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Chapter 1 : Lipid bilayer - Wikipedia

The planar bilayer lipid membrane, also known as lipid bilayer membrane, black lipid membrane or simply BLM(s), for short, has been investigated since its inception in , the details of which have been described in a monograph published in

The center of this bilayer contains almost no water and excludes molecules like sugars or salts that dissolve in water. The assembly process is driven by interactions between hydrophobic molecules also called the hydrophobic effect. An increase in interactions between hydrophobic molecules causing clustering of hydrophobic regions allows water molecules to bond more freely with each other, increasing the entropy of the system. This complex process includes non-covalent interactions such as van der Waals forces , electrostatic and hydrogen bonds. Schematic cross sectional profile of a typical lipid bilayer. There are three distinct regions: Although the head groups are neutral, they have significant dipole moments that influence the molecular arrangement. Despite being only a few nanometers thick, the bilayer is composed of several distinct chemical regions across its cross-section. These regions and their interactions with the surrounding water have been characterized over the past several decades with x-ray reflectometry , [4] neutron scattering [5] and nuclear magnetic resonance techniques. The first region on either side of the bilayer is the hydrophilic headgroup. This portion of the membrane is completely hydrated and is typically around 0. In phospholipid bilayers the phosphate group is located within this hydrated region, approximately 0. One common example of such a modification in nature is the lipopolysaccharide coat on a bacterial outer membrane, [7] which helps retain a water layer around the bacterium to prevent dehydration. TEM image of a bacterium. The furry appearance on the outside is due to a coat of long-chain sugars attached to the cell membrane. This coating helps trap water to prevent the bacterium from becoming dehydrated. Next to the hydrated region is an intermediate region that is only partially hydrated. This boundary layer is approximately 0. Within this short distance, the water concentration drops from 2M on the headgroup side to nearly zero on the tail core side. In human red blood cells , the inner cytoplasmic leaflet is composed mostly of phosphatidylethanolamine , phosphatidylserine and phosphatidylinositol and its phosphorylated derivatives. By contrast, the outer extracellular leaflet is based on phosphatidylcholine , sphingomyelin and a variety of glycolipids, [12] [13] In some cases, this asymmetry is based on where the lipids are made in the cell and reflects their initial orientation. There, it is recognised by a macrophage that then actively scavenges the dying cell. Lipid asymmetry arises, at least in part, from the fact that most phospholipids are synthesised and initially inserted into the inner monolayer: Flippases are members of a larger family of lipid transport molecules that also includes floppases, which transfer lipids in the opposite direction, and scramblases, which randomize lipid distribution across lipid bilayers as in apoptotic cells. In any case, once lipid asymmetry is established, it does not normally dissipate quickly because spontaneous flip-flop of lipids between leaflets is extremely slow. Certain types of very small artificial vesicle will automatically make themselves slightly asymmetric, although the mechanism by which this asymmetry is generated is very different from that in cells. This asymmetry may be lost over time as lipids in supported bilayers can be prone to flip-flop. The lipids with an unsaturated tail blue disrupt the packing of those with only saturated tails black. The resulting bilayer has more free space and is, as a consequence, more permeable to water and other small molecules. Lipid bilayer phase behavior At a given temperature a lipid bilayer can exist in either a liquid or a gel solid phase. All lipids have a characteristic temperature at which they transition melt from the gel to liquid phase. In both phases the lipid molecules are prevented from flip-flopping across the bilayer, but in liquid phase bilayers a given lipid will exchange locations with its neighbor millions of times a second. This random walk exchange allows lipid to diffuse and thus wander across the surface of the membrane. The phase behavior of lipid bilayers is determined largely by the strength of the attractive Van der Waals interactions between adjacent lipid molecules. Longer-tailed lipids have more area over which to interact, increasing the strength of this interaction and, as a consequence,

decreasing the lipid mobility. Thus, at a given temperature, a short-tailed lipid will be more fluid than an otherwise identical long-tailed lipid. An unsaturated double bond can produce a kink in the alkane chain, disrupting the lipid packing. This disruption creates extra free space within the bilayer that allows additional flexibility in the adjacent chains. Most natural membranes are a complex mixture of different lipid molecules. If some of the components are liquid at a given temperature while others are in the gel phase, the two phases can coexist in spatially separated regions, rather like an iceberg floating in the ocean. This phase separation plays a critical role in biochemical phenomena because membrane components such as proteins can partition into one or the other phase [23] and thus be locally concentrated or activated. One particularly important component of many mixed phase systems is cholesterol , which modulates bilayer permeability, mechanical strength, and biochemical interactions. Surface chemistry[edit] While lipid tails primarily modulate bilayer phase behavior, it is the headgroup that determines the bilayer surface chemistry. Most natural bilayers are composed primarily of phospholipids , but sphingolipids and sterols such as cholesterol are also important components. Other headgroups are also present to varying degrees and can include phosphatidylserine PS phosphatidylethanolamine PE and phosphatidylglycerol PG. These alternate headgroups often confer specific biological functionality that is highly context-dependent. For instance, PS presence on the extracellular membrane face of erythrocytes is a marker of cell apoptosis , [26] whereas PS in growth plate vesicles is necessary for the nucleation of hydroxyapatite crystals and subsequent bone mineralization. This barrier takes the form of a lipid bilayer in all known life forms except for a few species of archaea that utilize a specially adapted lipid monolayer. The nucleus, mitochondria and chloroplasts have two lipid bilayers, while other sub-cellular structures are surrounded by a single lipid bilayer such as the plasma membrane, endoplasmic reticula, Golgi apparatus and lysosomes. Many prokaryotes also have a cell wall , but the cell wall is composed of proteins or long chain carbohydrates , not lipids. In contrast, eukaryotes have a range of organelles including the nucleus , mitochondria , lysosomes and endoplasmic reticulum. All of these sub-cellular compartments are surrounded by one or more lipid bilayers and, together, typically comprise the majority of the bilayer area present in the cell. In liver hepatocytes for example, the plasma membrane accounts for only two percent of the total bilayer area of the cell, whereas the endoplasmic reticulum contains more than fifty percent and the mitochondria a further thirty percent. In response to a molecule such as a hormone binding to the exterior domain blue the GPCR changes shape and catalyzes a chemical reaction on the interior domain red. The gray feature is the surrounding bilayer. Neurotransmission and Lipid raft Probably the most familiar form of cellular signaling is synaptic transmission , whereby a nerve impulse that has reached the end of one neuron is conveyed to an adjacent neuron via the release of neurotransmitters. This transmission is made possible by the action of synaptic vesicles loaded with the neurotransmitters to be released. These vesicles fuse with the cell membrane at the pre-synaptic terminal and release its contents to the exterior of the cell. The contents then diffuse across the synapse to the post-synaptic terminal. Lipid bilayers are also involved in signal transduction through their role as the home of integral membrane proteins. This is an extremely broad and important class of biomolecule. It is estimated that up to a third of the human proteome may be membrane proteins. The HIV virus evades the immune system in part by grafting these proteins from the host membrane onto its own surface. A classic example of this is phosphatidylserine -triggered phagocytosis. Normally, phosphatidylserine is asymmetrically distributed in the cell membrane and is present only on the interior side. During programmed cell death a protein called a scramblase equilibrates this distribution, displaying phosphatidylserine on the extracellular bilayer face. The presence of phosphatidylserine then triggers phagocytosis to remove the dead or dying cell. Characterization methods[edit] Human red blood cells viewed through a fluorescence microscope. The cell membrane has been stained with a fluorescent dye. The two dark bands around the edge are the two leaflets of the bilayer. Historically, similar images confirmed that the cell membrane is a bilayer The lipid bilayer is a very difficult structure to study because it is so thin and fragile. In spite of these limitations dozens of techniques have been developed over the last seventy years to allow investigations of its structure and function. Electrical measurements are a

straightforward way to characterize an important function of a bilayer: By applying a voltage across the bilayer and measuring the resulting current, the resistance of the bilayer is determined. This resistance is typically quite high Ohm-cm2 or more [35] since the hydrophobic core is impermeable to charged species. The presence of even a few nanometer-scale holes results in a dramatic increase in current. Lipid bilayers cannot be seen in a traditional microscope because they are too thin. In order to see bilayers, researchers often use fluorescence microscopy. A sample is excited with one wavelength of light and observed in a different wavelength, so that only fluorescent molecules with a matching excitation and emission profile will be seen. Natural lipid bilayers are not fluorescent, so a dye is used that attaches to the desired molecules in the bilayer. Resolution is usually limited to a few hundred nanometers, much smaller than a typical cell but much larger than the thickness of a lipid bilayer. The pits are defects in the bilayer, exposing the smooth surface of the substrate underneath. Electron microscopy offers a higher resolution image. In an electron microscope , a beam of focused electrons interacts with the sample rather than a beam of light as in traditional microscopy. In conjunction with rapid freezing techniques, electron microscopy has also been used to study the mechanisms of inter- and intracellular transport, for instance in demonstrating that exocytotic vesicles are the means of chemical release at synapses. A new method to study lipid bilayers is Atomic force microscopy AFM. Rather than using a beam of light or particles, a very small sharpened tip scans the surface by making physical contact with the bilayer and moving across it, like a record player needle. AFM is a promising technique because it has the potential to image with nanometer resolution at room temperature and even under water or physiological buffer, conditions necessary for natural bilayer behavior. Utilizing this capability, AFM has been used to examine dynamic bilayer behavior including the formation of transmembrane pores holes [38] and phase transitions in supported bilayers. Because of this, the same scan can image both lipids and associated proteins, sometimes even with single-molecule resolution. This has been used to characterise the degree of order and disruption in bilayers using dual polarisation interferometry to understand mechanisms of protein interaction. Lipid bilayers are complicated molecular systems with many degrees of freedom. Thus atomistic simulation of membrane and in particular ab initio calculations of its properties is difficult and computationally expensive. Quantum chemical calculations has recently been successfully performed to estimate dipole and quadrupole moments of lipid membranes. This effect is particularly pronounced for charged species, which have even lower permeability coefficients than neutral polar molecules. When a cell or vesicle with a high interior salt concentration is placed in a solution with a low salt concentration it will swell and eventually burst. Such a result would not be observed unless water was able to pass through the bilayer with relative ease. The anomalously large permeability of water through bilayers is still not completely understood and continues to be the subject of active debate. This applies both to fats and organic solvents like chloroform and ether.

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Chapter 2 : Planar Lipid Bilayers

P. Koulen, in Membrane Science and Technology, 4 BILAYER LIPID MEMBRANES AS AN ANALYSYS TOOL TO INVESTIGATE THE PHARMACOLOGY OF POLYCYSTIN Bilayer lipid membranes were used to determine the biophysical properties of a previously uncharacterized intracellular calcium channel, polycystin-2, at the single channel level [14].

A sheet of plastic with a small hole in the center separates the two sides of the chamber. The bilayer is formed across this hole, separating the two chambers. The electrical properties of the bilayer can be measured by putting an electrode into each side of the chamber. First, a small aperture is created in a thin layer of a hydrophobic material such as Teflon. Typically the diameter of this hole is a few tens of micrometers up to hundreds of micrometers. To form a BLM, the area around the aperture is first "pre-painted" with a solution of lipids dissolved in a hydrophobic solvent by applying this solution across the aperture with a brush, syringe, or glass applicator. The most common solvent used is a mixture of decane and squalene. After allowing the aperture to dry, salt solution aqueous phase is added to both sides of the chamber. The aperture is then "painted" with a lipid solution generally the same solution that was used for pre-painting. Once the two sides of the droplet come close enough together, the lipid monolayers fuse, rapidly excluding the small remaining volume of solution. At this point a bilayer is formed in the center of the aperture, but a significant annulus of solvent remains at the perimeter. Indeed, this was one of the first clues that this technique produced a membrane of molecular-scale thickness. For this reason, electrical characterization is one of the most important methods used in conjunction with painted lipid bilayers. More advanced electrical characterization has been particularly important in the study of voltage gated ion channels. Membrane proteins such as ion channels typically cannot be incorporated directly into the painted bilayer during formation because immersion in an organic solvent would denature the protein. Instead, the protein is solubilized with a detergent and added to the aqueous solution after the bilayer is formed. The detergent coating allows these proteins to spontaneously insert into the bilayer over a period of minutes. Additionally, initial experiments have been performed which combine electrophysiological and structural investigations of black lipid membranes. The main problems associated with painted bilayers are residual solvent and limited lifetime. Some researchers believe that pockets of solvent trapped between the two bilayer leaflets can disrupt normal protein function. To overcome this limitation, Montal and Mueller developed a modified deposition technique that eliminates the use of a heavy non-volatile solvent. In this method, the aperture starts out above the water surface, completely separating the two fluid chambers. On the surface of each chamber, a monolayer is formed by applying lipids in a volatile solvent such as chloroform and waiting for the solvent to evaporate. The aperture is then lowered through the air-water interface and the two monolayers from the separate chambers are folded down against each other, forming a bilayer across the aperture. Typically, a black lipid membrane will survive for less than an hour, precluding long-term experiments. This lifetime can be extended by precisely structuring the supporting aperture, [9] chemically crosslinking the lipids or gelling the surrounding solution to mechanically support the bilayer. Supported lipid bilayers SLB [edit] Diagram of a supported bilayer Unlike a vesicle or a cell membrane in which the lipid bilayer is rolled into an enclosed shell, a supported bilayer is a planar structure sitting on a solid support. Because of this, only the upper face of the bilayer is exposed to free solution. This layout has advantages and drawbacks related to the study of lipid bilayers. One of the greatest advantages of the supported bilayer is its stability. SLBs will remain largely intact even when subject to high flow rates or vibration and, unlike black lipid membranes, the presence of holes will not destroy the entire bilayer. Because of this stability, experiments lasting weeks and even months are possible with supported bilayers while BLM experiments are usually limited to hours. One of the clearest examples of this advantage is the use of mechanical probing techniques which require a direct physical interaction with the sample. Atomic force microscopy AFM has been used to image lipid phase separation , [12] formation of

transmembrane nanopores followed by single protein molecule adsorption, [13] and protein assembly [14] with sub-nm accuracy without the need for a labeling dye. More recently, AFM has also been used to directly probe the mechanical properties of single bilayers [15] and to perform force spectroscopy on individual membrane proteins. Another example of a physical probe is the use of the quartz crystal microbalance QCM to study binding kinetics at the bilayer surface. Evanescent field methods such as total internal reflection fluorescence microscopy TIRF and surface plasmon resonance SPR can offer extremely sensitive measurement of analyte binding and bilayer optical properties but can only function when the sample is supported on specialized optically functional materials. Another class of methods applicable only to supported bilayers is those based on optical interference such as fluorescence interference contrast microscopy FLIC and reflection interference contrast microscopy RICM or interferometric scattering microscopy iSCAT. When the bilayer is supported on top of a reflective surface, variations in intensity due to destructive interference from this interface can be used to calculate with angstrom accuracy the position of fluorophores within the bilayer. In many cases, this resolution is all that is needed. After all, bilayers are very small only in one dimension. Laterally, a bilayer can extend for many micrometres or even millimeters. But certain phenomena like dynamic phase rearrangement do occur in bilayers on a lateral sub-micrometre lengthscale. A promising approach to studying these structures is near field scanning optical microscopy NSOM. But unlike AFM, NSOM uses an optical rather than physical interaction with the sample, potentially perturbing delicate structures to a lesser extent. Fluorescence micrograph of a supported bilayer on a substrate that has been patterned with a corral. This substrate was then sequentially exposed to two different populations of lipids dyed red and green. Although the populations were kept largely separated there was some intermixing at the interface as seen from the color gradient. Another important capability of supported bilayers is the ability to pattern the surface to produce multiple isolated regions on the same substrate. Creative utilization of the corral concept has also allowed studies of the dynamic reorganization of membrane proteins at the synaptic interface. Although supported bilayers generally do not directly touch the substrate surface, they are separated by only a very thin water gap. To quantify the diffusion coefficient and mobile fraction, researchers studying supported bilayers will often report FRAP data. Unwanted substrate interactions are a much greater problem when incorporating integral membrane proteins, particularly those with large domains sticking out beyond the core of the bilayer. Because the gap between bilayer and substrate is so thin these proteins will often become denatured on the substrate surface and therefore lose all functionality. In these systems the bilayer is supported on a loose network of hydrated polymers or hydrogel which acts as a spacer and theoretically prevents denaturing substrate interactions. Tethered bilayer lipid membranes t-BLM [edit] The use of a tethered bilayer lipid membrane t-BLM further increases the stability of supported membranes by chemically anchoring the lipids to the solid substrate. Gold can be used as a substrate because of its inert chemistry and thiolipids for covalent binding to the gold. Thiolipids are composed of lipid derivatives, extended at their polar head-groups by hydrophilic spacers which terminate in a thiol or disulphide group that forms a covalent bond with gold, forming self assembled monolayers SAM. The limitation of the intra-membrane mobility of supported lipid bilayers can be overcome by introducing half-membrane spanning tether lipids [32] with benzyl disulphide DPL and synthetic archaea analogue full membrane spanning lipids with phytanoly chains to stabilize the structure and polyethyleneglycol units as a hydrophilic spacer. Bilayer formation is achieved by exposure of the lipid coated gold substrate to outer layer lipids either in an ethanol solution or in liposomes. Additionally the spacer layer creates an ionic reservoir [34] that readily enables ac electrical impedance measurement across the bilayer. Vesicles[edit] Diagram of lipid vesicles showing a solution of molecules green dots trapped in the vesicle interior. A vesicle is a lipid bilayer rolled up into a spherical shell, enclosing a small amount of water and separating it from the water outside the vesicle. Because of this fundamental similarity to the cell membrane, vesicles have been used extensively to study the properties of lipid bilayers. Another reason vesicles have been used so frequently is that they are relatively easy to make. If a sample of dehydrated lipid is exposed to water it will spontaneously form vesicles. These naturally isolated vesicles are

composed of a complex mixture of different lipids and proteins so, although they offer greater realism for studying specific biological phenomena, simple artificial vesicles are preferred for studies of fundamental lipid properties. Since artificial SUVs can be made in large quantities they are suitable for bulk material studies such as x-ray diffraction to determine lattice spacing [39] and differential scanning calorimetry to determine phase transitions. To combat this problem researchers have developed the giant unilamellar vesicle GUV. GUVs are large enough several tens of micrometres to study with traditional fluorescence microscopy. Many of the studies of lipid rafts in artificial lipid systems have been performed with GUVs for this reason. However, GUVs are relatively fragile, time consuming to make and can only be produced in limited yield compared to SUVs. To circumvent these problems a microfluidic assembly line approach to GUVs was reported. In aqueous solutions, micelles are assemblies of amphipathic molecules with their hydrophilic heads exposed to solvent and their hydrophobic tails in the center. Micelles can solubilize membrane proteins by partially encapsulating them and shielding their hydrophobic surfaces from solvent. Bicelles are a related class of model membrane, [57] typically made of two lipids, one of which forms a lipid bilayer while the other forms an amphipathic, micelle-like assembly shielding the bilayer center from surrounding solvent molecules. Bicelles can be thought of as a segment of bilayer encapsulated and solubilized by a micelle. Bicelles are much smaller than liposomes, and so can be used in experiments such as NMR spectroscopy where the larger vesicles are not an option. Nanodiscs [58] consist of a segment of bilayer encapsulated by an amphipathic protein coat, rather than a lipid or detergent layer. Nanodiscs are more stable than bicelles and micelles at low concentrations, and are very well-defined in size depending on the type of protein coat, between 10 and 20 nm. Membrane proteins incorporated into and solubilized by Nanodiscs can be studied by a wide variety of biophysical techniques.

Chapter 3 : Polymer Supported Lipid Bilayers

BLMs (planar lipid bilayers) have been used in a number of applications ranging from basic membrane biophysics including transport, practical AIDS research, and 'microchips' studies, to the conversion of solar energy via water photolysis, to biosensor development using supported bilayer lipid membranes (s-BLMs), and to photobiology.

Spherical Liposomes General References S. Theory and Practice, Marcel Dekker, Inc. Ostro, Liposomes, Marcel Dekker, Inc. Biosensors and Biocomputers, Plenum Press, N. Passechnik, Bilayer Lipid Membranes: Cusz University Press, Romania, M. Wescott, Nature, ; J. Medical World News cover story , February 15,, pp. Bangham, Surrogate cells or Trojan horses, BioSystems, 17 6. Tien, Journal of General Physiology, 52 1, pt. Shimooka, Terada and T. Yager, Langmuir, 11 8 Acta, Kashchiev, Black Films, Contem. Physics, 27 Costa Pinto, and P. Lipids, 61, , No. Acta, 40 ; Vallejo and colleagues Acta, ; H. Pace, Nature, Surface Science, 41, , No. A, Naser, Planner A, Frackowiak D. A, , , No. Jakabovic, Rehacek, and M. Uhlar, Sensors and Actuators B, 19 C, 4 35 Hianik, Biologia, 51, Tien, Supramolecular Science, 4 ; J. Gregoriadis, Liposomes in drug targetting, Cell Biology, Knighton, Applied Optics, 34 Acta, 39 White, Science , Rational Design, Marcel Dekker, N. Venton, Methods of screening combinatorial libraries using immobilized or restrained receptors, J. B, , , No. Egorova, Disagreement between experimental potential changes and the Gouy-Chapman theory predictions for dilute 1: Phenomenology of a problem, Colloid J. The inspiration for lipid bilayer research, without question, comes from the biological world. This paper presents an overview of the origin of the lipid bilayer concept and its experimental realization, as well as the studies of our laboratory and recent research of others on the use of BLMs as models of certain biomembranes. In addition, we describe briefly our present work on supported BLMs as biosensors and molecular devices; the experiments carried out in close collaboration with colleagues on s-BLMs are delineated. Hooke, Royal Society Meeting, in T. Miller, London, , 3: Newton, Optics reprinted edition, Dover, NY, , pp. As viewed from experimental bilayer lipid membranes planar lipid bilayers and spherical liposomes , Elsevier, Amsterdam and New York, , pp. Langmuir, Monomolecular layers of fatty acids at air-water interfaces, J. Grendel, On bimolecular layers of lipoids on the chromocytes of the blood, J. Robertson, Unit Membrne Hypothesis, J. Published as a supplement in Circulation, 26 No. Wescott, Reconstitution of cell membrane structure in vitro and its transformation into an excitable system, Nature, ; J. Bangham, Surrogate cells or Trojan horses, BioEssays, 17 Physicochemical and Engineering Aspects, Tocanne, Organization and dynamics of the proteolipid complexes formed by annexin V and lipids in planar supported lipid bilayers, Biochemistry, 38 Barrow, Differential effects of cholesterol and oxidized cholesterol in egg lecithin bilayers, Biochimica et Biophysica Acta Biomembranes, Hong, Interfacial photochemistry of retinal proteins, Prog. Dryfe, Scanning electrochemical microscopy. Sukharev, Lipid and cell membranes in the presence of gadolinium and other ions with high affinity to lipids. A dipole component of the boundary potential on membranes with different surface charge, Membr. Ladha, The membrane-permeabilizing effect of avenacin A-1 involves the reorganization of bilayer cholesterol, Biophys. Goldman, Model-independent analysis of the orientation of fluorescent probes with restricted mobility in muscle fibers, Biophys. Zoratti, Helicobacter pylori vacuolating toxin forms anion-selective channels in planar lipid bilayers: Montecucco, Tetanus and botulinum neurotoxins: Sebo, An amphipathic alpha-helix including glutamates and is crucial for membrane translocation of adenylate cyclase toxin and modulates formation and cation selectivity of its membrane channels, J. Schubert, Solution structure and orientation of the transmembrane anchor domain of the HIVencoded virus protein U by high-resolution and solid-state NMR spectroscopy, Biochem. Yeaman, Membrane permeabilization by thrombin-induced platelet microbicidal protein 1 is modulated by transmembrane voltage polarity and magnitude, Infect. Procopio, Fatty acid transport across lipid bilayer planar membranes, Lipids, 35 Acta, 1 Nakajima-Iijima, Evaluation and comparison of ion permeation and agonist selectivities for N-methyl-d-aspartate receptor channels with different subunit compositions in bilayer

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Chapter 4 : Microtechnologies for membrane protein studies

Membranes consisting of zwitterionic lipids supported by agarose films were shown to be unstable, as observed by fluorescence microscopy, reflection interference contrast microscopy, and the impossibility of bilayer spreading (RÃ¤dler, J.; Strey, H.; Sackmann, E. Langmuir , 11, âˆ’) on the agarose surface.

This article has been cited by other articles in PMC. Abstract Despite the rapid and enormous progress in biotechnologies, the biochemical analysis of membrane proteins is still a difficult task. The presence of the large hydrophobic region buried in the lipid bilayer membrane transmembrane domain makes it difficult to analyze membrane proteins in standard assays developed for water-soluble proteins. To handle membrane proteins, the lipid bilayer membrane may be used as a platform to sustain their functionalities. Nonetheless, researchers are continuing to develop efficient and sensitive analytical microsystems for the study of membrane proteins. High-throughput and highly sensitive detection systems for membrane proteins are now becoming a realistic goal. Reactions of biomolecules contained in small volumes of buffer are efficiently controlled in sophisticated microfluidic systems, on the premise that the molecules are water-soluble. Membrane proteins are a class of proteins present in the plasma membrane of cells. They perform specific roles in the cell boundary, enabling cells to sense, communicate, and regulate in response to fluctuations in the outside world. The hydrophobic regions in the polypeptide chain of membrane proteins penetrate into the inner hydrophobic layer of the lipid bilayer. Thus, the presence of the large hydrophobic part makes the membrane proteins notoriously difficult to separate from the membrane and handle in water-based assay systems. To create a microanalysis system for studying membrane proteins, it is necessary to construct an efficient and easily useable platform to handle the plasma membrane or the lipid bilayer. We first introduce the technologies for the measurement of membrane protein functions expressed on intact cell membranes, and then introduce the technologies which utilize reconstituted artificial membranes. We have classified the use of reconstituted membranes into three major subcategories based on their forms: Here we especially focus on developments in the area of suspended bilayer systems, whereas applications of the other two categories are diverse and are discussed in other excellent reviews e. The membrane proteins to be discussed are categorized by function into three groups: Cell-membrane-based systems In nature, membrane proteins function in intact cell membranes. The most straightforward way is to directly examine the membrane proteins on isolated or cultured cells. In this section, we review the use of microstructures for the analysis of membrane proteins expressed on the plasma membrane. Ion channel recording The patch-clamping technique, which was introduced by Neher and Sakmann [12] in , is the most prominent technique in the history of membrane protein ion channel research. In this method, a sharp glass capillary patch-clamp electrode is pressed against the plasma membrane of the cell, and suction is applied to form a tight seal gigaohm seal between the membrane and the electrode. In the case of whole-cell recording, further suction is applied to rupture the membrane inside the capillary tip, providing electrical access to the inner space of the cell. The electric current and voltage across the membrane can be precisely controlled and measured using a patch-clamp amplifier Fig. By using other variations of this technique, one can record the ion flow through a single ion channel protein.

Chapter 5 : Model lipid bilayer - Wikipedia

Supported planar lipid bilayers based on alkanethiol-tethering chemistry are becoming increasingly important biomimetic materials. Hybrid bilayers containing thiol-derivatized alkane moieties plus natural lipids provide a biomimetic matrix that permits the successful reconstitution of membrane.

The lipid bilayer concept: Experimental realization and current applications H. Tien and Angelica Ottova Chapter 2: Boundary potentials of bilayer lipid membranes: Chain-ordering phase transition in bilayer: Kinetic mechanism and its physiological implications Dmitri Kharakoz Chapter 8: Coupling of chain melting and bilayer structure Thomas Heimburg Chapter 9: Water transport across membranes Peter Pohl Chapter Biopolymer induced structural modifications of lipid bilayers Sylvio May and A. Planar lipid bilayer analyses of bacterial porins: Reconstitution in planar lipid bilayers of ion channels synthesized in vivo and in vitro L. Multi-channel and single-channel investigation of protein and peptide incorporation into BLMs E. Structure and function of plant membrane ion channels reconstituted in planar lipid bilayers M. The mitochondrial ion channels in planar lipid bilayer and in native mitochondria Galina Mironova Chapter The use of liposomes to detect channel formation mediated by secreted bacterial proteins V. Vande Weyer, and J. Memory effect on ion channels by using BLM R. Symmetric and asymmetric planar lipid bilayers of various lipid composition A. Insights into ion channels from peptides in planar lipid bilayers Herve Duclohier Chapter Modulation of planar bilayer permeability by electric fields and exogenous peptides A. Gravitational impact on ion channels incorporated into planar lipid bilayers M. Advantages and disadvantages of patch-clamping M. Kelly and Dixon J. Pharmacology of intracellular calcium channels Peter Koulen Chapter Systems aspects of supported membrane biosensors I. Biosensors from interactions of DNA with lipid membranes U. Structure and electrochemistry of fullerene-lipid hybrid and composite materials Naotoshi Nakashima Chapter Analytical applications of bilayer lipid membrane systems Marek Trojanowicz Chapter Transmembrane voltage sensor J. Domains, cushioning, and patterning of bilayers by surface interactions with solid substrates and their sensing properties Ruxandra Vidu, Timothy V. Longo, and Pieter Stroeve Chapter Photosynthetic pigment-protein complexes in planar lipid membranes W. Simultaneous measurement of spectroscopic and physiological signals from a planar bilayer system Yoshiro Hanyu.

Chapter 6 : bilayers_liposomes

Abstract The lipid bilayer postulated as the basic structural matrix of biological membranes is widely accepted. At present, the planar bilayer lipid membrane (BLM) together with spherical lipid bilayers (liposomes), upon suitable modification, serves as a most appropriate model for biological membranes.

Not only do they serve as the protective boundary of cells and their internal organelles, they also organize and host major parts of the biochemical machinery for cellular communication and transmembrane transport. To study aspects of cellular membranes in a controlled manner, solid supported planar bilayers have served as reliable tools for many decades. They have been used in a large variety of studies ranging from fundamental investigations of membranes and their constituents to the dissection of cellular signaling mechanisms. However, there are limitations to these systems and recently a class of new systems in which the lipid bilayer is supported on a soft, polymer cushion has emerged. Here, we review the different polymer cushioned bilayer systems and discuss their manufacture and advantages. Introduction For the last three decades functionalization of interfaces with mimics of biological membranes has been an ongoing effort. These model-membrane systems have garnered much attention because they provide a useful and interesting interface between the biological world and man-made materials. Thus, they have great potential for basic membrane and cell-biology research as well as a variety of biotechnological and biomedical applications. The simplest of these membrane mimics is the solid supported lipid bilayer that in many ways behaves similar to free lipid membranes. A thin water layer between the substrate and the bilayer serves as lubricant that enables long range lateral diffusion of the lipids. Thus, they preserve the fluidity of biological membranes that is so central to many cellular functions. Since the first fabrication of a solid supported bilayer via successive deposition of two monolayers by Tamm and McConnell [1], solid supported bilayers have been instrumental in a wide variety of studies. This is in part because proteins or other membrane constituents can be placed on or in the membrane, thus providing a highly controlled environment for experimentation. For example, supported lipid bilayers have been used to investigate membrane bound signaling events of cells [8,9], study protein-lipid interactions on the single molecule level [5] and develop biosensor platforms []. However, a significant limitation of such traditional solid supported bilayers can be encountered when trying to incorporate transmembrane proteins into the supported membrane Figure 1. A typical solid supported bilayer will have an approximately 1 - 3 nm thick hydration cushion between the support and the bilayer. This does not provide sufficient space for the cytosolic domain of most transmembrane proteins and consequently the protein will contact the substrate surface, deform and eventually denature as indicated in Figure 1 b. To overcome this restriction and expand the use of supported lipid bilayers to other research areas and fields, a different type of bilayer support has been developed. In this alternative method a soft polymeric layer is introduced between the solid support and the artificial lipid membrane. The polymer layer provides a low friction interface for the lipid bilayer and any imbedded proteins. The system has proven its adaptability and has been utilized in such diverse applications as; membrane protein binding detection [13], electrophoretic accumulation studies [14,15], cellular cytoskeleton incorporation [16], and electro- Figure 1. Conventional solid supported lipid bilayer and its limitations. Both proteins and lipids are laterally mobile in such a scenario; b In contrast, transmembrane proteins with extended cytosolic domains have not enough space between the substrate and the bilayer. Thus, they will contact the substrate, deform and often denature which leads to loss of function. In addition, such proteins are also immobilized. Here, we discuss the options available for polymer bilayer supports and try to underscore the particular strengths and weaknesses of the different systems and methods. Polymer Supports for Lipid Bilayers Most common polymer supports have had their genesis in convenience. Popular biological techniques involve numerous polymerizing substances; consequently some have been adopted for use as membrane cushions. For a successful polymer based lipid bilayer cushion, the polymer must have some few specific characteristics. Firstly, they must be capable of

forming a thin layer with surface uniformity suitable for bilayer formation. Secondly, they would ideally have a well-defined elastic modulus that can be replicated at every iteration of the experiment. Thirdly, the polymer must be hydrophilic, and they must be relatively chemically inert so as not to cause unwanted reactions and interactions with the membrane. Due to their hydrophilicity such polymers typically have high water contents and are known as hydrogels. Hydrogels have refractive indices that deviate only slightly from that of the liquid used to hydrate them, this allows for good optical coupling between the hydrogel and the aqueous solution, giving aberration free imaging through the gels. The nature of self-assembly of amphiphillic molecules such as lipids dictates that there must be water present for the formation of a bilayer. Care must be taken when using polymers that have charged or polarized functional groups to ensure the attractive forces between these and the lipids are not too great. Typically polymer wetting ability is characterized by the contact angle of a water droplet on its surface. This can give some indication of a good polymer for a bilayer cushion application. Typical contact angles range from 30 - 70 degrees [20,21]. Polymer supports might be classified with respect to a variety of properties. A first possibility would be a distinction between copolymers such as styrene-acrylonitrile and nitrile rubber which are formed using two or more monomer species and homopolymers such as cellulose, PVC and polyethylene glycol which consist of only one monomer species. A thorough review of the literature reveals that polymers used for bilayer supports are overwhelmingly of the homopolymer variety although a clear advantage for their use is not obvious per se. Another possible distinction of polymer supported lipid membranes could be made between systems where the polymer layer s are formed independently of the bilayer and those that are formed through fusion of vesicles containing lipo-polymers. Another differentiation could be made between polymer supports that attach to the solid support just by adhesion and those polymers that are attached to the solid substrate through an intermediary binding molecule: These binding molecules need to have a functionalized domain for polymer attachment and can be either coated over the entire solid substrate when using independent polymer supports [24] or attached to the distal end of each polymer when using lipopolymer supports [25]. Yet, in this review we separated polymer supported bilayers into two main classes: A short summary of the different polymer systems are given in Table 1, while the chemical structures are summarized in Figure 3. Schematic of the two major classes of polymer supported lipid bilayers. Chemical structure of commonly used polymers for bilayer support. Independent Polymer Support Independent polymer supports are characterized by the fact that they have no direct linkage with the lipid bilayer. This allows for maximal flexibility with respect to polymer choice as well as deposition and manufacture procedures. The polymer in question can be spin coated on [26], deposited by sequential dipping [27] or, for chemically induced polymerization, polymerized while sandwiched between the substrate and a second solid layer with a nonreactive coating [28]. Following polymer preparation the lipid bilayer is deposited using one of three main techniques: Polyacrylamide Polyacrylamide see Figure 3 a is typically used in gel electrophoresis. In this application the gel structure is controlled by adjusting the ratio of acrylamide monomers and bis cross-linkers in the unpolymerized solution [29]. In an electrophoresis gel this ratio determines the average pore size and if used as membrane support polymer, this ratio can be used to control the elastic modulus of the gel typically between 1 and kPa [30]. This latter ability made polyacrylamide also very popular as a soft substrate material in studies of cellular biomechanics [31]. To prevent peeling, polyacrylamide requires that the solid substrate be coated with a bonding agent, typically alkylsilane, which covalently binds the cross-linked polymer to the glass [24]. To achieve a smooth surface Table 1. Polymers used as membrane supports. Once the nonreactive layer is removed the result is a uniform polymer surface suitable for bilayer deposition. This sandwiching technique is only possible because polymerization and crosslinking of the polyacrylamide is induced chemically and occurs over the time of minutes. In contrast to other polymer systems, the thickness of the crosslinked polyacrylamide gel can be easily controlled during production and thickness from tens to hundreds of micrometers can be achieved [32]. In comparison, other techniques give gel thicknesses in the tens to hundreds of nanometers range [29]. This wide range of thicknesses increases the number of potential applications for such a system. However, it should

be noted that the acrylamide monomer is a toxin that should be handled and processed with care in particular if live cells are involved in a study. It is hydrophilic and quite inert and thus provides a good substrate for biological studies [26]. It has some promise in the medical field due to its biocompatibility and biodegradability making it a good candidate as a scaffold for tissue engineering [33]. PLLA can be formed into a uniform support by spin coating a solution onto a solid substrate. The coating then gets annealed before use to complete the polymerization. This yields layer thicknesses in the nm range [26]. Having polymer layers this thin allows the use of sensitive optical techniques that rely on the use of objectives with high numerical aperture, such as; sum frequency generation vibrational spectroscopy [26], total internal reflection fluorescence and glancing angle illumination [34]. Cellulose Cellulose has been one of the most widely used polymers in modern history Figure 3 d. It is found naturally in plant cell walls and is the main constituent of paper and wood products. Cellulose has a diverse number of common uses from cellophane to wall paper paste to food filler. It is an inert hydrophilic polysaccharide, formed from dehydrated dextrose the right hand form of glucose. It can be formed into thin layers for bilayer support by first substituting their hydroxyl groups for a hydrophobic side chain; this allows them to be dissolved in organic solvents. Once dissolved they can be spin coated onto a substrate or formed into monolayers on a Langmuir trough and deposited onto a substrate; the thickness can be built up through repeated dipping [19,35]. A variety of cellulose derivatives exist, such as trimethylsilylcellulose TMSC and isopentylcellulosecinnamate IPCC , which provide different properties to the substrate such as solubility in nonpolar solvents and improved surface friction, respectively [19,35,36]. It has been shown that such cellulose derivatives can be modified post deposition via exposure to HCL vapor to create a hydrophilic surface with a hydrophobic core, this can change the electrical resistance of the bilayer which can be useful for ion channel studies [19,35,36]. Patterning permits close spatial control of the bilayer contents, and can be used to promote selective cell growth, to study membrane discrimination, or to isolate proteins or channels from each other [37]. Agarose Agarose is a polysaccharide most commonly found in agar, the gelatinous substance used for bacterial cell culture. It is derived from certain species of red algae and is used in such things as ice cream, the brewing process, as well as a food item in its own right [38]. In biological studies agarose is used to make a porous gel for microorganism motility assays [39]; the concentration of agarose in solution determines the final viscosity of the substance [40]. Agarose has been used as a polymer support for bilayers for the last 15 years [41,42]. It can be deposited on glass by brushing on a solution of agarose type VII in water, this is dried at room temperature, no further modifications are required [43]. This makes agarose arguably the simplest polymer supports to work with. Polyelectrolyte Cushions Another polymer cushioning system involves polyelectrolytes. These are polymers whose monomer subunits have an electrolyte group. The electrolyte groups will dissociate when exposed to an aqueous solution leaving the polymer with a net charge. To form a bilayer cushion the polyelectrolyte is deposited onto the substrate which is typically charged in a layer by layer fashion [44,45]. The substrate is repeatedly dipped between two polyelectrolyte solutions; one a polycation such as Poly diallyldimethylammonium chloride , one a polyanion such as poly 4-venyl-benzenesulfonic acid, Figure 3 c [27]. Each dipping causes a monolayer of polyelectrolyte to be adsorbed on the surface through electrostatic attraction and reverses the charge on the surface leaving it ready for the next layer. This layer by layer method is inexpensive, easy and gives excellent thickness control, down to single nanometer precision [27]. Bilayer deposition is then dependent on the relative charges in the system, for a positively charged final polyelectrolyte layer negatively charged lipids are required to get total coverage. This electrostatic coupling may make polyelectrolyte cushions a poor choice for membrane dynamics studies but a good choice for ion channel studies. Surface patterning can be carried out by making use of the electrostatics to selectively layer certain sections through micro contact printing [27]. This approach has the advantage of providing a chemical contrast as opposed to a topographical contrast for membrane patterning [27]. Coupled Membrane Polymer Systems Coupling between the bilayer and the polymer support is usually achieved by the use of lipopolymers.