CHAPTER 2

LITERATURE REVIEW

2.1 BACTERIORHODOPSIN

2.1.1 Halobacterium Salinarum

Halobacterium salinarum is an extremely halophilic (salt loving) single celled primitive micro-organism that belongs to the domain archea of the three-domain system namely bacteria, archea and eucaryota. It thrives in hyper- saline environment such as natural salt lakes and salt evaporation ponds, which has salt concentration of about 4 – 5 Molar. The cell of the bacterium is in the shape of rod with a typical dimension of three micrometers long and half a micrometer wide. It also has one or two bundles of flagella that derive from the cell pole which the bacterium uses to move around [18].

The membranes of *Halobacterium* can be divided into three fractions, a yellow fraction, a red fraction and a purple fraction. The yellow fraction is the protein rich walls of gas vacuoles which is used to regulate the cell pressure at different depths in the water. The red fraction consist of all the necessary enzymes for cellular respiration and the synthesis of ATP, carotenoid and porphyrin pigments. Hence, in the environment of high concentration of oxygen and dim light, the bacterium survives with normal respiration process.

Whenever the oxygen concentration drops in its environment and with the presence of lots of sunlight the bacterium grows the purple membrane patches. The patches are in oval shape and about one micrometer in diameter. It occupies more than half the surface of the membrane of the bacterium. The purple membrane is composed primarily of protein molecules called bR which exist in a two dimensional crystalline array

embedded in lipid bilayer. It is estimated that a single purple membrane patch contains about 20,000 molecules of bR.

bR is the center of photosynthesis. Upon absorbing a quantum of photon, bR pumps a proton from the inside to the extracellular side of the membrane. The proton or the potential energy gradient across the membrane which is called the proton motif force (PMF) drives the synthesis of Adenosine Triphosphate (ATP) by use of ATPase complex. ATP is the primary source of energy, for virtually every cellular activity of a living cell. Thus the synthesis of bR by the *Halobacteria* ensures its survival in harsh environment. However, this unique anaerobic ATP manufacturing system could not be considered as true photosynthesis. The bacterium obtains energy from light but they do not use light to synthesize food (carbohydrate) and they cannot fix carbon dioxide. *Halobacteria* is heterotrophic, as such they still need source of organic food for survival.

Four different rhodopsin like pigments can be found in the bacterium (Figure 2.1). The first two are the sensory rhodopsins, rhodopsin I and rhodopsin II, and the other two are bacteriorhodopsin and halorhodopsin, which are used to drive photosynthesis. The latter two are the most extensively researched due to their potential usage in modern technology.

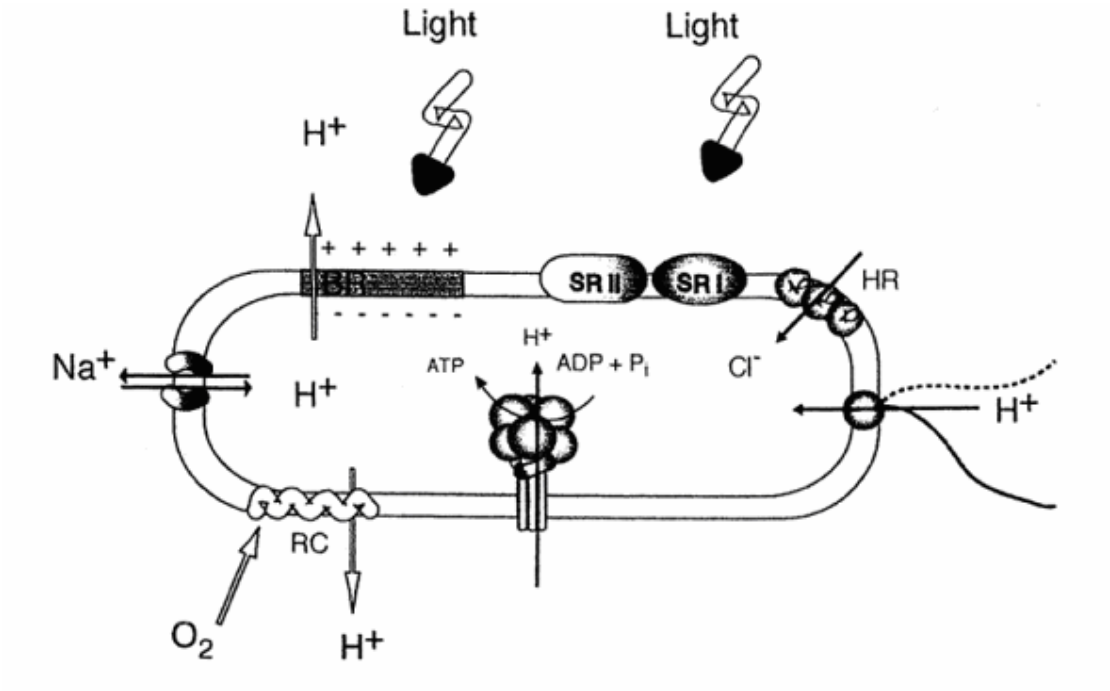

Figure 2.1 *Proton transfer processes related to bioenergetic and photosensory processes in Halobacterium salinarum. bR – bacteriorhodopsin; HR – halorhodospin ; SRI, SRII -photosensory pigments I and II; RC – respiratory chain. (From Bickel-Sandrkotter et al. 1996)*

2.1.2 Sensory Rhodopsins

The discovery of the sensory rhodopsins are much attributed to the researches at the University of Texas. They discovered that special Halobacterium mutants that lacked bacteriorhodopsin were still able to respond to light. Subsequently they found that the H. Salinarium used two retinal-based pigments to control its movement and named them sensory rhodopsin I and sensory rhodopsin II. In the condition where oxygen, carbohydrate and other food sources are abundant, the bacterium grows normally and moves away from sunlight to prevent damage caused by photo-oxidation. The sensory rhodopsin II which strongly absorbs blue green light, is used to control this response. In fact it is the only rhodopsin that significantly found in the bacterium in these conditions.

When the oxygen level is low and the bacterium can no longer sustain normal cellular respiration, the bacterium synthesizes the bacteriorhodopsin, halorhodopsin and the sensory rhodopsin I. By absorbing red-orange light, the photosynthetic rhodopsins perform a unique form of photoshyinthesis so that the bacterium can survive in the anaerobic conditions. It is the sensory rhodopsin I that drives the cell towards the red-orange light so as to maximize photosynthesis. Rhodopsin I is also used by the bacterium to swim away from ultraviolet radiation which can cause cellular damage.

2.1.3 Photosynthetic Rhodopsins.

As discussed above the bacterium makes the two photosynthetic rodhopsins, halorhodopsin and bacteriorhodopsin in order to survive in anaerobic condition. While both are similar in its functions, they are used for two different purposes. Bacteriorhodpsin is a light driven proton pump while the latter is a light activated Chloride ion pump. In its typical habitat where the sodium chloride concentration outside the cell is very high, is balanced by a very high internal concentration of potassium chloride (KCl). The cells normally expel sodium ions and take up potassium ions to maintain this balance. Halobacterium cells must take up potassium ions more rapidly than they expel sodium ions in order to grow (take up water and increase in volume). As the cells take up potassium ions, they must also take up chloride ions; this is an energy-requiring process because the inside of the cell is more negatively charged than the outside. Thus Halorhodopsin uses the energy of sunlight to drive the important process of chloride ion uptake so that cells can grow.

Since bacteriorhodopsin is the main subject of this study, its structure, function, characteristics and use in technology is elaborated in the next section.

2.1.4 Bacteriorhodopsin

2.1.4.1 Discovery and Structure

Bacteriorhodopsin was first discovered in the early 1970s by D. Oesterhelt and W. Stoeckenius [19]. It was not just a new discovery of new membrane protein but also was a relatively simple alternative photosynthesis system.

As mentioned above, bacteriorhodopsin is found in the purple membranes of the *Halobacterium Salinarium*. Naturally occurring in two dimensional crystal array, the purple membrane consist of many thousands of repeated hexagonal unit cells, where each unit cell contains three bR molecules (bR trimer) and lipid molecules (Figure 2.2). The bR molecules are closely packed as such they make up 75 % of the total mass of the purple membrane and the balance 25% are taken up by lipid molecules. A typical dimension of a single bR molecule in its native from is 25 X 35 X 45 Angstrom [20].

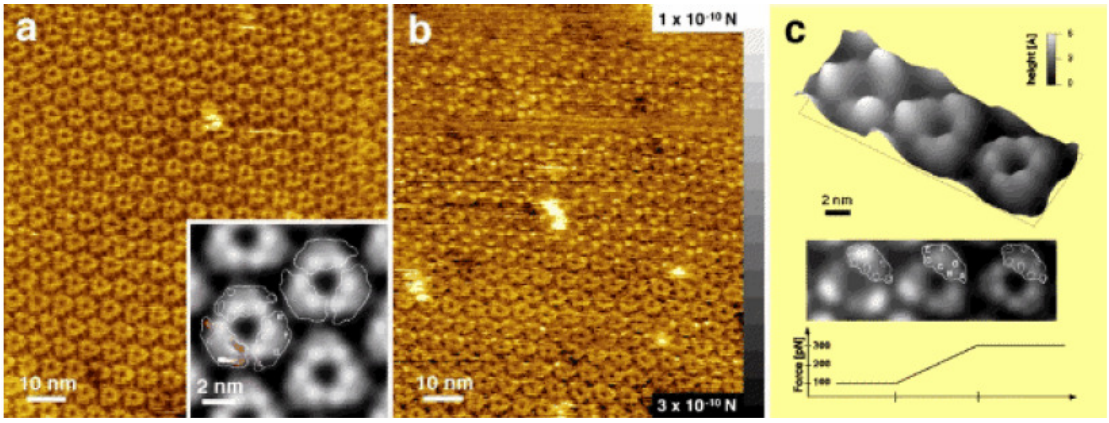

Figure 2.2: *AFM image with a high resolution of the topography of the purple membrane containing bR and lipids [21]*

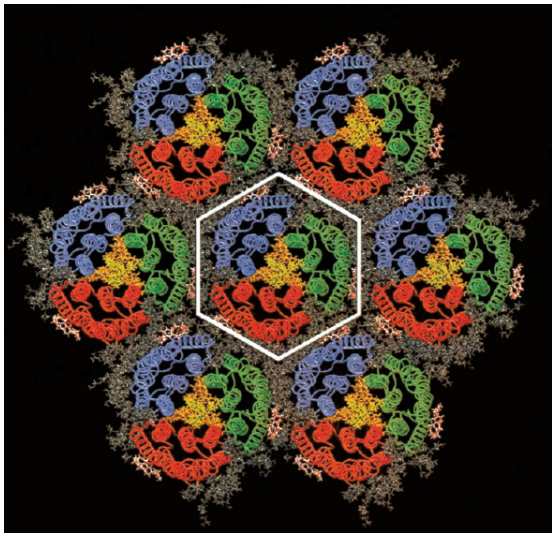

Figure 2.3*: bR Trimer in 2-Dimensional Hexagonal cell[67]*

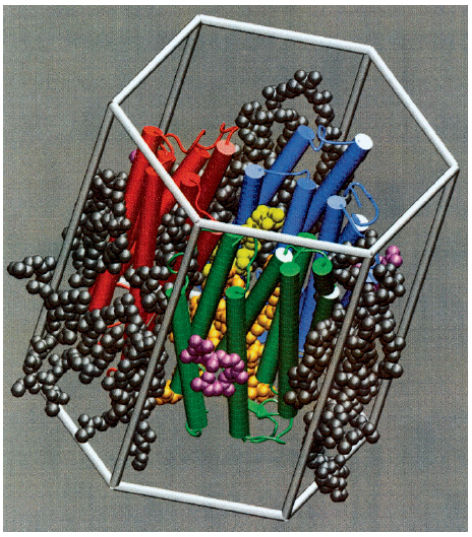

Figure 2.4: *Unit cell of bR in 3-Dimensional View[67]*

First attempt to describe the three dimensional detailed structure was made by be Henderson et al in 1975 using 7 Å resolution electron microscopy [20]. Later the resolution was further refined using 3 Å resolution cryo-electron microscopy (Henderson et al 1990 [22]; Griogrieff et al 1996 [23]; Kimura et al 1997 [24]) then to 1.9 Å resolution using X-ray diffraction (Belrahli et al 1999[25]) and finally to 1.55 Å (Luecke et al 1999 [26]). The detailed three dimensional structure is shown in figure 2.3 and 2.4.

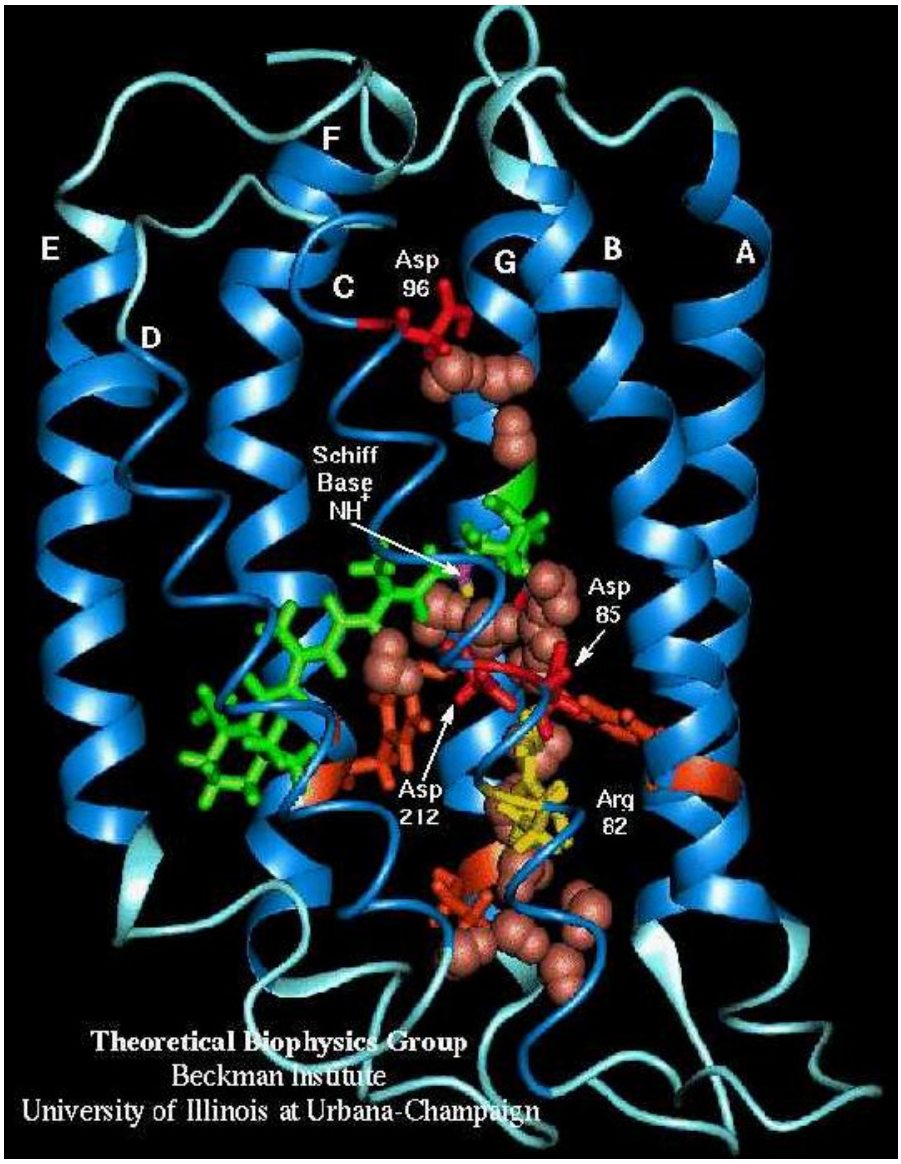

Figure 2.5: *Seven Helical structure of Bacteriorhodopsin with the retinal shown in green*

The 248 amino acid polypeptides of bR consist of seven helical segments(A through G), each spanning and enclosing an all trans-retinal or the chromophore (Figure 2.5). Helices A, B, C and D are inclined at small angels while helices E, F and G are almost perpendicular to the plane of the membrane [27]. The retinal is bound to the middle of helical G, near the NH_2 group of lysine residue and forms a positively charged Schiff

base. Upon absorbing light, the retinal undergoes a photoisomerization process in which the C_{13}-C_{14} bond isomerizes from "linear" all-trans to "bent"13-cis retinal (Figure 2.6).

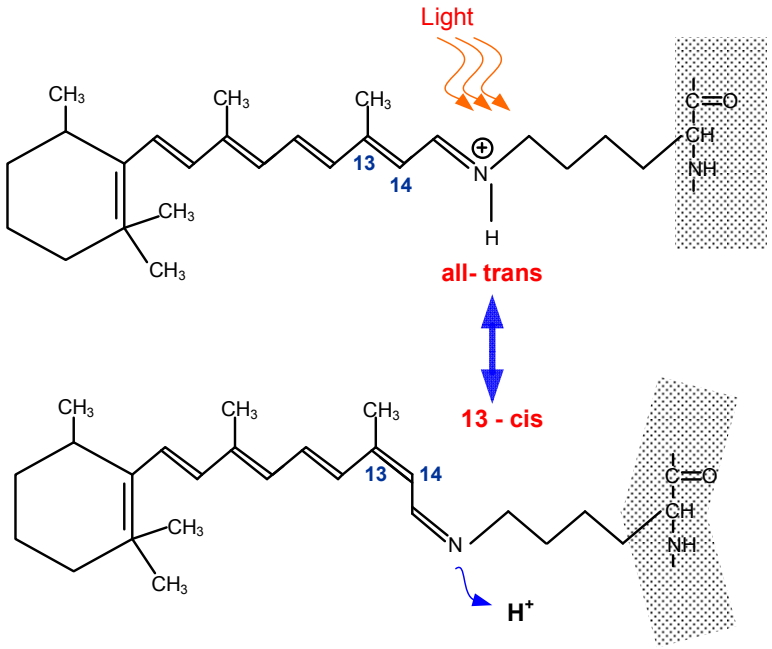

Figure 2.6: *Photoisomerization of retinal from all-trans to13- cis*

The 13-cis retinal is at higher energy intermediate and has lower affinity for the proton. As such a proton is released from the Schiff base and passes through the protein to the extracellular side of the membrane. On the other hand, the all-trans retinal is at its ground state and has higher affinity for the proton. Thus the Schiff base takes up a proton from the cytoplasmic side of the membrane and slowly stretches back to the "linear" all-trans retinal. This cycle is repeated many times and a proton gradient is developed across the membrane. As discusses earlier, the proton gradient is then used to synthesis ATP molecules which is crucial for the survival of the Halobacterium Salinarium.

So far we have only discussed the proton pumping cycle in brief. However the cycle consists of a complex photochemical cycle called the photocycle, in which the bR goes through a distinct intermediate states of J,K,L, M and O.

2.1.4.2 Photocycle

Although many femtosecond spectroscopic experiments have been conducted to elucidate the key kinetics of the photocycle process, there are still many aspect of the process yet to be discovered. However, the generally accepted scheme of the photocycle is ground state bR, intermediates of J,K,L,M,N and O and back to ground state bR. This is schematically shown in figure 2.7 below.

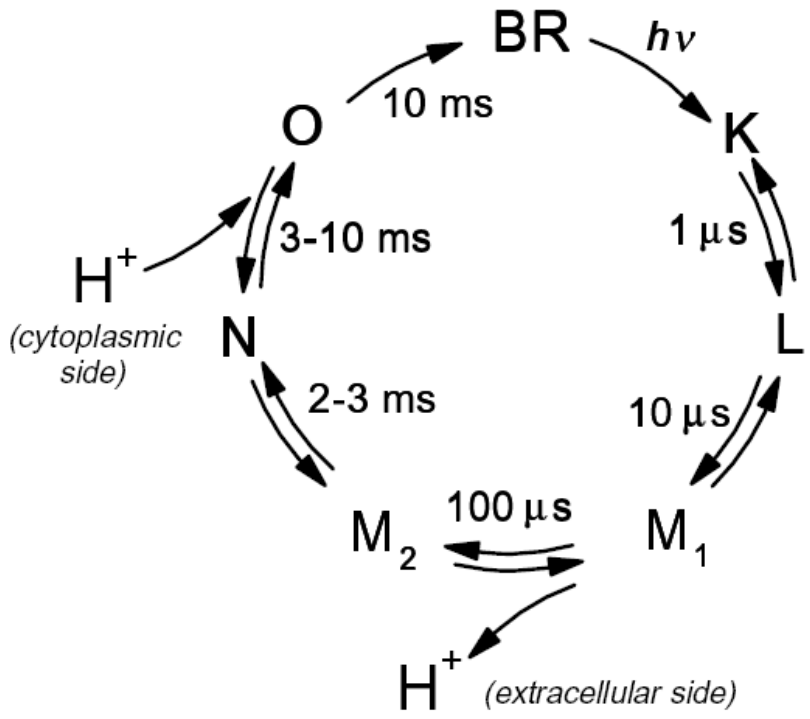

Figure 2.7: *Photocycle of bR with the approximate relaxation times of the reactions at room temperature*

We have seen that the photoinduced isomerization around the C13=C14 double bond which converts all-trans to 13-cis retinal kick starts the photocycle process. It is an

extremely fast (200-500 fs) process and indeed is the fastest chemical reactions in nature. The photon absorbed at this initial process has a maximum wavelength (λ_{max}) at 568 nm. J (λ_{max} 625 nm), is the first electronic intermediate structure that formed. It is likely that the retinal is already transformed to 13-cis configuration. Since J is extremely unstable, it is not considered as the primary intermediate state (and not shown on the photocycle schematic).The red-shifted intermediate K is the first primary intermediate state. It has the maximum absorption at 590 nm and formed within 3 picoseconds. The K to L (λ_{max} 550 nm) intermediate takes place within 1 micro second. In the L state the Schiff base looses its proton while Asp-85 receives the proton and becomes protonated.

The deprotonated Schiff base can be detected through its blue shifted absorption maximum near 412 nm and its various forms are called as the M states. Though the M intermediates itself is quite complex, there are two equilibrium states M_1 and M_2 exist. The L to M_1 transition takes approximately 10 micro seconds while M_1 to M_2 takes 100 microseconds. At the end of M_2 equilibrium the proton have been released into the extra cellular side.

The Schiff base is subsequently becomes red shifted upon reprotonation from the cytoplasmic side which corresponds to the N intermediate. This takes about 2-3 milliseconds. The Schiff base is protonated but still in the 13-cis isomeric state. In the N to O transition, reprotonation of Asp 96 from the cytoplasmic side and thermal reisomerization of the Schiff base back to all-trans takes place. Finally the O state recovers back to the initial bR state within 10 milliseconds. Also during this the Asp85 deprotonates and the proton release group in the extracellular channel is reprotonated. With this, the bacteriorhodopsin is ready to repeat the cycle as long as it is illuminated.

2.1.4.3 Application of bR

Though being a protein molecule, a wide range of practical applications have been found for bR due to its exceptional quality such as mechanically robust, chemically and functionally stable in extreme conditions like high temperatures and wide range of pH[28-30]. Below are some of the outstanding properties of bR [31] :-

- Simplicity in its function
- High quantum efficiency of 64%
- Stable toward high temperatures of up to 140^{o} C
- Stable towards exposure to sunlight for years
- Stable towards wide range of pH, 0 -12
- Extremely thin – thickness of 5 nm

The above mentioned extraordinary properties of bR makes it an excellent material to be utilized in many modern applications such as holography, optical computing and memories, parallel data processing, spatial light modulators artificial retina and photovoltaic generators. The potential gradient generation by the proton pumping activity upon illumination can be used as photovoltaic generator [32-33], photosensors / photodetecters [34-35] and artificial retina [36-37]. The photochromic effect has been successfully utilized for the creation of holographic memories [38-41], spatial light modulators [42-43] and many more.

2.2 LANGMUIR FILMS AND LANGMUIR-BLODGETT FILM DEPOSITION

2.2.1 Introduction

In the recent years the demand for smaller and smaller devices with higher speed and performance is ever increasing. In this regard the development of thin film technology plays crucial role in the process of realizing molecular level devices. There are various techniques available to produce mono molecular level thin films, such as thermal evaporation, sputtering, electrodeposition, molecular beam epitaxy, adsorption from solution, Langmuir-Blodgett (LB) and self-assembly. Among these, the LB-technique is one of the most promising method as it provides true monomolecular assemblies for various applications. Some of the advantages of using LB technique over others are;-

i) It enables precise control of the monolayer thickness
ii) Enable homogenous deposition of monolayer film over large areas
iii) Possibility of making multilayer structures with varying layer composition
iv) Low cost
v) Increase in thermal, physical and chemical stability of deposited thin film

2.2.2 Short History of LB- films

In 1774, American statesman, Benjamin Franklin reported to the British Royal society that little oil dropped on a large pond, spread amazingly covering about half an acre area of the ponds' surface [44]. He suspected that the oil film on the water represents a layer of one molecular thick but however no quantitative calculations were attempted.
About 100 years later a German woman called Agnes Pockles [45] developed a basic surface balance in her kitchen sink where she determined surface contamination as a function of area of the surface for different oils and published it in Nature 1891.

It was Irwing Langmuir who first to perform a systematic studies on floating monolayers on water in the late 1910's and early 1920's. As early as 1920 he reported the transfer of fatty acid molecules from water surfaces onto solid supports [46]. These studies led to him being awarded the Nobel prize. However, several years later, Katherine Blodgett first detailed the sequential monolayer transfer on to solid substrate. These build-up monolayer are therefore referred to as Langmuir-Blodgett(LB) films [47]. It took almost half a century before scientist all around the world to realize the importance and potential of this unique technique in many fields of research. Currently the LB is one the most widely used technique to produce monolayer films especially with biomembrane and organic based materials.

2.2.3 Fundamentals of Surface Chemistry

In order for one to understand how the Langmuir Blodgett technique works, it is important to know the fundamentals of surface chemistry and its underlying theories.

The first and foremost important property that one must understand is the surface tension or surface free energy. Forces acting on molecules in the bulk and the molecules on the surface are totally different. In the bulk a molecule is surrounded by other molecules in all direction and experiences equally balanced force from its' surrounding molecules. On the other hand, molecules on the surface act as an interface between two phases – i.e. liquid-air phases. At one side, the molecules were subjected to a strong attractive force from the molecules below in the bulk, while weak or no force from the top or the gaseous phase (Figure 2.8) [48].

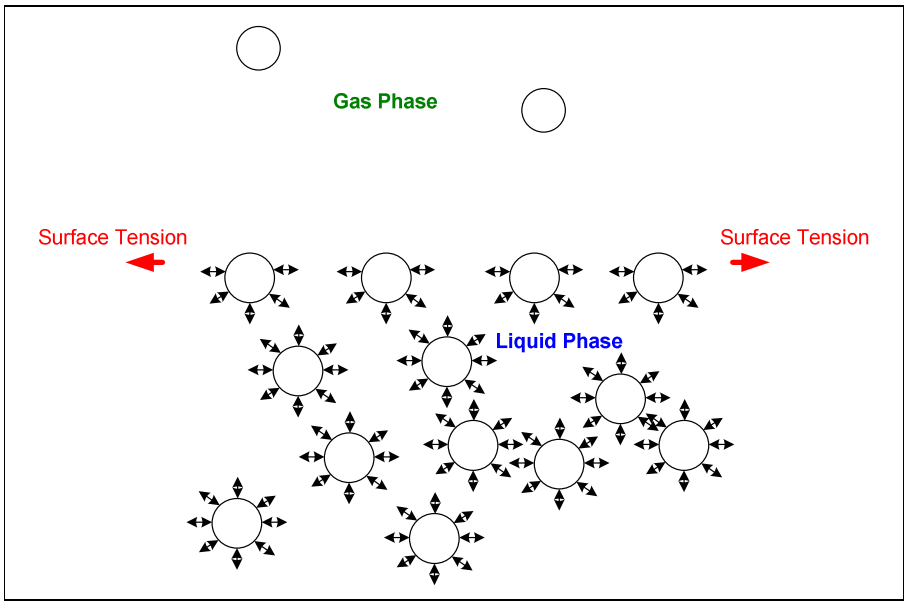

Figure 2.8: *Schematic illustration of the interaction of molecules at a gas-liquid interface and the bulk.*

As a net result of this condition, there will be always a free or surface free energy exists at the surface. The surface free energy also can be considered as a line force that acting on the surface molecules, thus called the surface tension γ. The surface tension can be quantified as force per unit length and the common unit is mN/m. In terms of thermodynamic equilibrium, the surface tension is the partial derivatives of the free energy functions *F* with respect to the area A of the surface.

$$\gamma = \left(\frac{\partial F}{\partial A} \right)_{T,V,n_i}$$

Table 2.1 below shows some typical two phase inter-phase system and the value of surface tension. The molecular interaction in a polar liquid such as water is strong which is reflected in the high value of the surface tension. As such water makes the most suitable liquid to be used as sub-phase.

Table 2.1: *Some two phase inter-phase systems and the surface tension value*

Interface system	γ (mN/m)
Water - air	72.8
Ethanol - air	22.3
Hexane - air	18.4
Hexane - water	51.1
Decane - water	52.2

Amphiphilic molecules are molecules that contain both hydrophobic and hydrophilic groups in them. The hydrophobic part usually consists of hydrocarbon or fluorocarbon chains, while the hydrophilic part consists of a polar group. (-OH, -COOH, -NH3+, -PO4 -(CH2)2NH3+ etc.). They have unique molecular interactions due to the hydrophobic interaction between the tail group and the hydrophilic or electrostatic interaction between the head or polar groups.

Surfactants or surface active molecules have at least one hydrophobic tail group and one hydrophilic head group [49]. When comes in contact with a polar liquid such as water, the hydrophobic group will repel the water, while the hydrophilic group gets attracted to water. As such the surfactant molecules orient themselves in a manner where the polar head group is immersed in the water and the long hydrocarbon chain is pointing towards air (away from water) as shown in Figure 2.9.

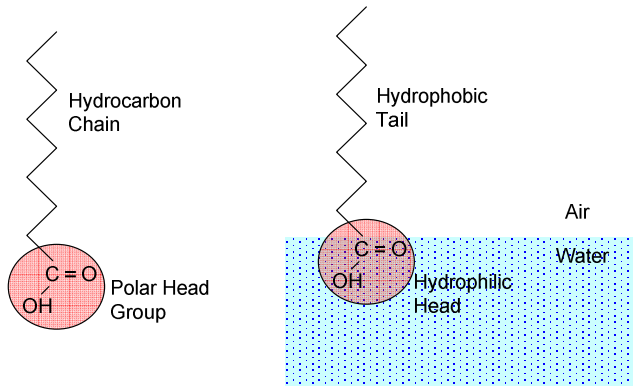

Figure 2.9: *A schematic illustration showing the components of an amphiphile (left), and the orientation of an amphiphile adopted at an interface (right).*

Many of these surfactants are insoluble in water. However, with the help of a volatile and water insoluble solvent, they can be dissolved and easily spread on a water surface to form an insoluble monolayer at the air/water interface (Figure 2.10). Their presence even in a small concentration on the surface of water causes decrease in the surface tension of the water. The driving force behind this is the reduction of the free energy of the system.

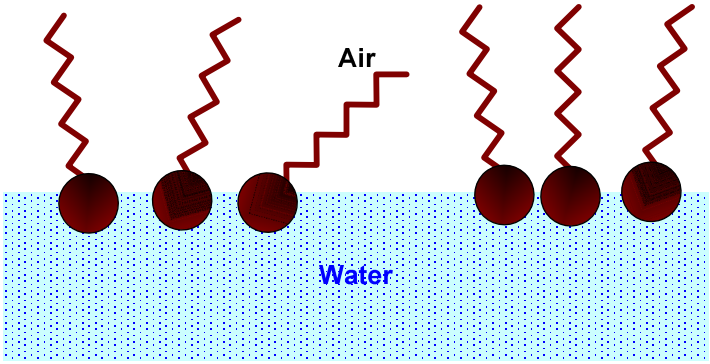

Figure 2.10: *A schematic illustration showing a spread monolayer at the air/water interface.*

The balance between the hydrocarbon chain and the polar head plays a crucial role in forming a stable insoluble monolayer film on the air/water interface. If the hydrocarbon chain is too short, the surfactant molecules will dissolve in to water phase or tend to

form micelles (which are also water soluble). On the other hand, if the length of the chain is too long, the amphiphiles tends to crystallize on the water surface. Both conditions prevent the formation of monolayer film on the air/water interface.

It is difficult to determine the optimal length for the hydrocarbon chain because its film forming ability also depends on the polar part of the amphiphile. Furthermore, the amphiphile has to be soluble in some organic solvent which is highly volatile and water insoluble (chloroform or hexane is commonly used).

2.2.4 Surface Pressure

As discussed earlier air/water interface possesses excess free energy originating from the difference in the molecular interaction between the surface molecules and those in the bulk. This interfacial free energy is accessible by measurements of the surface tension.

When a solution of surfactant in a water insoluble solvent is placed on a water surface, the solution spreads immediately to cover the available area. As the solvent evaporates, the surfactant molecules are left on the water surface forming a monolayer film. The presence of surfactants on the water surface, reduces its' surface tension. The difference between the surface tension with and without the presence of surfactant molecules [50] on the water surface is defined as the surface pressure π :-

$$\pi = \gamma - \gamma_o = -\Delta\gamma$$

Where γ is the surface tension in absence of a monolayer and γ_o the surface tension with the monolayer present.

2.2.5 The Langmuir Film Balance

With a set up of Langmuir film balance as shown in Figure 2.11 below, it is possible to measure the surface pressure of the monolayer film. A basic construction of the Langmuir film balance is consists of trough, movable barriers and a tensiometer [50].

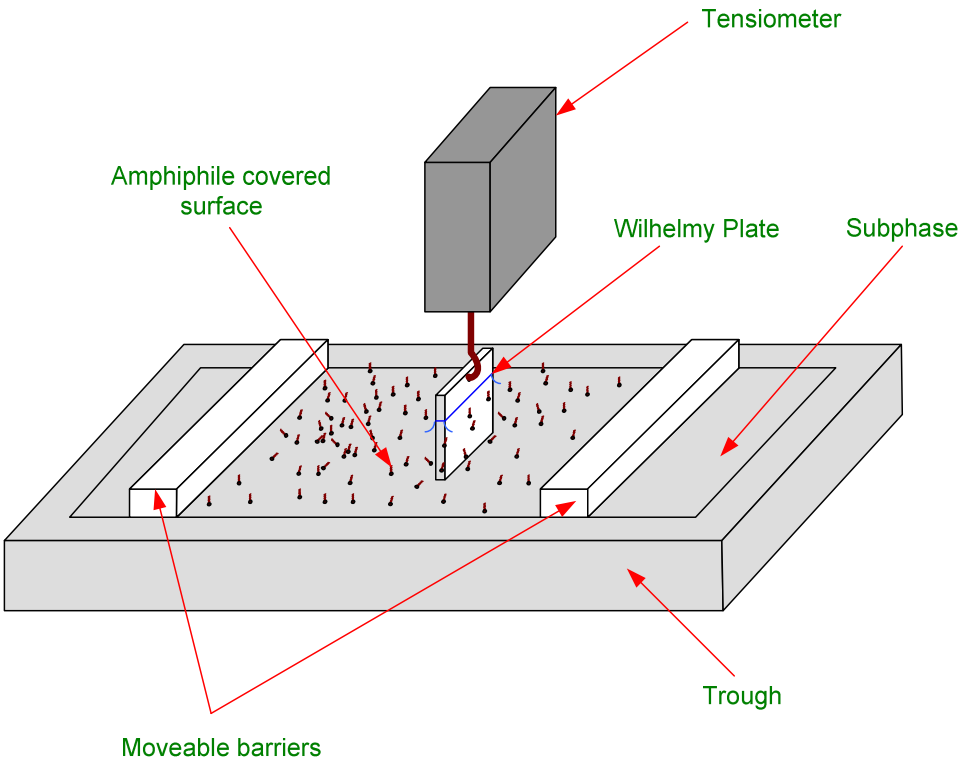

Figure 2.11: *Schematic illustration of a Langmuir film balance with a Wilhelmy plate electrobalance measuring the surface pressure, and barriers for reducing the available surface area.*

The trough which is holding the subphase is usually made of Teflon, a material which is highly hydrophilic to prevent any leakage of the subphase over the edges. The movable barriers are used to sweep across the surface of the subphase to vary the surface area of the monolayer film. These are usually made of Delrin, also a hydrophilic material and heavy enough to prevent any leakage of the monolayer beneath the barrier. The tensiometer is the device that measures the surface pressure of the monolayer as it is get

compressed by the two barriers. The tensiometer uses the Wilhelmy plate method to measure the surface pressure. In this method a plate usually made of platinum or filter paper is suspended so that it is partially submerged in the subphase. The forces acting on the plate is then determined and converted into surface pressure.

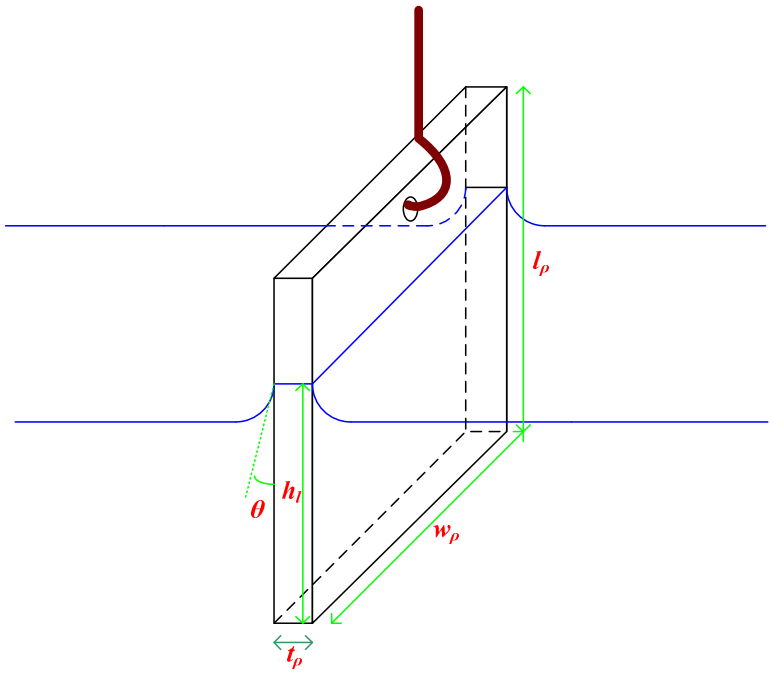

Figure 2.12: *A Wilhelmy plate partially immersed in subphase*

A rectangular plate with dimensions l_ρ, w_ρ and t_ρ, of material ρ, is immersed to a depth h_l in a liquid of density l (Figure 2.12).The forces acting on the plate are gravity, surface tension and buoyancy. The gravity and surface tension are acting downward while the buoyancy upward. Therefore the net force acting on the plate is given by

$$F = \rho_p g l_p w_p t_p + 2\gamma(t_p w_p)(\cos\theta) - \rho_l g t_l w_l h_l$$

Where ρ_p is the liquid surface tension, θ is the contact angle of the liquid on the solid plate and g is the gravitational constant. The surface pressure is calculated by measuring the change in F for a stationary plate between a clean surface and the same surface with

a monolayer present. If the plate is completely wetted by the liquid (i.e. $\cos\theta = 1$) the surface pressure is then obtained from the following equation:

$$\pi = -\Delta\gamma = -\left[\frac{\Delta F}{2\left(t_p + w_p\right)}\right]$$

if $w_\rho >> t_\rho$, then

$$\pi = -\frac{\Delta F}{2w_p}$$

From the formula above we can deduce that the thinner the plate, the more accurate is the measurement. The sensitivity of the tensiometer can thus be increased by using a very thin plate. By coupling the plate directly to a sensitive electrobalance, the force acting on the plate can be determined by measuring the changes in the mass of the plate.

The monolayer can also be held at a constant surface pressure, which is enabled by a computer controlled feedback system between the electrobalance and the motor responsible for the movements of the barriers.

2.2.6 Surface Pressure - Area Isotherms

The characteristics of the monolayer material on the water surface are analyzed by measuring the changes in surface tension in terms of the surface area per molecule(surface area available to each molecule) upon compressing the monolayer. Since the process of compressing is carried out at a constant pressure it is known as the surface pressure – area isotherm or simply “isotherm”. Repeated compressions and expansions may be necessary to achieve a reproducible trace.

The shape of the isotherm provides the two dimensional signature of the characteristics of the molecules that make up the monolayer film. However many physical parameters such as the temperature, composition and pH of the subphase, the barrier speed, the solvent used to dissolve the surfactants and the relative humidity, may change the shape of the isotherm. As such it is crucial to perform the isothermal compression in a controlled environment such as clean room.

For example, various monolayer states exist depending on the length of the hydrocarbon chain length and the magnitude of other cohesive and repulsive forces existing between head groups. An increase in the chain length increases the attraction between molecules, condensing the π-A isotherm. On the other hand, if an ionizeable amphiphile is used the ionization of the head groups induces repulsive forces that tend to oppose the phase transitions.

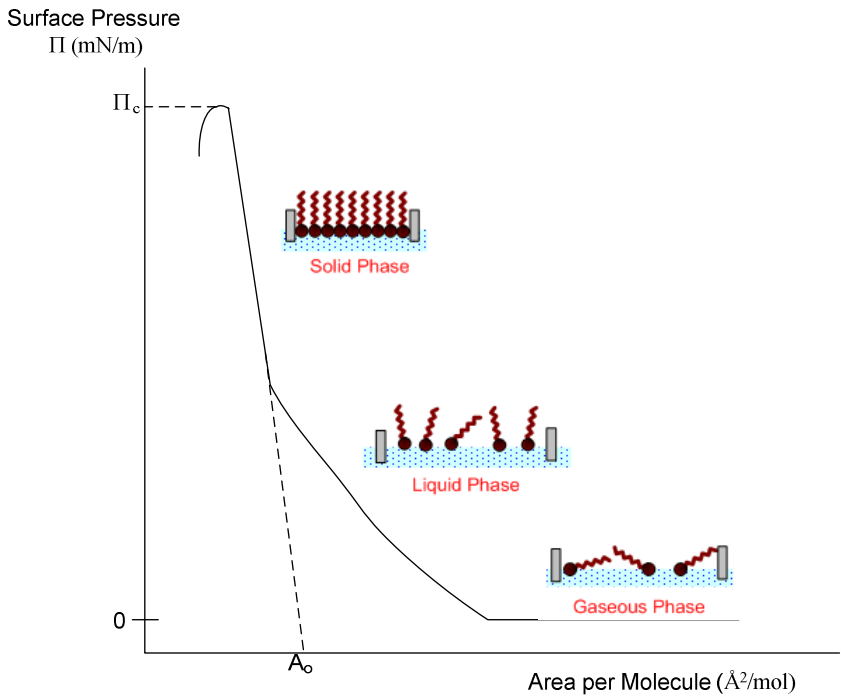

Figure 2.13: *Schematic π -A-isotherm and orientation of the molecules in different phases.*

A typical surface pressure π - area per molecule *A* isotherm is shown in Figure 2.13. It is apparent that there are a number of distinct regions or phases from the isotherm. Initially, when the surfactants are transferred on to the surface of subphase, the available area for the monolayer is large. The distance between adjacent molecules is large and their interactions are weak. The molecules can be considered to be in two-dimensional gaseous phase. Under this condition the molecules have very little effect on the surface tension of the subphase [49].

As we compress the surfactant molecules on the subphase, the mean distance between molecules starts to reduce and they begin to exert stronger forces among them. The surface pressure of the system starts to increase and it can be considered to be in two dimensional liquid phase.

Further compression will cause the molecules to become even closer and compactly packed. The surface pressure starts to increase steeply and linearly. The molecules are now in two dimensional solid phase. With continued closing the barrier, the forces acted upon the molecules become too strong for confinement in two dimensions and the molecules start to slip either into the subphase or onto the monolayer. The surface pressure corresponding to this point is called the Collapse pressure π_c. The film irreversibly loses its mono-molecular form.

2.2.7 Deposition of Langmuir Blodgett Films

At the solid phase stage, the floating molecules are compactly packed and the surface pressure is high enough to ensure sufficient cohesion in the monolayer. With a proper mechanism the monolayer film can be deposited on to a solid substrate which later can be used for characterizing or for fabricating mono-molecular devices. The deposition is usually done by simply dipping a solid substrate up and down through the monolayer while simultaneously keeping the surface pressure constant by a computer controlled feedback system between the electro balance, measuring the surface pressure and the barrier moving mechanism. It is crucial that the surface pressure where the deposition process takes place is high so that the attractive force between the molecules is high enough to prevent them from falling apart during the transfer process. This also ensures the buildup of a homogeneous film.

Repeating successively the dipping and withdrawing process allow us to coat multilayer structures consist of hundreds of layers. These multilayer structures are commonly called Langmuir-Blodgett or simply LB films.

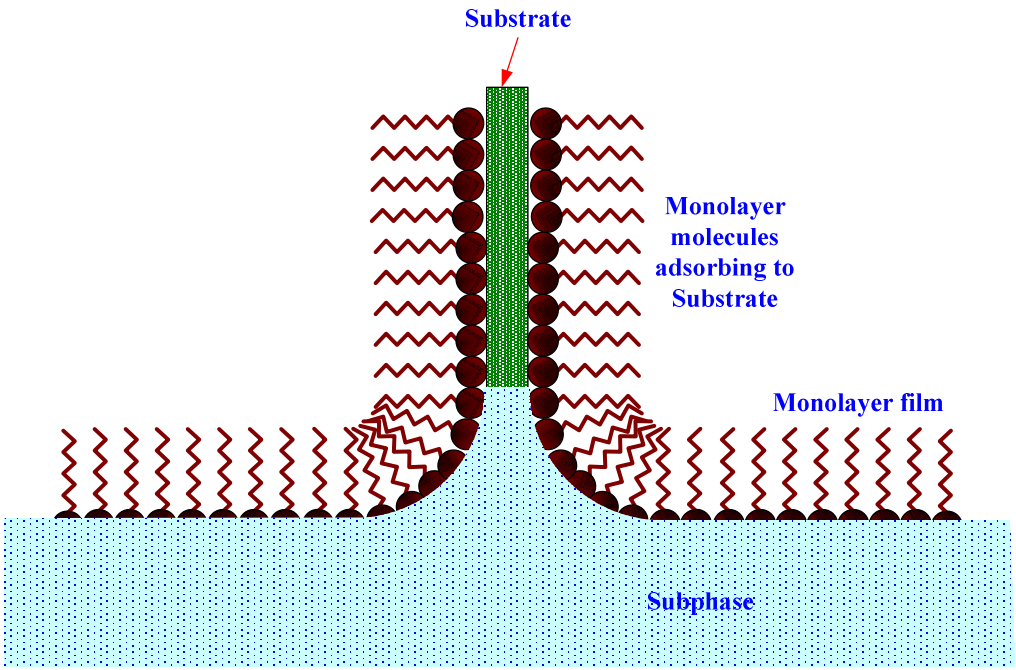

Figure 2.14: *Deposition of a floating monolayer on a solid substrate*

The surface pressure value that gives the best results depends on the nature of the monolayer and is usually established empirically. However, amphiphiles can seldom be successfully deposited at surface pressures lower than10 mN/m, and at surface pressures above 40 mN/m, molecular collapse and film rigidity often pose problems.

The monolayer film is transferred on to the substrate by the action of adsorption of the amphiphile molecules on to the surface of the substrate (Figure 2.14). There are two types of surface exist, either hydrophobic (HOPG, silanized SiO2 etc.) or hydrophilic(glass, SiO2 etc.). Depends on the type of the surface, few types of depositions are possible.

Deposition onto a hydrophobic surface always takes place during the down ward stroke where the hydrocarbon tails adhere to the hydrophobic surface (Figure2.15). With the substrate covered with the amphiphile molecules in such a way that the polar heads

protruding out, the substrate becomes hydrophilic. Now, if we withdraw the substrate from the subphase, the second layer with the polar head adhering to the polar head, a bilayer film is produced. As such with a hydrophobic substrate, it's always even (2, 4, 6, …) number of layers deposited.

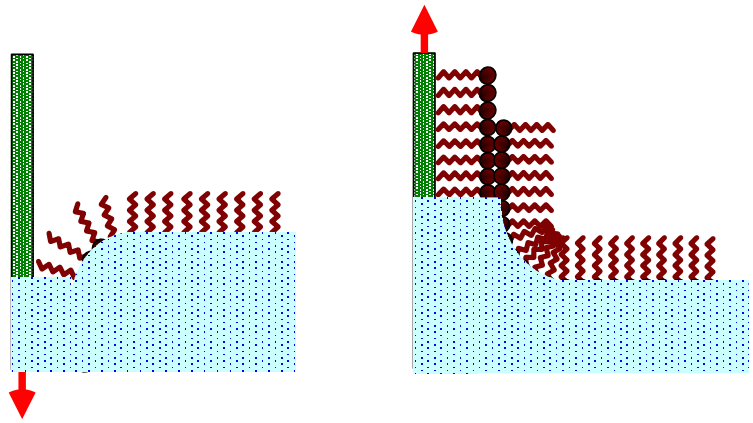

Figure 2.15: *Deposition on hydrophobic substrate*

In the case of hydrophilic substrate, deposition only takes place on the first up ward stroke because the hydrophobic tails are repelled by the hydrophilic substrate as it is immersed in the water (Figure 2.16). With the polar head adhering to the substrate's surface and the hydrocarbon tails protruding out, it becomes hydrophobic surface. When the substrate is dipped again, the second layer where the hydrocarbon tails adhering to hydrocarbon tail is produced. Hence with hydrophilic substrate, always odd (1, 3, 5, …) number layers is deposited.

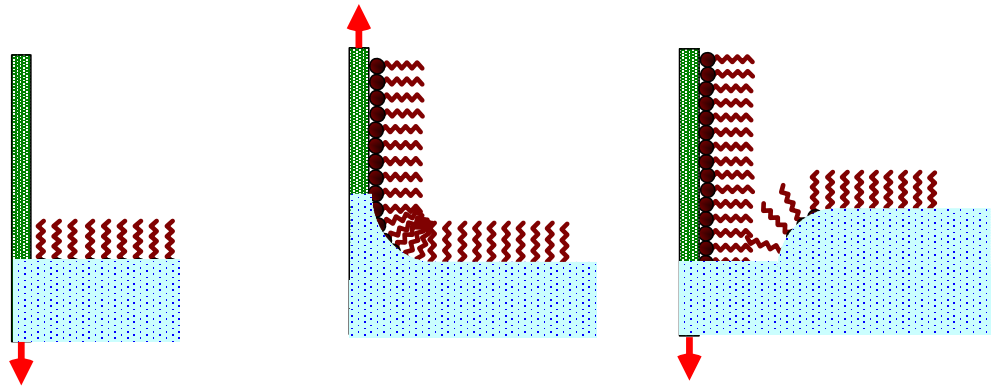

Figure 2.16: *Deposition of on hydrophilic substrate*

Different types of multilayered configurations can be produced by successive deposition of the monolayers on the same substrate. The most common type is the Y-type which is produced by depositing the amphiphile molecules in 'head to head and tail to tail' configuration. There are two other type of deposition have be observed, 'X' and 'Z' type [51-52] (Figure 2.17).

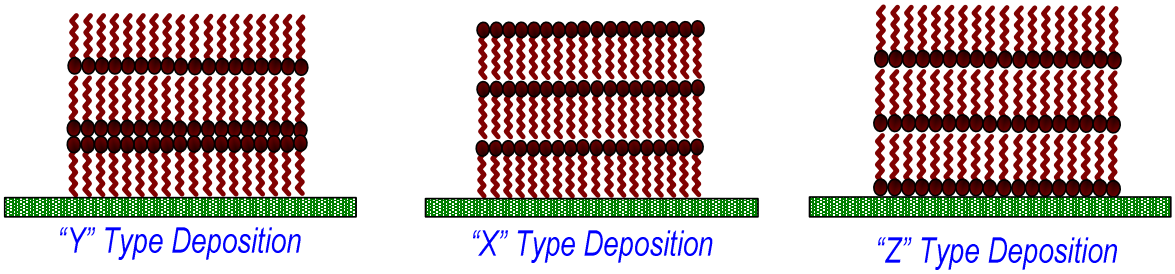

Figure 2.17: *Different types of deposited LB films*

When the monolayer deposits only in the up (the head group attached to the substrate) direction it is called Z-type and when deposits in the down (tail group attached to the substrate) direction it is called X-type. Intermediate structures are sometimes observed for some LB multilayers and they are often referred to be XY-type multilayers.

However there is no complete understating of why some molecules will deposit in the X, Y or XY type.

Transfer Ratio (TR) is an important measure of the quantity and quality of the deposited film. It is defined as the ratio between the area of monolayer removed from the subphase at constant pressure to the area of substrate. For ideal transfer the TR should be equal to 1.

An alternative way to deposit the monolayer is the Langmuir-Schaeffer (LS) technique. This technique differs from the vertical technique described above only in the sense that the solid substrate is horizontally lowered in contact with the monolayer.

2.3 Previous Works on bR- Langmuir Blodgett Film and Scope of This Study

Earlier we have discussed that most of the bR based molecular prototype devices were configured in a monolayer to multilayered thin film on a solid substrate and there are various ways of incorporating bR into thin films [7-12]. Among these techniques, the Langmuir-Blodgett (LB) seems to be one of the most suitable as it able to produce closely packed, highly oriented thin film. Also in section 2.2 we learned that LB method involves floating stable amphiphilic molecules on an air-water interface which will then be compressed to a predetermined surface pressure to form a well distributed and compact monolayer film which then can be transferred onto almost any solid substrate. Since bR is more hydrophilic molecule [53], it can easily penetrate into the water sub-phase. As such, in many research works [54-56] the solubility of bR in water have been reduced by using a certain concentration of salt solution of either Sodium Chloride (NaCl) or Potassium Chloride (KCl) as subphase. However once deposited on to solid substrate, salt crystals were formed and removal of the crystals by any mean leaves holes in the monolayer which reduces the quality of monolayer. In other research works, bR suspension of certain concentration is mixed with certain ratios of organic solvents such as dimethylformamide (DFM), hexane and Soya phosphatidylcholine (PC) to form a complex emulsion of spreading solution which acts as a stabilizing medium to form a stable membrane-like bR film on a pure water sub-phase [57-59]. Almost in all the methods mentioned, sonication was used as a mean of mixing the solutions with bR suspension. However we do not know the degree of damage done to the delicate molecules of the bR by sonication. As such our first objective of this work is to prepare the bR solution and the spreading emulsion without the use of sonication. Howard H. Weetal et al [59] have successfully mixed bR suspension with hexane to produce a spreading emulsion that produces stable floating layer of bR molecules on

pure deionized water subphase. They also successfully transferred a functionally active bR monolayer film on to an ITO slide. As such, in this study we have followed the method proposed by Howard H. Weetal et al to prepare a bR suspension- hexane spreading emulsion with slight modification. Instead of sonication, we proposed agitation and a simple method of forced mixing the bR suspension and hexane to prepare the complex emulsion of spreading solutions.

The process of depositing thin film on a substrate is a vital process in producing good quality monolayer (LB) film, consequently a highly functional device. To achieve this, the most optimum condition for making the deposition is when the bR molecules are compactly packed at the sub-phase surface. Generally this condition can be identified as the condensed solid phase from the surface pressure – area isotherm. However identifying the solid phase from the isotherm is not easy as the phases are not well defined for bR. The compressibility modulus *K* which gives the information on the molecular arrangement can be calculated directly from the isotherm. Furthermore different phases are easily distinguishable from the *K* versus area plot, thus the compact solid phase [60-61]. Mario Method et al [17] used the *K* and the surface potential and found that both maximizes at a same surface pressure of 15 mN/m. They concluded that mechanically and electrically that 15 mN/m is the ideal surface pressure for film deposition. However, one of the important properties that have not been considered for the conclusion of the optimum condition is the thermodynamic properties. So far there were no references available to what is the thermodynamically ideal condition for bR thin film deposition. Therefore the second objective of our studies is to calculate thermodynamic properties such as Gibbs Free Energy, Enthalpy and Entropy of compression for the bR thin film. From the calculated values, we can determine thermodynamically optimum condition for the film deposition. The deposited film then

will be subjected to various spectroscopic studies to ascertain the molecular order and functionality.

2.3.1 Thermodynamic Properties of Langmuir Film

Since there were no literatures available on the thermodynamic properties of bR-LB thin film, in this section we have only discussed the general theory and some literature work done on other Langmuir film.

Thermodynamics is the study of parameters that describes the macroscopic properties of a system, such as temperature, pressure, concentration, and volume. In the case of two dimensional (2-D) system, it is the study of the surface pressure, area per molecule and the surfactant concentration.

The Gibbs free energy is a thermodynamic potential calculated from an isothermal, isobaric thermodynamic system. A system will spontaneously progress from a specified initial state to a hypothetical final state if the Gibbs free energy of the final state is lower than that of the initial state. The Gibbs free energy of compression is related to the Enthalpy of compression $\Delta H_c(\pi)$ and Entropy of compression $\Delta S_c(\pi)$ through:-

$$\Delta G_c(\pi) = \Delta H_c(\pi) - T\Delta S_c(\pi) \quad \text{----------(1)}$$

Enthalpy H is the sum of the internal energy and the product of pressure and volume of a system (surface pressure and area in the case of 2-D system). However the enthalpy change ΔH is a more useful parameter, as it consists of the change in the internal energy of the system and work that the system does to its surrounding. By definition ;-

$$\Delta H = \Delta U + \Delta(\pi A) \quad \text{----------(2)}$$

For the isothermal compression $\pi A = 0$, thus $\Delta H = \Delta U$. This implies that, the work done to compress the monolayer film isothermally is used to increase the internal energy of the monolayer film.

Entropy *ΔS* of a system is a measure of molecular disorder, or molecular randomness. As a system becomes more disordered, the positions of the molecules become less predictable and the entropy increases. For example the entropy of a substance is lowest in the solid phase and highest in the gas phase. In the case of monolayer film in the air-water interphase, the compression entropy, ΔS_c gives information on molecular rearrangement process that occur during compression and therefore the degree of order that the monolayer contains.

There are two thermodynamic models [49, 62-63] that have been used to calculate thermodynamic quantities of a monolayer at air water interphase as a function of compression from the π – A isotherm. They allows the calculation of the Gibbs free energy of compression as below :-

$$\Delta G_c(\pi) = -\int_{\pi_i}^{\pi_f} A_{bR}(\pi) d\pi - \int_{\pi_i}^{\pi_f} A_{water}(\pi) d\pi \text{ ----------(3)}$$

Since we reset the surface pressure of water to zero during the isothermal experiment, the integral part for water can be ignored, so the above equation is reduced to :-

$$\Delta G_c(\pi) = -\int_{\pi_i}^{\pi_f} A_{bR}(\pi) d\pi \text{ ----------(4)}$$

The negative sign in the formula above implies that work is done to the system by the barriers during compression.

2.3.2 Spectroscopic Characterization

2.3.2.1 UV-Vis Spectroscopy

In the process of fabricating/building a mono molecular thick film of biomaterial, it is essential to ensure that the molecules are still functionally active. Otherwise the bioelectronic device to be built using the monolayer film will not function as expected. To ascertain that bR molecules are still functionally active, many researches have [14,64] conducted the UV-Vis absorption spectroscopic measurements.

The light adaptation properties shown as the maximum absorption peak at 570nm wavelength (λ_{max}) is the key indication that the photoreaction process is taking place or in other words, the bR molecules are still functioning well. However there have been slight variations in the λ_{max} absorption peak reported by different researches due to the different film forming materials and techniques used. In this work, we have also included the UV-Vis spectroscopy studies to conclude if the deposited film is functionally active.

Ultraviolet radiation has wavelengths of 200-400 nm while visible light has wavelengths of 400-800 nm. UV rays are naturally harmful to living cells. As such the *Halobacterium salinarum*, has its own mechanisms to avoid the harmful UV rays in nature. Due to these reasons, we have opted to run the UV-Vis scanning only in the range of 400 – 700 nm

2.3.2.2 Structural Characterization

Structural studies provide excellent view on the molecular arrangement, orientation and distribution of the deposited monolayer film [14, 19, 66]. Scanning Electron Microscopy (SEM) is a type of electron microscope capable of producing high resolution images of a samples' surface revealing details about 1 to 5 nm in size. Due to the way these images are created, SEM micrographs have a very large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. SEM on this work were carried out to provide a basic idea on the level of close packing achieved on the monolayer film deposited at the mechanically and thermodynamically most optimal condition.

2.3.2.3 Film Thickness Measurement

Film thickness is a very important parameter in the characterization of Langmuir films. Thickness of a single bR molecule have been successfully determined to be 4.5 nm, using X-ray crystallographic method [22]. There are many methods available to determine the bR LB film, however in this work Auger Electron Spectroscopy (AES) and Surface Profiler methods were used to determine the thickness of the deposited bR film.