Polymer-Supported Membranes: Physical Models of Cell Surfaces

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Abstract

The functional modification of solid surfaces with plasma membrane models has been drawing increasing attention as a straightforward strategy to bridge soft biological materials and hard inorganic materials. Planar model membranes can be deposited either directly on solid substrates (solid-supported membranes), or on ultrathin polymer supports (polymer-supported membranes) that mimic the generic role of the extracellular matrix and the cell surface. The first part of this review provides an overview of advances in the fabrication of polymer-supported membranes. The middle section describes how such thin polymer interlayers can physically modulate the membrane–substrate contact. The last section introduces several methods to localize membranes and membrane proteins. Finally, some ideas are presented on combining supported membrane concepts with semiconductor technology toward applications in materials science.

Keywords: *adhesion, biological, cellular, film, polymer, sensor.*

Introduction

Lipid membranes are a major constituent of biological membranes, which act as the outer boundary of cells and organelles. Owing to their amphiphilic nature, lipid molecules assemble into a bilayer in water in order to minimize the contact of hydrophobic chains to the surrounding water; this property enables physicists to describe them within the framework of liquid-crystal theory.[1] In biological membranes, lipid molecules do not possess short-range order and can diffuse within the membrane plane. Various peripheral and integral proteins and carbohydrates can be bound to or incorporated into these approximately 5-nm-thick "fluid" membranes.

These features enable membranes to act as switchable gateways, controlling flow into and out of cells or organelles. In other words, they act as smart filter materials. For example, organelles serve as microcontainers to confine biochemical processes inside the cell. Similarly, plasma membranes block most toxic substances from entering the cell while simultaneously allowing special nutrients, wastes, and metabolites to selectively pass to the outside environment. Furthermore, many important biochemical processes occur at membrane surfaces via interactions between various membrane proteins. However, if one looks at biological membranes as a material, they are complex assemblies of a number of molecular machines that we cannot reassemble piece by piece. Furthermore, interactions with intra- and extracellular networks, such as the cytoskeleton and the extracellular matrix, add to the complexity, making it difficult to directly investigate the structures and functions of biological membranes. In order to design model systems with a reduced number of components, phospholipid bilayers deposited onto solid substrates (solid-supported membranes) have been the most commonly used experimental cell surface model and have allowed us to gain insight into immune reactions and cell adhesion processes over the past 20 years.[2–8]

Supported membranes are prepared by the direct deposition of lipid monolayers or bilayers on solid or polymer surfaces to yield macroscopically large areas—on the order of a square centimeter. Bilayer deposition can occur by four methods:[9] successive transfer of lipid monolayers, fusion of lipid vesicles, single bilayer spreading, and the solvent exchange method. In the first method, the monolayer transfer from the air–water interface using a Langmuir film balance is laborious but also advantageous for the fabrication of asymmetric bilayers. In the second method, lipid vesicles are deposited onto the substrate from suspensions. By adjusting the interaction between the membranes and the surface, the vesicles can rupture and form membrane patches that fuse into continuous bilayers. In the third method, single-bilayer spreading is achieved simply by depositing a lipid reservoir onto the solid followed by the spontaneous spreading of a single bilayer over the surface. In the fourth method, lipid membranes are formed by the exchange of solvents from alcohols (e.g., isopropanol, ethanol) to aqueous buffers. Although a trace of alcohol might remain between head groups and hydrophobic tails, this method is less laborious than other methods for forming continuous membranes. Interestingly, supported membranes retain the intrinsic "fluid" property to self-heal local defects while achieving excellent mechanical stability.[10–12] The combination of fluidity and stability on solid substrates offers distinct advantages over freestanding, solvent-free lipid bilayers (so-called "black" lipid membranes) or spherical lipid vesicle suspensions. Planar supported membranes indeed open a possibility to use various physical characterization techniques that are difficult or impossible to use with other model systems. For example, surface-sensitive methods such as total interference fluorescence,[13,14] attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR),[15] surface plasmon resonance,[16] and x-ray and neutron scattering[17–19] can be used to probe the structural and dynamic properties of membranes on solid supports.

With the aid of biochemical tools and genetic engineering, supported membranes can be functionalized with various membrane-associated proteins. One of the commonly used methods is the spreading of "proteoliposomes" (phospholipid vesicles incorporating transmembrane proteins, such as ion channels or membrane spanning receptors). Another convenient method is to prepare supported membranes incorporating "anchor" lipids with biotin and chelator head groups, and then couple engineered proteins that have the specific "tags" to those anchor lipids. This provides a powerful tool for coupling a

variety of biomolecules to supported membranes to create complex experimental models of cell surfaces. An early study by Brian and McConnell et al.[2] demonstrated that the supported membranes with transmembrane antigens can stimulate cytotoxic T cell lymphocytes.[13] Later, Grakoui et al.[7] revealed that initiation of the immune response depends on dynamic recognition and interaction during the contact between T cells and the antigen-presenting cell, which is the so-called immunological synapse. These studies clearly demonstrated that lateral diffusivity of the lipid molecules in the membrane must be maintained in order to probe such dynamic processes. Moreover, using cells or vesicles in conjunction with supported membranes offers opportunities for the study of the physical principle of cell adhesion.[8,20,21]

Polymer-Supported Membranes

Although many experimental studies have benefited from the use of solid-supported membranes, these membranes have some fundamental drawbacks. The artificial membrane and its solid support are in close proximity (Figure 1a), typically 0.5–2 nm,[18,22,23] which leaves a water reservoir that is usually not sufficient to prevent protein subunits from coming into direct contact with the bare substrate. This causes serious risks of denaturation of membrane proteins, particularly for transmembrane proteins and cell adhesion receptors, whose functional extracellular domains can extend out of the membrane several tens of nanometers.

Many of these problems can be overcome by separating the membrane from the solid substrate with a soft polymeric spacer layer typically less than 100 nm thick.[9,24] As demonstrated in the following section, this approach successfully reduces the frictional coupling between membrane-incorporated proteins and the substrate surface and, therefore, reduces the risk of protein denaturation.[9,24–26] Here, several classes of polymer-supported lipid membranes are introduced. This will be followed by a discussion of the manipulation and patterning methods, which can benefit studies to determine the functions of membrane-associated proteins.

Membranes on Polymer "Cushions"

One major class of polymer supports is often referred to as "polymer cushions," on which lipid membranes can be deposited (Figure 2a). In this scenario, there is no chemical link between the lipid molecules and the polymer films, but interplays of attractive and repulsive physical forces determine the minimum of the interaction potential between the membrane and the substrate. The polymer supports act as a lubricating layer between the membrane and the substrate and assist self-healing of local defects in the membrane, thus allowing uniform coverage over macroscopically large substrates.

One of the most important criteria when choosing polymer materials is that the supported membrane needs to be thermodynamically and mechanically stable. That is, the deposition of a lipid bilayer onto a hydrated polymer support should result in a reduction in the Gibbs free energy. Let us consider the thermodynamics of multiple layers consisting of a solid substrate, a hydrated polymer support, a lipid membrane, and water. The free energy per unit area can be given as newtons per meter (N/m), which is a unit of surface tension σ. In order to achieve homogeneous coverage of the surface with a membrane, the sum of the free energies from individual interfaces (i.e., the tensions at the solid/polymer interface γ_{SP}, the polymer/membrane interface γ_{PM}, and the membrane/liquid interface γ_{ML}) must be smaller than that of the free energy of the direct solid/liquid contact γ_{SL}:

$$\gamma_{SL} - (\gamma_{SP} + \gamma_{PM} + \gamma_{ML}) \geq 0. \quad (1)$$

Although polymer films and lipid membranes possess not only viscosity but also elasticity and differ from classical Newtonian liquids, this free-energy description is analogous to the homogeneous coverage of solid with a liquid film (called "complete wetting"), which can be characterized by a positive spreading coefficient,

$$S = \gamma_{SV} - (\gamma_{SL} + \gamma_{LV}) \geq 0, \quad (2)$$

where γ_{SV}, γ_{SL}, and γ_{LV} are tensions at the solid/vapor, solid/liquid, and liquid/vapor interface, respectively.[27] This clearly indicates that hydrophilic polymers must be chosen for the deposition of lipid bilayers, and hydrophobic polymers for lipid monolayers.

On the other hand, if one considers the interplay of various interfacial forces such as electrostatic, van der Waals, hydration repulsion, and polymer-induced (entropic) force, the interaction between the membrane and the surface needs to remain "repulsive." This means that the polymer support must keep the membrane away from the deep potential minimum governed by van der Waals attraction, which can be calculated to be less than 1 nm in the case of membrane–substrate contacts. From this context, polymer cushions mimic the characteristic feature of the extracellular matrix and carbohydrate layers rendering the cell surface (glycocalyx), which maintain distinct distances (typically 10–100 nm) between neighboring cells and between cells and tissue surfaces, respectively. This indeed suggests the existence of the second potential minimum created by the presence of hydrated biological polymer interlayers.

However, since measuring each force contribution is not practical, one should take an analytical concept to describe the sum of the net effect of the various interfacial forces, called disjoining pressure.[28] From a thermodynamic viewpoint, the disjoining pressure can be defined as the first derivative of the work that one has to do to alter the membrane–substrate distance. Note that no work is necessary to alter the distance if the membrane and the surface are physically decoupled (i.e., no overlap of long-range force fields). Similar

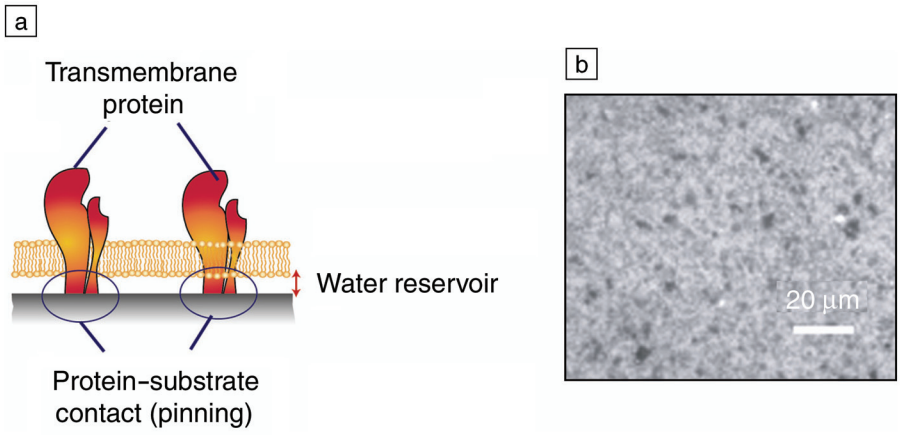

Figure 1. (a) Schematic illustration of a solid-supported membrane with transmembrane proteins that directly contact the solid surface due to the thin water reservoir between the membrane and the substrate. (b) Fluorescence image of a solid-supported membrane with human platelet integrin $\alpha_{IIb}\beta_3$ (labeled with a fluorescent dye), exhibiting immobile patches.

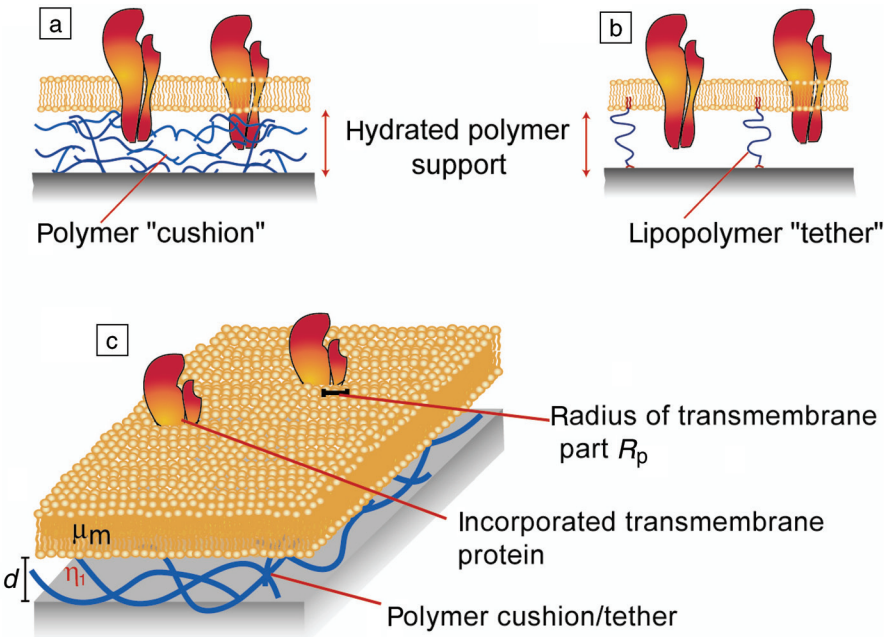

Figure 2. Supported membranes on (a) a polymer "cushion" and (b) polymer "tethers." (c) Compared with transmembrane proteins in solid-supported membranes, both polymer supports effectively modulate the membrane–substrate interaction and improve the homogeneity of the protein distribution, the lateral diffusivity, and biological activity of transmembrane proteins. Symbols: d *is thickness,* μ_m *is the two-dimensional membrane viscosity, and* η_1 *is the viscosity of the interlayer.*

to finite cell–cell distances found in nature, distinct membrane–substrate distances in the presence of polymer cushions were experimentally determined using neutron reflectivity,[29] indicating the presence of the second potential minimum.

Membranes on Polymer "Tethers"

An alternative strategy for separating lipid membranes from their solid substrates is to incorporate lipids with macromolecular head groups (so-called "lipopolymer tethers") into the proximal layer (Figure 2b). The head groups act not only as spacers to increase the substrate–membrane distance, but also as the lubricating layer (as polymer cushions do) to prevent direct contact between transmembrane proteins and solid substrates. The choice of spacers can be based on a wide range of linear macromolecules, including oligo(ethylenoxide)[30–32] and poly(ethylenoxide),[33] and oligopeptides[34] with surface coupling groups such as silanes and thiols. One important aspect of fabricating polymer spacers is to have a uniform polymer chain length with minimal polydispersity indices. From this viewpoint, living cationic polymerization of poly(2-oxazoline)s yields polymers with precisely controlled polymer chain lengths. Lipopolymer tethers with such well-defined chain lengths[35,36] allow for quantitative investigations of the effect of spacer length and lateral spacer density on structures and functions of supported membranes.[35] Flexibility in controlling spacer length and lateral spacer density enables one to fine-tune both the membrane–substrate distance and the viscosity of the polymer layer, both of which are crucial to regulating the lateral diffusivity and function of transmembrane proteins.

Physical Modulation of Membrane–Substrate Contact

As outlined above, the major motivation for using a polymer interlayer is to avoid direct, nonspecific contact between transmembrane proteins and solid substrates. Here, I will give several examples to demonstrate how the physical contact between membrane proteins and solid substrates can be modulated by the presence of polymer interlayers.

Homogeneity, Diffusivity, and Biological Function of Transmembrane Proteins

When lipid vesicles incorporating transmembrane proteins with large extracellular domains, such as integrin and ATPase, are spread directly on solid substrates, they often exhibit inhomogeneous lateral distribution (Figure 1b). These patches of fluorescently labeled proteins are not healed by prolonged incubation time or annealing at elevated temperature. In fact, fluorescence recovery after photobleaching (FRAP) experiments indicate that fluorescently labeled human platelet integrin $\alpha_{IIb}\beta_3$ exhibits no sign of lateral diffusion of integrin in solid-supported membranes.[37] In contrast, when the same integrin-doped vesicles are spread on a regenerated cellulose film with a thickness of 10 nm, the lateral protein distribution was homogeneous over the entire substrate, showing no remarkable defects.[37] In the case of polymer-tethered membranes, integrin-doped vesicles are spread on a predeposited, dry proximal layer. In the absence of lipopolymer tethers or in the presence of short spacers (monomer number $n = 14$), the lateral protein distribution is very inhomogeneous. The elongation of polymer chain length shows a significant improvement in the homogeneity of protein distribution; no remarkable defects are found for $n > 33$.[35]

The homogeneous protein distribution accomplished in both polymer-cushioned and polymer-tethered membranes suggests that the transmembrane proteins can diffuse in polymer-supported membranes. The FRAP technique enables us to measure both the lateral diffusion coefficient D and the fraction of mobile protein. Following the Einstein relation, the drag coefficient f is given by $f = k_BT/D$, where k_B is the Boltzmann constant and T is the absolute temperature. However, as is obvious from the asymmetric boundary conditions at two sides of the membrane (Figure 2c), the lateral diffusion of proteins in supported membranes cannot be treated by the classical continuum hydrodynamic model of Saffman and Delbrück, which assumes a "free" lipid membrane as a two-dimensional continuum with a symmetric boundary condition.[38] Evans and Sackmann[39] modified this theory by taking the interfacial drag σ proportional to the velocity of a diffusant v into consideration: $\sigma = b_s v$, where b_s is the intrinsic frictional coefficient between the membrane and the substrate. The frictional coefficient b_s depends upon the viscosity η_l and thickness d of the interlayer: $b_s = \eta_l/d$. Following the modified theory, the drag coefficient f can be expressed as a function of the dimensionless particle radius ε of the diffusant:

$$f = 4\pi\mu_m\left(\frac{1}{4}\varepsilon^2 + \frac{\varepsilon K_1(\varepsilon)}{K_0(\varepsilon)}\right) = \frac{k_BT}{D}, \qquad (3)$$

where μ_m is the two-dimensional membrane viscosity, and K_0 and K_1 are modified zero and first-order Bessel functions

of the second kind. The dimensionless particle radius ε is defined as a function of the radius R_p of the transmembrane part of a protein (interacting with the membrane matrix):

$$\varepsilon = R_p\sqrt{b_s\mu_m}. \quad (4)$$

The dimensionless particle radius ε can be obtained analytically from the dimensionless particle mobility:

$$m = 4\pi\mu_m/f. \quad (5)$$

From these relationships, ε depends upon f, R_p, b_s, and μ_m. The drag coefficient f can be calculated from the measured diffusion coefficient D; the frictional coefficient b_s can be calculated in a quantitative manner if R_p and μ_m can be approximated from the number of transmembrane helices and the phase diagram of the membrane, respectively. For instance, in the case of integrin in polymer-cushioned membranes, $\varepsilon = 2.8$ can be calculated from the diffusion constant D (and thus, dimensionless mobility m). As integrin has two α-helices spanning the membrane, the radius R_p of integrin can be approximated to 0.64 nm. If one takes the two dimensional viscosity of the membrane $\mu_m = 1.6 \times 10^{-10}$ N m^{-1} s,[40] the frictional coefficient of $b_s = 3 \times 10^9$ N m^{-3} s can be calculated. Moreover, owing to the ability to flexibly adjust both the viscosity η_l and thickness d of the interlayer (both of which affect b_s), polymer-tethered membranes can offer advantages over polymer-cushioned membranes, where viscosity is fully determined by the properties of the polymer material.

The advantage of using polymer supports can also be seen from the biological functions of incorporated cell receptors like integrin. When the interaction between the integrin-doped polymer-supported membranes and giant vesicles exposing integrin-specific ligands was monitored by micro-interferometry, the adhesion free energy was ~10 times higher than the adhesion energy obtained with solid-supported membranes containing the same amount of integrin receptors.[37] The binding energy between integrin and the ligand calculated from the lateral protein density was comparable to the one calculated from the dissociation constant, suggesting that the integrins maintain the native adhesion function in the polymer-supported membranes.

Native Supported Membranes, Tuning Interfacial Contact

In the fabrication of artificial supported membranes, purified transmembrane proteins are first stabilized in surfactant micelles, then incorporated into lipid vesicles before spreading onto solid or polymer-coated substrates. Despite some successful reports on directed (orientation-selective) protein incorporation,[41] many surfactants used for protein purification (such as Triton X-100) disrupt the membrane, which makes it difficult to determine the lateral density of incorporated proteins with the right orientation.[37]

These problems can be overcome if one can spread native cells or vesicles extracted from cells and organelles (microsomes) onto planar substrates instead of artificial vesicles. Here, polymer interlayers are preferred over bare solid substrates, owing to their ability to finely tune the cell–surface contact (i.e., surface tensions and the interplay of interfacial forces, mentioned in the previous section). In fact, as adult animal cells are rendered with negatively charged sialic acid residues, they are not adherent to bare glass/quartz slides. The first successful deposition of natural cells was reported for human erythrocyte "ghosts" (red blood cells after removal of their cytoplasm) spread over regenerated cellulose cushions. Incubation of ghost cells for 60 min resulted in polymer-supported native membranes that appear defect-free and expose the cytoplasmic domain.[42] The simplicity of the method and the precise control of membrane orientation seem to result from the optimized cell–surface contact. Cellulose cushions have also been used for spreading other native membrane extracts such as sarcoplasmic reticulum membranes extracted from muscle cells.[43] Other types of cells or cell membrane extracts (homogenized membranes) are now being examined for the creation of natural polymer-supported membranes.

In contrast to the compatibility of cellulose films to artificial and natural membranes, strong polyelectrolytes (highly charged polymers) do not seem to be suited for the fabrication of natural supported membranes. Although there are some successful reports of the deposition of artificial lipid bilayers on strong polyelectrolytes such as poly(lysine) and poly(styrene sulfonate), incubation of ghost cells on poly(lysine) layers results in inhomogeneous patches of "pinned" membranes, which can be explained as the "dewetting" of negatively charged membranes on too strongly attractive surfaces (Figure 3a).[42] Such a finding suggests a strong demand for polymer films whose physical properties can be flexibly tuned via external stimuli (Figure 3b), for example, exposure to light and slight changes in temperature and pH[29,44,45] in near-physiological conditions. From this context, a combination of supported membrane concepts and polymer/materials

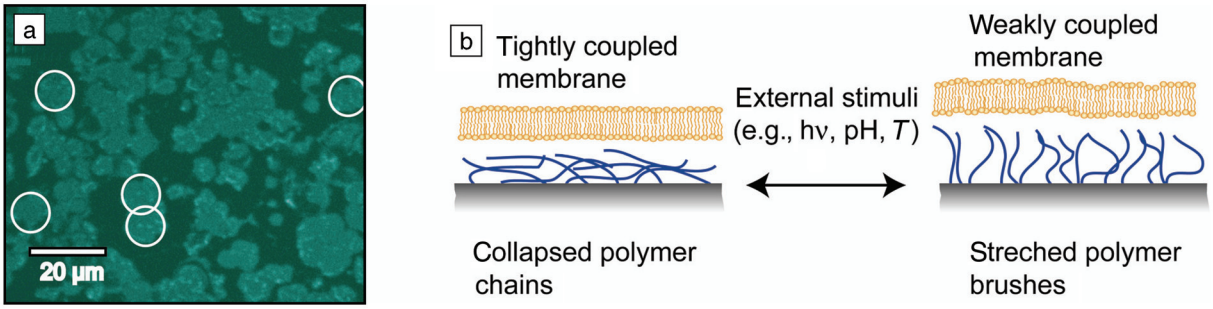

Figure 3. (a) The wrong choice of polymer materials can cause dewetting of cell membranes. An immunofluorescence image of human erythrocyte membranes spread on a strong polyelectrolyte film (the cytoplasmic domain of transmembrane protein is labeled with a fluorescent antibody) exhibits patches of "pinned" membranes. White circles coincide with ruptured patches of individual cells. (b) Schematic illustration of the strategy to fine-tune the membrane–substrate interaction by modulating the polymer chain conformation via external stimuli (e.g., light, temperature, and pH). Symbols: h *is Planck's constant,* ν *is the light frequency, and* T *is the absolute temperature.*

chemistry would open a new possibility for stress-free deposition of various biological membranes.

Lateral Confinement of Membranes and Membrane Proteins

In plasma membranes, lipids and membrane proteins do not always mix homogeneously, but rather exhibit heterogeneous domains of different length scales whose cooperative interactions regulate (or even amplify) overall functionality. They are not always relatively static, like lipid rafts and protein clusters, but can be dynamic, as found for the accumulation of ligand–receptor pairs near focal adhesions[46] and immunological synapses.[7] Localization of membranes and membrane proteins in a confined geometry is one of the key steps needed for applications of supported membranes in (bio)materials science, such as parallel screening of membrane-active analytes, targeting membrane proteins[47] by antibodies or drugs, or combining membranes with semiconductor devices. Progress in micropatterning and manipulation of solid-supported lipid membranes was summarized by Groves and Boxer.[12]

In the next two sections, several methods to localize polymer-supported membranes and manipulate the incorporated proteins will be introduced, some of which take the same principle as those established for solid-supported membranes, while others utilize the advantages of polymer supports.

Manipulation by Electric Fields

Charged molecules (lipids and proteins) incorporated in or bound to lipid membranes can be accumulated or separated within the membrane environment by lateral electric fields. Using solid-supported membranes, previous studies successfully achieved the manipulation of charged lipids embedded in membranes,[48,49] proteins attached to them,[50] and adsorbed DNA molecules.[51] A recent study demonstrated that artificial vesicles tagged to the distal layer of a solid-supported lipid membrane can be used as transporters of synthetic vectors by applying electric fields.[52] In electric manipulation of supported membranes, one major advantage of polymer-supported membranes relative to solid-supported membranes would be their ability to reduce frictional coupling between proteins and the lipid matrix and to electrically decouple the proximal lipid layer from the solid substrate beyond the screening length. Moreover, sophisticated membrane diffusion barrier geometries that can realize Brownian ratchet effects[53] would be very promising to separate molecules with only subtle differences in mobility under electric fields.

Micropatterning of Membranes

An alternative method for spatially localizing membranes and membrane proteins into restricted geometry (e.g., arrays of membrane patches of a defined size) is the micropatterning of supported membranes.[12,54] Micropatterned supported membrane arrays can be used to investigate cell adhesion and growth[55] as well as to screen various antibodies, viruses, or drugs that target membranes.[47]

In the case of solid-supported membranes, micrometer-sized patterns can be obtained using photolithography[56,57] and microcontact printing of the membranes themselves.[58] An alternative strategy applicable for solid-supported[59,60] and polymer-supported membranes[43] is either to deposit or to print grid-like diffusion barriers (e.g., metal, metal oxide, and water-soluble proteins) onto the support surface. These barriers then effectively prevent the membrane constituents from diffusing across, thus isolating the membrane within individual compartments. Here, microstructuring of polymer supports can provide "enforced contrasts" in membrane–substrate interactions. For example, inspired by the observation that erythrocyte membranes spread readily on cellulose films but not on glass slides,[42] position-selective spreading of ghost cells was achieved by incubating cells on micropatterned cellulose films,[61] which utilizes the unique advantage of polymer-supported membranes to create the micropatterns of native supported membranes (Figure 4).[43]

Design of Membrane-Based Hybrid Materials

Many pathogens and drugs are known to target transmembrane proteins and membrane-bound proteins, or they sometimes even interact directly with lipid membranes themselves. Defined model membranes play a key role in understanding the protein recognition processes, as well as in realizing fast screening of disease agents or drugs. To reach this goal, a wide range of systems and physical techniques have been developed for evaluating functions of membrane-associated proteins in supported membranes in a quantitative manner. Fluorescence-based methods in particular have yielded valuable insights, owing to their very high sensitivity, and are widely used for probing various biochemical processes. Alternatively, the supported membrane concepts can complementarily bridge biological molecules and advanced semiconductor technology, which suggests a possible design of new bio–semiconductor hybrids.

This strategy is inspired by the fact that both native plasma membranes and artificial lipid bilayers possess high electrical resistance and behave essentially as insulators. Thus, in this section, I will focus on recent developments offered by supported membranes on semiconductor electrodes for electrochemical detection of membrane protein functions. As described in the following, polymer supports play active roles to match the membrane–surface interaction. By deposition of supported

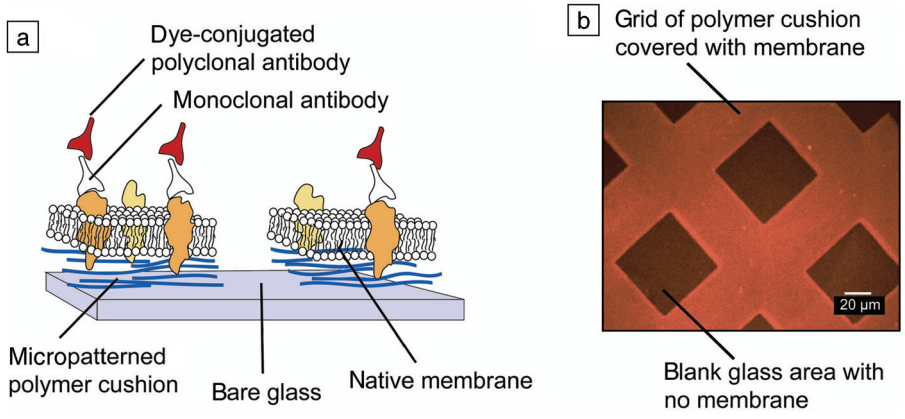

Figure 4. (a) Schematic illustration and (b) immunofluorescence image of a micropatterned ghost cell membrane that labels specific protein subunits with dye-conjugated antibodies. The contrast of membrane–substrate contact between the polymer-coated area and the bare glass substrate can be introduced by lithographic structuring of the polymer cushion, which allows position-selective confinement of cell membranes.

membranes onto semiconductor electrodes, two basic measurement strategies are possible: monitoring membrane conductance associated with the transport of ions (conductive sensing, see Figure 5a), and monitoring changes in membrane surface potential associated with membrane function (capacitive sensing, see Figure 5b).

Conductive Sensors

The first strategy, conductive sensing, can be used on both metal and semiconductor electrodes as an alternative to the traditional patch clamp experiments, which are widely used in electrophysiology.[63] The first crucial step in patch clamp experiments is to seal a fine glass micropipette to a whole cell or small patch of membrane and monitor transmembrane dc current of picoampere order through single channels. However, despite its great achievements, this method often suffers because of the mechanical instability of the clamped membranes. Thus, if the high membrane resistance (the so-called "giga-seal," on the order of 1 MΩ cm^2) necessary for electric detection of the small current signals can be achieved, supported membranes on planar electrodes can be an alternative for studying ion channel functions, owing to their high mechanical stability. Conductive sensing of the activity of ion channels embedded in supported membranes has been reported using gold[30,64,65] and semiconductor[66–68] electrodes. These electrodes have been characterized using ac impedance spectroscopy. The complex impedance signal, obtained by measuring the current through the system as a function of frequency, can be analyzed to calculate the electrochemical properties (resistance and capacitance) of individual layers, that is, the membrane, electrode, and electrochemical double layers in the electrolyte. Here, the advantage of using polymer supports is to reduce the density of local defects and therefore the noise level coming from the leakage current. In fact, on ITO electrodes, polymer-supported membranes have an electric resistance ~5–50 times higher than that of membranes deposited directly onto ITO.[67] As the electrochemical property of a hydrated polymer film is almost identical to that of the bulk aqueous electrolyte, the system can be generalized as an electrolyte–membrane–electrolyte–semiconductor (EMES) multilayer (Figure 5a). The time resolution of ac impedance spectroscopy, typically 10–60 min, can be improved to the microsecond regime by using time-resolved Fourier transform impedance spectroscopy[69] and a more direct readout of current signals using semiconductor transistors.[70,71] As another alternative, recent studies also demonstrated that membranes spanning arrays of small cavities (with openings of submicrometer to several micrometers' width)[72,73] can achieve a higher membrane stability for dc current recording.

Capacitive Sensors

The second strategy, called capacitive sensing, can only use semiconducting electrodes. Here, a hydrophobic polymer cushion not only provides a fluid environment to fabricate a defect-free lipid monolayer, but also acts as an insulating layer with a high electric resistance amounting to 20 MΩ cm^2. Thus, the entire system (the electrolyte, lipid monolayer, hydrophobic polymer support, and semiconductor) can be treated as an analogue of a metal–insulator–semiconductor (MIS) setup (Figure 5b), as the electrolyte acts as a conductor ("metal") and the polymer-supported lipid monolayer as an insulator. The system allows detection of the charging and de-charging of the head groups of membrane lipids by monitoring changes in the semiconductor space-charge capacitance. The sensitivity of this biological MIS setup depends upon the potential drop across the insulator, that is, the polymer film thickness. From this context, the layer-by-layer deposition of nanometer-thick polymer films, such as Langmuir–Blodgett deposition of polymer monolayers, offers the precise optimization of the sensitivity. A recent study indicated that the bio-MIS can reach a sensitivity of up to 1 e^{-1} /30 nm^2 when the process is optimized.[62] If one considers the size and net charge of typical membrane-bound proteins, the sensitivity accomplished on a bulk ITO electrode may even be sufficient to detect the binding of proteins to the membrane surface on the single-molecule level.

Conclusions and Perspectives

Ultrathin polymer supports (typical thickness, ~10 nm) that mimic the characteristic features of the extracellular matrix and the cell surface (glycocalyx) in biological membranes allow for the fine adjustment of the physical interaction at the membrane–solid interface. The lateral distribution, diffusivity, and biological

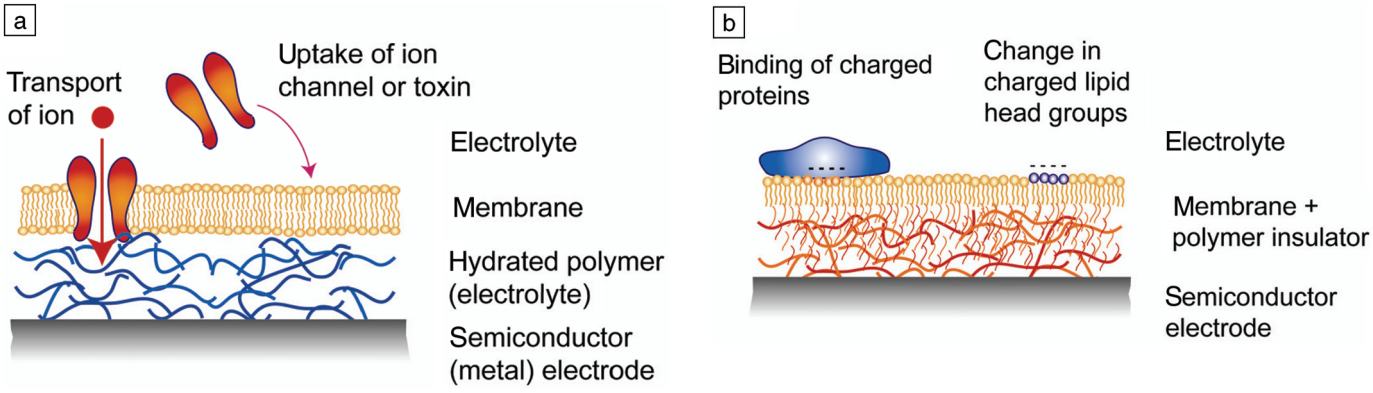

Figure 5. Prototypes of membrane-based hybrid materials for electrochemical biosensors. Polymer supports play an active role in flexible adjustment of membrane–substrate interaction to minimize the leakage current that determines the signal-to-noise ratio. (a) A conductive sensor based on a polymer-supported membrane on a metal/semiconductor electrode for detecting uptake of ion channels and toxins into the supported membrane and evaluating the channel activity after incorporation. (b) A capacitive sensor consisting of a highly resistive polymer-supported lipid monolayer on a semiconductor electrode, which is an analogue of a metal–insulator–semiconductor (MIS) setup. Changes in the surface charge density by charging of lipids or coupling of charged proteins can be detected quantitatively via changes in the semiconductor space-charge capacitance, which can reach sufficient sensitivity (1 charge per 30 nm^2)[62] for sensing protein binding on a single-molecule level.

function of the transmembrane proteins assembled are improved by the presence of polymer supports, which opens possibilities for controlling, organizing, and studying the properties and functions of biological membranes and membrane-associated proteins.

Polymer-supported membranes can be patterned and manipulated for local immobilization of membrane proteins within a confined geometry. The optimization of the membrane–substrate contact has enabled the fabrication of several prototypes of membrane–semiconductor hybrids that seem promising for the label-free detection of protein–protein recognition.

Based on these achievements, (polymer-) supported membranes on semiconductor devices suggest exciting new applications, particularly once it is possible to locally detect signals from individual (or small numbers of) proteins and enzymes. For instance, the fabrication of micropatterned supported membranes containing ion channels with an area ranging from a few hundred square micrometers to ~1000 μm^2 each is realistic. The deposition of such membrane patterns on arrays of field-effect transistors with a comparable sensor area, as presented in Figure 6, will allow parallel monitoring of the channel activity from individual compartments. Polymer supports allow both the tuning of membrane–substrate contact and matching of the position and length scale of membrane patterns and transistor arrays. Moreover, the combination of these membranes with microfluidic devices would provide a powerful tool for high-throughput screening of membrane-targeting pathogens and drugs. Although some of these concepts are technologically available now, it should be noted that the results presented here are intended as a proof of principle, and are still far from real technological applications. However, the complementary combination of supported membranes and semiconductor technology, especially with the aid of soft polymer supports, will result in new materials that bridge two worlds: soft biological materials and hard semiconductor devices.

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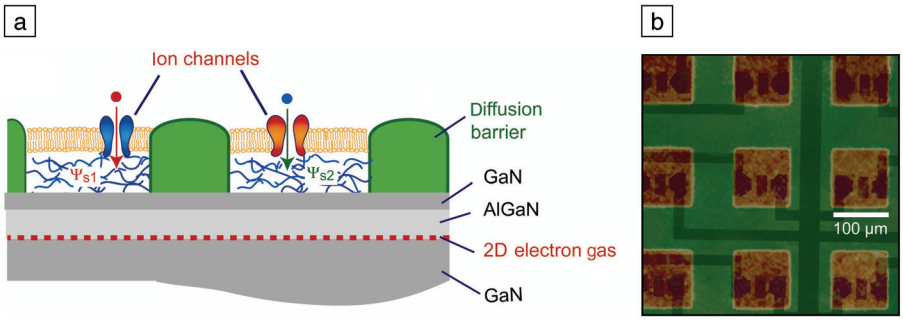

Figure 6. (a) A potential hybrid material combining a supported membrane and a high-electron-mobility transistor. Changes in potentials near the surface Ψ_s resulting from the transport of charged species can be sensed by changes in the carrier density (i.e., the current signal) in a two-dimensional electron gas layer 30–100 nm below the surface. (b) A false overlay of a fluorescence image of micropatterned native supported membranes on an optical microscopy image of a GaN transistor array. Individual patches of native membranes (labeled with red antibody) are isolated by diffusion barriers (labeled with green dye) stamped onto the surface. The transistor arrays can be seen as the shadowed area. (Courtesy of Dr. M. Eickhoff, Technical University of Munich.)

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