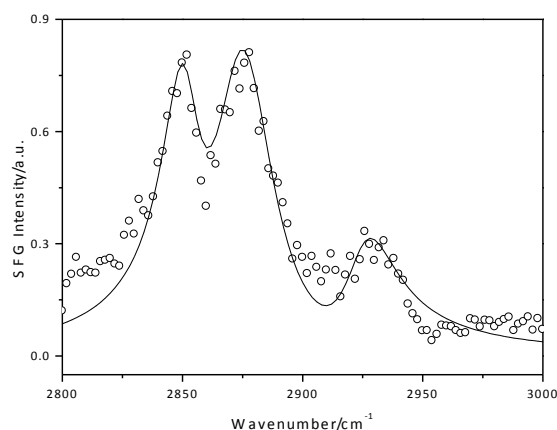


Figure 4.14 SFG spectrum of maltose adsorbed on the fused quartz surface from 0.01 M maltose solution in Ar gas. The polarization combination used was ssp.

Figure 4.14 shows the SFG spectrum of the fused quartz surface in contact with 0.01 M maltose solution and dried in Ar gas. Three peaks at 2845, 2877, and 2928 cm^{-1} were due to the C–H stretching of the maltose molecule. Based on the results, the changes in the SFG spectrum (Figure 4.12 (b)) came from the formation of small bilayer domains and the adsorption of maltose molecules.

However, no C–H stretching vibration was observed when trehalose was adsorbed on the fused quartz surface from 0.1 M trehalose solution, as shown in Figure 4.15. Weak SFG signals $> 2950\text{ cm}^{-1}$ in the SFG spectrum were due to the OH stretching vibration of water. No SFG signal of the CH_2 groups was observed possibly because of low coverage or the random adsorption structure of trehalose molecule. However, the trehalose concentration used in Figure 4.15 was higher than the maltose concentration used in Figure 4.14. Thus, no CH signal was observed may be because of the randomly oriented CH_2 groups of the trehalose molecule.

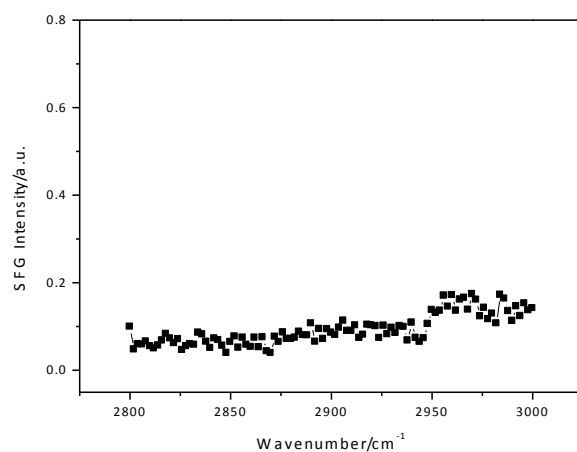


Figure 4.15 SFG spectrum of trehalose adsorbed on fused quartz surface from 0.1 M trehalose solution in Ar gas. The polarization combination used was ssp.

To discuss the molecular structure on the surface, several low-energy conformations of trehalose and maltose were considered, as shown in Figure 4.16. Figure 4.16 shows several possibilities of the low-energy conformations of trehalose ((a), (b), and (c)) and maltose ((d), (e), and (f)) in vacuum.³⁵⁻³⁸

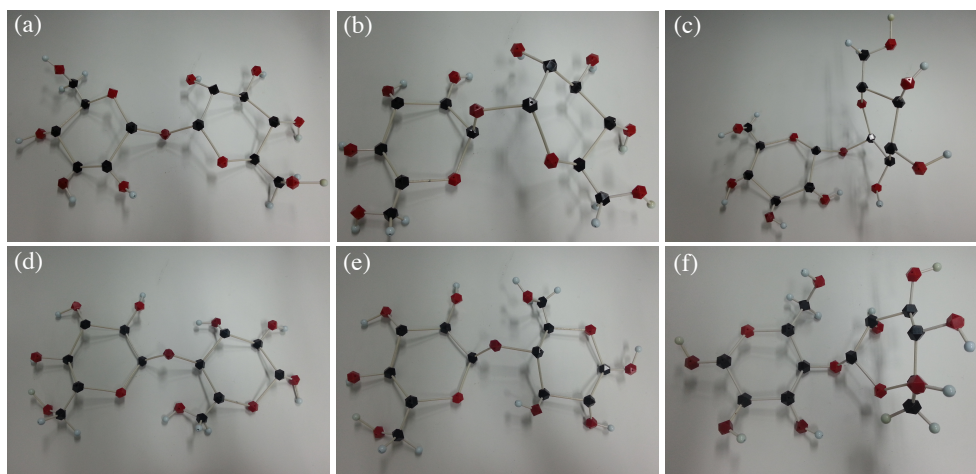


Figure 4.16 Molecular models of low-energy conformations of trehalose (a), (b), and (c) and maltose (d), (e), and (f).

If the adsorption structures of trehalose and maltose on a hydrophilic surface are considered, hydrogen bonds formed between the molecule and surface. Six hydrogen

bonds may form between OH groups and the hydrophilic surface in trehalose configuration (a) and (b), and four hydrogen bonds may form in trehalose configuration (c). Trehalose configuration (a) was more stable than others because of its flatter molecular structure and increased formation of hydrogen bonds. The more stable maltose structure was shown in maltose configuration (d). Figure 4.17 shows the most possible adsorption structures of trehalose and maltose on a quartz surface.

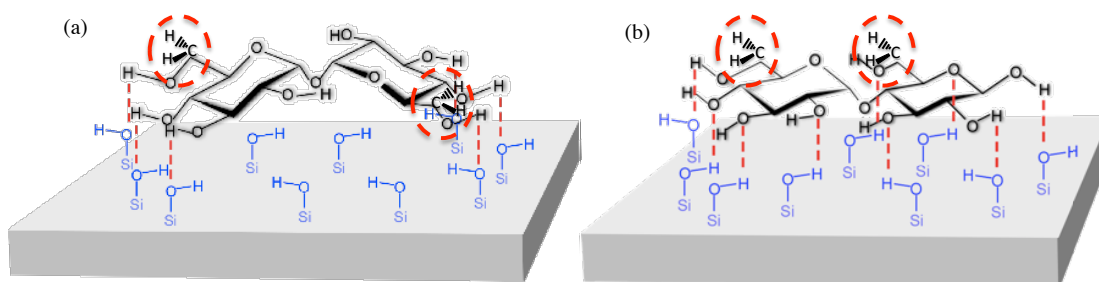


Figure 4.17 Adsorption structure of (a) trehalose and (b) maltose on the fused quartz surface.

In Figure 4.17 (a), trehalose was formed by an $\alpha(1\rightarrow1)$ -glucoside bond between two glucose units. Two CH_2 groups were pointed in the opposite direction, which canceled their respective SFG signals. Two glucoses that compose maltose were joined by an $\alpha(1\rightarrow4)$ -glucoside bond, as shown in Figure 4.17 (b), which resulted in two CH_2 groups pointing in the same direction and giving a SFG signal.

Concentration-dependent experiments were carried out using the same experimental sequences in the following experiment. When the DMPC bilayer was in contact with 0.1 and 1.0 M maltose solution and dried by Ar gas, weak CH peaks were observed, as shown in Figures 4.18 (a) and (b).

Figure 4.18 shows that weak CH signals possibly form maltose molecules, and DMPC bilayers were observed even when high maltose concentration was introduced. No CH signals from trehalose molecule were observed when trehalose concentration increased, as shown in Figure 4.5. Changes in the adsorption structure of trehalose and maltose were also observed when they interacted with the DMPC bilayer.

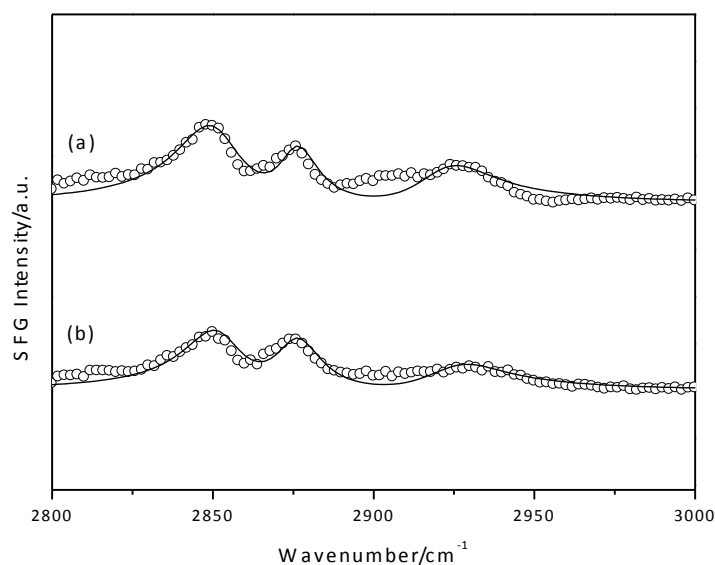


Figure 4.18 SFG spectra of DMPC-covered fused quartz surface in Ar gas after contact with (a) 0.1 and (b) 1 M maltose solutions. The polarization combination used was ssp.

AFM measurements were carried out to confirm these changes in SFG spectra. Figures 4.19 (a) and (b) show the SiO₂ surfaces on which DMPC bilayers formed, followed by immersion in 0.1 and 1.0 M maltose solutions and drying by N₂ gas. DMPC membranes with a single bilayer height of 6 nm (a) and a four-layer height of 12 nm (b) were present on the SiO₂ surface, and the size of DMPC membranes increased when maltose concentration increased. These results suggest that the weak SFG signal was due to the presence of the DMPC bilayer and the adsorption of maltose molecules.

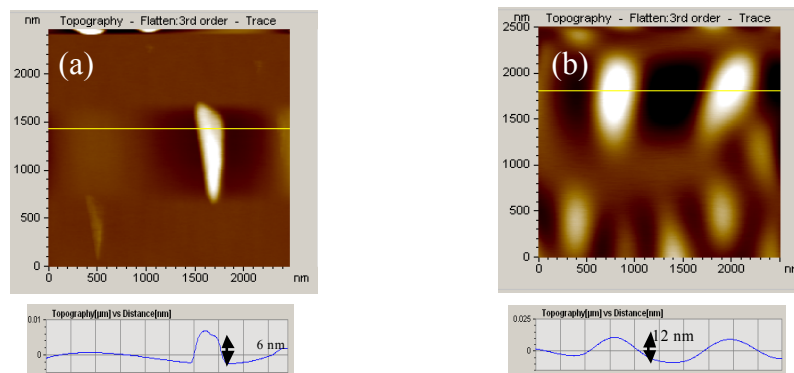


Figure 4.19 AFM images of SiO₂ surfaces after incubation in 0.1 mg mL⁻¹ of 100 nm filtered DMPC vesicle suspension and immersing in (a) 0.1M and (b) 1M maltose solution and drying by N₂ gas. The surface cross section profiles are shown at the bottom of each image.

ATR-FTIR measurements were carried out in the same conditions as described in 4-2-2 and 4-2-3 to determine which species were adsorbed on the surface. Figure 4.20 shows the ATR-FTIR spectra of the oxidized Si(111) surface on which the DMPC bilayer formed, followed by immersion in glucose solution and drying by N₂ gas.

Figure 4.20 (a) shows the ATR-FTIR measurements in the C=O stretching region (1550 cm⁻¹ to 1850 cm⁻¹) of the DMPC bilayer without contact with maltose solution (curve I) and the DMPC bilayer in contact with 0.01, 0.1, 0.5, and 1 M maltose solution (curves II to V) in N₂ gas. Only one peak at 1737 cm⁻¹ was observed in curve I because of non-hydrogen-bonded carbonyls. No shift was observed at 1737 cm⁻¹ when the maltose concentration increased. These results were similar to the results in Figures 4.7 (a) and 4.11 (a), which confirm that the hydrogen bond between the disaccharide and the headgroup of phospholipids was small. Thus, hydrogen bond formation was not the main reason for the stability of the bilayer structure in dry state. The peak at 1650 cm⁻¹ began to appear when the DMPC bilayer made contact with 0.1 M maltose solution (curve III). The intensity of this peak increased when the maltose concentration increased (curves III to V). As in the previous study, this increased intensity was attributed to the OH bending of maltose molecules.

Figure 4.20 (b) shows the ATR-FTIR measurements in the CH (2800 cm^{-1} to 3000 cm^{-1}) and OH stretching regions (3000 cm^{-1} to 3800 cm^{-1}) of the DMPC bilayer without contact with maltose solution (curve I) and the DMPC bilayer in contact with 0.01, 0.1, 0.5, and 1 M maltose solution (curves II to V). Two strong peaks at 2851 ($\text{CH}_2(\text{s})$) and 2919 cm^{-1} ($\text{CH}_2(\text{as})$) and one weak peak at 2960 cm^{-1} ($\text{CH}_3(\text{as})$) were observed in curve I. These results were the same as those in Figures 4.7 (b) and 4.11 (b), which show that the DMPC monolayer was present on the surface. A small OH peak was observed when 0.01 M maltose solution was introduced. However, this peak was not significantly different from that in the C–H stretching region in curve II, which indicates that a few maltose molecules and the majority of the DMPC monolayer were adsorbed because of low maltose concentration. The intensity of both CH and OH peaks increased when the maltose concentration increased, which can be attributed to the adsorption of maltose molecules. These results show that large bilayer or multilayer DMPC domains were on the surface in dry state when the maltose concentration increased.

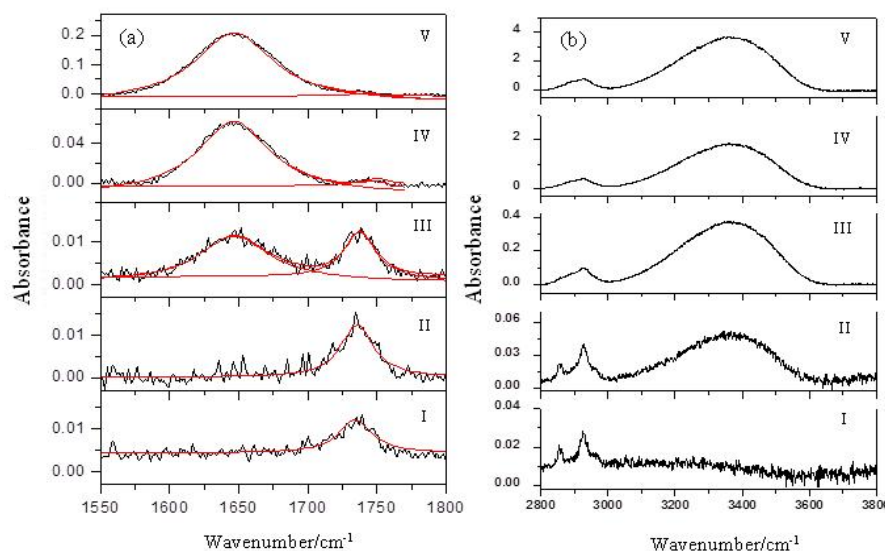


Figure 4.20. ATR-FTIR spectra in (a) C=O stretching vibration region and (b) CH and OH stretching regions of the DMPC bilayer-covered oxidized Si(111) surface obtained in N_2 without contact with maltose solution (curve I) and after contact with 0.01, 0.1, 0.5, and 1 M maltose solution (curves II to V).

These results were similar to those of trehalose stabilizing the DMPC bilayer in dry state, which suggests that maltose was also effective in preserving the bilayer structure when water was removed. Maltose effectively preserves liposomes compared with monosaccharides.^{39,40} However, hydrogen bond formation stabilizes dry liposomes.

4-2-5 Comparison of Three Saccharides

Figure 4.21 shows that the relative SFG intensity of $\text{CH}_3(\text{s})$ of the DMPC molecule layer depends on saccharide concentration. SFG intensity was normalized by the peak height of $\text{CH}_3(\text{s})$ at 2876 cm^{-1} obtained without introducing saccharides (trehalose, glucose, or maltose). The intensity of the $\text{CH}_3(\text{s})$ peak was very high without introducing saccharides, showing a highly ordered DMPC monolayer adsorbed on the fused quartz surface in dry state. The intensity of the $\text{CH}_3(\text{s})$ peak decreased to almost 0 when trehalose was introduced, as shown in the closed square. The decreased intensity of $\text{CH}_3(\text{s})$ suggests bilayer formation based on the above results. A similar result was also observed when maltose was introduced, as shown in the closed triangle. Based on Figures 4.12 and 4.18, the peak at 2875 cm^{-1} possibly come from DMPC and maltose molecules. The decrease in peak intensity was still observed when maltose concentration increased, which suggests that the coverage of the DMPC bilayer increased. Only a slight decrease in the intensity of $\text{CH}_3(\text{s})$ was observed when glucose was introduced, which suggests low coverage of the DMPC bilayer on the surface. The SFG spectra indicate that trehalose and maltose may form a uniform bilayer on the surface. However, glucose is ineffective in ensuring the stability of the DMPC bilayer in dry state.

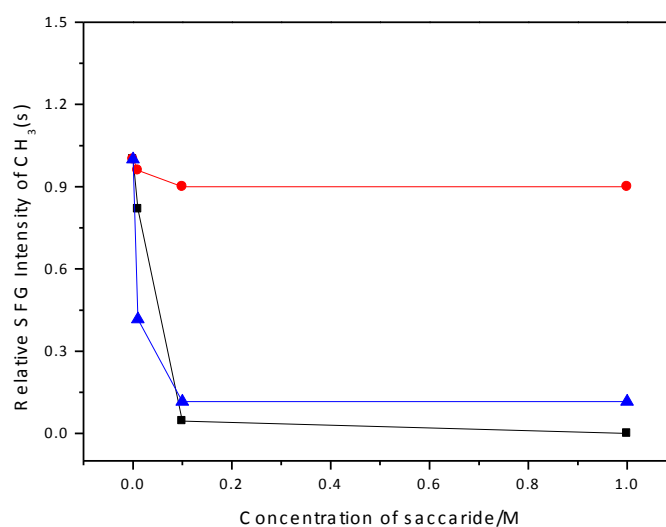


Figure 4.21 Relative SFG intensity of the $\text{CH}_3(\text{s})$ of the DMPC molecule layer depends on saccharide concentration. Closed square is trehalose, closed triangle is maltose, and closed circle is glucose.

Figure 4.22 shows the coverage of the DMPC bilayer depending on the saccharide concentration. This coverage was estimated based on the AFM images in Figures 4.6, 4.10, and 4.19.

The coverage of the DMPC bilayer increased up to 30% when trehalose was introduced (closed square). The coverage of the DMPC bilayer increased only to 10% and 3% when maltose (closed triangle) and glucose (closed circle) were introduced, respectively. Trehalose was the most effective saccharide on the stability of the DMPC bilayer in dry state. Glucose was the least effective.

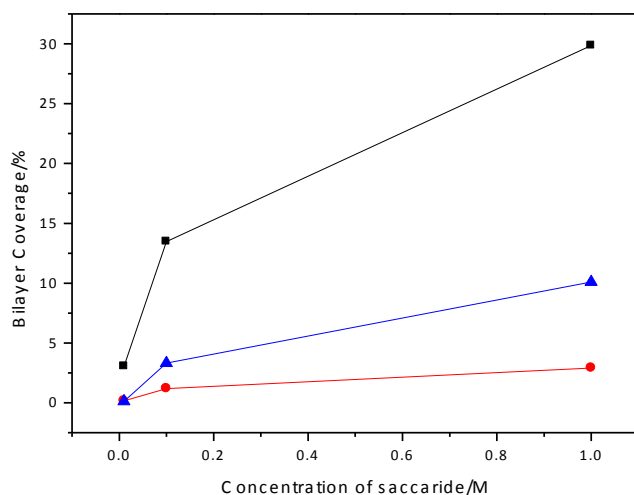


Figure 4.22 Coverage of DMPC bilayer depending on the saccharide concentration. This coverage was calculated based on AFM images in Figures 4.6, 4.10, and 4.19. Closed square is trehalose, closed triangle is maltose, and closed circle is glucose.

The difference between trehalose and maltose on the stabilization of DMPC bilayer was possibly caused by the differences in adsorption structure, as discussed in Figure 4.17. The volume and chemical structure were effective in stabilizing the DMPC bilayer in dry state.

To study the mechanism of the saccharide effect on the stability of the DMPC bilayer in dry state, ATR-FTIR measurements were carried out. Results are shown in Figure 4.23. Figure 4.23 shows that the wavenumber of C=O stretching vibration depends on the saccharide concentration. Almost no shift was observed, depending on the trehalose, maltose, and glucose concentrations, which suggests that the hydrogen bond formation was small. Thus, the volumetric effect was proposed to explain the mechanism of the saccharide effect on the stability of the DMPC bilayer in dry state.

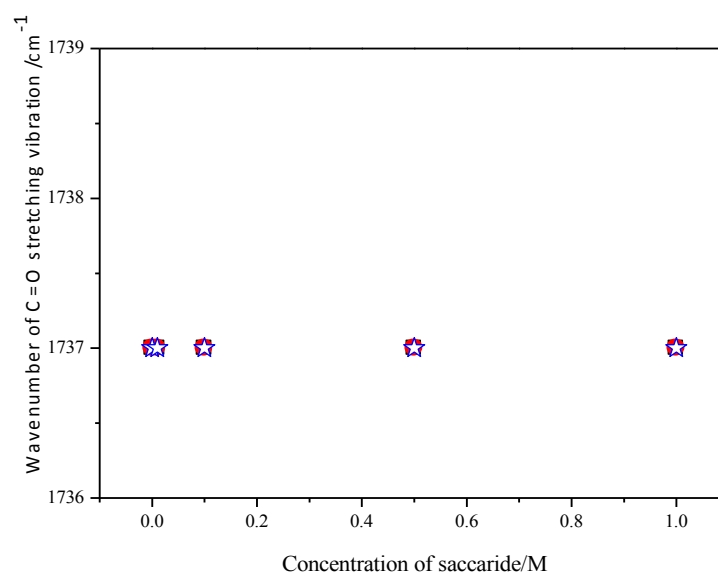


Figure 4.23 Wavenumber of C=O stretching vibration depends on the saccharide concentration. Closed square is trehalose, opened triangle is maltose, and opened star is glucose.

4-3 Conclusion

The stability of the DMPC bilayer in dry state was studied using SFG, AFM, and ATR-FTIR measurements. The DMPC bilayer was found to be stable in D₂O. However, the DMPC bilayer was broken and formed an ordered monolayer when D₂O was removed. The stability of the DMPC bilayer increased when saccharide was introduced. Moreover, the size of the saccharide was effective in stabilizing the DMPC bilayer in dry state. Disaccharides were more effective in stabilizing the DMPC bilayer domain in dry state, whereas small-sized monosaccharides (e.g., glucose) were less effective. Based on the ATR-FTIR results, hydrogen bond formation between saccharide and phospholipid headgroups was minimal. Thus, the volumetric effect was proposed to explain the stabilization of the DMPC bilayer in dry state, and the model is shown in Figure 4.24.

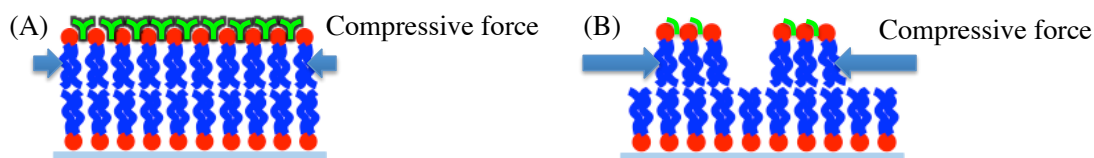


Figure 4.24 Volumetric effect model of (a) disaccharide insertion into DMPC bilayer and (b) monosaccharide insertion into DMPC bilayer.

The volume of disaccharides was suitable for insertion into the headgroup of phospholipids and effectively reduced the packing defects, thereby stabilizing the bilayer domain in dry state (Figure 4.24(a)). However, the volume of monosaccharides was too small to provide enough space between the headgroups of phospholipids. Thus, the packing defects were not significantly reduced (Figure 4.24(b)). The monosaccharide was less effective than the disaccharide in stabilizing the DMPC bilayer structure in dry state. In a previous study, the disaccharide with a molecular weight of 342 g/mol remains between bilayers during dehydration, whereas solute exclusion occurs when the molecular weight exceeds 1000 g/mol.¹⁴ Thus, the stabilization of bilayer in dry state was affected by the solute volume.

In this study, the difference between trehalose and maltose in stabilizing the DMPC bilayer was found. This difference is possibly due to the difference in chemical structure between trehalose and maltose. Thus, the volume and chemical structure were effective in stabilizing the bilayer structure in dry state.

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Chapter 5

Dynamics of Molecules within Free Standing SDS

Membrane

5-1 Introduction

Mimicking the cell membrane is a challenging topic, in which the processes that occur in the cell membrane and its potential applications in biomimetic systems can be studied. Conventional membrane mimetic systems are made of supported bilayer lipid membranes on solid and liquid surfaces, micelles, vesicles, and biopolymers.¹⁻⁴ The architecture of these systems is based on the assemblies of amphipathic molecules in water as they occur in the natural environment. By contrast, soap bubbles constitute a different class of model membranes, in which the orientation of surfactant molecules is opposite that in a lipid bilayer, i.e., the hydrophobic parts pointing to the air and water molecules between the hydrocarbon layers. The possibility of incorporating additional molecules into these films provides new techniques to study molecular properties and chemical reactions in thin soap films. The fundamental challenge lies in obtaining thin stable soap films for scientific and practical applications, ranging from membrane biophysics (including transport, practical AIDS research, and microchip studies) to biosensors that use supported bilayer lipid membranes.⁵

The key to stable soap films is to understand the time evolution of these films and the structural changes before the film collapses. One traditional investigative approach on the stability of soap film is to make a film from a soap solution and study various time-independent and time-dependent properties.⁶⁻⁸ Soap solutions can form stable bubbles and films. Either the surface of the soap solution or the soap film

consists of an amphiphilic monolayer that has a hydrophilic headgroup and a hydrophobic tail. The hydrophilic part prefers to be surrounded by water because of the attraction between the hydrophilic headgroup and the water molecules. By contrast, the hydrophobic part moves away from the water environment, causing the hydrocarbon tails to point out from water. A typical soap molecule is SDS. The positive metallic sodium ions are dispersed throughout the bulk fluid in the soap solution or film. The surface of soap bubbles is composed of soap molecules that are largely separated by water molecules. The film immediately thickens after it forms. Once the film forms, it will start to become thin because the water will start to drain from the film. The film thickness will decrease until a final equilibrium thickness is reached, provided the film does not rupture. Study of the thinning of vertical foam film has revealed various aspects of time evolution of film drainage, leading to a Newton black film.^{9,10} UV-vis and IR spectroscopic methods have been used to study the time-dependent changes in soap film thickness and to obtain information on the structure and chemical composition of films.^{5,6,11} However, the real-time change of the structure and arrangement of soap and water molecules during bubble thinning remain unclear. To study these structural changes, the stability of bubbles needs to be improved.

In this study, SDS was used to investigate the time evolution of the stability of soap membrane under different concentrations of 1-dodecanol and trehalose. The time-dependent structures of water and SDS molecule were studied using FTIR measurements. The effects of 1-dodecanol and trehalose in stabilizing SDS membrane were also studied using time-dependent FTIR measurements. 1-Dodecanol was effective in stabilizing the SDS membrane because of the decrease in the water evaporation rate. By contrast, trehalose had a different effect in the stabilization of the SDS membrane. Based on the study in Chapter 4, the effect of trehalose in stabilizing the SDS membrane is possibly caused by the insertion into the SDS headgroup, which can reduce the packing defects and stabilize the SDS membrane.

5-2 Results and Discussion

5-2-1 Stability of SDS Membrane and Effect of Trehalose

SDS membranes were prepared from a repeatedly boiled solution of 0.11 M SDS. The details of this preparation procedure are described in Chapter 2. The stability of these membranes was found to be more enhanced compared with that of the membrane obtained from the ordinary SDS solution. As described in previous chapters, trehalose can effectively stabilize the membrane in dry state. Thus, the effect of trehalose on the stability of SDS membrane was also studied.

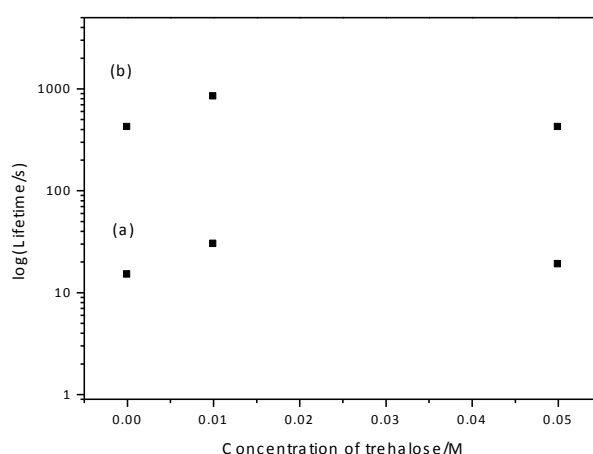


Figure 5.1 Stability of SDS membrane obtained from (a) the ordinary SDS solution and (b) a repeatedly boiled solution of SDS depending on trehalose concentration.

Figure 5.1 (a) shows the lifetime of SDS membrane obtained from an ordinary SDS solution. The lifetime was measured from the start of SDS membrane formation until its collapse. The SDS membrane collapsed after 15 s without the addition of trehalose. The lifetime increased when trehalose was added, but increased to twofold (30 s) when the trehalose concentration increased to 0.01 M. A slight lifetime decrease was observed when the trehalose concentration was further increased to 0.02 M. A similar trend of increasing stability, which was affected by adding trehalose, was also observed in Figure 5.1 (b). Figure 5.1 (b) shows the lifetime of SDS membrane obtained from a repeatedly boiled solution of SDS. The SDS membrane collapsed after 420 s without adding trehalose, but increased when

trehalose was added. The lifetime of SDS membrane obtained from a repeatedly boiled SDS solution (420 s) was significantly longer than that of SDS membrane obtained from an ordinary SDS solution (15 s). The enhanced stability of SDS membranes remains unclear. The enhanced stability is possibly due to the presence of 1-dodecanol, which is generated by SDS hydrolysis upon prolonged boiling.¹² To study the effect of 1-dodecanol and trehalose on the stability of SDS membrane, the ordinary SDS solution was used in the following experiments.

5-2-2 Effect of 1-Dodecanol on the Stability of SDS Membrane

5-2-2-1 Concentration Dependence for the Lifetime

To study the effect of 1-dodecanol on the stability of SDS membrane, 0.11 M SDS was dissolved in Milli-Q water without the repeated boiling procedure and various concentrations of 1-dodecanol were added. Figure 5.2 shows the relationship between the lifetime (obtained from the start of SDS membrane formation until its collapse) of 0.11 M SDS membrane and the 1-dodecanol concentration.

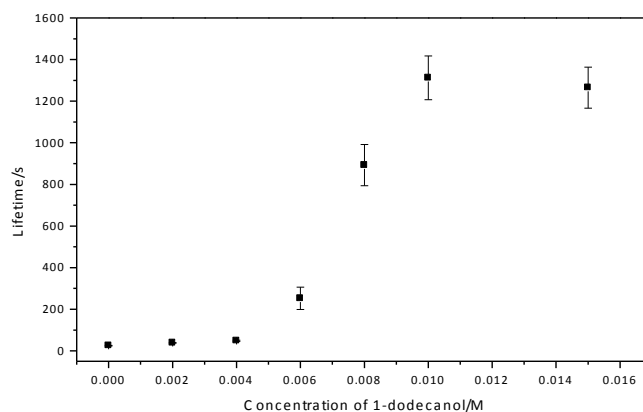


Figure 5.2 Stability of SDS membrane depends on 1-dodecanol concentration.

The results show that the SDS membrane collapsed within 30 s without 1-dodecanol, whereas the lifetime of SDS membrane slightly increased when a small amount of 1-dodecanol was introduced. The lifetime of SDS membrane dramatically increased when the 1-dodecanol concentration increased to 0.008 M, and the maximum lifetime reached 1400 s when 0.01 M 1-dodecanol was introduced. SDS

and 1-dodecanol did not mix well when the 1-dodecanol concentration further increased to 0.15 M. A cloudy solution was obtained in that mixture, which was also reported in a previous study.¹² Thus, in the present study, 0.01 M or a relatively lower 1-dodecanol concentration, which can obtain a clear solution, was used for FTIR measurement.

5-2-2-2 Time-dependent FTIR

To study the effect of 1-dodecanol on stabilizing SDS membrane, time-dependent FTIR measurements were carried out, as shown in Figure 5.3. Figure 5.3 (a) shows the time-dependent FTIR spectra of SDS membrane. The first spectrum was obtained immediately after the membrane was formed (t_0). The time duration to obtain one spectrum was 5 s. The absorbance gradually decreased and finally became 0 because of the collapse of the SDS membrane.

One peak at 1645 cm^{-1} was observed, which was possibly caused by the OH bending of water molecule. A strong OH peak at 3400 cm^{-1} and a relatively weak shoulder at 3217 cm^{-1} were observed in the OH stretching region. Based on previous studies, the band at 3400 cm^{-1} was attributed to randomly oriented asymmetric O–H stretching vibration, whereas that at 3217 cm^{-1} corresponded to the well-ordered, tetrahedrally coordinated, symmetric O–H stretching mode of water.¹³⁻¹⁵ Two weak peaks at 2855 and 2926 cm^{-1} were observed, as shown in the inset figure of the expanded C–H stretching region, which could be assigned to the symmetric C–H stretching of the methylene group ($\text{CH}_2(\text{s})$) and the asymmetric C–H stretching of the methylene group ($\text{CH}_2(\text{as})$), respectively.^{11,16,17} These C–H peaks originated from the SDS molecule in the SDS membrane.

The time-dependent FTIR spectra of the SDS membrane containing 0.005 and 0.01 M 1-dodecanol are shown in Figures 5.3 (b) and 5.3 (c), respectively. Compared with the spectra in Figure 5.3 (a), more spectra were observed when the 1-dodecanol concentration increased because the lifetime of SDS membrane increased by introducing 1-dodecanol. The details of the increase in lifetime by introducing 1-dodecanol are discussed below.

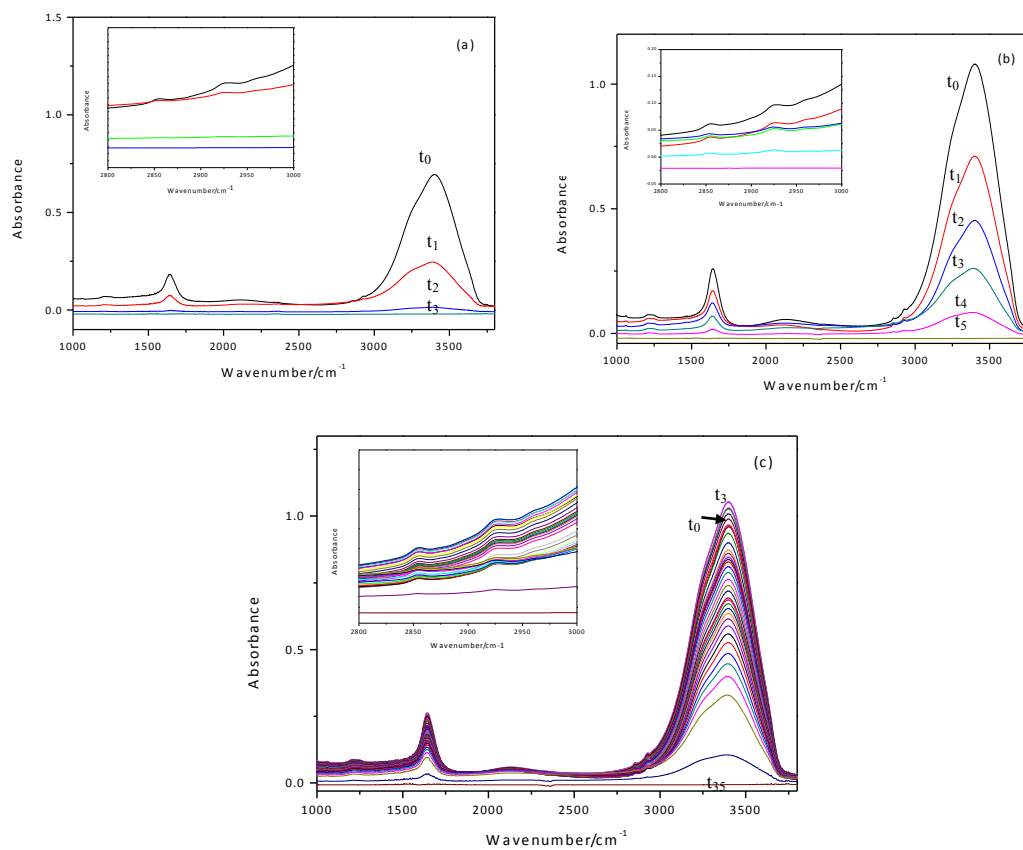


Figure 5.3 Time-dependent FTIR spectra of (a) SDS membrane, (b) SDS membrane with 0.005 M dodecanol, and (c) SDS membrane with 0.01 M dodecanol. Each spectrum in (a) and (b) is the average of four scans, which took 5 s. The first spectrum was obtained at t_0 , and the final spectrum was obtained at t_3 in (a) and t_5 in (b), respectively. Each spectrum in (c) is the average of 16 scans, which took 20 s. The first spectrum was obtained at t_0 . A slight increase in absorbance was obtained from t_0 to t_3 . A gradual decrease in absorbance of the subsequent spectra was obtained from t_3 to t_{35} . The insert spectrum is the expanded C–H stretching region. More spectrum curves were obtained in (b) and (c) because of the longer lifetime of SDS membrane.

(1) O-H stretching vibration

Figure 5.4 shows the time-dependent absorbance change in O–H stretching vibration at 3400 cm^{-1} of the SDS membrane, which contained only SDS without 1-dodecanol (opened triangle), SDS with 0.005 M 1-dodecanol (opened star), and SDS with 0.01 M 1-dodecanol (opened circle).

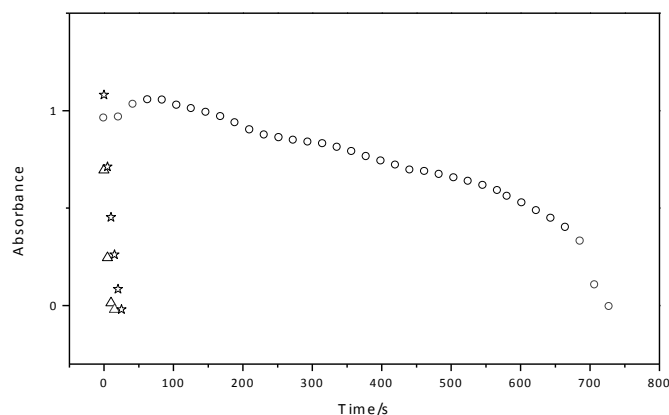


Figure 5.4 Time-dependent absorbance change in O–H stretching vibration at 3400 cm^{-1} of SDS membrane, which contained only SDS without 1-dodecanol (opened triangle), SDS with 0.005 M 1-dodecanol (opened star), and SDS with 0.01 M 1-dodecanol (opened circle).

According to the Beer-Lambert law, the fraction of monochromatic light that is transmitted through an absorbance system is expressed through the following equation:

$$\frac{I_t}{I_0} = 10^{-\varepsilon Cd} = e^{-\alpha Cd} \quad (5-1)$$

Where I_t and I_0 are the intensities of the incident light and the transmitted light, respectively. ε is the decadic absorption coefficient. α is the equivalent constant in the expression, using base e as the natural absorption coefficient. C is the concentration of the absorber. d is the depth of the absorber through which the light beam passed. The absorbance is defined as:

$$A = -\log_{10} \frac{I_t}{I_0} = \alpha Cd \quad (5-2)$$

Based on Equation (5-2), the change in the absorbance that was observed in Figure 5.4 had a linear relationship with the water concentration and the SDS membrane thickness. The decrease in the absorbance was attributed to the water loss and the decrease in the SDS membrane thickness.

The absorbance of the O–H stretching vibration of SDS without 1-dodecanol

sharply decreased to zero within 20 s. The absorbance became zero because of the collapse of SDS membrane. The result shows that the water loss and the decrease in the SDS membrane thickness were very fast. However, the decrease in the absorbance slightly slowed down when 0.005 M 1-dodecanol was added, but dramatically slowed down when 0.01 M 1-dodecanol was added. This result is possibly due to the water evaporation rate, which slowed down with the addition of 1-dodecanol. According to the previous report, the vapor pressure of water containing 0.11 M SDS was 3.169 KPa, whereas the vapor pressure of pure 1-dodecanol was 0.003 KPa.¹⁸

According to Raoult's law, once the components in the solution reach equilibrium, the total vapor pressure p of the solution is:

$$p = p_A^*x_A + p_B^*x_B \quad (5-3)$$

Where p_A^* and p_B^* are the vapor pressures of pure components A and B , respectively. x_A and x_B are the mole fractions of components A and B in the mixture solution, respectively. The vapor pressure of 0.01 M 1-dodecanol in 0.11 M SDS solution calculated using Equation (5-3) was 3.168 KPa. Only a slight decrease in vapor pressure was induced by adding 1-dodecanol. The small decrease in vapor pressure was not the main reason for the retarded absorbance observed in Figure 5.4.

As reported by James-Smith et al., a 23% increase in the amount of SDS that partitioned to the hexadecane/water interface when dodecanol was added to the hexadecane as a co-surfactant was compared with the case in which no dodecanol was added. Dodecanol significantly enhanced SDS adsorption at the interface because it interacted with SDS micelles, which resulted in more SDS molecules to go to the surface.¹⁹ A model was used to describe that the formation of the packed SDS monolayer, which was induced by 1-dodecanol, as shown in Figure 5.5. Figure 5.5 (a) shows that the SDS monolayer adsorbed on the membrane surface and micelles formed inside the membrane. The adsorption of 1-dodecanol, which is insoluble, was favored on the surface. The SDS micelles were dragged to the surface by the interaction between the SDS headgroup and the 1-dodecanol headgroup, which

resulted in the increased adsorption of SDS molecules on the membrane surface and the formation of packed monolayers on the surface (Figure 5.5 (b)).

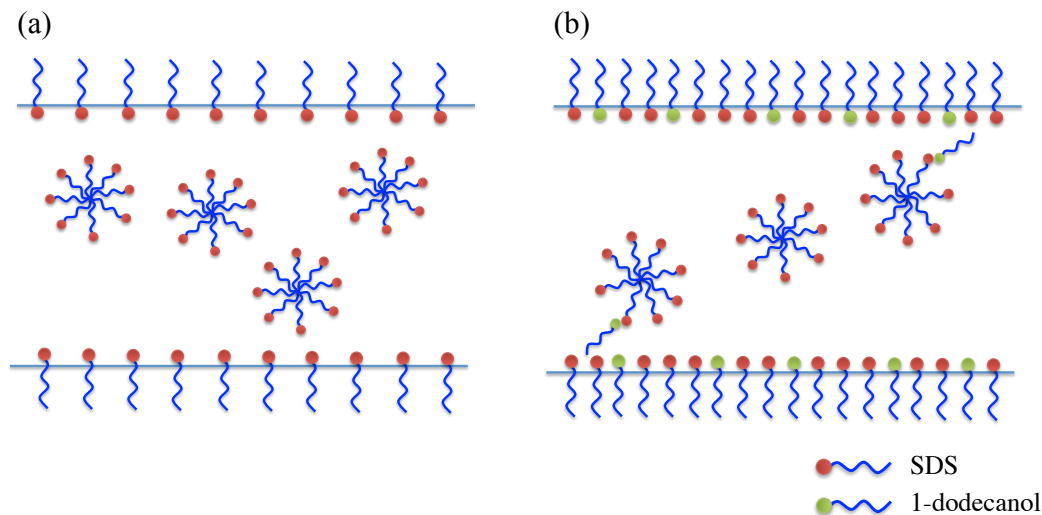


Figure 5.5 Model of SDS membrane (a) without 1-dodecanol and (b) with 0.01 M 1-dodecanol.

In the present experiment, the SDS concentration was 0.11 M, which was higher than the CMC of SDS (0.0082 M at 25 °C). SDS micelles already formed in the solution. Thus, the decrease in the absorbance dramatically slowed by adding 1-dodecanol obtained in Figure 5.4. This result is possibly caused by the increase in SDS molecules adsorbed on the membrane surface and the formation of packed SDS monolayers, which slowed water evaporation.

The SDS membrane thickness in Figure 5.4 was calculated using the equation:²⁰

$$A(\nu) = \log_{10} \frac{I_t(\nu)}{I_0(\nu)} = \frac{4\pi}{2.3} \nu k(\nu) d \quad (5-4)$$

Where $A(\nu)$ is the absorbance at wavenumber ν , $k(\nu)$ is the absorption coefficient, and d is the membrane thickness. Figure 5.6 shows the time-dependent change in the thickness of SDS without 1-dodecanol (opened triangle), SDS with 0.005 M 1-dodecanol (opened star), and SDS with 0.01 M 1-dodecanol (opened circle).

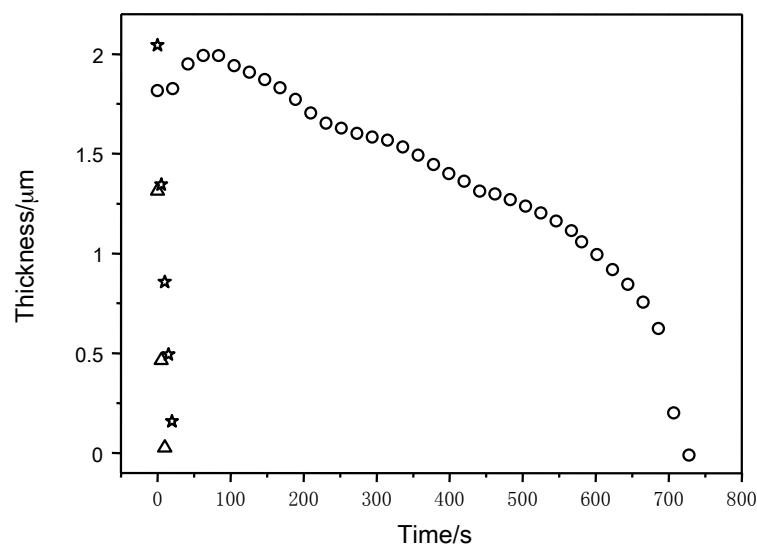


Figure 5.6 Time-dependent change in the thickness of the SDS membrane, which contained only SDS without 1-dodecanol (opened triangle), SDS with 0.005 M 1-dodecanol (opened star), and SDS with 0.01 M 1-dodecanol (opened circle).

The opened triangle in Figure 5.6 shows that the SDS membrane thickness abruptly decreased from 1.3 μm to 0 μm . However, the decrease in this thickness slightly slowed when 0.005 M 1-dodecanol was added, as shown in the opened star. The opened circle shows that the SDS membrane thickness initially increased from 1.8 μm to 2 μm (from 0 s to 63 s), and then gradually decreased to 0 μm when 0.01 M 1-dodecanol was added. The increase in thickness is possibly caused by the gravity effect, whereas the decrease is possibly caused by water evaporation, as shown in Figure 5.7. Figure 5.7 (a) shows the SDS membrane thickness (including SDS and 0.01 M 1-dodecanol) immediately after the membrane formed. The IR beam passed through the center of the membrane. The thickness was uniform at this state. However, the shape of the SDS membrane changed because of gravity, which caused the thickness of the center of SDS membrane to increase slightly, as shown in Figure 5.7 (b). As reported by Josiah Willard Gibbs, the higher regions of a vertical soap film are thinner than the lower regions because the cross-section of the film is wedge-shaped.²¹ Thus, the soap film became increasingly thinner because of water evaporation, as shown in Figure 5.7 (c).

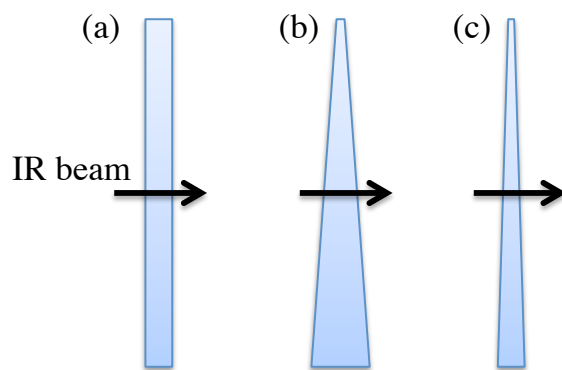


Figure 5.7 Schematic image of the time-dependent change in thickness within the SDS membrane that contained 0.01 M 1-dodecanol (a) immediately after the SDS membrane formed, (b) until 63 s, and (c) after 63 s. The IR beam always passes through the center of the SDS membrane.

The closed square in Figure 5.8 (a) shows the peak frequency of O–H stretching vibration at around 3400 cm^{-1} of SDS membrane, SDS with 0.005 M 1-dodecanol, and SDS with 0.01 M 1-dodecanol. Almost no shift was observed depending on the 1-dodecanol concentration.

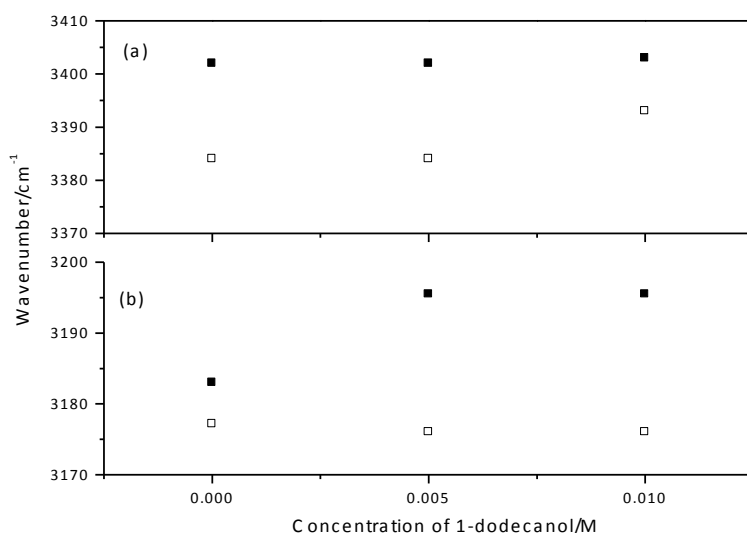


Figure 5.8 Peak frequency of O–H stretching vibration at around (a) 3400 cm^{-1} and (b) 3170 cm^{-1} of the SDS membrane depending on 1-dodecanol concentration. Closed square is the frequency observed at t_0 , and opened square is the frequency observed at the time immediately before the SDS membrane collapsed. The times before the SDS membrane broke for each sample, which contained 0, 0.005, and 0.01 M 1-dodecanol, were 10, 20, and 686 s, respectively.

The closed square in Figure 5.8 (b) shows the peak frequency of the O–H

stretching vibration at around 3170 cm^{-1} of SDS membrane, SDS with 0.005 M 1-dodecanol, and SDS with 0.01 M 1-dodecanol. A blue shift was observed when 1-dodecanol was added. These results suggest that the water structure became less ordered because of the introduction of 1-dodecanol.²²⁻²⁴ The opened squares in Figures 5.8 (a) and (b) were the frequencies observed immediately before the SDS membrane collapsed. Both the peaks at around 3400 and 3170 cm^{-1} shifted to lower wavenumbers before the membrane collapsed, which suggests that the water structure became more ordered at this state.

(2) C–H stretching vibration

To determine the change in the SDS concentration within the SDS membrane, Equation (5-2) can be written as follows:

$$\frac{A_{CH}}{A_{OH}} = \frac{\alpha_{CH} C_{CH} d}{\alpha_{OH} C_{OH} d} \quad (5-5)$$

Where α_{CH} and α_{OH} are the absorption coefficients of SDS molecule and water, respectively. C_{CH} and C_{OH} are the concentrations of SDS molecule and water, respectively. d is the SDS membrane thickness. The time-dependent SDS concentration can be plotted by the absorbance ratio, as shown in Figure 5.9(a).

Figure 5.9 (a) shows the time-dependent ratio between the absorbance of asymmetric C–H stretching of the methylene group and asymmetric O–H stretching of the SDS membrane, which contained 0.01 M 1-dodecanol. The ratio showed almost no change until 600 s, which indicates that the SDS concentration within the SDS membrane was almost the same against time. The ratio increased after 600 s, which was possibly caused by the increase in the SDS concentration. This increased ratio may also be caused by the orientation change in the SDS molecule of the SDS membrane.

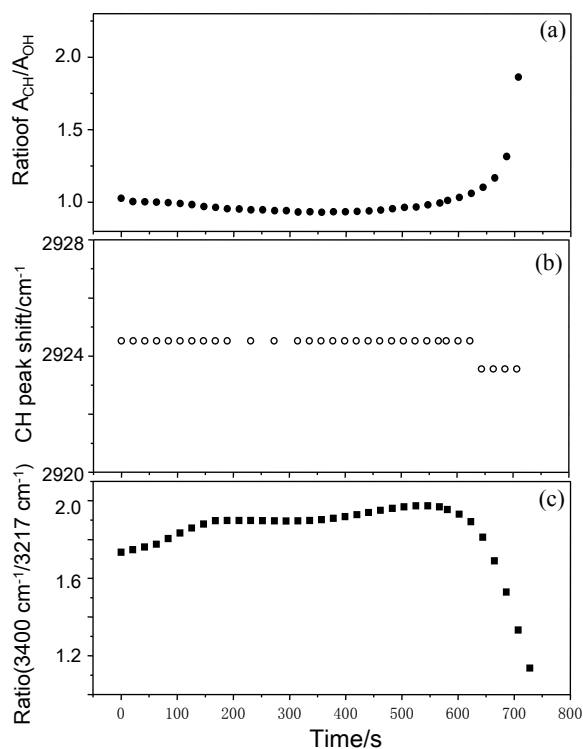


Figure 5.9 Time dependence of (a) the absorbance ratio of asymmetric stretching of CH₂ and asymmetric OH stretching and (b) the peak frequency of CH₂ (as) and (c) the ratio of O–H stretching vibration (3400 cm⁻¹/3217 cm⁻¹) of SDS membrane, which contained 0.01 M 1-dodecanol.

A model used to explain the effect of interfacial molecular structure changes for the FTIR absorbance is shown in Figure 5.10.

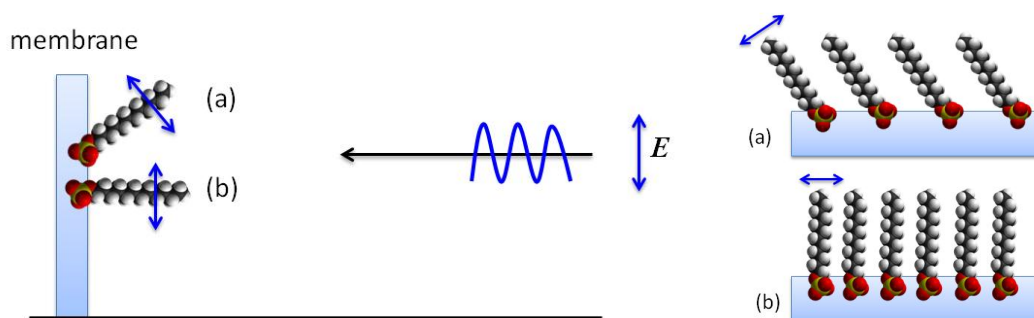


Figure 5.10 Schematic image of electric field vector and transition dipole moment of molecule. (a) and (b) show the adsorbed molecule in different orientations.

The electric field of the IR beam was parallel to the membrane surface, as shown in Figure 5.10. The adsorbed SDS molecule had a long alkyl chain, which consisted of CH₂ groups pointed out from the membrane surface. The CH₂ groups had a transitional dipole moment oriented perpendicular to the axis of the alkyl chain, as shown in Figure 5.10.

According to Fermi's golden rule, the integral intensity A of the absorption band of the normal mode is proportional to the probability per unit time of the transition between an initial state i and a final state j , as shown below:

$$A \propto E^2 |\langle j | \mathbf{p} | i \rangle|^2 \cos^2 \theta \quad (5-6)$$

where $|\langle j | \mathbf{p} | i \rangle|$ is the transitional dipole moment and θ is the angle between vectors $\mathbf{E}$ and $\mathbf{p}$. Maximum absorption is observed when $\mathbf{E}$ is parallel to the transition dipole moment, as shown in Figure 5.10(b). In Figure 5.10(b), a stronger absorbance of a perpendicularly oriented molecule can be observed compared with (a).

Figures 5.9 (b) and (c) show that the orientation of molecules changed before the membrane collapsed. Figure 5.9 (b) shows the time dependence of CH₂ (as) peak frequency of SDS membrane, which contained 0.01 M 1-dodecanol. The result shows that the C–H stretching vibration shifted to a lower wavenumber after 600 s. Christopher E. D. Chidsey et al. reported that the peak position for the CH₂ (as) mode of a crystalline polymethylene chain (2920 cm⁻¹) is 8 cm⁻¹ lower than that for the liquid state (2928 cm⁻¹), and the shift in peak frequency to a lower wavenumber indicates the change from solid phase to liquid phase.²⁵ The red shift in Figure 5.9 (b) indicates that the SDS molecule was in a more ordered structure.^{26,27} Thus, the increase in the ratio observed in Figure 5.9 (a) can be explained by the increase in concentration and the orientation change in SDS molecule.

Figure 5.9 (c) shows the time-dependent ratio of the O–H stretching vibration of 3400/3217 cm⁻¹. The ratio was almost constant until the time reached around 600 s, and the ratio subsequently decreased after 600 s. The decrease in the ratio was suggested to be attributed to the O–H stretching vibration from 3217 cm⁻¹, which

corresponds to the increased strength of the well-ordered water structure.¹³⁻¹⁵ Thus, the water structure became more ordered after 600 s. In addition, a more ordered SDS molecule structure, which was observed immediately before SDS membrane collapse, was due to the improved arrangement of water molecules.

5-2-2-3 Discussion

The structure of the SDS membrane can be discussed using a model, as shown in Figure 5.11. Figure 5.11 (a) shows the structures of water and SDS molecule at the time immediately after the membrane formed. The hydrophilic headgroups of SDS and 1-dodecanol molecules were adsorbed on the membrane surface, whereas the hydrophobic tails were directed outward. The water molecules that were near the membrane surface had a more ordered structure, whereas the water molecules that were present in the middle of the membrane were more randomly oriented.

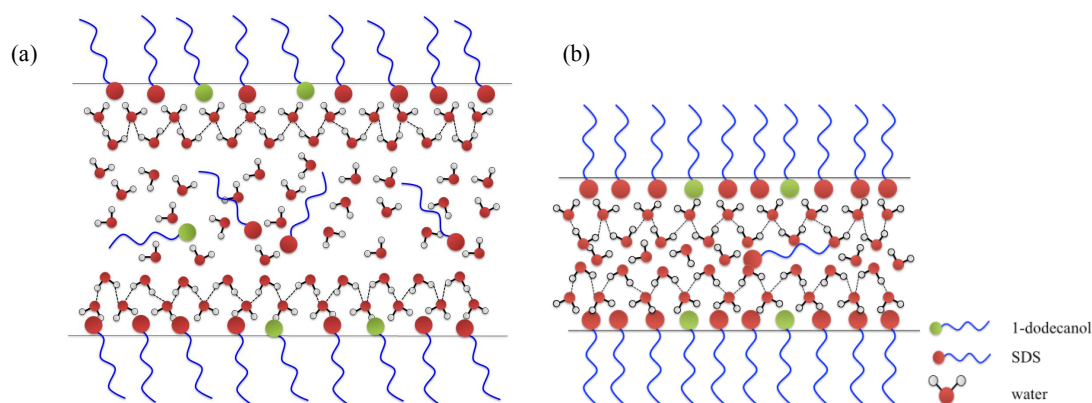


Figure 5.11 Model of the structure of SDS membrane (a) immediately after the membrane formed (t_0) and (b) immediately before membrane collapse.

Given the loss of water and the decrease in thickness of the SDS membrane, a very thin SDS membrane was observed immediately before membrane collapse, as shown in Figure 5.11 (b). Fewer water molecules were found in the middle of the SDS membrane because of the decrease in membrane thickness and amount of water. However, the proportion of ordered water molecules increased, which suggests that the structure of water became more ordered on the membrane surface. This ordered water structure may support the order of SDS molecules.

5-2-3 Effect of Trehalose on the Stability of SDS Membrane

5-2-3-1 Concentration Dependence for the Lifetime

The opened circle in Figure 5.12 shows the stability of the 0.11 M SDS membrane depending on the 1-dodecanol concentration. The results show that the SDS membrane collapsed after 15 s, whereas the lifetime of this SDS membrane dramatically increased to 1400 s when 0.01 M 1-dodecanol was added. The effect of 1-dodecanol on the lifetime of the SDS membrane was discussed in 5-2-2.

The closed square in Figure 5.12 shows the stability of SDS membrane, which contained 0.11 M SDS without 1-dodecanol, depending on the trehalose concentration. The results shows that the SDS membrane collapsed after 15 s, whereas the lifetime of this SDS membrane increased when trehalose was introduced.

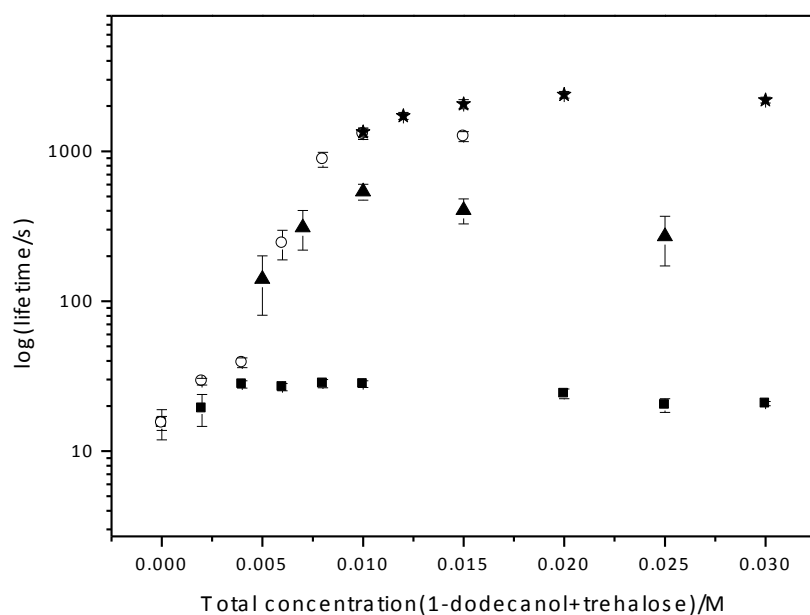


Figure 5.12 Stability of SDS membranes, which contained SDS (0.11 M) without 1-dodecanol (closed square), 0.005 M 1-dodecanol (closed triangle), and 0.01 M 1-dodecanol (closed star) depending on the trehalose concentration. Opened circle shows the stability of the 0.11 M SDS depending on the 1-dodecanol concentration.

Figure 5.12 shows the stability of the SDS membrane, which contained SDS (0.11 M) and 1-dodecanol (0.005 M in closed triangle and 0.01 M in closed star) depending on the trehalose concentration. The results show that the SDS membrane

collapsed after 150 s when 0.005 M 1-dodecanol was included. In addition, the lifetime of this SDS membrane increased when trehalose was introduced. Similar results were observed when the 1-dodecanol concentration increased to 0.01 M. The SDS membrane collapsed after 1350 s, and a longer lifetime of this SDS membrane was obtained when trehalose was added. The results also show that trehalose was effective in stabilizing the SDS membrane.

5-2-3-2 Time-dependent FTIR Measurement

To clarify the effect of trehalose on stabilizing the SDS membrane, time-dependent FTIR measurements were performed, as shown in Figure 5.13.

Figures 5.13 (a) and (b) show the time-dependent FTIR spectra of SDS membrane with 0.005 M 1-dodecanol and various trehalose concentrations (0.005 M in (a), 0.01 M in (b)). The first spectrum was obtained at the time immediately before the membrane formed (t_0). One spectrum was obtained in 5 s. The absorbance gradually decreased and became zero because of SDS membrane collapse.

One peak at 1645 cm^{-1} was observed because of the O–H bending of water molecules. A strong O–H peak at 3400 cm^{-1} and a relatively weak shoulder at 3217 cm^{-1} were observed in the O–H stretching region. Based on previous studies, the band at 3400 cm^{-1} was attributed to randomly oriented, asymmetric O–H stretching vibration, whereas the band at 3217 cm^{-1} corresponded to the well-ordered, tetrahedrally coordinated, symmetric O–H stretching mode of water.¹³⁻¹⁵ Two weak peaks at 2855 and 2926 cm^{-1} were observed, as shown in the inset figure of the expanded C–H stretching region, which can be assigned to $\text{CH}_2(\text{s})$ and $\text{CH}_2(\text{as})$, respectively.^{11,16,17} These C–H peaks originated from the SDS and 1-dodecanol molecules within the SDS membrane.

The time-dependent FTIR spectra of SDS membrane with 0.01 M 1-dodecanol and various trehalose concentrations (0.005 M in (c) and 0.01 M in (d)) are shown in Figures 5.13 (c) and (d), respectively.

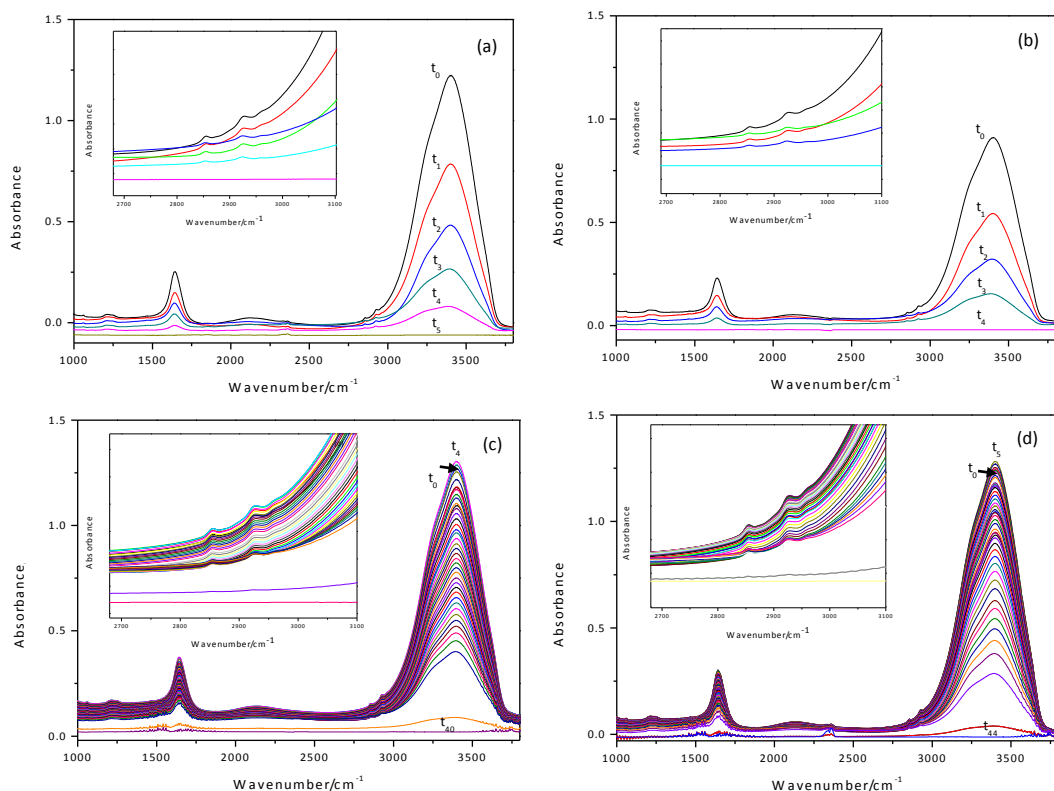


Figure 5.13 Time-dependent FTIR spectra of SDS membrane with 0.005 M 1-dodecanol ((a) and (b)) and SDS membrane with 0.01 M 1-dodecanol ((c) and (d)), and various trehalose concentrations. The trehalose concentration used in the experiment were 0.005 M ((a) and (c)) and 0.01 M ((b) and (d)). Each spectrum in (a) and (b) is the average of 4 scans, which took 5 s. The first spectrum was obtained at t_0 in (a) and (b), and the final spectrum was obtained at t_5 in (a) and t_4 in (b), respectively. Each spectrum in (c) and (d) is the average of 16 scans, which took 20 s. The first spectrum was obtained at t_0 in (c) and (d). A slight increase in absorbance was obtained from t_0 to t_4 in (c) and t_0 to t_5 in (d). A gradual decrease in absorbance of the subsequent spectra was obtained from t_4 to t_{40} in (c) and t_5 to t_{44} in (d), respectively. Insert spectrum is the expanded C-H stretching region.

(1) O-H stretching vibration

The opened symbols in Figure 5.14 (a) show the time dependence of absorbance change of the O-H stretching vibration at 3400 cm^{-1} of the SDS bubble, which contained SDS (0.11 M) and 0.005 M 1-dodecanol and the trehalose concentration from 0 M to 0.01 M. To observe these absorbance changes depending on trehalose concentration, the x-axis in (a) was changed to $\log(\text{time/s})$, as shown in the opened symbols in Figure 5.14 (b).

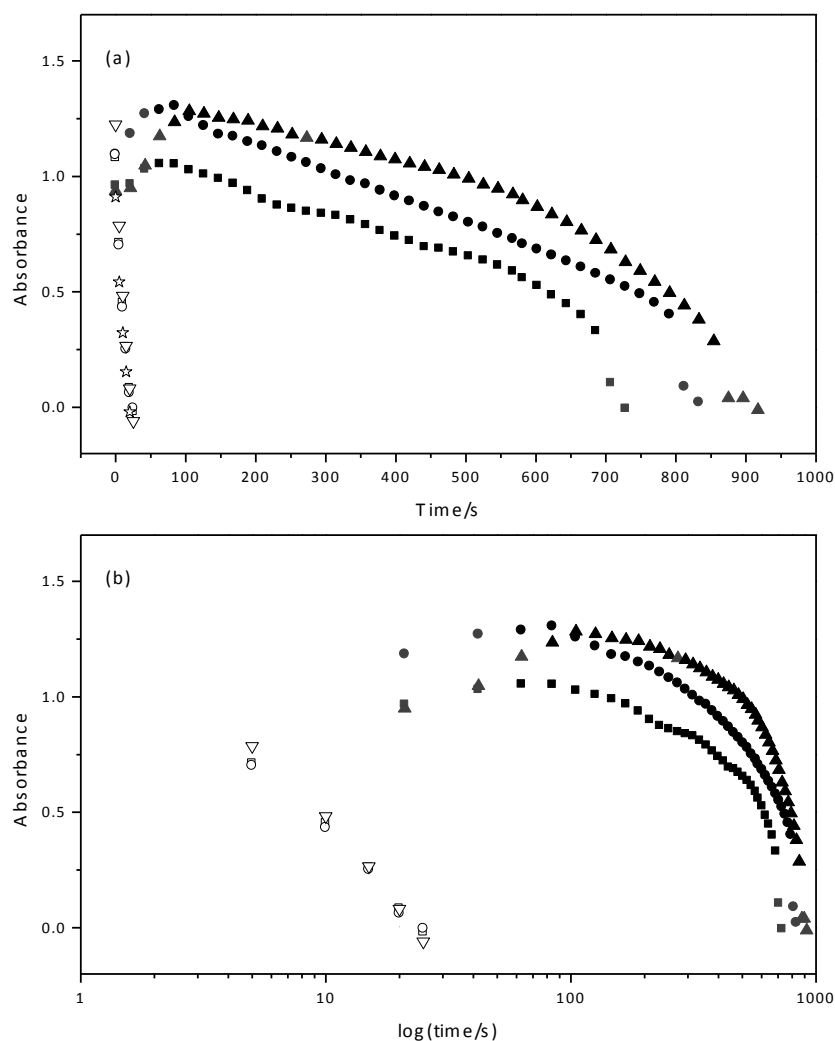


Figure 5.14 (a) Time-dependent absorbance changes in OH stretching vibration at 3400 cm⁻¹ of SDS membrane, which contained SDS with 0.05 (opened symbol) and 0.01 M (closed symbol) 1-dodecanol and various trehalose concentrations. The trehalose concentrations used in (a) were 0 (closed and opened square), 0.005 (closed and opened circle), and 0.01 M (closed and opened triangle). (b) Change from x-axis (time/s) in (a) to log(time/s).

The opened square in Figure 5.14 (b) shows that the absorbance of the O–H stretching vibration of SDS membrane (0.11 M SDS and 0.005 M 1-dodecanol) without trehalose decreased to zero after 25 s because of the SDS membrane collapse. The time-dependent absorbance change was almost the same with or without trehalose, as shown in the opened symbols in Figure 5.14 (b). This effect may be caused by the sudden decrease in absorbance. Thus, providing detailed information is difficult. To understand whether trehalose was effective in stabilizing the SDS

membrane, 0.01 M 1-dodecanol was added to obtain a longer lifetime.

The closed symbols in Figure 5.14 (a) show the time-dependent absorbance change in the O–H stretching vibration at 3400 cm^{-1} of the SDS membrane, which contained 0.11 M SDS and 0.01 M 1-dodecanol, and the trehalose concentration ranged from 0 M to 0.01 M. The closed square shows that the absorbance of the O–H stretching vibration of SDS membrane (0.11 M SDS and 0.01 M 1-dodecanol) without trehalose decreased to zero after 728 s because of the SDS membrane collapse. The time-dependent decrease in absorbance resulted in no significant change when trehalose was added; however, the lifetime increased. As studied in Chapter 4, the effect of trehalose on stabilizing the lipid bilayer in dry state was due to the volumetric effect.²⁸⁻³⁰ Trehalose can separate lipid molecules by insertion into the headgroup of lipid molecules in dry state, which can effectively reduce the packing defect and stabilize the bilayer structure. In this study, the stability of SDS membrane increased when trehalose (0.005 M to 0.01 M) was added. Based on the discussion in Chapter 4, the effect of trehalose in stabilizing the SDS membrane is possibly caused by the insertion into the SDS molecule headgroup, which can effectively avoid SDS molecule packing and improve stability.

The time-dependent water evaporation was dominated by 1-dodecanol after the SDS membrane formed, whereas the effectiveness of trehalose became more apparent immediately before the membrane collapsed.

The closed square in Figure 5.15 (a) shows the peak frequency of O–H stretching vibration at around 3400 cm^{-1} of the SDS membrane with 0.11 M SDS, 0.01 M 1-dodecanol, and trehalose (0, 0.005, and 0.01 M). Almost no shift was observed depending on the trehalose concentration.

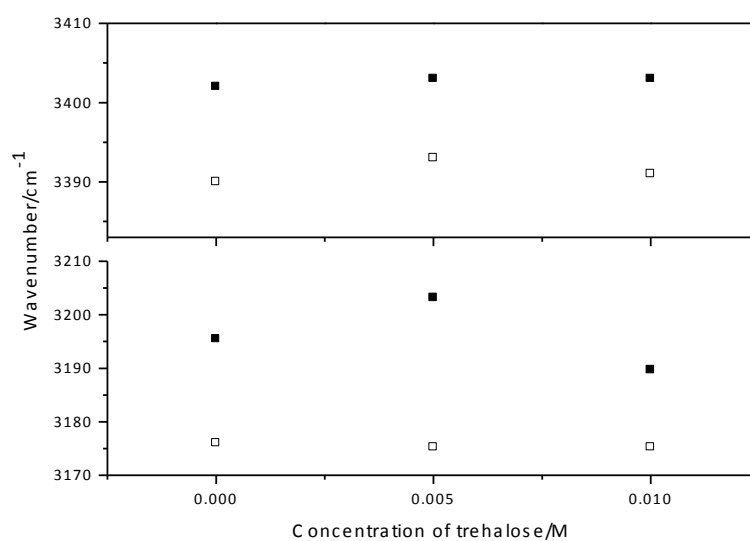


Figure 5.15 The peak frequency of OH stretching vibration at around (a) 3400 cm^{-1} and (b) 3170 cm^{-1} of SDS membrane which contained 0.11 M SDS and 0.01 M 1-dodecanol depending on trehalose concentration. Closed square is the frequency observed at t_0 and opened square is the frequency observed at the time just before SDS membrane broken. Here, the time just before SDS membrane collapse for each membrane which contained 0, 0.005 and 0.01 trehalose were 686, 812 and 896.

The closed square in Figure 5.15 (b) shows the peak frequency of O–H stretching vibration at around 3170 cm^{-1} of SDS membrane with 0.11 M SDS, 0.01 M 1-dodecanol, and trehalose (0, 0.005, and 0.01 M). A blue shift was observed when 0.005 M trehalose was added, which suggests that the water structure became less ordered. However, a red shift appeared when the trehalose concentration further increased to 0.01 M, which suggests the presence of water in a more ordered structure. Based on the previous discussion, when the water structure became ordered, the SDS membrane thickness decreased. A relatively thinner SDS membrane was observed when the trehalose concentration increased.

The opened squares in Figures 5.15 (a) and (b) were the frequencies observed immediately before the SDS membrane collapsed. Both the peaks at around 3400 and 3170 cm^{-1} shifted to a lower wavenumber before the membrane collapsed, which suggests that the water structure became more ordered.

(2) C–H stretching vibration

Figure 5.16 (a) shows the time-dependent absorbance ratio between asymmetric C–H stretching of CH₂ (as) and asymmetric OH stretching of SDS membrane, which contained SDS, 0.01 M 1-dodecanol, and various trehalose concentrations (0 M in opened triangle, 0.005 M in opened square, and 0.01 M in opened circle). Similar results were obtained, as discussed in Figure 5.9. The ratio was almost constant until around 700, 800, and 500 s for each sample, respectively, followed by a subsequent increase, which suggests that the SDS molecule concentration possibly increased before membrane collapse. This increase in ratio may be due to the structural change in SDS molecule, as previously discussed. Figure 5.16 (b) shows the time-dependent peak frequency of CH₂ (as) of the SDS membrane, which contained SDS, 0.01 M 1-dodecanol, and various trehalose concentrations (0 M in opened triangle, 0.005 M in opened square, and 0.01 M in opened circle). Results show that no shift was observed until around 700, 800, and 500 s for each sample, respectively, whereas CH₂ (as) shifted to a lower wavenumber before membrane collapse. Thus, the ratio in Figure 5.16 (a) increased because of the increase in concentration and the more ordered structure of SDS molecule. Figure 5.16 (c) shows the time-dependent ratio of the OH stretching vibration of 3400/3217 cm⁻¹. The ratio was almost constant for each sample, but decreased after 700, 800, and 500 s, which suggests that the water structure became more ordered. Thus, a more ordered SDS molecule structure, which was observed before SDS membrane collapse, was caused by the improved arrangement of water molecules.

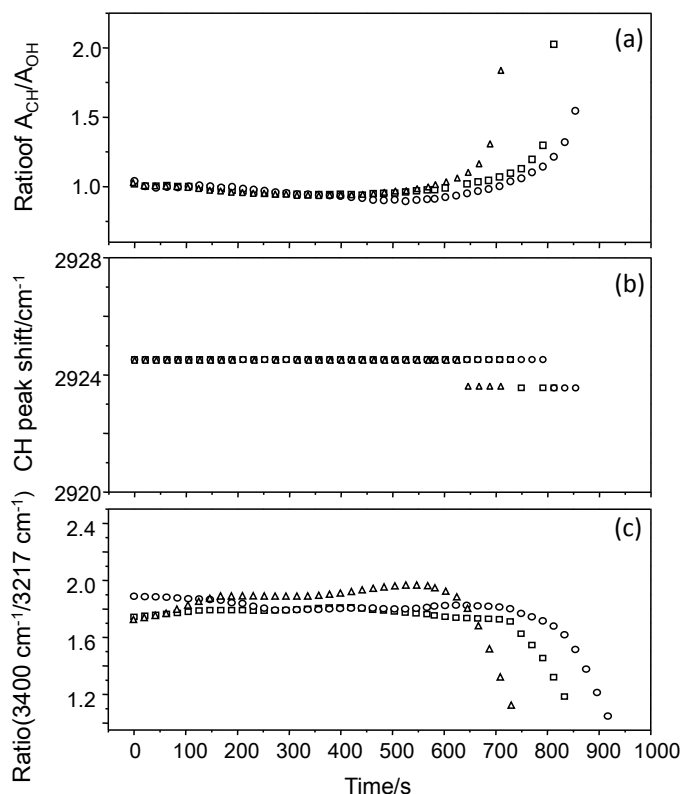


Figure 5.16 Time dependence of (a) the absorbance ratio of asymmetric stretching of CH_2 and asymmetric OH stretching and (b) the peak frequency of CH_2 (as) and (c) the ratio of O–H stretching vibration ($3400\text{ cm}^{-1}/3217\text{ cm}^{-1}$) of SDS membrane, which contained SDS and 0.01 M 1-dodecanol and various concentration of trehalose. The concentrations of trehalose were as follows: 0.005 M (opened square in (a), (b) and (c)), 0.01 M (opened circle in (a), (b) and (c)) and 0.02 M (opened triangle in (a), (b) and (c)).

5-2-3-3 Discussion

The molecular structure of the SDS membrane with trehalose had no significant difference than that without trehalose. Trehalose did not affect the molecular structure, but increased the SDS membrane lifetime. As studied in Chapter 4, trehalose inserted into the lipid bilayer headgroup and stabilized the bilayer structure via the volumetric effect. In this study, trehalose was possibly inserted into the SDS molecule headgroup, which could effectively avoid SDS molecule packing and stabilize the SDS membrane.

The structure of the SDS membrane and the effect of trehalose on the stability of SDS membrane can be discussed using a model, as shown in Figure 5.17.

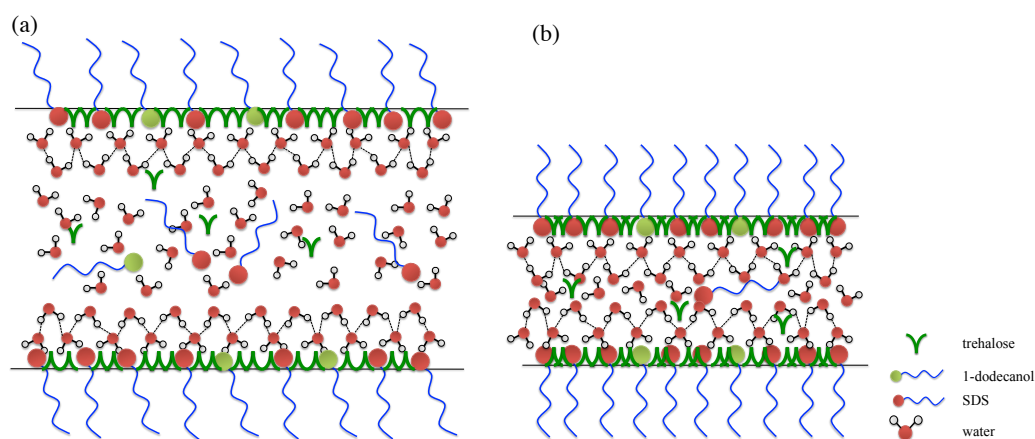


Figure 5.17 Model of the structure of SDS membrane at (a) immediately after the membrane formed (t_0) and (b) immediately before membrane collapse.

Figure 5.17 (a) shows the structures of water and SDS molecule immediately after the membrane was formed. The hydrophilic headgroups of SDS and 1-dodecanol molecules were adsorbed on the membrane surface, whereas the hydrophobic tails were directed outward. The water molecules that were near the surface of the membrane were in a more ordered structure. By contrast, the water molecules that were present in the middle of the membrane were more randomly oriented. When trehalose was inserted into the SDS molecule headgroup, no obvious affect was observed since the majority of water molecules were present at this state. Given the water loss and reduced thickness in the SDS membrane, a very thin SDS membrane was observed immediately before membrane collapse, as shown in Figure 5.17 (b). The ordered and packed molecule layer was adsorbed on the surface, and trehalose inserted in between may stabilize the SDS membrane via the volumetric effect. The effect of trehalose became obvious at this state.

5-3 Conclusion

The stability of SDS membrane under different concentrations of 1-dodecanol and trehalose was studied using FTIR spectroscopy. The SDS membrane was broken

within several seconds, whereas its stability dramatically increased when 0.01 M 1-dodecanol was introduced. The effect of 1-dodecanol in stabilizing the SDS membrane was caused by the decrease in the water evaporation rate. Trehalose was also effective in stabilizing the SDS membrane. Based on the study in Chapter 4, the effect of trehalose in stabilizing the SDS membrane was possibly caused by the insertion into the SDS molecule headgroup to reduce the packing defects. This effect became more obvious when the membrane became very thin. To clarify this effect, a shorter integration time for each spectrum was used in this study. More spectra were obtained to analyze the structural changes at this state.

The time-dependent structural changes in water and SDS molecules on the membrane surface were also studied. The ratio of $3400\text{ cm}^{-1}/3217\text{ cm}^{-1}$ decreased against time before membrane collapse. This observation suggests that the water structure was more ordered. The peak frequency of the stretching vibration of CH_2 (as) shifted to a lower wavenumber, which indicates that SDS molecule had a more ordered structure before membrane collapse.

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Chapter 6

General Conclusion and Future Prospects

In the present thesis, the structures of planar supported monolayer and bilayer depending on different environment and the stability of lipid bilayer and soap membrane were studied by SFG as well as AFM and FTIR spectroscopy.

In Chapter 1, the introduction of lipid monolayer, bilayer and a freestanding soap membrane were given. The stabilization of lipid membrane in dry state was reviewed and the theory of SFG spectroscopy was introduced.

In Chapter 2, the brief description of the experimental details such as materials, experimental instruments including femtosecond and picosecond SFG, FTIR and AFM measurements and the details of sample preparation were given.

In Chapter 3, adsorption behavior of sodium stearate molecules at fused quartz/solution interface was studied by measuring SFG spectra in sodium stearate aqueous solution, followed by under Ar gas, in pure water, and under Ar gas flow again. Strong peaks due to CH stretching of CH₃ group and a small peak due to CH stretching of CH₂ group appeared under Ar gas flow, showing the presence of the stearic acid monolayer with very high conformational order. However, no SFG signal was detected when it was contact with water, showing that the structure of stearic acid monolayer was totally disordered in water. Time dependent SFG measurements showed that the adsorption of stearic acid molecules on the fused quartz surface was very fast and completed within 5 s. Furthermore, it was found that the well ordered stearic acid monolayer was formed only below critical micelle concentration (CMC).

In Chapter 4, the effect of saccharides on the stability of DMPC bilayer in dry state was studied by SFG, ATR-FTIR and AFM measurements. Results showed that while disaccharide was very effective for stabilizing DMPC bilayer domain in dry state, smaller size monosaccharide was less effective, showing that the size of

saccharide is the dominant factor for stabilizing DMPC bilayer in dry state. Hydrogen bond formation between saccharide and phospholipids headgroup was small as proved by ATR-FTIR measurement. Thus, volumetric effect was proposed to explain the mechanism of stabilization of DMPC bilayer in dry state. The difference between trehalose and maltose for stabilizing DMPC bilayer in dry state was also discussed.

In next study, it is very important to clarify the difference of trehalose and maltose on the stabilization of bilayer structure in dry state.

In Chapter 5, the stability of freestanding SDS membrane was studied by FTIR measurements. It was found that the stability of SDS membrane was increased by introducing 1-dodecanol and trehalose. The decrease of the evaporation rate of water is considered to be the origin for stabilizing SDS membrane by 1-dodecanol. The effect of trehalose was discussed based on the study in Chapter 4 that trehalose might be inserted into the headgroup of SDS molecule to reduce the packing defect, and therefore, stabilize SDS membrane. The molecular structure within SDS membrane depending on time was also studied. It was found that the water structure became more ice-like before membrane collapse, and this ordered structure of water supported for the ordering of SDS molecule in the membrane.

The structure of 1-dodecanol within SDS membrane can be studied by using deuterium 1-dodecanol which can separate FTIR signal from SDS molecule. Furthermore, the role of trehalose on the stabilization of SDS membrane will be further clarified.