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[30] Spectroscopic and analytical data for these products were in accord with those reported.[2, 5]

Reconstitution of Channel Proteins in (Polymerized) ABA Triblock Copolymer Membranes[**]

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Mathias Winterhalter

Lipid bilayers are the basic constituent of biological membranes. The lipids serve as a fluid matrix for membrane or membrane-associated proteins, which are responsible for various key functions such as signaling or transport. Many of these membrane proteins are pharmacologically important or have biotechnological potential. For such applications one has to immobilize them in an artificial membrane system. This creates a biosensor which can be used, for example, for rapid drug screening. The great advantage of planar freestanding films is the direct access to both sides of the membrane. Thus, these can be applied to carry out, for example, conductance measurements to monitor transport processes across membranes or to detect minor changes on reconstituted channel-forming membrane proteins. An early model system for freestanding films was the so-called “black lipid membranes”, named as such because during the thinning out of the membrane the intensity of the reflected light vanishes and the membrane appears black in the reflected light. In addition to their biological functions, lipid membranes have unique material properties; for instance, they are extremely flexible but at the same time mechanically very stable. In contrast, artificial, freestanding membranes were always fragile and thus of little technological interest. Above a certain size, supported lipid membranes contain defects, which rules out conductance measurements as a recording technique. Herein we present a new type of matrix for membrane proteins. This material is stable and defect-free and allows the formation of giant dense planar membranes.

Similar to conventional lipids, amphiphilic block copolymers may also form membrane-like superstructures in aqueous solution.[1–6] Hence they can be regarded as higher molecular weight analogues of lipids. Moreover, the high diversity of block copolymer chemistry may lead to a plethora of new artificial membrane structures inaccessible with conventional lipids. For example, it is possible to vary the molecular weight, the block–length ratio, the chemical constitution, or even the molecular architecture of these molecules. Owing to their larger size and their slower dynamics, amphiphilic block copolymers may lead to signifi-

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cantly more stable aggregates than those formed from conventional lipids.[1–3, 5–8] Additionally, the structure of such aggregates is considerably strengthened if the individual block copolymers are interconnected through covalent bonds to form giant supermacromolecules.[3, 4, 6] Recently we reported the synthesis of a new type of amphiphilic ABA triblock copolymer.[3] The polymer consisted of a flexible, hydrophobic poly(dimethylsiloxane) (PDMS) middle block and two water-soluble poly(2-methyloxazoline) (PMOXA) side blocks. Additionally, the ends of this PMOXA-PDMS-PMOXA triblock copolymer carried methacrylate groups which allow a crosslinking polymerization. The phase behavior of aqueous solutions of the PMOXA-PDMS-PMOXA triblock copolymer ($M_{n,PMOXA} = 1800$ Da, $M_{n,PDMS} = 5400$ Da; $M_w/M_n = 1.7$) between 0°C and 90°C is shown in Figure 1. At block copolymer concentrations above 48 wt% the system forms a homogeneous lamellar liquid crystalline phase. The lamellar phase was directly imaged by cryo-transmission electron microscopy (cryo-TEM). The samples were prepared as thin films (thickness below 500 nm) by depositing a drop of the solution on a polymer-carbon-coated TEM grid and blotting it with filter paper. The samples were then vitrified by plunging them into liquid ethane. Imaging was performed at temperatures below −160°C with a few micrometers underfocus to increase phase contrast. A representative cryo-TEM image of the lamellar phase formed at 50 wt% triblock copolymer is also shown in Figure 1. The dark ordered lines are interpreted as the triblock copolymer membranes observed from the side. The lamellar spacing can be estimated to be about 20 nm at this composition and the hydrophobic thickness of the lamellae formed by the PDMS blocks can be estimated to be approximately 10 nm. This is in good agreement with capacitance measurements on freestanding triblock copolymer membranes which yielded a hydrophobic thickness of 10 nm.[6] Below 48 wt% polymer, the system shows a broad miscibility gap where the lamellar phase coexists with excess water. That means that over the whole composition range the basic morphological unit are lamellae with a hydrophobic PDMS core and a hydrated PMOXA corona. It has to be emphasized that the phase behavior of the triblock copolymer is similar to that of typical bilayer-forming lipids like lecithin.[9]

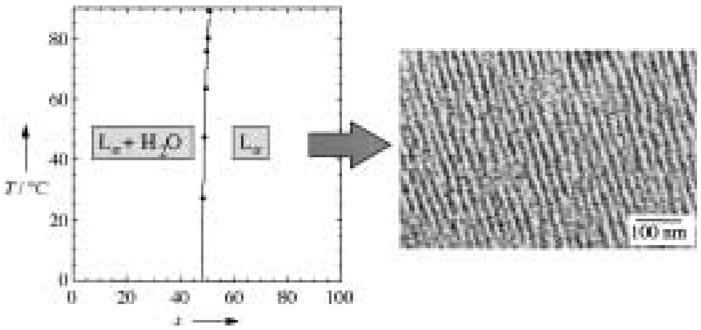

Figure 1. Phase diagram of the PMOXA-PDMS-PMOXA triblock copolymer in water, and Cryo-TEM image of the lamellar phase formed at $x = 50$; x = polymer fraction in water in wt%; L_α = lamellar liquid crystalline phase.

Recently we showed that in dilute aqueous solution this PMOXA-PDMS-PMOXA triblock copolymer forms vesicular structures that consist of spherically closed triblock copolymer membranes.[3] Furthermore, freestanding films with an area of up to 1 mm^2 and a thickness of 10 nm could be formed.[6] Interestingly, the methacrylate end-groups could be polymerized within these self-assembled structures under preservation of the characteristic membrane structure. As a result of the crosslinking polymerization, the individual triblock copolymer molecules are covalently linked together, which leads to a considerable mechanical stabilization of the membranes. Since the polymerizable groups of the macromonomers are attached to the very ends of the hydrophilic blocks, the hydrophobic PDMS middle block preserves a certain mobility within the membrane despite the crosslinking reaction.[3, 6, 10, 11]

Conventional black lipid membranes are frequently employed as model membranes to reconstitute transmembrane proteins (Figure 2).[12, 15] Here planar freestanding PMOXA-PDMS-PMOXA triblock copolymer membranes with an area of 0.02 mm^2 were prepared by using an adapted standard procedure known from conventional low molecular weight lipids.[6, 13, 15] The block copolymer membranes are usually thicker than conventional lipid bilayers due to the larger size of the underlying block copolymer molecules.[3, 5, 6] The hydrophobic–hydrophilic pattern of membrane proteins is naturally optimized with respect to the thinner biological

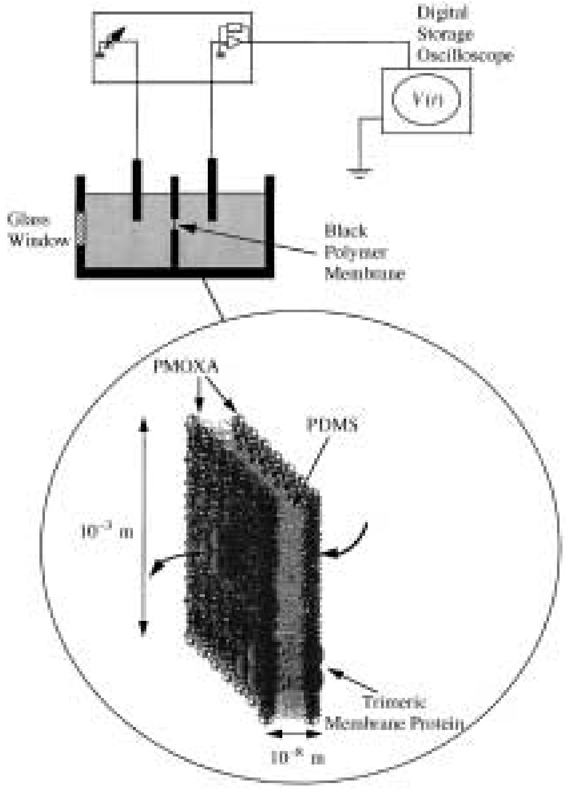

Figure 2. Schematic representation of a protein-containing (polymerized) PMOXA-PDMS-PMOXA triblock copolymer membrane (bottom) and of the experimental setup used to record the conductance across such membranes (top).

membranes and the hydrophobic part of, for example, channel-forming proteins may therefore be too small to fit through the polymer membranes. This raises the question as to whether and when to what extent the proteins preserve their activity within a block copolymer membrane. On the other hand, the high flexibility and the conformational freedom of the polymer molecules may allow a block copolymer membrane to adapt to the specific geometric and dynamic requirements of membrane proteins without considerable loss of free energy. In such a case it is expected that the protein will remain functional.

For the reconstitution experiments we used the well-characterized bacterial porins OmpF and maltoporin as model systems.[14–17] Both porins are transmembrane proteins that form trimeric channels in the outer membrane of Gram-negative bacteria. These water-filled channels allow passive diffusion of small solutes like ions, nutrients, or antibiotics across the membrane. The incorporation of such channels into planar freestanding films can be monitored directly by conductivity measurements.[12, 15]

A first set of experiments was devoted to whether such transmembrane proteins can indeed be reconstituted in a preformed PMOXA-PDMS-PMOXA triblock copolymer membrane. For this purpose about $1-5\,\mu$L of a porin stock solution (about $0.2-1.5\,\text{mg}\,\text{mL}^{-1}$ in 1% octyl-polyoxyethylene, 1 mM NaN_3, 100 mM NaCl, and 2.5 mM Hepes, pH 7.6) was added on both sides of the chamber (each having a volume of 5 mL). The incorporation of porin into the triblock copolymer membranes was favored by applying a potential of 20 mV across the membrane. The conductance across the freestanding film was measured by using Ag/AgCl electrodes and a current amplifier (BLM120, Biologic, Claix, France; see Figure 2).

Figure 3 shows the characteristic time course of the conductance across the triblock copolymer membrane in the presence of OmpF. Surprisingly despite the extreme thickness of this polymer membrane a few minutes after the addition of the porin from the stock solution the conductance increases in a stepwise manner due to multiple OmpF trimer insertions in agreement with previous measurements.[18] In natural lipid membranes each OmpF trimer contributes a conductance of 2 nS under the given experimental conditions (buffer, temperature).[18] Under the assumption that the conductance of a porin channel in the block copolymer membrane is the same as in a natural lipid membrane, an insertion of three OmpF trimers is deduced from the conductance increase of 6 nS in Figure 3.

We also tested the voltage-dependent closure of reconstituted OmpF;[12, 19, 20] no closure was observed up to 200 mV. Similar results were reported for OmpF in conventional solvent-containing black lipid films.[20] The occurrence of the voltage gating seems to depend significantly on the preparation procedure of the membrane. For soft membranes the OmpF porin will close only at very high voltages. In contrast solvent-free membranes which are stiffer, close at about 100 mV. However, the solvent-free preparation method failed with the PMOXA-PDMS-PMOXA triblock copolymer.

PMOXA-PDMS-PMOXA triblock copolymers carry methacrylate groups at both ends which can be polymerized within the membranes by a UV-induced free radical polymerization. Then the individual triblock copolymers are all interconnected through covalent bonds, which freezes in the whole superstructure. The formation of a covalently cross-linked network structure can be expected to change both the lateral mobility and the conformation of the triblock copolymer molecules within the membrane. Therefore, after polymerization protein insertion is inhibited.

In a second series of experiments we used a second bacterial porin, LamB protein or maltoporin. Maltoporin forms very narrow channels of about 150 pS at 1M KCl[15, 18] and possesses stereospecific binding sites for maltooligosaccharides inside the aqueous channels which enhances passive diffusion of the sugars.[14–16, 21, 22] Using the above-mentioned set-up (Figure 2), we monitored directly the incorporation of maltoporin into the polymer membranes. Titration of sugar drives the sugar into the channels in a concentration-dependent manner and causes the channels to close. The maltooligosaccharide affinity can be obtained from the decrease in conductance as a function of sugar concentration.[15]

Figure 4 shows the time course of the conductance of a maltoporin-containing triblock copolymer membrane. For comparison the conductance of a protein-free membrane is also included. Initially the conductance of the maltoporin-

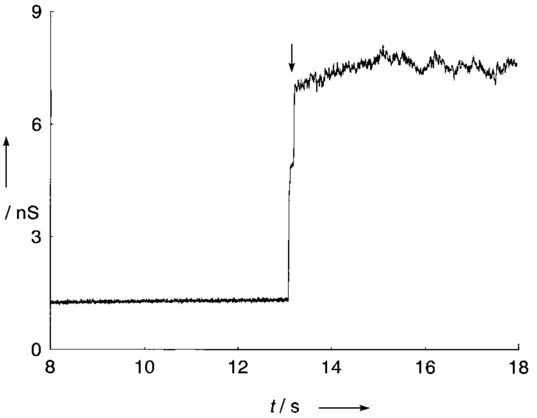

Figure 3. Characteristic time course of the conductance across a PMOXA-PDMS-PMOXA triblock copolymer membrane in the presence of the bacterial porin OmpF. The arrow marks the OmpF insertion.

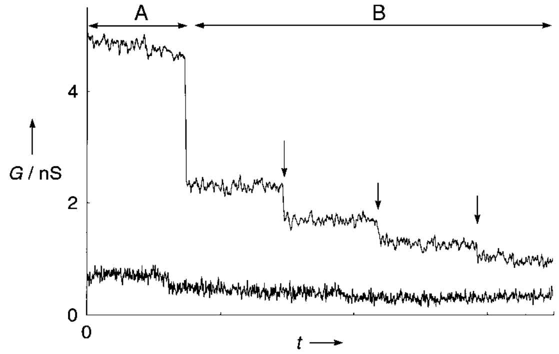

Figure 4. Conductance of a maltoporin-containing PMOXA-PDMS-PMOXA triblock copolymer membrane (upper curve) and a protein-free membrane (lower curve) A) before and B) after polymerization as well as on the stepwise addition of 6 μL of a 10^{-1}M maltohexaose solution (vertical arrows).

containing triblock copolymer membrane is about 4 nS higher than that of the protein-free membrane. This suggests that 27 maltoporin trimers are inserted. The same preparation was subsequently polymerized with UV light. Interestingly the conductance decreases considerably. This could reflect a closure or an expulsion of some of the channels during the crosslinking reaction, probably due to internal stress occurring in the membrane during the polymer chain reaction which may lead to a steric contraction of the hydrophilic blocks of the polymers.[3, 6] Such a steric contraction should depend sensitively on the length of the hydrophilic blocks. This remains to be clarified. This argumentation is also supported by the slight decrease in conductance observed in the protein-free membrane during the crosslinking reaction (see Figure 4). Presumably, the polymerization induces a reorganization within the films that allows small membrane defects to be healed.

The remaining maltoporin-trimers in the polymerized PMOXA-PDMS-PMOXA triblock copolymer membrane were subsequently titrated with maltooligosaccharides (maltodextrin). Figure 4 shows the conductance of the polymerized membrane during stepwise addition of 6 µL of a 10^{-1} M maltohexaose solution. Analysis of the conductance data in the usual way[15, 18] gave a binding constant of $K = 7100\,\mathrm{M}^{-1}$ between the proteins and the sugar. The sugar affinity constants for maltoporin within the polymerized triblock copolymer membrane were the same and in good agreement with previous investigations on maltoporin in conventional lipid membranes.[12, 15] Evidently, the conformation of the protein is not influenced by the surrounding membrane and its functionality is fully preserved.

The present investigations show clearly that a functional reconstitution of membrane proteins can be achieved in completely artificial ultra-thin films. This opens the possibility to benefit from the enhanced stability and diversity of such block copolymer aggregates and to incorporate membrane proteins into such a complete artificial polymer membrane. The resulting protein–polymer hybrid materials can be expected to possess great potential for applications in the area of diagnostics, sensor technology, protein crystallization, and even drug delivery.

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Direct Observation of the Lower Critical Solution Temperature of Surface-Attached Thermo-Responsive Hydrogels by Surface Plasmon Resonance**

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Affinity biosensing is an important method for the investigation of biochemical binding processes: it allows the monitoring of biomolecular interaction in real time and therefore enables the user to deduce kinetic constants from experimental data.[1] One of the most popular transduction principles in affinity biosensing is surface plasmon resonance (SPR).[2] The excitation of surface plasmons in thin metal films—usually gold—by totally internal reflected light can be observed as a minimum in the intensity of the reflected light at a certain angle of incidence Θ_{SPR}, the resonance angle. Changes of the refractive index in close proximity to the gold surface lead to a shift in the resonance angle. SPR offers the advantage of interaction measurements in real time and does not require labeled analytes.[3]

An important prerequisite for the success of affinity biosensing is a chemically tailored sensor interface. Typically, hydrogels are employed as a biocompatible matrix to prohibit the denaturation and to maintain the unique functions of biomolecules which are covalently immobilized at sensor

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