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# Monolayers of Carbohydrate-Containing Lipids at the Water-Air Interface

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Additional information is available at the end of the chapter

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## Abstract

Glycolipids are important members of the glycoconjugate family that are distributed on cell surfaces and are important in aspects of cellular behavior including signal transduction, protein trafficking, cell surface recognition and cell adhesion. Errors in the synthesis or mutations of these glycoconjugates are often linked with various human pathological conditions. The complex nature of their molecular structures coupled with the complexity of cellular structure make their study a challenging process, which can be simplified by fabrication of model membrane systems. Liposomes and monolayers of lipids at the air-water interface are two of the most frequently used model membrane systems. Techniques for fabrication of monolayer models and methods used for their studies are discussed with a focus on glycolipids.

**Keywords:** glycoconjugates, glycosphingolipids, gangliosides, monolayer, membrane

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## 1. Introduction

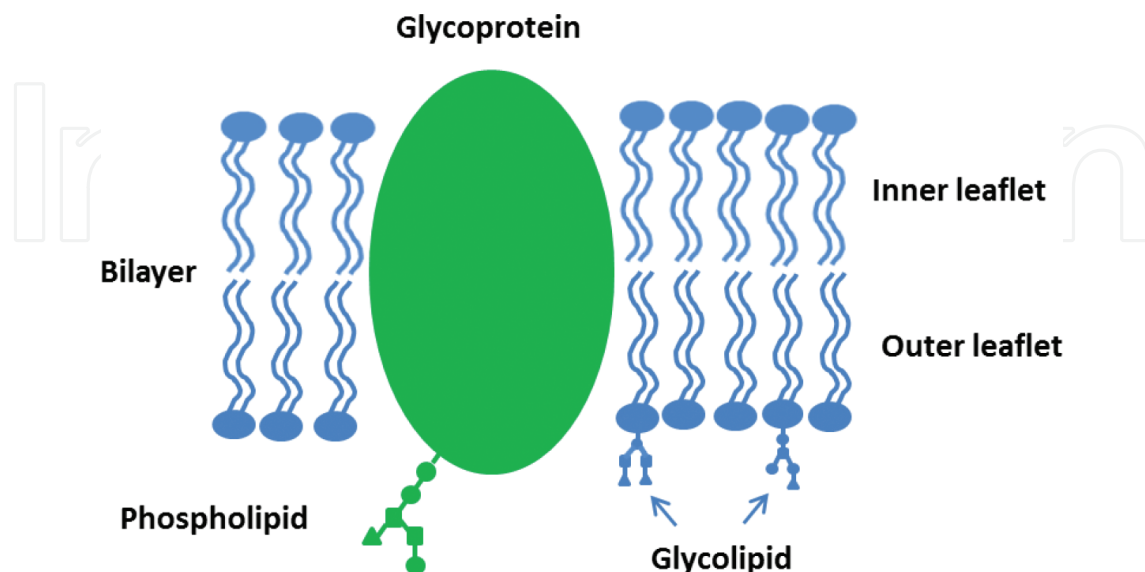
Biological membranes are the boundaries that separate interiors of cells from their external environment. The composition of biological membranes is complex; they are made of lipid bilayers [1, 2] with a wide array of components depending on the type, function and age of the cell [3]. Carbohydrates are a key structural feature of cell membranes. In cell membranes, carbohydrates are mostly found covalently attached with other biomolecules, these pairs are termed glycoconjugates. Glycoconjugates are compounds in which one or more carbohydrate units are covalently linked to a biomolecule such as a protein or a lipid [4]. Depending upon the counterparts to which the carbohydrates are linked, glycoconjugates are classified as glycoproteins, glycolipids, glycosaminoglycans or proteoglycans. These glycoconjugates

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are involved in regulating biological activities such as fertilization, host-pathogen recognition, immunity and immune response, and in cancers where changes in glycosylation are commonly observed [5].

The complex nature of biomembranes makes them challenging to study [6], see **Figure 1**. This complexity necessitated development of simpler model systems, which would mimic native membranes but at the same time would give control over parameters such as structure, composition, size and facilitate monitoring of molecules of interest [7]. The majority of model systems are tailored to incorporate the bilayer structure of biological membranes. These bilayer model systems can be arranged in two-dimensions on a solid support or can form three-dimensional spherical structures (supported or free in solution) as in liposomes [7–9]. Liposomes may be small, large, or giant in size and can have either one (unilamellar) or multiple (multilamellar) lipid bilayers. Liposomes are the subject of intense clinical interest where they are being studied as a vehicle for drug delivery [10–12]. Alternatively, one can fabricate monolayers of biomolecules as mimetic model systems. The compositions of monolayers are chosen to mimic one of the two leaflets of a biological membrane. One can then study the changes these systems would go through when they interact with external stimuli, which could be pathogens such as bacteria or viruses, proteins, or changes in environmental factors as temperature, pressure or pH. The results of such studies may then be extrapolated to natural biological membrane systems.

The lateral organization of lipids and cholesterol in cell membranes is important for cellular functions, especially cell signaling activities. Lipid rafts [13, 14], which are aggregates of sphingolipids and cholesterol, are also known to incorporate glycolipids [15] and host key cell signaling proteins such as glycosylphosphatidylinositol (GPI)-anchored proteins. Studies in which the lipid organization is both perturbed and also observed in living cells under culture conditions are challenging. Exposure to agents such as methyl  $\beta$ -cyclodextrin that perturbs the



**Figure 1.** A representation of the complex nature of cell membranes.

cholesterol content of membranes will disrupt lipid rafts, alter lipid organization, and affect cell behavior [16]. Using two-photon microscopy, liquid-ordered and liquid-disordered (raft-like) domains were observed in living cells including macrophages [17]. The removal of 9.5% of the cholesterol from live RAW264.7 cells affects the cells morphology by removing membrane protrusions, while adding more cholesterol increases the number of cell-cell contact points. The fluorescence data show a shift toward a greater population of liquid-disordered domains. Two-photon fluorescence imaging has also been used to show that cholesterol depletion in live macrophages caused disappearance of lipid rafts and that restoration of cholesterol restored the raft organization [18]. Another example concerns the culturing of hippocampal neurons, in which depletion of cholesterol resulted in a number of directly observable effects including the loss of many synapses and dendritic spines and the internalization of AMPA receptors [19]. The glycolipid ganglioside GM1 was contained within these rafts. An opportunity exists for further study of the effect of perturbation of lipid constitution on lipid organization and hence on cell behavior in living cells in culture.

This chapter will give insights into methods used for designing and studying monolayer model systems in general along with some pertinent experimental results. The primary component of the monolayer chosen for discussion here are glycolipids. However, the methods and theories described are not limited to glycolipids or biological systems and in principle can be adapted to many other interfacial systems.

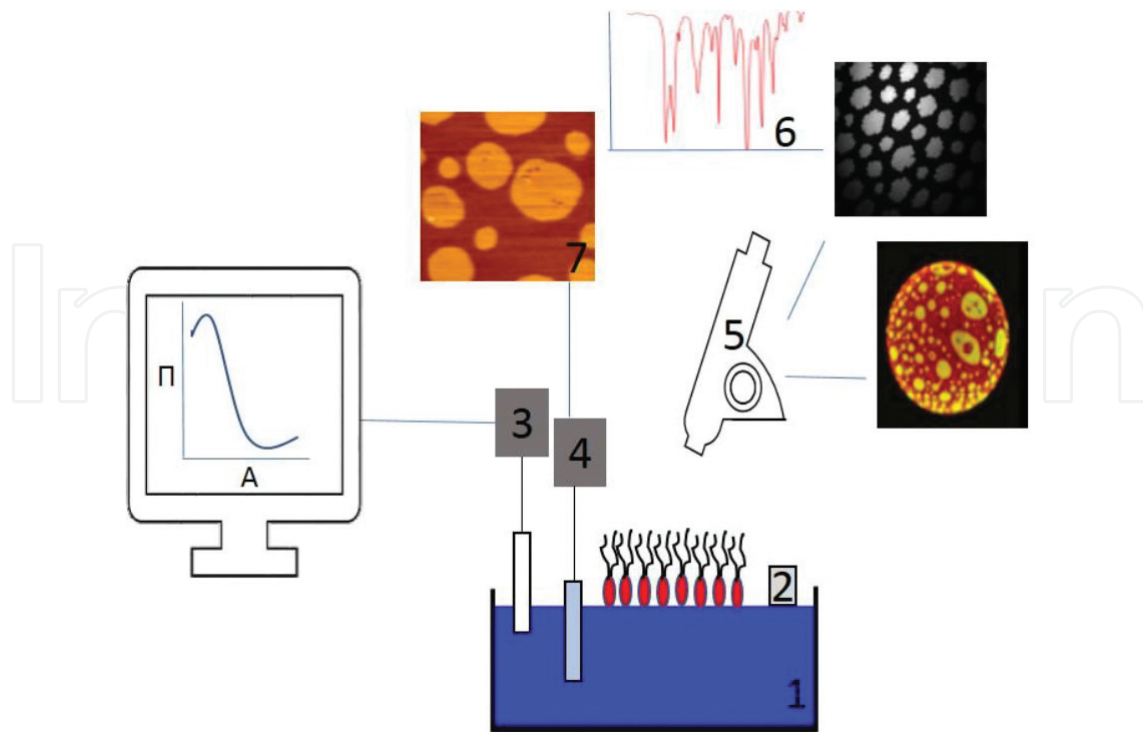
## 2. Fabrication of monolayer of lipids as membrane models

Lipids are amphiphilic molecules consisting of a hydrophilic head-group and a hydrophobic tail made of one or more hydrocarbon chains that may be saturated or unsaturated.

Monolayers are assembled by depositing droplets of lipid solution onto the water surface and subsequently waiting for the solvent to evaporate. The molecules spread out while the solvent evaporates. An example of a good spreading solvent is chloroform, although not all lipids are soluble in chloroform and sometimes mixed solvents with an alcohol must be used. Once deposited on the water surface, the polar or charged head-group orients towards the water surface and the hydrophobic tail(s) aligns away from the water. The lipid molecules get spread uniformly over the water surface forming a monomolecular thick film called a Langmuir monolayer named after Irving Langmuir [20], who pioneered this technique together with Katharine B. Blodgett.

Selection of solvent is critical for uniform spreading of monolayer. An ideal solvent should be volatile, chemically inert, relatively pure and with enough solubilization power to dissolve the solutes under study. Care must also be taken to make sure that the solvents are insoluble in the subphase [21]. Chloroform, cyclohexane, benzene, hexane, and mixtures with acetone, ethanol or methanol are some commonly used solvents. Water or buffer solutions of various composition and pH are used as the subphase.

The depositions are carried out in a Langmuir-Blodgett (LB) trough, depicted in **Figure 2** where some of the main monolayer techniques are also schematically depicted. The basic

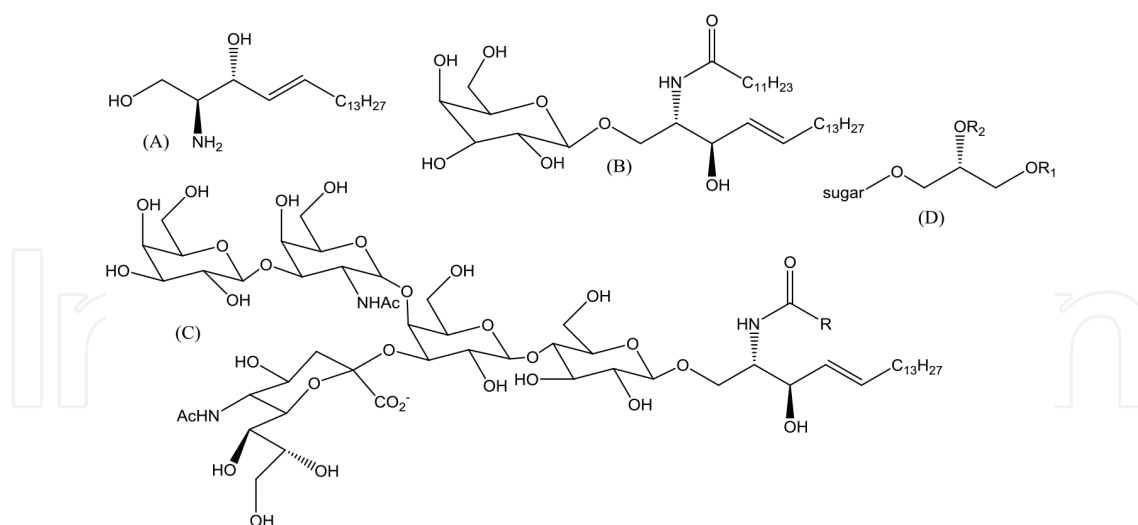


**Figure 2.** Schematics of Langmuir-Blodgett trough along with some associated measurement techniques. (1) Trough with subphase and deposited monolayer, (2) side view of barrier, (3) surface pressure transducer with Wilhelmy plate, (4) dipping system with a solid support, (5) microscopic measurements (BAM, fluorescence), (6) spectroscopic measurements, and (7) AFM image of transferred monolayer on solid support.

components of the LB trough are a Teflon trough which holds the subphase, a barrier which helps compress the spread monolayer to a targeted area or surface pressure at specified compression rates, a surface pressure transducer for measurement of surface pressure and a dipper which helps in transferring the monolayer film onto a solid substrate. Some details on the mechanism of transfer will be discussed later in the chapter. The trough can be accessorized with temperature, pH and surface potential sensors. It can also be coupled with optical and spectroscopic instruments such as a fluorescence microscope, a Brewster angle microscope or an infrared spectrometer which help in visualization and characterization of the monolayers.

### 3. Biological significance of glycolipids

The notions of how biomolecules are arranged in membranes have been continuously evolving. If one is to attempt building a timeline depicting major events in the development of biological membrane models, one could divide it into three periods: pre Singer-Nicolson, Singer-Nicolson and post Singer-Nicolson [22]. Among the various models proposed, two that stand out are fluid-mosaic [23] and lipid raft [24]. The fluid-mosaic model proposed by Singer and Nicolson pictured the membrane as a lipid bilayer, predominately of phospholipids, embedded within which were transmembrane proteins. The texture of the matrix was hypothesized to be like a viscous fluid which would allow the translational diffusion of the embedded proteins. Although



**Figure 3.** Structures of (a) D-erythro-sphingosine, (b) N-dodecanoyl-β-D-galactosylceramide, (c) ganglioside GM1, and (d) a glycolipid.

thoughtful considerations were taken to address thermodynamic limitations and attempts were made to correlate the experimental evidence with the proposed model, there were some anomalies hinting at the presence of regions in the membrane where the lipids would behave differently, i.e. were different in composition and/or phase. To explain these discrepancies a new hypothesis was proposed which suggested that certain lipids within the cell membrane have unique properties which would allow them to self-associate and form segregated regions which were named “lipid rafts”. Originally it was proposed that these rafts were made of sphingolipids and cholesterol and functioned as platforms for trafficking proteins; however, it was later found that there was more to lipid rafts than trafficking of proteins [25].

Glycolipids are an important group of raft forming lipids. Their ability to aggregate together to form microdomains indicates their involvement in various cellular activities. Glycolipids can broadly be divided into two major categories-glycosphingolipids (GSLs) and glycolipid, the first one being widely present in animal cells and the latter in plant and microbial cells with an exception of sulfated glycolipids called seminolipids which are found in mammalian testis [26]. The major difference between these two classes of glycolipids is their lipid moiety. While GSLs have ceramide as their lipid component, which is made of an aminoalcohol base (sphingoid base) and fatty acid joined by an amide bond, glycolipids have diacylglycerol as their lipid component. The sugar units are attached to GSLs through glycosidic linkage to hydroxyl groups at the C-1 carbon of the ceramide. In glycolipids the glycosylation occurs at the C-3 hydroxyl group of glycerol, see **Figure 3**. From here on we attempt to understand what structural features of these glycosylated lipids gives them their unique property to cluster and form microdomains.

### 3.1. Glycosphingolipids

As mentioned above, GSLs have ceramide as their lipid moiety. Ceramides can have a variety of structures depending upon the sphingoid bases and the fatty acid combinations. This variability in the structure of ceramide adds diversity to GSLs and further diversity is

Series	Symbol	Core structure			
		IV	III	II	I
globo	Gb	GalNAc $\beta$ 3Gal $\alpha$ 4Gal $\beta$ 4GlcCer			
isoglobo	iGb	GalNAc $\beta$ 3Gal $\alpha$ 3Gal $\beta$ 4GlcCer			
ganglio	Gg	Gal $\beta$ 3GalNAc $\beta$ 4Galp4Gcer			
lacto	Lc	Gal $\beta$ 3GlcNAc $\beta$ 3Gal $\beta$ 4GlcCer			
neolacto	nLc	Gal $\beta$ 4GlcNAc $\beta$ 3Gal $\beta$ 4GlcCer			
mollu	Mu	GlcNAc $\beta$ 2Man $\alpha$ 3Man $\beta$ 4GlcCer			
artho	At	GalNAc $\beta$ 4GlcNAc $\beta$ 3Man $\beta$ 4GlcCer			

**Table 1.** Series of glycosphingolipids.

added because of possible variations in the saccharide units. Often GSLs are classified based on their saccharide units, that can range from a single to 20 or more carbohydrate residues [27]. Most of GSLs have a neutral core structure which is used for their classification into different series (**Table 1**). Roman numerals are assigned, starting from the ceramide end while referring to a particular residue of the core and an Arabic numeral superscript is given to indicate the position at which a substituent is attached if any are present [28]. GSLs are further subclassified as neutral, sulfatides or gangliosides [29]. Gangliosides are sialylated GSLs. Gangliosides are written using Svennerholm abbreviations, where the first letter G stands for ganglioside, the number of sialic acid residues is denoted by a letter, defined as M-mono, D-di, T-tri and Q-tetra, and is followed by a number which represents the order of migration on thin layer chromatography.

GSLs can participate in both donating and receiving hydrogen bonds through the hydroxyls of the sphingoid base, fatty acids, carbohydrates and the acylamide group. Because of this hydrogen bonding ability, GSLs can cluster together to form rigid highly organized domains on the surface of the biomembrane. These clusters of GSLs often have signal transducer proteins, growth factors or adhesion receptors organized in them and are involved in carbohydrate dependent intercellular adhesion, which triggers the signaling transducers leading to modification of the cellular phenotype. These GSL enriched domains that are involved in GSL-dependent cell adhesion and signaling are termed “glycosynapses” [30]. Glycosynapses differ from other membrane domains such as caveolae and lipid rafts in that neither of these microdomains are involved in carbohydrate dependent cell to cell adhesion.

The major form of glycoconjugates found in animal brains are glycolipids which includes galactosylceramide (GalCer), its 3-O-sulfated form sulfatide and gangliosides. GalCer and sulfatide make up a significant portion of myelin lipid and gangliosides are found in neuronal plasma membrane [31]. Inherent defects in the biosynthesis and catabolism of gangliosides results in neurodegenerative diseases. So far very few incidences of diseases caused by mutations of genes responsible for synthesis of gangliosides have been reported [32, 33]. Inherited defects in catabolism of gangliosides are well documented. Defects in catabolism of gangliosides

results in their accumulation inside lysosomes known as gangliosidoses. Gangliosidoses can occur in any age group, although most patients showing the symptoms are infants. Tay-Sachs disease is caused by deficiency of the enzyme  $\beta$ -hexosaminidase A which causes the lysosomal accumulation of GM2 gangliosides and is an example of gangliosidoses [34]. Gaucher and Fabry disease are other examples of GSL storage diseases in which lysosomal accumulation of neutral GSLs occurs [35].

### 3.2. Glycoglycerolipids

Glycoglycerolipids constitute a major portion of the lipids found in chloroplasts of plants and in cyanobacteria with digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), and sulfoglycolipid sulfoquinovosyl diacylglycerol (SQDG) composing more than 80% of total lipid composition [36]. In general, organisms performing oxygenic photosynthesis tend to have higher percentage of galactolipids. Many different glycoglycerolipids besides galactolipids are found in bacteria where they contribute to membrane stability and survival of bacterial species in phosphate limited environments.

Glycoglycerolipids have been explored for their biological activities for the past few decades. Natural and synthetic analogs of MGDGs, DGDGs and SQDGs have been studied for their antitumor [37–39], antiviral [40–42], antifungal [43], anti-inflammatory [44, 45] and other biotechnological applications [46, 47].

### 3.3. Glycophosphatidylinositol

Glycosylphosphatidylinositols (GPI) are complex structures to which the C-terminus of proteins gets attached during their post-translational modification [48]. All GPI have a core glycan structure sandwiched between an ethanolamine phosphate linker, bridging the C-terminal of the protein with the highly conserved glycan core, and a phosphatidylinositol (PI) group. The fatty acids of the PI moiety attach the GPI to the cell membrane. So far more than 200 proteins have been found to be anchored by a GPI to the cell surface and more than 20 GPI structures have been elucidated [49]. Some of the proteins attached to GPI anchor are enzymes like alkaline phosphatase (APase), acetylcholinesterase (AChE) and 5'-nucleotidase, complement defense proteins like decay accelerating factor (DAF or CD55), CD59, and mammalian antigens like Thy-1, or protozoan antigens like variant surface glycoprotein (VSG) found on the cell surface of *Trypanosoma* [48].

Unlike GPI-anchored proteins not much is known about biological functions of GPI anchors, apart from their role as a membrane anchor for proteins. Given the complexity and diversity of their structures, they are thought of as being involved in many different biological functions but there are not sufficient experimental evidences to draw definitive conclusions [50]. However, there are several studies implicating their involvement in sorting of proteins in the lipid raft and in signal transduction [51–53]. Other studies have shown that the structure and conformation of proteins change upon binding to GPI anchors [54, 55]. Besides this physiological role, deficiency of GPI anchors on red blood cells causes a chronic pathological disorder paroxysmal nocturnal hemoglobinuria (PNH) [56].



### 3.4. Lipopolysaccharides

Lipopolysaccharides (LPS), also referred to as endotoxins, are a major component of the outer membrane of gram negative bacteria and are essential for maintaining the structural integrity of the membrane. LPS have three components: lipid A, core oligosaccharide and O-antigen polysaccharide. Lipid A is the active component and under normal conditions consists of  $\beta(1-6)$  linked glucosamine disaccharides. The diglucosamine backbone is phosphorylated and decorated with multiple fatty acids anchoring them in the outer leaflet of the bacterial cell membrane. Lipid A, when released from the cell, is recognized by pattern recognition receptor TLR4 triggering cytokine synthesis. At low level of endotoxins, the innate immune system eliminates it, but at high concentrations it can prove fatal [57].

## 4. Methods used to study glycolipids at the water-air interface

Given the significance of glycolipids in cellular processes and their association with chronic diseases, it is important to seek a clear understanding of the biophysical and biological properties of these glycolipids. In this section, we attempt to address some of the techniques which might be helpful and a survey of studies done using these methods will be discussed. Each method has pros and cons associated with it and therefore two or more complimentary methods are often employed together for the complete assurance of the result observed.

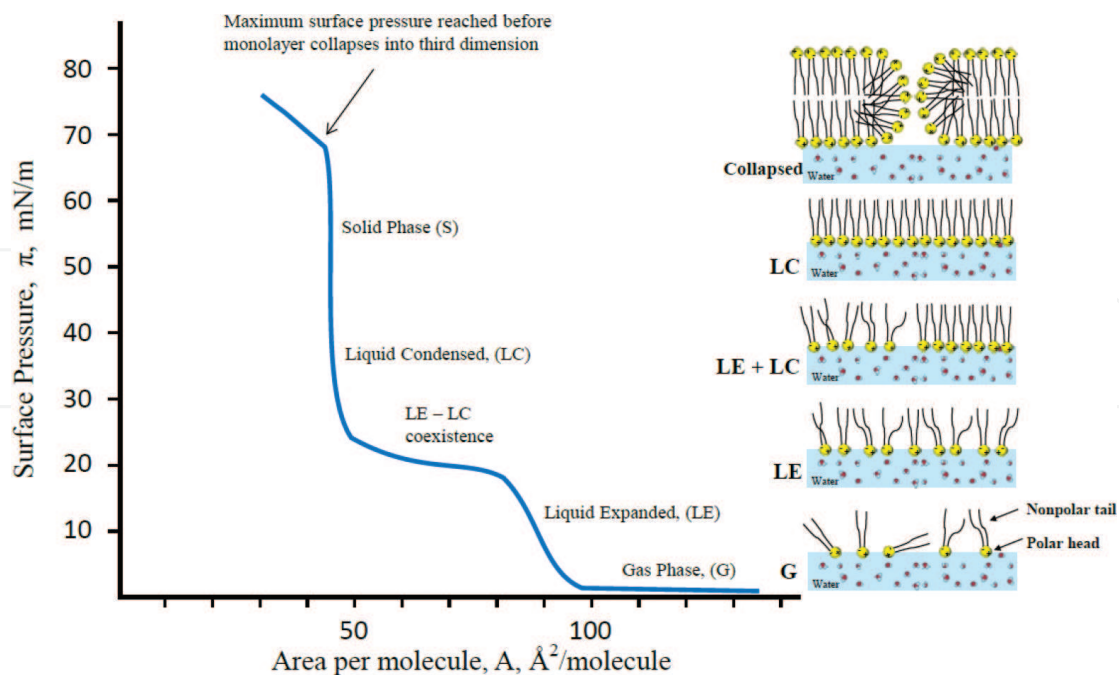
### 4.1. Surface pressure isotherms

The plot of surface pressure,  $\Pi$ , versus molecular area,  $A$ , at constant temperature as the monolayer film is compressed, by closing the barrier at constant rate, after initial deposition and solvent evaporation is known as an isotherm. Surface pressure-area isotherms provide information about molecular packing, molecular stability, phase transitions and compressibility of phases. Isotherms recorded at various temperatures helps to obtain phase diagrams [21]. The data from  $\Pi$ - $A$  isotherms can also be used for various thermodynamic calculations.

Water molecules on the surface are under tension due to imbalance of the force compared to the bulk where each molecule is attracted by equal force from all direction. The surface tension decreases when a monolayer is deposited at the air-water interface. The difference in surface tension before and after monolayer deposition is known as surface pressure,  $\Pi$ , which is given by the relation

$$\Pi = \gamma_0 - \gamma \quad (1)$$

where  $\gamma_0$  and  $\gamma$  are surface tension in the absence and presence of monolayer, respectively. Surface pressure is measured by the Wilhelmy plate method in which a plate made of platinum or thin filter paper is contacted with the water and changes in downward force are measured. A schematic  $\Pi$ - $A$  isotherm is shown in **Figure 4**. Monolayers can exhibit a range of



**Figure 4.** Schematic showing a pressure area,  $\Pi$ - $A$  isotherm with the major phases and transitions shown for a single component lipid monolayer.

phases and phase transitions upon compression [58]. At high molecular areas there can be a gas-like phase in which molecules are widely separated and the surface pressure is very low. Upon compression, a liquid-like phase can be entered that is known as the liquid-expanded (LE) phase in which molecules are closer together and ordered like a two-dimensional liquid. A liquid-condensed (LC) phase in which molecules are well-ordered as in a two-dimensional liquid crystal, and may be oriented vertically or tilted, is entered upon further compression. Other phases are possible and the sequence of phases seen on compression depends on temperature and other conditions. Coexistence regimes between phases, such as LE + LC, are often encountered. Kinks or flat regions in an isotherm signal these phase transitions for single component monolayers. At very high surface pressures, two-dimensional solid-like phases can be observed for some compounds. Compression of the monolayer beyond its stability limit results in collapse into three-dimensional structures.

#### 4.2. Surface potential measurement

$\Pi$ - $A$  isotherms mainly provide information about later stages of monolayer compression when the molecules are closer together. Surface-potential measurement allows one to probe a Langmuir monolayer at higher surface area before it has been significantly compressed. Surface potential vs. area,  $\Delta V$ - $A$  isotherms, have a region in the higher range of molecular area which marks the initial rise of the surface potential and is attributed to aggregation of microdomains present in the monolayer [59]. In addition to these advantages, surface potential measurements are used to gain information about molecular orientation, to calculate dipole moment, surface charge density and interfacial thickness.

Two common methods applied to measure surface potential are the vibrating electrode and the ionizing electrode. The vibrating electrode method also known as Kelvin probe method uses a plate-like electrode placed at a certain distance above air-water interface. The electrode is connected to the reference electrode placed in the subphase. The electrode above the interface is periodically vibrated during measurements. The ionizing electrode method employs the same measurement setup as the vibrating plate method. In this method, the electrodes are coated with  $\alpha$ -emitters such as  $^{241}\text{Am}$  or  $^{210}\text{Po}$  to increase the conductivity of the air gap [60].

#### 4.3. Transfer as Langmuir-Blodgett film for study by atomic force microscopy

The deposited monolayer can be transferred to a solid support. For example,  $\text{CaF}_2$  plates are used for transmission infrared spectroscopy while germanium silicon and ZnSe plates are used for internal reflection infrared spectroscopy [21]. The substrate most commonly used for atomic force microscopy (AFM) studies is freshly cleaved mica, because of its atomically flat surface. AFM is helpful for the visualization of coexisting phases with great resolution, which can be down to a few nanometers, and not readily visualized by other methods. Care must be taken while analyzing the micrograph that the structures visualized are a true representation of what was on the deposited monolayer and are not artifacts created during the transfer process.

The monolayer can be transferred to a solid support by Langmuir-Blodgett deposition which is carried out as a vertical transfer or by Langmuir-Schaefer transfer which is carried out as a horizontal transfer. The Langmuir-Blodgett transfer method can be performed in a number of ways depending upon the nature of the substrate (hydrophobic or hydrophilic) and the number of layers desired. For a hydrophilic substrate, the transfer is performed by immersing the substrate into the subphase prior to spreading the monolayer. The monolayer is then compressed to a desired surface pressure and the substrate is lifted out of the subphase at a suitable speed while maintaining a constant surface pressure. A series of immersion and emersion cycle can be performed to generate multilayers. In the horizontal transfer method, the substrate is held horizontally above the compressed monolayer, lowered until it makes contact with the water surface and is then lifted gently thus transferring the monolayer onto the substrate.

#### 4.4. Fluorescence microscopy

Fluorescence microscopy is another important method that is often used for studying interfacial behavior of monolayers. Even though the images from AFM help visualize the structure of monolayers at a near molecular level resolution they only provide snapshots of the continuous process and require transfer to a substrate. Fluorescence microscopy has an edge over AFM or any other static visualization method, as it provides a real time picture of the events as they occur.

Fluorescence microscopy provided the first evidence of coexisting domains in the plateau region of  $\Pi$ -A isotherms of Langmuir monolayers. In fluorescence microscopy, the monolayer

is doped with an amphiphilic fluorescent probe that has different solubility in two surface phases resulting in contrast when excited using light from an arc lamp or a laser. Despite the usefulness of fluorescence microscopy, it has a few drawbacks. First the fluorophores added, although in trace amount, can alter the original monolayer if their concentration is too high. Secondly, observation of more highly ordered phases is difficult because they reject the fluorescent molecular probe [59]. Also, problems may occur due to dissolution of the probe into the subphase.

#### 4.5. Brewster angle microscopy

The problems associated with fluorescence microscopy due to the addition of fluorescent dye can be avoided with Brewster angle microscopy (BAM) as it does not require any probe molecule. When a light beam is directed onto a water surface a portion of it is reflected, and the reflected light intensity depends upon the angle of incidence. For plane polarized light, there exists a certain angle of incidence at which no reflection occurs known as the Brewster angle. For water at room temperature the Brewster angle is 53°. For monolayer study at the air-water interface, light is directed onto the surface at the Brewster angle and the reflected light is observed using a CCD camera. The monolayer on the water surface has a different refractive index and this leads to violation of the Brewster angle condition and increased reflectivity so the monolayer domains will appear bright [61].

#### 4.6. X-ray reflectivity and scattering

X-rays provide a very sensitive tool for the study of monolayers by virtue of their small wavelength of few Å. Unlike visible light, reflection of X-rays from a denser medium coming from rarer (gas) medium at a certain critical angle are totally reflected without any diffraction into the denser medium (solid or liquid) [60]. Grazing incident X-ray diffraction (GIXD) has been an important technique used for investigating *in situ* structural arrangement of amphiphiles for more than two decades. In GIXD the incident angle,  $\alpha$  is slightly below the critical angle. The small incident angle is desirable for larger penetration of X-rays. Two commonly used X-ray techniques are X-ray reflectivity (XRR) and diffraction (XD).

XRR provides data that can be used to estimate the thickness of the monolayer,  $d$  calculated using Bragg's law:

$$n\lambda = 2d\sin\theta \quad (2)$$

Besides thickness of monolayer, XRR also provides information about the electron density distribution perpendicular to the interface and the roughness of the monolayer. XRR data can be used to model thickness of different segments of the monolayer. XD provides information about the two-dimensional arrangement of the molecules on the surface and the tilt angles of the molecules.

#### 4.7. Infrared spectroscopy

Infrared Spectroscopy is another important technique widely used to characterize the conformation and orientation of monolayers transferred onto the solid support or *in situ* at the air-water interface. It is desirable to carry out the experiments *in situ* to avoid problems due to artifacts during transfer. Infrared reflection absorption spectroscopy (IRRAS) and polarization modulated (PM)-IRRAS are two versions of Fourier transform infrared spectroscopy (FTIR) frequently employed for the study of Langmuir monolayers [62].

In IRRAS, the sample is irradiated with an IR beam and the intensity of the reflected beam is measured as a function of wavelength. The measurements can be carried out with p- and s-polarized light at various angles of incidence above or below the Brewster angle. If the samples are on metal substrates, a “surface selection” rule is followed which states that only vibrational dipole moments oriented perpendicular to the substrate are observed. IRRAS has a disadvantage while studying Langmuir films, the strong absorption of water vapor conceals the spectral regions with desired molecular information. PM-IRRAS, which is insensitive to IR absorption of water vapor, was introduced to avoid such problems. In PM-IRRAS, the incident beam is alternated between s- and p-polarization at a frequency of tens of kHz [63] and differential reflectivity is calculated. Besides these two IR spectroscopic techniques, there are others such as surface-enhanced infrared-absorption spectroscopy (SEIRA) and attenuated total reflection ATR-FTIR spectroscopy.

### 5. Thermodynamics of mixed monolayers at the water-air interface

The monolayer deposited can be made of two or more surface active molecules at varying composition. In this chapter, we are concerned with mixed monolayers of glycolipids and other membrane components including membrane phospholipids such as dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylethanolamine (DPPE) and others. Three main types of interactions exist between such molecules in the mixed monolayer, van der Waal attractions between the hydrocarbon chains, and hydrogen-bonding and ionic interaction between the head-groups. These interactions, especially the ionic interactions, determine the stability of the mixed monolayer and are also responsible for the deviations from ideality in mixing. A quantitative study of such forces can be done by applying the concepts of thermodynamics.

The excess Gibbs free energy of mixing,  $\Delta G^{exc}$  is one such measure which helps determine the stability of the mixed monolayer.  $\Delta G^{exc}$  can be determined by using the following relation.

$$\Delta G^{exc} = \int_0^\pi (A_{1,2} - x_1 A_1 - x_2 A_2) d\pi. \quad (3)$$

$A_{1,2}$  is mean molecular area of the mixed monolayer,  $A_1$  and  $A_2$  are the molecular areas of individual monolayer components, and  $X_1$  and  $X_2$  are their mole fractions, respectively. The

term inside parenthesis is the excess surface area. Negative values of  $\Delta G^{exc}$  indicate favorable interactions resulting in a more stable monolayer and positive  $\Delta G^{exc}$  indicates unfavorable interactions and possible phase separation. Furthermore, one could calculate a free energy of mixing,  $\Delta G^{mix}$  using the following equations:

$$\Delta G^{mix} = \Delta G^{id} + \Delta G^{exc} \quad (4)$$

$\Delta G^{id}$  is the ideal value that can be calculated using

$$\Delta G^{id} = RT(x_1 \ln x_1 + x_2 \ln x_2) \quad (5)$$

where  $R$  and  $T$  are gas constant and temperature respectively. Phase separation may also occur upon compression in mixed monolayers, resulting in coexisting phases of different composition. Occurrence of critical points and azeotropes in two-dimensions is also possible.

## 6. Survey of studies of monolayers containing glycolipids

### 6.1. Gangliosides

Gangliosides have been the subject of many studies as monolayers and we survey some of the reported results. In an early study using surface pressure isotherms of gangliosides GM1, GM2, GM3, GD1a, GD3 and GT1, it was found that increasing the number of sialic acid residues caused an increase in the prevalence of the LE phase and that the surface pressure isotherms shifted to higher molecular areas and the monolayers became more compressible [64]. This trend was attributed to increasing electrostatic repulsions with introduction of additional negatively charged sialic acids in the structures. In a related study using surface pressure and surface potential measurements, it was found that the strength of interaction of  $\text{Ca}^{2+}$  ions in the subphase with gangliosides depended on the number and arrangement of the sialic acid units [65]. A study using surface pressure isotherms of mixed monolayers of GM1, GD1a, and GT1 with phospholipids showed positive deviations from ideality at  $30 \text{ mN m}^{-1}$  for DPPE, a phosphatidylinositol and a phosphatidylserine, but negative deviations from ideality for mixtures with DPPC [66]. In contrast, the interaction of gangliosides with neutral glycosphingolipids was found to be favorable. Mixed monolayers of ceramide or of glucosyl ceramide with gangliosides were found to show favorable interactions, while those of lactosyl ceramide and gangliosides showed immiscibility [67]. The activity of the enzyme phospholipase  $A_2$  injected into the subphase against mixed monolayers of dilauroylphosphatidylcholine and gangliosides GM1, GD1a, and GT1b was found to be strongly inhibited [68]. Gangliosides were also found to inhibit the activity of phospholipase C against model membrane systems including monolayers [69]. The sensitivity of monolayer parameters to trace impurity of peptide materials in isolated gangliosides has been emphasized along with provision of methods for rigorous purification [70].

Gangliosides are known for their ability to form microdomains in biological membranes. The partitioning of ganglioside, GM1 in phase separated DOPC/DPPC LB films transferred to

freshly cleaved mica at the approximated physiological pressure of  $37 \text{ mN m}^{-1}$  and varying concentration of GM1 ranging from 0.2 to 4 mol% was studied using AFM [71]. It was found that below 1 mol% GM1 preferred the DPPC LC phase as evident from nanometer scale patches of round and elongated shapes at the center and periphery of DPPC macrodomains. At 3 mol% concentration of GM1 the size of DPPC macrodomains decreased and patches in their center and on their periphery were more pronounced, and further increasing the concentration to 4 mol% resulted in separation of macrodomains into smaller domains and the elongated patches were arranged around their boundary forming a fence-like structure. According to the authors, this was the first direct determination of distribution of GM1 in phase separated lipid mixtures. A similar study was conducted in DPPC and 2:1 DPPC/cholesterol monolayer. For DPPC monolayer, the experiments were conducted in a pure gel phase (at  $45 \text{ mN m}^{-1}$ ) and a mixture of LE and LC phase (at  $7 \text{ mN m}^{-1}$ ) while DPPC/cholesterol monolayers were in a homogenous liquid-ordered phase at both lower and higher pressure [72]. Ganglioside rich microdomains that were small and circular were observed at the center and the edges of LC and gel phase that coalesced at higher ganglioside concentration to form filamentous structures in the center and larger patches around the edges. In the case of liquid-ordered 2:1 DPPC/cholesterol monolayer, the addition of GM1 gave rise to small round domains of varying diameter (50–150 nm) which coalesced to give long filaments that covered 30–40% of the monolayer surface when the GM1 concentration was increased to 10 mol%. The results indicated that biologically relevant concentrations of GM1 led to formation of microdomains in the model membranes, which is suggestive of their raft forming nature in cholesterol-rich regions of biological membranes.

The influence of subphase ionic strength on mixed monolayers of GM1 and either stearyl-oleoyl-phosphatidylcholine (SOPC) or DPPC was analyzed by surface pressure measurements [73]. Both phospholipids chosen had similar zwitterionic head-groups, but SOPC exists in a fluid LE phase and DPPC exist in a condensed phase at higher surface pressure. It was observed that mixed monolayers were more expanded on buffer than on pure water. The author suggested that a change in GM1 orientation at the interface was responsible. The binding of wheat germ agglutinin (WGA), a dimeric lectin recognizing GM1, with the monolayer was studied. The binding of WGA to GM1 was reduced in the mixed monolayer with DPPC as compared to that with SOPC, attributed to a higher packing density of the SOPC monolayer.

The structure of the two-dimensional mixed monolayer of glycolipid, GM1 + DPPE at air-water interface was studied using GXID and XRR [74]. Pure DPPE, GM1 and mixtures of 5, 10, and 20 mol% of GM1 with DPPE were studied. It was observed that GM1 was accommodated within the DPPE matrix without distorting the in-plane and out-of-plane packing structure. The observed results were in contrast with the previous finding in which the lipids with hydrophilic head groups altered the packing of the phospholipid monolayer. Based on their observation, the author suggested that X-ray scattering technique in combination with monolayer bearing GM1 as a probe can be utilized for studying interaction of proteins such as amyloid  $\beta$ , myelin-based protein and cholera toxin.

Another important ganglioside found predominantly in the early immature nervous system of mammals and birds is GD3. GD3 constitutes 3–8% of total ganglioside in adult human brain and is vital for cell growth and proliferation. A combined AFM-thermodynamic analysis was performed to study the aggregation process of GD3 in DPPC [75]. The results obtained revealed a very different aggregation behavior of GD3 from those observed for GM1. In contrast to GM1, GD3 molecules were found to be miscible with DPPC. The excess Gibbs free energy values, calculated for four mole fractions ( $X = 0.2, 0.4, 0.6$  and  $0.8$ ) of GD3, were all negative with a minimum at  $X = 0.4$ . The mean molecular area showed negative deviations indicative of attractive interactions. AFM images did not show any significant changes in domain diameter or height in the LC region of DPPC up to  $X = 0.4$ . A significant morphological change was observed at  $X = 0.6$ , a compact LC domain of  $\approx 2 \mu\text{m}$  in diameter from which  $\approx 0.14 \mu\text{m}$  wide stripes were extended. This trend became more pronounced at  $X = 0.8$ , with the disappearance of stripes and formation of a filamentous network covering the whole surface. The authors suggested that the appearance of stripes indicated a critical point in the phase diagram [76].

Gangliosides are able to act as receptors for binding bacterial toxins and mediate their entry into cells. For example, cholera toxin binds specifically to GM1, whereas tetanus toxin and botulinum neurotoxin A have strong binding affinity for trisialogangliosides. The in-plane and out-of-plane structure of pure and mixed monolayers of GT1b, a trisialoganglioside, in DPPC/DPPE were investigated using GIXD and XRR measurements [77]. These studies showed that the phospholipids were able to incorporate up to 20 mol% of GT1b without any phase separation. The finding suggested the binary monolayers can be employed as a model for the study of toxin membrane binding and penetration.

Much remains to be learned about the lateral organization of gangliosides and how it influences the neighboring lipids. As evident from the above examples of binary DPPC/GM1 mixtures, GM1 has a condensing effect on DPPC, but not enough is known about which interactions are responsible for this effect. This effect has been credited to the complimentary geometrical structures of GM1 and DPPC, intermolecular hydrogen-bonding between the sugar groups and to alignment of the dipole moment of DPPC with the negative charge on the sialic acid residue. Recently, a study was conducted to understand the impact of gangliosides on the surrounding lipids and see if previous knowledge of the GM1/DPPC system can be used for generalization over a range of other gangliosides [78]. Three gangliosides with the same ceramide backbone but different numbers of sialic acid were investigated. A trend seen in GM1/DPPC, of condensation at lower concentration ( $<20 \text{ mol}\%$ ) followed by fluidization at higher concentration ( $20 \text{ mol}\%$ ), was observed, but less DPPC was required to condense gangliosides of larger cross-sectional area. A model was proposed to explain the observed result in which the authors took into account two competing factors: electrostatic repulsion between sialic acid groups and their perpendicular dipole moments,  $\mu$ . It was suggested that with the increase in number of sialic acids the effect of a positive perpendicular dipole moment,  $\mu$  is more pronounced and hence requires a smaller proportion of DPPC with negative  $\mu$  to stabilize a proportionally small electrostatic repulsion.



## 6.2. Cerebrosides

The interaction of cerebrosides with cholesterol in mixed monolayers has been investigated [79]. A lactosylceramide with a C7 chain, and maltosylceramides with C8 and C18 chains were studied in mixed monolayers with cholesterol; also studied were GalCer and GlcCer from bovine brain. Cholesterol was found to condense the dihexosyl cerebroside monolayers, shifting the isotherms to lower molecular areas. Cholesterol oxidase served as a probe of the cerebroside-cholesterol interactions by injecting the enzyme into the subphase. The molecular area increased as more cholesterol in the monolayer was oxidized. Stronger interaction with the lipid served to protect the cholesterol from oxidation. It was found that cholesterol interacted more strongly with the monolayers containing dihexosyl ceramides than those contain monohexosyl ceramides. In another study, mixed monolayers of porcine galactocerebrosides with palmitic acid were examined [80]. The excess Gibbs free energy of mixing was negative for most compositions indicating favorable interactions attributed to head-group hydrogen-bonding, and palmitic acid condensed the cerebroside monolayers. BAM showed a gas + LC coexistence with palmitic acid and cerebrosides forming a homogeneous mixture.

Mixed monolayers of cerebrosides bearing glucosyl head-groups mixed with cholesterol or with cholesteryl sulfate were examined by surface pressure isotherms, surface potential isotherms, and fluorescence microscopy [81]. The surface pressure and surface potential isotherms were analyzed as a function of composition resulting in the conclusion that these cerebrosides were not miscible with cholesterol but were miscible with cholesteryl sulfate. Fluorescence microscopy images supported these conclusions. The same group reported a study of six cerebrosides extracted from the blue sea star *Linckia laevigata* [82]. Mixed monolayers with DPPC were examined by surface pressure and surface potential isotherms and fluorescence microscopy and miscibility of the cerebrosides with DPPC was established. It was found that the nature of the hydrocarbon chain had a significant influence on the surface potential.

The thermodynamic behavior and structure of monolayers of three galactocerebrosides was examined using surface pressure isotherms, BAM, GIXD, and IRRAS [83]. The high prevalence of galactocerebrosides in myelin membranes motivated the study. The galactocerebrosides contained a galactose head-group, a sphingosine backbone, and a fatty acid chain that was varied between C24 (GalCer C24:0), C24 hydroxylated on the 2-position (GalCer C24:0 (2-OH)), and a C24 with a double bond at position 15 (GalCer C24:1). The isotherms of GalCer 24:0 and GalCer 24:1 showed plateaus indicating LE + LC phase coexistence above 38 and 20°C, respectively. In contrast, the GalCer 24:0 2-OH derivative at all temperatures showed direct transition from a gas-like phase to a LC phase, with the condensation effect assigned as due to additional interactions of the 2-OH groups. BAM showed appearance of flower-like domains on compression of GalCer 24:0, of round domains on compression of GalCer 24:1 which also showed a kinetic overshoot in the compression isotherms at the LE to LC transition. Using a two-dimensional analogy of the Clapeyron equation and the phase transition pressures found in the isotherms as a function of temperature,  $\Delta H$  for the phase change was estimated. Together, for GalCer 24:0, the GIXD and IRRAS data confirmed a rigid phase with hydrogen-bonding between head-groups of neighboring molecules.

AFM has been applied in a few studies of cerebroside monolayers. Bovine brain cerebroside were spread as monolayers and first examined on compression by fluorescence microscopy [84] revealing formation of branched, fractal-like domains of a LC phase. Transfer of the monolayers onto mica and imaging by AFM revealed the presence of rod-like structures that were concluded to be single bilayer nanotubes based on their size. Cerebroside extracted from *Bohadschia argus* (sea cucumber) were investigated by surface pressure and surface potential isotherms, BAM, fluorescence microscopy and AFM [85]. Miscibility of the cerebroside with DPPC was confirmed and surface pressure isotherms as a function of composition suggested a negative azeotrope in the phase diagram. AFM on the pure cerebroside showed circular domains of LC and LE phase coexisting. BAM and fluorescence microscopy confirmed miscibility of the cerebroside and DPPC.

### 6.3. Globosides

Monolayers of globotriaosylceramide (Gb3), which contain two galactose units and a glucose unit, were examined by surface pressure isotherms, BAM, XRR, and GIXD [86]. Analogs of Gb3 with variable acyl chains (22:0, 22:1, 14:0) and the lysolipid form were studied in mixtures with DSPC and DPPE. The isotherms and BAM observations indicated that the molecules were miscible in the monolayer. Electron density profiles were calculated from the XRR data and in-plane structure determined by GIXD. The carbohydrate region was found to extend into the water, by 10 Å for the 4:1 DSPC/Gb3 mixture, and also was extended for the 4:1 DPPE/Gb3 mixture. The thickness of the monolayer alkyl chains was correlated with the length of the Gb3 acyl chains. For mixed monolayers with DSPC, a segment of the carbohydrate of Gb3 was located within the phospholipid head-group region, an observation that has implications for Gb3 binding of Shiga toxin. Longer acyl chains on the Gb3 analog resulted in greater carbohydrate exposure in the subphase.

Surface pressure isotherms were used to study mixed monolayers of stage-specific embryonic antigen-1 (SSEA-1) and DPPC [87]. The SSEA-1 was isolated from Japanese quail intestine and contained a mixture of chain lengths on the ceramide. A LE to LC phase transition was observed in the mixed monolayers. The isotherms as a function of composition showed behavior resembling that of a negative azeotrope which indicated favorable interactions between SSEA-1 and DPPC.

### 6.4. Lipopolysaccharides

The outermost membranes of gram negative bacteria contain lipopolysaccharides. Lipid A, a glucosamine based phospholipid serves as a hydrophobic anchor for LPS. Kdo (3-deoxy-D-manno-oct-2-ulosonic acid) domains are present in the structure as well possibly additional core and O-antigen sugars. Monolayers of LPS have been investigated. The smallest LPS that are active are known as Re-LPS. The miscibility of Re-LPS with monolayers of DPPC was studied using fluorescence microscopy [88]. The fluorescent lipid probe 1-palmitoyl-1-[12-[(7-nitro-2-1,3-benzoxadiazole-1-yl)amino]dodecanoyl]phosphatidylcholine (NBD-PC) was used at 1 mol%. The surface pressure for monolayers of pure Re-LPS began rising around 400 Å<sup>2</sup> molecule<sup>-1</sup> upon

compression. The variation of the collapse pressure with composition provided evidence that Re-LPS and DPPC were miscible. Plots of mean molecular area vs. composition at three surface pressures all showed negative deviations from ideality and hence evidence for attractive interactions between Re-LPS and DPPC. Pure monolayers of DPPC show a coexistence region between a LE and a LC phase in which distinct microdomain formation occurs. Addition of increasing amounts of Re-LPS increased the transition pressure and caused a decrease in domain size along with a change to less distinct shapes. Pure monolayers of Re-LPS did not show a phase transition upon compression. Addition of lung surfactant protein A beneath mixed monolayers of Re-LPS and DPPC induced segregation and domain formation. In a later study, Re-LPS was extracted from *Salmonella* Minnesota strain R595 and studied by surface pressure isotherms and by XRR and GIXD [89]. On pure phosphate buffer solution of pH 7.2, the surface pressure for LPS began to rise at  $370 \text{ \AA}^2 \text{ molecule}^{-1}$ ; addition of 50 mM  $\text{CaCl}_2$  to the subphase reduced this area to  $315 \text{ \AA}^2 \text{ molecule}^{-1}$  as the divalent counterions cross-linked the sugar units of LPS molecules. As the surface pressure increased, the monolayer became thicker and there was a change in the conformation of the sugar head-groups. At higher surface pressures, the hydrocarbon chain packing became more oblique and the size of ordered domains became smaller.

Monolayers of LPS extracted from *Pseudomonas aeruginosa* were studied using surface pressure measurements and found to be stable [90]. When monovalent or divalent salts were added to the subphase, LPS molecules adopted a compact conformation. Addition of increasing amounts of  $\text{CaCl}_2$  to the subphase destabilized the monolayer and caused the collapse pressure to decrease. Upon compression to  $45 \text{ mN m}^{-1}$ , surface pressure relaxation to about  $43 \text{ mN m}^{-1}$  was seen over a period of about 90 min. The RcLPS form of LPS, whose structure contains seven sugars of the inner and outer core polysaccharide, was extracted from *Escherichia coli*, and used to form monolayers that were studied using surface pressure, BAM, XRR and neutron reflectivity, and GIXD [91]. The surface pressure isotherm showed a steady increase and no obvious phase transition, and the BAM observation showed a homogeneous surface. At  $20 \text{ mN m}^{-1}$ , a monolayer thickness of  $41 \text{ \AA}$  was calculated. A hexagonal oblique arrangement of hydrocarbon chains was observed at all surface pressures. The calculated arrangement of rcLPS at the air-water interface shows that the molecules are overall tilted by  $15\text{--}29^\circ$  and that the tails occupy a thickness of  $12 \text{ \AA}$ , and that the inner carbohydrate and amide and ester linkages occupy  $14 \text{ \AA}$  of thickness and the outer entirely carbohydrate part of the head-group occupies  $15 \text{ \AA}$  of thickness.

Interaction of plasticins with LPS monolayers was studied [92]. Plasticins are linear antimicrobial peptides with a repeated GXXXG motif where G is glycine and X is any amino acid. The interaction of plasticins with mixed monolayers of LPS (both smooth LPS and Re-LPS) and phospholipids was studied to gain insight into how the antimicrobial peptide interacts with bacterial membranes. A combination of surface pressure measurements, BAM, GIXD and AFM performed on films transferred to mica was applied. Both plasticins studied were highly surface active. Smooth LPS formed unstable monolayers but Re-LPS formed stable monolayers. The monolayers appeared homogeneous to BAM. The cationic plasticin was able to significantly penetrate the LPS monolayers. Plasticin insertion was able to introduce disorder into the monolayers, as seen by changes in the X-ray correlation lengths.

Glycopeptidolipids (GPLs) are present in their bacterial cell wall of mycobacteria. In one study, the GPLs were extracted from mycobacteria and studied in mixed monolayers with phospholipids [93]. Three GPLs were studied as monolayers and found by surface potential measurements to undergo a conformational change upon compression but not a phase transition. Addition of GPL dispersions beneath egg phosphatidylcholine monolayers resulted in insertion of GPL into the monolayer as registered by significant surface pressure increases. A subsequent study used PM-IRRAS to study mixed monolayers of GPLs with 1,2-di(perdeuterio-palmitoyl)phosphatidylcholine [94]. It was concluded that phase segregation occurred between the GPLs and the phospholipid, driven by the ability of GPLs to form  $\beta$ -sheet structures, and that the phase segregation was more pronounced for the GPLs that were more heavily glycosylated.

### 6.5. Synthetic glycolipids

In addition to studies of the monolayer behavior of natural occurring carbohydrate containing lipids, significant effort has been devoted to the study of synthetic derivatives, some being structural analogs of naturally occurring lipids and others being new structures. For example, changes in the structure of ganglioside and sphingosine derivatives have been found to alter the activity of phospholipase enzymes against mixed monolayers containing dilauroylphosphatidylcholine [95]. In these experiments, enzyme is injected into the subphase with the monolayer maintained at a surface pressure of  $12 \text{ mN m}^{-1}$  and the decrease in molecular area due to hydrolysis of the ester bond of the phospholipid is followed as a function of time. These studies illustrate the role of gangliosides and glycolipids in regulating enzyme-based signaling at membrane surfaces. The surface pressure and surface potential behavior of these derivatives was studied in detail separately [96]. One of the structural changes investigated was removal of the sialic acid from ganglioside GM1 resulting in asialo-GM1, which was found to form a LC phase not seen for monolayers of GM1. Surface potential data revealed that monolayers of asialo-GM1 had a significantly larger dipole moment perpendicular to the water surface than did monolayers of GM1.

Mixed monolayers of DMPC and derivatives of N-acetylglucosamine were formed and their interaction with the lectin wheat germ agglutinin were examined. It was observed that the synthetic glycolipids were miscible with DMPC at higher surface pressures, and that the lectin could only bind to derivatives with a spacer group between the hydrocarbon chains and the sugar [97]. The binding of the lectin to the monolayer was seen to result in a significant increase in surface pressure for the derivatives with a long enough spacer. Subsequently, the interaction of monolayers of synthetic glycolipids bearing either N-acetyl-D-glucosamine or L-fucose with three lectins (wheat germ, *Ulex europaeus* I, and *Lotus tetragonolobus* agglutinins) was examined. Mixed monolayers of the synthetic glycolipids with DMPC were able to bind lectins injected into the subphase resulting in an increase in surface pressure with time and a shift of the monolayer isotherms to higher molecular areas [98].

Synthetic glycolipids derived from glycerol bearing two hydrocarbon chains, a triethyleneglycol spacer, and an N-acetyl-D-glucosamine head-group were used to form monolayers, with the

increase in the alkyl chain length to C16 resulting in a highly organized surface arrangement [99]. Monolayers containing one of these glycolipids together with immunoglobulin G were successfully formed by spreading from vesicle dispersions [100]. The presence of the immunoglobulin G in transferred monolayers was subsequently confirmed by infrared spectroscopy, and it was proposed that on compression the immunoglobulin G re-orientates from a flat to standing orientation [101]. Monolayers of these derivatives were studied in mixed monolayers with phospholipids by GIXD, and it was found that addition of the glycolipid reduced the correlation length and hence the extent of ordering for the phospholipid component [102].

A homologous series of dialkylglycerylethers and their  $\beta$ -D-glucoside and  $\beta$ -D-cellobioside derivatives were studied as monolayers. It was found that introduction of the sugars expanded the monolayers, and acted in opposition to the effect of increasing chain length [103]. Using pentaerythritol as a building block, a gemini glycolipid was synthesized with two C16 alkyl chains and two N-acetyl- $\beta$ -D-glucosamine units [104]. Surface pressure-area isotherms showed that monolayers of the compound underwent an expanded to condensed phase transition. In a related study, glucoside lipids were created with a single hydrocarbon chain and either one or two glucose units in the head-group, presented in a branched geometry [105]. The bivalent glucoside achieved maximal binding to lectin at a lower surface fraction than did the monovalent glucoside lipid, when studied in mixed monolayers with an analog lacking sugar units.

Some studies in which glycolipid systems were transferred onto solid supports have been reported. Synthetic glycolipids bearing lactose, Lewis X, and sialyl Lewis X were synthesized containing partially fluorinated chains [106]. Mixed monolayers with phospholipids were observed by fluorescence microscopy and found to display phase separated microdomains. The monolayers were transferred onto silanized glass slides and it was found that adherence of Chinese hamster ovary cells to the supported monolayers varied with the composition and extent of microdomain formation. Langmuir-Blodgett films containing polydiacetylene derivatives that undergo a color change upon a binding induced conformational change are of interest for development of biosensors. Dioctadecyl glyceryl ether- $\beta$ -glucosides (DGG) were used to form mixed monolayers with 10,12-pentacosadiynoic acid (PCDA) or tricoso-2,4-diyynoic acid (TCDA) [107]. BAM showed that mixed monolayers with TCDA could be uniform, but those with PCDA showed phase separated domains. The excess Gibbs free energy of mixing was determined under varied subphase conditions. Another study reported mannosyl derivatives of PCDA in which BAM revealed highly structured dendritic like domains indicating the presence of a highly ordered phase [108]. Absorbance spectroscopy, carried out directly at the water-air interface, showed a shift from blue to red upon irradiation of the monolayers.

Synthetic derivatives of galactosyl ceramides with varied chain length between 11 and 33 carbons were synthesized and used to prepare monolayers, the phase behavior of the monolayers varied with overall chain length becoming more condensed with increasing chain length [109]. Synthetic glycolipids based on glycerol with two hydrocarbon chains and one, two, or three lactose units as the head-group were used to make monolayers and the interfacial viscosity was measured. With one lactose unit, a highly viscoelastic monolayer was obtained, with two a more fluid monolayer was observed, and with three a transition from viscous to elastic was observed [110]. In this study, the glycolipid monolayers were considered as a model for the cellular glycocalyx.

## 7. Conclusions

The study of monolayers containing glycolipids provides many insights into the molecular arrangement of glycolipids in membranes. The physical state of the monolayers influences the interaction of glycolipids with binding partners, which can be studied directly by introducing these partners into the subphase. The modulation of these binding interactions is significant to the membrane biochemistry of glycolipids, and monolayers at the water surface provide a uniquely convenient and controllable environment in which to conduct such studies.

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