Polymer-supported membranes as models of the cell surface

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Lipid-bilayer membranes supported on solid substrates are widely used as cell-surface models that connect biological and artificial materials. They can be placed either directly on solids or on ultrathin polymer supports that mimic the generic role of the extracellular matrix. The tools of modern genetic engineering and bioorganic chemistry make it possible to couple many types of biomolecule to supported membranes. This results in sophisticated interfaces that can be used to control, organize and study the properties and function of membranes and membrane-associated proteins. Particularly exciting opportunities arise when these systems are coupled with advanced semiconductor technology.

Biological membranes are vital components of all living systems, forming the outer boundary of living cells or internal cell compartments (organelles). They consist largely of a lipid bilayer that imparts a fluid character. Proteins embedded in the bilayer and carbohydrates attached to its surface facilitate communication and transport across the membrane. These features enable membranes to act as important filters: processes (some of which may be incompatible) are confined to the organelles they occur in, and toxic substances are kept out of the cell. But specific nutrients, wastes and metabolites can pass through the membranes of organelles and cells to reach their destination. In addition, many important biological processes are regulated at membrane surfaces, through interactions between peripheral and integral membrane proteins.

The complexity of biological membranes and their interactions with intra- and extracellular networks make direct investigations difficult. For this reason, artificial model membranes have played an important part in unravelling the physical and chemical characteristics of membranes and how these contribute to membrane function. For almost 20 years, phospholipid bilayers deposited onto solid substrates (so-called 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[1–7]. These model systems are readily prepared by directly depositing lipid monolayers or bilayers onto solid surfaces to yield large areas (of the order of cm^2) that maintain excellent mechanical stability without losing their fluid nature[8–10]. This combination of fluidity and stability on planar surfaces offers distinct advantages over freestanding 'black' lipid membranes or spherical lipid vesicles because it makes it possible to carry out experiments and use analytical methods that are difficult or impossible to use with other model systems. For example, methods such as total interference fluorescence[11,12], nuclear magnetic resonance (NMR)[13], Fourier-transform infrared spectroscopy[14], surface plasmon resonance[15] and X-ray and neutron scattering[16–18] can all be used to probe the structural and dynamic properties of supported membranes.

It is easy to make supported membranes functional by using membrane-associated proteins. This can be achieved in two ways. One method is to spread vesicles containing reconstituted integral proteins, such as ion channels or membrane-spanning receptors, onto planar substrates. The other method is to prepare supported membranes and incorporate 'anchor' molecules and then couple engineered proteins to those anchors. When combined with protein engineering and bioorganic chemistry, these techniques are powerful tools for creating complex experimental cell-surface models that allow us to probe processes that are difficult or even impossible to study otherwise.

Supported membranes containing reconstituted proteins have already provided imformation about several important biological processes. For example, an early study directly demonstrated that the recognition of antigen-carrying cells by T cells — a crucial feature of the immune response — requires antigens to be associated with the major histocompatibility complex[11]. Later work[6] revealed that initiation of the immune response depends on dynamic features of the recognition and interaction process that creates the immunological synapse (the supramolecular contact between the T cell and the antigen-presenting cell). Crucial to the success of this study was the preservation of the lateral fluidity of the lipids within the supported membranes, illustrating that they can serve as artificial surrogate cell surfaces to probe dynamic aspects of membrane function. Moreover, using cells or vesicles in conjunction with supported membranes offers opportunities for examining cell adhesion[19,20] or the mutual interaction of the proteins that mediate membrane fusion in intracellular vesicle transport and exocytosis[21].

Solid-supported membranes have some fundamental drawbacks. These arise from the proximity of the artificial membrane and the bare solid surface onto which it is deposited. The membrane–substrate distance is usually not sufficiently large to avoid direct contact between transmembrane proteins incorporated in the membrane and the solid surface. This problem is particularly serious when working with cell-adhesion receptors, whose functional extracellular domains can extend to several tens of nanometres. This problem can be avoided by separating the membrane from the solid substrate using soft polymeric materials that rest on the substrate and support the membrane. This approach significantly reduces the frictional coupling between membrane-incorporated proteins and the solid support, and hence the risk of protein denaturation[9,21–23].

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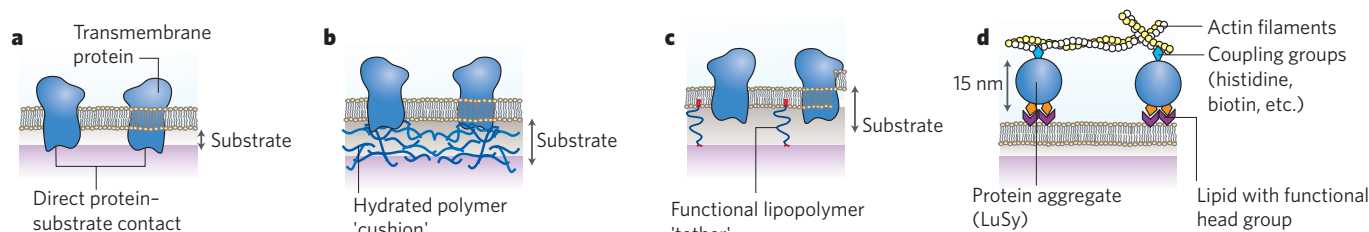
a Transmembrane protein
Direct protein–substrate contact
Substrate

b Hydrated polymer 'cushion'
Substrate

c Functional lipopolymer 'tether'
Substrate

d 15 nm
Actin filaments
Coupling groups (histidine, biotin, etc.)
Protein aggregate (LuSy)
Lipid with functional head group

Figure 1 | **Supported membranes.** The schemes show a solid supported membrane (**a**), and membranes that are supported using a polymer cushion (**b**) or lipopolymer tethers (**c**). Transmembrane proteins are shown in red, membranes in yellow and polymer supports in blue. The thickness of the water reservoir between the membrane and the substrate, distilled H$_2$O, can be adjusted when using polymer supports. With both types of polymer-supported membrane, the lateral distribution of transmembrane proteins is homogeneous, with proteins tending to exhibit higher diffusivity and improved activity than when incorporated in solid supported membranes. In the latter, immobile patches caused by the direct protein–solid contact can often be observed. The scheme (**d**) illustrates actin filaments coupled to a supported membrane using protein aggregates, such as lumazin synthase (LuSy) exposing linker groups (for example, biotin and histidine). The linkers allow attachment of LuSy to membranes containing complementary coupling groups (for example, the chelator complex). Remaining linkers then attach actin filaments, keeping them about 15 nm (the diameter of the capsid) above the membrane surface.

Here, we review different classes of polymer-supported lipid membrane and the various methods for manipulating and patterning them. We also outline the unique opportunities offered by these systems for studying the function of membrane-associated proteins and for practical applications such as protein purification and screening.

Polymer-supported membranes

Supported membranes are readily obtained by depositing phospholipids bilayers onto solid surfaces, with the space between the bilayer and substrate 'bathed' in aqueous solution. In the case of solid-supported membranes, the artificial membrane and its solid support are close together (Fig. 1a). They typically approach each other to within 5–20 Å (refs 13, 17, 24, 25), which leaves a water reservoir that is usually not sufficient to prevent protein subunits from coming into direct contact with the bare substrate. Such direct contact can be avoided by using polymer supports of typically less than 100 nm thickness that 'cushion' or 'tether' the membrane.

Membranes on polymer 'cushions'

When using a polymer to 'cushion' a supported membrane (see Fig. 1b), it should ideally act as a lubricating layer between the membrane and the substrate. This will assist self-healing of local defects in the membrane over macroscopically large substrates (~cm^2) and allow the incorporation of large transmembrane proteins without the risk of direct contact between protein subunits and the bare substrate surface.

One of the most important criteria when choosing an appropriate polymer is that the supported membrane must be thermodynamically and mechanically stable, which calls for careful adjustment of the wetting behaviour of the surface-hydrated polymer interface, the surface-membrane interface and the membrane-hydrated polymer interface[22,26]. The stratified films are only stable if there is complete wetting between the surface and the hydrated polymer, and between the membrane and the hydrated polymer[27,28]. Moreover, the interaction between the membrane and the surface needs to be repulsive; if it is attractive, the layered system is unstable. This can result in dewetting, giving rise to regions of tight local contact between the membrane and surface (so-called pinning centres). In this regard, polymer cushions mimic the extracellular matrix and the cell-surface glycocalyx, which maintain a relatively high osmotic pressure to keep distinct distances (of typically 10–100 nm) between neighbouring cells and between cells and tissue surfaces. Nonspecific contacts caused by van der Waals attraction, which are effective over distances up to about 3 nm, are thus effectively suppressed.

A particularly versatile material for generating polymer cushions is regenerated cellulose[29], which can be made into films with flexibly adjustable thickness and wetting properties. The effectiveness of polymer cushions is illustrated with membranes supported on 5-nm-thick cellulose films and containing cell receptors of the integrin family (human platelet integrin $\alpha_{IIb}\beta_3$). When probing the interaction between these membranes and giant vesicles exposing integrin-specific ligands, the adhesion free energy for the interaction is 3–10-fold higher than the adhesion energy obtained in analogous experiments using solid-supported membranes[30] and comparable to the value inferred from the integrin–ligand dissociation constant. Integrins thus seem to fully retain their mobility and native functionality when incorporated in polymer-supported membranes.

Tethers and spacers

In an alternative strategy that separates lipid bilayers from their solid substrates, lipids with macromolecular head groups (so-called lipopolymer tethers) are incorporated into the lipid layer (Fig. 1c). The head groups act as spacers that control the substrate–membrane distance and, in common with polymer cushions, prevent direct contact between transmembrane proteins and their solid substrates. The spacers can be based on a wide range of compounds, including oligo(ethyleneoxide)[31–33] and poly(ethyleneoxide)[34], and oligopeptides with thiol groups[35]. Living cationic polymerization of poly(2-oxazoline)s yields polymers with precisely controlled monomer numbers, which are attractive as spacers with well-defined lengths[36,37] for relatively straightforward investigations of the effect of spacer length and lateral spacer density on membrane structure and function[36]. The ability to flexibly adjust spacer length and lateral spacer density makes it possible to finely tune the membrane–substrate distance and the viscosity of the polymer layer, both of which control the lateral diffusivity and function of transmembrane proteins. This can offer advantages over the use of polymer cushions, where viscosity is completely determined by the material properties of the polymer support itself (see also Box 1).

Another intriguing possibility offered by macromolecular spacers is the attachment of large proteins or protein complexes to supported membranes. If the proteins are attached such that they retain lateral mobility while avoiding non-specific attractive van der Waals contact with the model membrane, then their assembly and interactions can be studied. Particularly attractive macromolecular spacers for this purpose are proteins and their aggregates. For example, the bacterial enzyme lumazin synthase (LUSY) forms a stable 60-mer capsid with a diameter of 15 nm. The monomers can be fused with different linkers (such as histidine and biotin) by expression in bacteria, which enables simple attachment of the assembled capsid to membranes carrying compatible chelator units (see Fig. 1d). Protein complexes can then be coupled to the prepared membrane using the remaining LuSy linkers. An initial demonstration of the system looked at the self-assembly of actin, showing that reversible and controlled binding and unbinding of filaments is possible[38]. When using supported membranes directly, electrostatic

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Box 1 | Diffusion

One of the main benefits of using polymer-supported membranes is that they reduce frictional coupling between proteins and the substrate surface. This makes it possible to easily manipulate functional membrane constituents using tangential electrical fields or temperature gradients, as the drift velocity of proteins is proportional to the passive diffusion coefficient D_v. Here, measurements of diffusion coefficient D can provide valuable information on the frictional drag acting on proteins.

Macromolecular diffusion in supported membranes differs from that in free membranes (described by Saffman and Delbrück in ref. 86) as a consequence of the frictional tension σ exerted by the solid surface on the diffusing particle, the diffusant. The frictional tension is determined by the viscosity η_c and the thickness h of the hydrated polymer layer, and increases with the velocity ν of the diffusant[85]:

$$\sigma = (\eta_c/h)\nu.$$

If external frictional forces other than the tension exerted by the surface are negligible, the particle mobility depends only on the frictional tension and the friction within the membrane (with viscosity η_m). In such a case, the diffusion coefficient depends on the radius R_P of the diffusant according to the well known Saffman–Delbrück logarithmic law[86]:

$$D = (k_B T/8\pi\eta)\ln(R_p^2\eta_c/\eta_m),$$

where k_B is the Boltzmann constant, T is an absolute temperature, and η is the viscosity of water. For ultrathin polymer cushions ($h = 10$–100 nm), the viscosity η_c (and therefore the frictional force σ) is high, and the diffusion coefficient depends very sensitively on the radius of the diffusant:

$$D \approx k_B T h/\eta_c R_p^2.$$

If the thickness of the polymer supporter is known, measurements of passive diffusion coefficients thus enable the inference of either the viscosity of polymer layer η_c or the radius of transmembrane domains R_p (refs 48, 87).
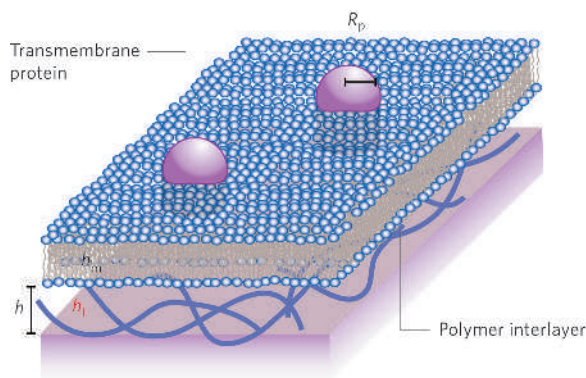
Box 1 Figure | Lateral diffusion of transmembrane proteins in a polymer supported membrane. Here, proteins are assumed to be cylindrical particles, whose transmembrane part has a radius R_p. In the case of ultrathin polymer cushions (h=10–100 nm), the passive diffusion constant D can be used as an indicator to calculate either the viscosity of polymer layer η_c or the radius of transmembrane domains R_p.

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interactions result in irreversible and ill-defined binding between actin and the supported membrane[39].

Supporting native membranes

Nature stringently controls the orientation and the population of transmembrane proteins. It can even dynamically adjust plasma-membrane composition in response to events or stimuli, as demonstrated by the increase in the fraction of a particular protein class (band III proteins) seen upon transfection of a human erythrocyte. Replicating this degree of control using supported membranes is difficult. Transmembrane proteins are usually first stabilized in surfactant micelles and then incorporated into lipid vesicles, which are then used to create supported membranes. Functional assays indicate that
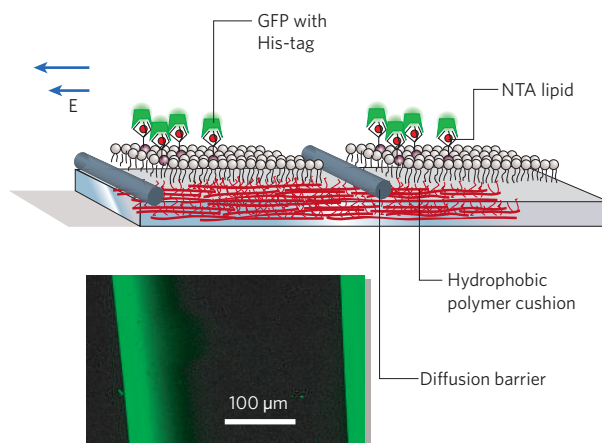

Figure 2 | Electrophoretic accumulation. Electrophoretic accumulation of green fluorescent protein (GFP) with histidine tags (His–EGFP) coupled to a polymer-supported membrane containing 2mol% of chelator lipids. When using hydrophobic cellulose polymer supports, the diffusivities of proteins coupled to membrane lipids are comparable to those of the membrane lipids themselves (1–2 $\mu m^2\, s^{-1}$). The accumulation of GFP over distances of several hundreds of μm can be easily achieved by a d.c. electric field of only 10 V cm^{-1} for 30–60 min.

this method can result in directed (orientation-selective) protein incorporation[21,40], but many of the surfactants used for protein purification disrupt the membrane so that the incorporated proteins are randomly orientated[30]. Although the protein:lipid molar ratio of cell membranes is typically 1:500 and can be realized in artifical vesicles with small proteins, it is difficult to go beyond a ratio of 1:5,000 when incorporating large transmembrane proteins.

These problems can be overcome by spreading native cells onto planar substrates, as first demonstrated with human erythrocyte 'ghosts' (cells with their intracellular components removed) and 10-nm-thick, hydrated cellulose cushions. An hour's incubation yielded polymer-supported native membranes that seemed to be defect-free and exposed the cytoplasmic domain[41]. The ease and simplicity of the process result from careful initial adjustment of the wetting properties of the system overall, to ensure that membrane spreading is thermodynamically favourable. By contrast, using highly charged polymer films induces dewetting, with cell membranes strongly pinned to the solid substrate[41]. Cellulose cushions have also been used for spreading other native membrane extracts such as microsomes from the sarcoplasmic reticulum[42], and there is no reason to assume that spreading of other types of cell or cell-membrane extract (patches) would not be amenable to this approach as well. We anticipate that when used in conjunction with biochemical assays and suitable detection and monitoring techniques, these systems will allow us to directly probe the intracellular membrane leaflet and processes occurring at or near it.

Manipulating membranes

A characteristic feature of membranes is their fluidity: individual constituents are able to diffuse freely within the membrane plane. As a result, lipids and transmembrane proteins need not always mix uniformly, but are able to cluster to form microdomains. The domains can be relatively static, as seen with lipid rafts and protein clusters, for example, or they can be dynamic, with the accumulation of ligand–receptor complexes mediating adhesion or signalling processes. A distinctive feature of microdomains is that they allow cooperative interactions that enhance overall functionality. For instance, the adhesion of cells onto the walls of venules (small veins) is initiated at several sites within the contact zone, followed by recruitment and accumulation of ligand–receptor pairs to those sites to generate focal-adhesion domains and establish firm adhesion[43]. Polymer-supported membranes retain much of their fluidity, which makes it possible to create not only static but also dynamic domains.
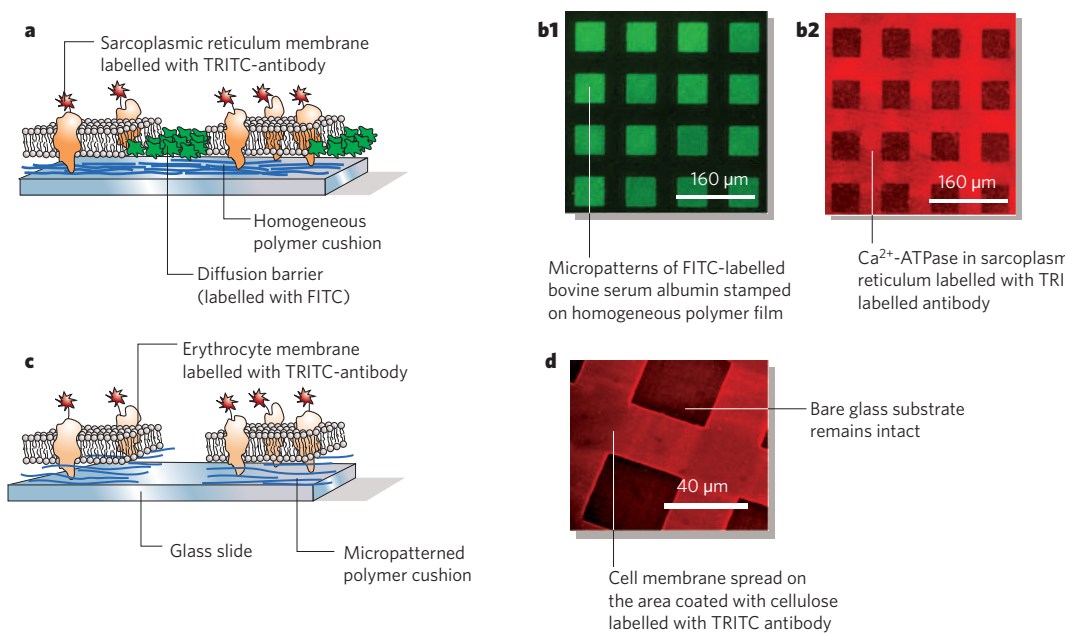
Figure 3 | Membrane patterning. a, Sarcoplasmic reticulum membranes confined by diffusion barriers, established by the micro-contact printing of water-soluble protein (bovine serum albumin labelled with FITC, **b**1) on an homogeneous cellulose film. **b**, The cytoplasmic domain of Ca^{2+}-ATPase is visualized with TRITC-labelled antibody (**b**2). **c**, Incubation of human erythrocyte ghosts with lithographically micropatterned cellulose films results in selective spreading of cell membranes on the area coated with cellulose. **d**, Cytoplasmic domain of the proteins in the erythrocyte membrane (band III) is visualized with antibodies conjugated with a fluorescent dye (TRITC).

However, these domains are typically several orders of magnitude larger than those occurring in natural membranes. Rather than mimicking biological processes involving microdomains, these systems are of interest for practical applications such as protein purification and parallel screening.

Electrophoretic accumulation

If a supported membrane contains charged species, these can be accumulated or separated within the membrane environment by applying lateral electric fields. Charged lipids embedded in membranes[44,45], the proteins attached to them[46] and the adsorbed DNA molecules[47] have all been manipulated in this manner. An intriguing extension involves attaching artificial vesicles with short tethers to lipid headgroups within solid-supported membranes, which allows for transport of synthetic vectors by applying electric fields[48].

It is the fluid nature of membranes that allows electric fields to work well in altering the distribution of membrane molecules, an effect that might be exploited in electrophoretic separations. When attempting to electrically manipulate lipids, lipid-like proteins and lipid-anchored proteins, crucial issues are the defect density and long-range connectivity of the membranes, with defects being particularly problematic as they can lead to permanent 'traps' that cannot be overcome by electrical fields. Membranes supported on glass have so far proven useful for avoiding these problems, given that many molecules are easily manipulated using these systems. But the approach fails with transmembrane proteins, which are essentially impossible to move in glass-supported bilayers. Extending electrophoretic manipulation to this class of protein is likely to be feasible only if membranes are supported on polymer cushions that avoid contact between membrane protein and substrate.

The proximity between a solid-supported membrane and its support surface is not only problematic when trying to move transmembrane proteins, but also increases epitactic coupling and therefore reduces lateral mobility (see also Box 1)[49]. Polymer cushions can be modified with suitable fluid hydrophobic chains to reduce mechanical coupling between the distal lipid-membrane leaflet and the polymer, thus avoiding electrostatic coupling between the proximal membrane leaflet and the substrate. The effectiveness of this strategy is apparent when comparing covalently grafted alkylsilane monolayer surfaces and suitably designed polymer cushions: lipid monolayers deposited on the latter can have more than 10 times larger lateral diffusivities[50]. With hydrophobic cellulose polymer supports, proteins coupled to membrane lipids exhibit diffusivities almost independent of protein weight and comparable to the diffusivities of the membrane lipids themselves ($1–2\ \mu m^2\ s^{-1}$). That is, the frictional drag acting on tethered proteins is dominated by the drag acting on the lipid that anchors proteins to the membrane surface. This facilitates easy accumulation of even relatively large proteins that are tethered to membranes and spread over areas stretching over several hundreds of micrometres, using a d.c. electric field of only 10 V cm^{-1} (see also Fig. 2). For comparison, electric fields typically used in gel electrophoresis are about an order of magnitude stronger. Ongoing membrane separation will of course give rise to inevitable electro-osmotic forces that counteract the electric driving force on the proteins[46,51], but enriching the membrane with counterions partly overcomes this problem (J. Hermann, M. Fischer, S. G. Boxer & M.T., unpublished results). To achieve separation of molecules exhibiting only subtle differences in mobility, sophisticated membrane-diffusion-barrier geometries that exploit geometrical brownian ratchet effects[52] might prove effective.

Patterning membranes

Rather than manipulating the constituents within a membrane, spatial control can also be introduced through patterning to create static and well defined membrane domains or corrals[10,53]. Micropatterned-domains allow investigations of membrane discrimination[54], whereas domain arrays offer attractive opportunities for parallel screening of membrane-active analytes, such as antibodies or drugs targeting membrane proteins[55].

In the case of solid-supported membranes, micrometre-sized patterns can be generated by using a mask to spatially control the photochemical crosslinking of polymerizable lipids[56] and through micro-contact printing of the membranes themselves[57]. In an alternative and particularly versatile strategy applicable to solid-supported[58,59] as well as polymer-supported[42] systems, grid-like diffusion barriers are micro-contact printed using hydrophobic species that attach to the support surface. These barriers then effectively isolate the subsequently deposited membrane into corrals (see also Fig. 3a, b). Patterning is also readily achieved by exploiting wetting contrasts, such as the observation that erythrocyte membranes spread readily on cellulose films but not on glass slides[41]. After micropatterned cellulose films with appropriate wetting contrasts have been created[60], it is straightforward to selectively deposit cell membranes onto the pre-formed patterns[42] (Fig. 3c, d).
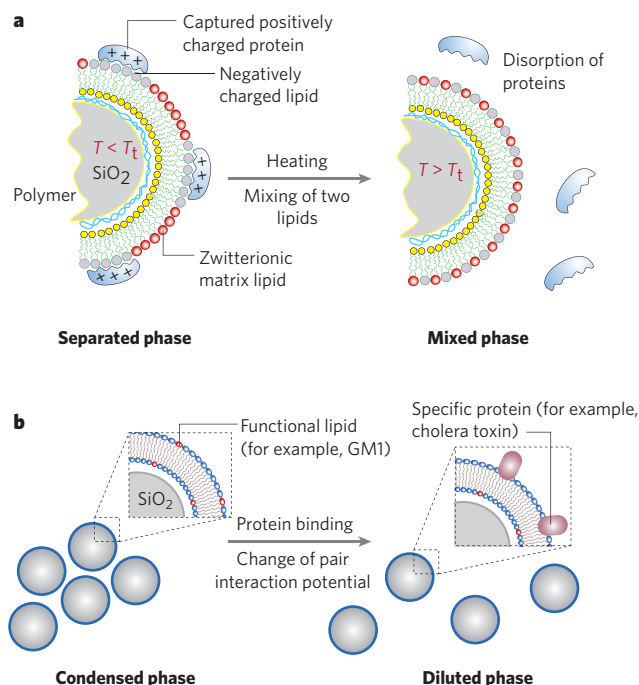
Figure 4 | Membranes on beads. a, Principle of 'phase-transition chromathography'. Polymer-coated silica particles are pre-coated with membranes composed of charged and uncharged (zwitterionic) lipids. At low temperature in the gel phase, lateral phase separation creates charged microdomains that electrostatically trap oppositely charged proteins present in an analyte mixture. The trapped proteins can be released by increasing temperatures at which the charged lipid microdomains dissolve. **b**, Use of colloidal phase transition as a label-free detection method for membrane processes. The pair-interaction potential between membrane-coated silica beads is adjusted using an appropriate lipid composition so that the system is poised near its phase transition. Interactions between analytes and membrane components (such as between cholera toxin and ganglioside GM1 in the membrane) change the interaction potential between the beads. Therefore, the system undergoes a phase transition, changing its macroscopic colloidal organization from a condensed to a diluted phase. The overall effect is that the analyte–membrane interaction is amplified, allowing for label-free detection. (See ref. 63 for full details.)

Supported membranes for applications

Transmembrane proteins and membrane-associated proteins are targeted in many infectious diseases. Membrane models play an important part in unravelling the fundamental cellular processes involved and in screening for pathogens or drug candidates. A wide range of membrane systems and detection methods have been developed for this purpose, although polymer-supported membranes are only starting to be used in this context. We will therefore focus here on two general approaches and illustrate their scope with examples that use solid-supported membranes as well as polymer-supported membranes. One of these approaches involves membranes placed on beads or microparticles, which offer some unique advantages for a range of applications. The second approach we will cover aims to electrochemically detect membrane-protein function by using metal and semiconductor electrodes as substrates. In this setup, the electrical properties of the polymer supports play an active role in system design.

Membranes on beads and microparticles

Membranes are easily deposited on silica beads or polymer-coated beads by fusing the beads with lipid vesicles. Bead diameters typically range from 3 to 30 μm, which is comparable to the diameter of cells and about a thousand times larger than the membrane thickness (5–7 nm). The lateral packing and ordering of the lipids on the beads is thus comparable to that of cell membranes.

Some of the advantages of planar-supported membranes for detailed structural and functional investigations are inevitably lost when membranes on beads, but these systems offer a number of other important advantages. For instance, when analytes need to interact with membrane surfaces or membrane-bound agents, the increase in surface area associated with placing membranes on microparticles will significantly increase detection efficiency and speed. Suspensions of membrane-coated microparticles can also be concentrated in any given detection volume; this effect significantly improves the signal of spectroscopic techniques such as NMR[13], which enables investigations of structural as well as dynamic membrane features.

Like their planar supported counterparts, supported membranes on microparticles are mechanically stable (compared with, say, lipid vesicles) and easily adapted to various functions. Unlike the former, they are readily mixed with reaction or culture media. This attractive combination of features makes it possible to use the particles as reagents. For example, planar supported membranes were used in a study that used membranes containing GPI-anchored neuroligin on silica particles to show that the clustering of β-neuroligin is sufficient to trigger the recruitment of synaptic vesicles[61]. The simple fact that membrane-coated beads are mechanically stable and discrete micrometre-sized entities makes it possible to capture them in laser traps[62]. This offers increasingly sophisticated manipulation possibilities (such as the ability to create dynamic three-dimensional arrays) that might lead to unusual applications.

As the approaches mentioned above illustrate, membranes placed directly on microparticles can be used in many different ways. But only if the membranes are supported on polymers is it possible to fully preserve membrane fluidity while presenting a large surface area to analytes. This combination may extend the scope of these systems for high-throughput screening and protein purification. For instance, in one method that makes use of the fluidity of polymer-supported membranes, charged peripheral proteins are trapped and released by using the thermotropic phase transitions exhibited by some lipid mixtures[62]. Here, hydrogel-covered silica particles are coated with lipid membranes containing charged and uncharged lipids. When placed in a chromatography column at low temperature, the charged lipids cluster into domains that can bind oppositely charged target proteins. Heating the column above the phase-transition temperature dissolves the charged lipid domains, which in turn reduces the electrostatic attraction between the membrane and the charged proteins and hence releases them from the beads (Fig. 4a). This method allows the separation of proteins with similar molecular masses but different net charges.

Microparticles can behave not only as mechanically stable discrete entities but also as colloidal particles that exhibit rich colloidal phase behaviour, which can be exploited to monitor molecular interactions[63]. This requires careful adjustment of membrane composition so that the pair-interaction potential between individual membrane-coated beads poises the system near the transition between a dispersed and condensed phase. As illustrated in Fig. 4b, small membrane perturbations (such as an analyte of antibody binding to a membrane protein) will change the inter-bead potential and induce marked changes in the macroscopic organization of the colloidal phase. This effect enables amplified, label-free detection of cell-membrane processes with high sensitivity[64].

Native cell membranes can also be deposited on silica microparticles. In fact, the use of microparticles makes it possible to rapidly isolate plasma membranes from intracellular elements[65–67] as an essential first step towards the analysis of protein composition. This isolation method is particularly attractive because it yields membrane-coated particles with free access to the protoplasmic membrane surfaces for further biochemical studies. In principle, it should be straightforward to use native membranes placed on beads much like their counterparts. For example, they could be used in conjunction with the colloidal phase-transition assay to study protein recognition on the protoplasmic surface of cell membranes.
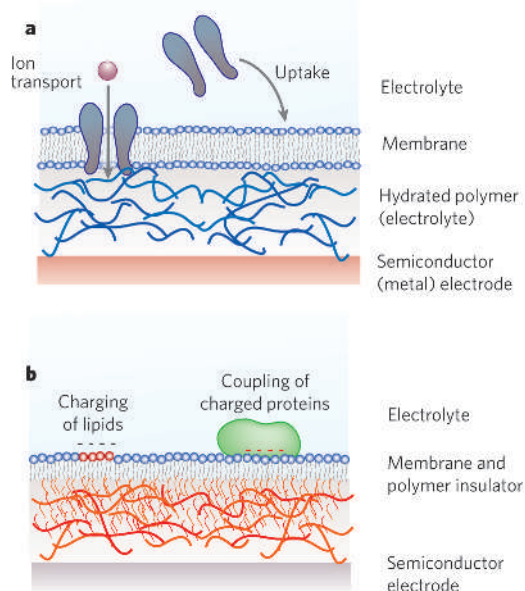
Figure 5 | Electrochemical detection schemes. Electrochemical biosensors using polymer-supported membranes deposited on electrode surfaces. **a**, Using a supported membrane deposited on a hydrated polymer cushion, which electrochemically behaves as an electrolyte, uptake of ion channels and toxins into the supported membrane and their functions can be detected as a change in membrane conductivity. Dynamic Fourier analysis of electrochemical impedance spectra improves the time resolution (to a millisecond order) when monitoring signals from individual ion channels. **b**, The electrolyte–insulator–semiconductor (EIS) structure, which consists of a highly resistant hydrophobic polymer cushion and a supported lipid monolayer. Changes in the surface charge density due to charging of lipids or coupling of charged proteins can be detected quantitatively by monitoring changes in the semiconductor space charge capacitance. Note that when using metal electrodes, the 'total' capacitance cannot be measured with a sensitivity as high as that achieved when using an ITO electrode (one electron per 30 nm^2). The sensitivity achievable with ITO electrodes is sufficient to detect binding of charged peripheral proteins on a single molecular level.
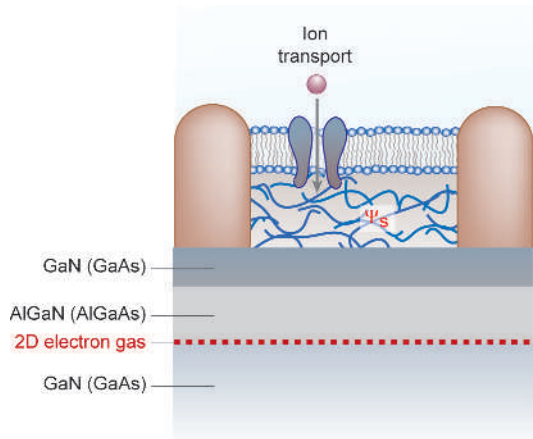

Figure 6 | Membrane–semiconductor systems. The contact between supported membranes and semiconductors can be optimized using suitable polymer supports, whereas patterning techniques ensure the position and area occupied by the supported membranes is matched with the position of semiconductor heterostructure devices (on the basis of GaAs/AlGaAs or GaN/AlGaN). In such an array, each domain could, in principle, allow for independent local sensing of protein functions. The illustration shows changes in the potential near the surfaces caused by selective ion transport through a channel protein. The potential change can be detected with high sensitivity as a change in the carrier density (that is, the current signal) in a two-dimensional (2D) electron gas layer buried 30–100 nm below the semiconductor surface.

Electrochemical detection of protein function

So far, a number of techniques have been used successfully to investigate the function of membrane-associated proteins, often in a quantitative fashion. Fluorescence-based methods in particular have yielded valuable insights owing to their unrivalled sensitivity, which allows detection down to the true single-molecule level. Although there is no doubt that fluorescence detection will play an important role in studies of protein functions in polymer-supported membranes, we focus here on electrochemical methods. These exploit the fact that lipid bilayers possess an intrinsically high electrical resistance and behave essentially as insulators, with the choice of polymer support providing a means to optimize different measurement configurations. In principle, supported membranes deposited on semiconductor electrodes are amenable to two basic measurement strategies: monitoring membrane conductance associated with the transport of ions (conductive sensing; see Fig. 5a), and monitoring changes in membrane surface potential associated with membrane function (capacitive sensing; see Fig. 5b).

In fact, conductive sensing has a long tradition in biology: physiologists widely use the patch-clamp technique[68] (which involves sealing a fine glass micropipette to a whole cell or small patch of membrane) to monitor the activity of a single or a few ion channels by measuring the associated small (order of picoampere) d.c. currents across the membrane. The method has been crucial for a number of great achievements in our understanding of ion channels, but it is time consuming and limited by the mechanical instability of the clamped membranes. The limited stability of clamped whole cells or membranes is particularly problematic when trying to perform more demanding experiments. The observation that support surfaces containing arrays of cavities with openings of several micrometres significantly improve membrane stability is therefore exciting[69]. Such a set-up has already led to a more comprehensive characterization of structural changes in single-ion channels through simultaneous fluorescence imaging and DC current recordings, revealing that gramicidin dimer formation correlates with channel activity[70].

Difficulties associated with traditional patch-clamp experiments might also be avoided by placing supported membranes with high mechanical stability onto planar electrode surfaces, provided a high specific membrane resistance (the so-called 'giga-seal', of the order of $1\,M\Omega\,cm^{-2}$) necessary for electrical detection of the small current signals associated with ion channel activity can be achieved. Although the current passing through a membrane in a d.c. field has not been measured in such systems, a.c.-impedance spectroscopy does enable conductive sensing of the activity of ion channels embedded in supported membranes placed on gold[32,71,72] and semiconductor[73–76] electrodes. The complex impedance signal, obtained by measuring the current through the system as a function of frequency, provides information about the electrochemical properties (resistance and capacitance) of the entire system (the membrane, electrode and electrochemical double layers in the electrolyte). In the case of a supported lipid bilayer deposited on a hydrated cellulose cushion (Fig. 5a), the electrochemical property of the polymer film is almost identical to that of the bulk aqueous electrolyte, so the system can electrochemically be treated as an electrolyte–membrane–electrolyte–semiconductor (EMES) multilayer. This makes it possible to determine the electric resistance of the membrane component, which provides quantitative information about selective ion transport via ion channels and carrier proteins incorporated in the membrane. When using indium tin oxide (ITO) electrodes and polymer-supported membranes[74] with reduced local-defect densities, electric resistances are about 5–50 times higher than can be achieved with solid-supported membranes[75,77]. As a result, a.c.-impedance spectroscopy with a time resolution of typically 10–60 min is possible; the time resolution can be improved to the millisecond regime by using time-resolved Fourier transform impedance spectroscopy[78] and a more direct readout of current signals using semiconductor transistors (see also below)[79–82].

A hydrophobic cellulose cushion not only provides a fluid environment for a lipid monolayer but also acts as an insulating layer with

a high specific electric resistance of up to 20 MΩ cm^2. A lipid mono-layer in contact with an electrolyte and deposited on such a cushion that is sitting on a semiconductor surface thus constitutes an analogue of a metal–insulator–semiconductor (MIS) set-up (Fig. 5b), with the electrolyte acting as the conductor ('metal') and the lipid monolayer and polymer cushion as the insulator. As the film thickness can be adjusted with nanometre accuracy, the potential drop across the poly-mer insulator can be controlled precisely. The system can thus be tuned so that it is possible to detect the charging and de-charging of the head groups of membrane lipids by monitoring changes in the semi-conductor space charge capacitance. When optimized, this simple device should reach a sensitivity of about $1 \times e \, 30 \, nm^{-2}$ (ref. 50). When considering the size and net charge of typical peripheral proteins, such a sensitivity might well allow the detection of protein binding to the membrane surface on the single-molecule level.

A look to the future

Supported membranes can be patterned and manipulated in many ways to tune their architecture and physical properties for optimal immobilization of peripheral and integral membrane proteins, and to tune the communication between the membrane itself and supporting surfaces. We anticipate that using semiconductors as supports will result in a range of exciting new applications (see also Fig. 6), particu-larly once it is possible to locally detect signals from individual or small numbers of proteins and enzymes. Imagine depositing membrane micropatterns, doped with different ion channels, on semiconductors that form arrays of field-effect transistors with a sensor area of a few $10 \, \Omega \, m^2$ each; such a system would allow parallel monitoring of the channel activity in each membrane corral. Combined with microfluidic devices that enable controlled delivery of analytes to individual corrals, this would provide a powerful tool for high-throughput screening.

Crucial to realizing this vision is the availability of highly sensitive, low-dimensional semiconductor structures. With recent advances in band-gap engineering technology, such structures are now increas-ingly available and ready for use in novel detection schemes. For example, two-dimensional electron gases buried 10–100 nm beneath a semiconductor surface exhibit exquisite sensitivity to small changes in surface-dipole moments and solvent polarities[83,84]. In fact, first steps towards exploiting the opportunities offered by advanced semi-conductor devices have already been taken: the carrier density (that is, conductance) in quantum wires made biologically functional with biotin groups shows a high sensitivity to binding of streptavidin[85]. Moreover, semiconductor field-effect transistors have been used suc-cessfully to monitor the activities of neuronal cells[79,80] and cardiac myocytes[82]. Admittedly, these results serve so far mainly as a proof of principle of what might be possible. But we are excited and confident about the possibilities that can arise from combining supported-membrane technology and semiconductor engineering to develop label-free methods for the detection of protein functions at the mol-ecular level. ∎

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