Eur Biophys J (1997) 25: 249–259

ARTICLE

H. Sigl · G. Brink · M. Seufert · M. Schulz · G. Wegner
E. Sackmann

Assembly of polymer/lipid composite films on solids based on hairy rod LB-films

Received: 2 February 1996 / Accepted: 28 October 1996

Abstract The present work deals with the assembly of multilayers or rod-like polymers with hydrophobic side chains (called hairy rods) and their potential application as ultrathin polymer cushions for the build-up of self healing supported membranes on various solids (Si/SiO_2-wafer, gold covered substrates). Three types of hairy rods were studied: Isopentyl cellulose (IPC), phtalocyaniatopolysiloxane with mixed alkane side chains (PCPS) and trimethylsilane cellulose (TMCS). Detailed analysis of the thickness of supported multilayers as a function of the number of deposited monolayers with ellipsometry, near infrared surface plasmon resonance (NIR-SPR), a quartz crystal microbalance (QCM) and reflection interference contrast microscopy (RICM), show that the basic building blocks of hairy rod multilayers are bilayers with the hydrophobic surfaces of the monolayers facing each other. Continuous and stable firms of hairy rods can be deposited if the hydrophobicities of the solid surface and the monolayer are matched. It is demonstrated by lateral diffusion measurements (using photobleaching techniques) that continuous phospholipid bilayers can be deposited onto multilayers of rigid rods of TMCS after hydrophilization by cleavage of trimethylsilane side chains in HCl-vapour, while stable lipid monolayers can be deposited onto hydrophobic surfaces of rigid rod layers. NIR-SPR allows the observation of double band reflectivity curves at interfaces separating different surface layers and thus offers the possibility of differential detection of ligand binding at the interface of differently functionalized domains.

H. Sigi [1] · G. Brink · E. Sackmann (✉)
Physik-Department E22 (Biophysics Laboratory),
TU München, D-85747 Garching, Germany

M. Seufert · M. Schulze · G. Wegner
Max-Planck-Institut für Polymerforschung,
Ackermannweg 10, D-55021 Mainz, Germany

Present address:
[1] FAST Multimedia AG, Munich, Germany

Key words Supported membranes · Soft interfaces · Biosensors · Polymer films · Biomembranes

Abbreviations BSA, Bovine Serum Albumin · DMPC, Dimyristoyl Phosphatidylcholine · DMPE, Dimyristoyl Phosphatidylethanolamine · FRAP, Fluorescence Recovery after Photobleaching · IPC, Isopentyl Cellulose · LB, Langmuir-Blodgett technique for the deposition of thin films · NBD-DMPE, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) dimyristoyl-L-α-phosphoethanolamine) · NIR-SPR, Near infrared surface plasmon resonance – Surface plasmon resonance real time monitoring of thin films with a light wavelength of 1.3 μm · OTS, Octadecyltrichlorsilane · PCPS, Phtalocyaninatopolysiloxane · QCM, Quartz Crystal Microbalance · RICM, Reflection Interference Contrast Microscope · TMCS, Texylmethylc Cellulose

1. Introduction

The present work was motivated by efforts to bio-functionalize solids by supported membranes which are separated from the solid surface by ultrathin and soft hydrated polymer films (cf. Sackmann 1996 for a review). It is hoped that defect-free planar membranes, showing self healing effects similar to free bilayers, can be deposited in this way. Direct deposition of lipid bilayers on solids leads to rather high defect densities as demonstrated with capacitance measurements based on impedance spectroscopy (Stelzle et al. 1993). The defects are most probably a consequence of the surface roughness of the substrates resulting in abrupt changes in the local curvature of the membranes as well as of roughness-induced reduction in membrane fluidity which prevents self healing of local ruptures. Unfortunately these defects form strong nonspecific binding sites for proteins, which is most probably a consequence of hydrophobic pockets being exposed to the aqueous phase. Smoothening of solid surfaces by soft hydrated polymer cushions is expected to overcome these difficulties. Defect free supported lipid layers would allow one to build more

sensitive sensors based on the detection of ligand binding by electrical impedance measurements, since defects induce pores which form high conductivity short circuits. Finally, the polymer-lipid composite films would allow the reconstitution of membrane spanning proteins under non-denaturing conditions and facilitate the separation of receptors or the accumulation of proteins by two-dimensional electrophoresis (Stelzle et al. 1992; Dietrich et al. 1995).

One major problem encountered is the stability of supported polymer/lipid films. They exhibit a strong tendency for dewetting, owing to the Van der Waals attraction between the lipid layer and the substrate (Elender and Sackmann 1994), or tend to destabilize by vesiculation of bilayers deposited by monolayer transfer. The latter instability is most likely a consequence of local inhomogeneities of the weakly cross-linked soft polymer films (Kühner et al. 1994).

Recently, a new class of rod-like polymers with flexible side chains attached along the long axis (called hairy rods) has been synthesized. They form monolayers at the air/water interface and can be assembled into layers of well defined thickness on solids by the LB-technique (Wegner 1993; Seufert et al. 1995). The main purpose of this present work was to study the potential application of multilayers of hairy rods as polymer cushions for supported lipid monolayers or bilayers.

In the first part we used different techniques to study systematically the conditions for the deposition of stable and continuous (defect-free) monolayers and multilayers of hairy rods onto different substrates, including (1) glass, (2) glass hydrophobized by the deposition of octadecylsilane (OTS) monolayers, (3) glass covered by gold films and (4) free and OTS-covered Si/SiO_2-wafers. The techniques included ellipsometry, near infrared surface plasmon resonance (NIR-SPR), and reflection interference contrast microscopy (RICM).

We show that continuous monolayers of hairy rods on hydrophilic substrates remain stable in contact with air if they are deposited by pulling the substrates out of the aqueous phase, showing that the side of the monolayer facing water is highly hydrophilic. Monolayers deposited with this hydrophilic side onto hydrophobic surfaces exhibit many defects after exposure to air due to dehydration caused by the adhesion. Multilayers may be deposited onto hydrophobic substrates (OTS-covered glass or gold) which are continuous and well ordered provided the substrate is alternatingly dipped-into and pulled-out of the subphase of the film balance. It is postulated that owing to an amphiphilic conformation of the rods with respect to their long axes the basic building block of the multilayers is the bilayer. We assume that the amphiphilic structure is induced by the interaction of the molecule with its asymmetric environment: the air-water interface.

In the third part we deposited (1) lecithin monolayers onto supported hydrophobic multilayers of the hairy rods and (2) lecithin bilayers onto multilayers hydrophilized by cleavage of the hydrophobic side chains in HCl vapor. For this purpose cellulose with trimethylsilane sidegroups was used. The continuity and fluidity of the supported membranes are demonstrated by lateral diffusion measurement using the spot photobleaching technique following Kühner et al. (1994).

Of considerable practical interest is our finding that double reflectivity curves are observable by NIR-SPR by focusing the laser beam onto the step from a free to a multilayer-covered area on the substrate. This opens the possibility of increasing the sensitivity of sensors by differential measurement of ligand binding to differently functionalized surfaces.

2. Materials and methods

Materials

Three types of hairy rod molecules were studied: (1) Isopentyl cellulose (IPC) with the structure shown in Fig. 2, (2) phtalocyaninatopolysiloxane (PCPS) with the structure shown in Fig. 3 and (3) trimethylsilyl cellulose, a cellulose derivative like IPC, where all –OH groups are substituted by $–Si(CH_3)$ groups.

Substrates

As glass substrates we used microscopy cover glasses (2.5 cm^2). The silicone wafers for ellipsometry were provided by Wacker Chemie, Burghausen (Germany) and were polished to a roughness of about 3 Å. They were thermally oxidized.

For the surface plasmon resonance experiments Si/SiO_2 wafers polished on both sides and exhibiting a resistivity of R >2000 Ohm from Freiberger Elektronikwerkstoffe (Germany) were used. They were first covered with a 20 Å thick layer of titanium and then with a 500 Å thick gold film by evaporation. The titanium was required to stabilize the gold films.

The cover glasses and Si/SiO_2 wafers were rendered hydrophobic by deposition of a monolayer of octadecyltrichlorosilane ($n\text{-}C_{18}H_{37}SiCl_3$) from Sigma (Deisenhofen, Germany) as follows: after careful cleaning of the substrates they were suspended for 2 min in a solution of 0.1% OTS dissolved in 80 : 20 chloroform : n-hexadecane under mechanical shaking. They were then washed twice for 2 min in pure chloroform baths and subsequently kept for 12 h in a drying box at 75 °C. Many details about silanization of solid substrates with OTS can be found in a recent systematic study (Brozoska et al. 1994).

Monolayer experiments and film deposition

For the monolayer experiments we used a home made large film balance with a trough of 45 cm length and 9 cm width. It was equipped with a Wilhelmy balance for surface pressure measurements and a temperature control system.

The Wilhelmy system was calibrated with arachidic acid monolayers. The speed of area change was about 10Å^2/min.

For the deposition of hairy rod monolayers a smaller film balance with a trough of 19 cm length and 3 cm width was used. It was equipped with a film lift. During the monolayer transfer by vertical motion of the substrate the lateral pressure was kept constant by appropriate variation of the area with the barrier. This pressure regulation was computer-controlled.

The phospholipid monolayers were transferred to the substrates following Tamm and McConnell (1985). For the deposition of monolayers facing the substrate with the hydrophilic face the substrates were pulled out vertically from the aqueous phase into air with the film lift. The substrate was moved with a speed of 0.1 mm/s. For the deposition of films facing the substrate with their hydrophobic side the substrates were dipped horizontally from the air into the water subphase with the hydrophobic surface kept parallel to the plane of the monolayer (cf. Kühner et al. 1994 for details and references).

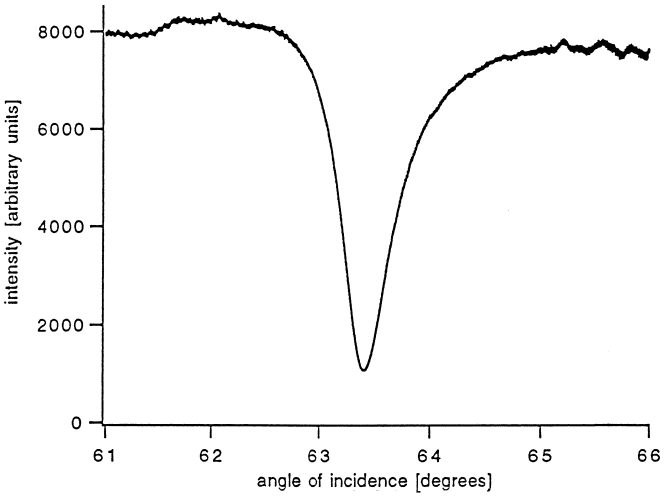


Fig. 1 Record of reflectivity of NIR-light of $\lambda = 1.3$ μm by a gold covered glass substrate as function of the angle of incidence φ. The gold film was 50 nm thick. The drop in reflectivity corresponds to the excitation of surface plasmons. The measuring parameter is the angel of minimum reflectivity φ_{min}

Methods of structural characterization

Photobleaching. For the measurement of lateral diffusion coefficients by the fluorescence recovery after photobleaching technique (FRAP) we used the fluroescence microscope described previously (cf. Kühner et al. 1994). Bleaching was performed with a 4 W argon laser (Coherent) and the bleaching spot diameter was about 6.5 mm.

Ellipsometry. These studies were performed with a home made ellipsometer attached to a film balance which was described previously (Frey and Sackmann 1993).

Near infrared surface plasmon resonance (NIR-SPR). The home made NIR surface plasmon spectrometer (working at 1.3 μm) was designed and built in the Munich laboratory. The principle of the method and the details of the instrument were described previously (Brink et al. 1995). A typical NIR-SPR-spectrum is shown in Fig. 1 to demonstrate the sharpness of the spectra in the near-infrared region. The linewidth is proportional to the wavelength of the incident light and is thus sharper in the NIR than in the visible. An outstanding advantage of NIR-SPR is that it can be applied for silicon based devices since both Si and water are transparent in the 1.3–1.7 μm wavelength range.

Quartz crystal microbalance (QCM). A transversal cut quartz crystal microbalance with a resonance of 20 MHz was used in a oscillator-driven circuit. The details of the instrument are described in Kößlinger et al. (1992).

Reflection interference contrast microscopy (RICM). The RICM observations were performed with a Zeiss Axiomat inverted microscope equipped with an antiflex objective. The method has been described in detail previously (Rädler et al. 1993).

3. Results

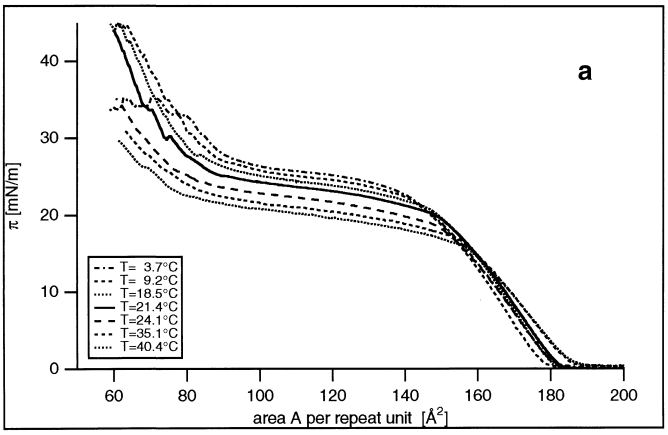
3.1 Isotherms of IPC- and PCPS-monolayers

Figures 2 and 3 show pressure-area isotherms of the hairy rod polymers isopentyl cellulose (IPC) and phtalocyaninatopoly(siloxane) (PCPS), respectively. In both cases one observes breaks in the pressure-versus-area curves at pressures of about 20 mN/m where the lateral compressibility increases abruptly. For IPC a further break arises at increasing pressure where the compressibility decreases again abruptly before the film collapses at 30–40 mN/m. Thus a plateau-like transition arises which is significantly shifted to higher pressures with decreasing temperature. For PCPS the isotherms could not be followed to higher pressures since the film became so rigid that our Wilhelmy pressure measuring system failed. Such measurements were, however, performed previously (Kalacher et al. 1990).

For both types of films we found strong hysteresis effects if the films were compressed above the lower break point. In order to ensure that the monolayers were transferred from the same equilibrium state for each preparation all films were deposited from pressures below the break points.

3.2 Characterization of supported multilayers of hairy rods by ellipsometry, QCM, NIR-SPR and reflection interference contrast microscopy (RICM)

In order to characterize the structure and smoothness of supported multilayers of hairy rods and their application



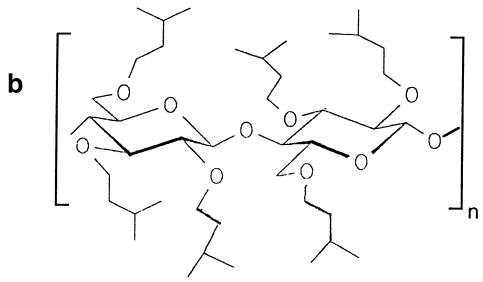
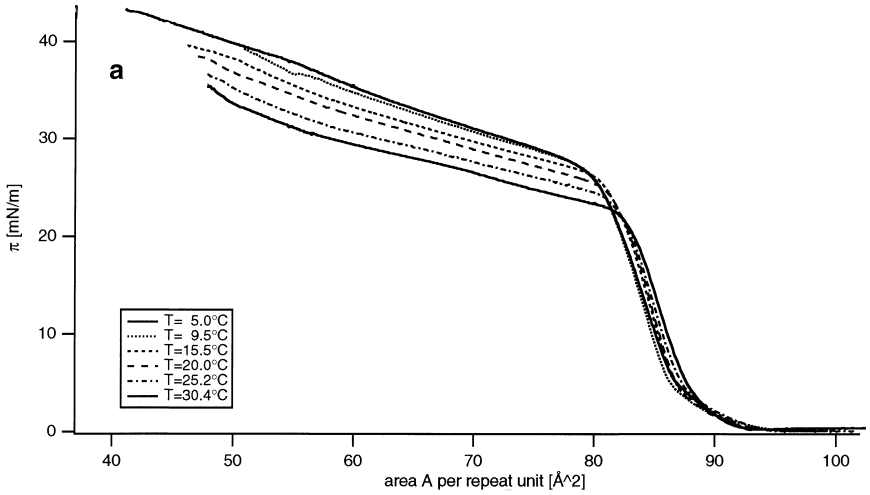


Fig. 2 **a** Pressure area isotherms (observed during compression) of isopentyl cellulose (cf. scheme in **b**) for temperatures indicated. The abscissa gives the area per cellulose repeat unit. Note that transition pressure of the plateau region decreases with increasing temperature. **b** Repeat unit of hairy rod composed of isopentyl cellulose. The degree of substitution is 2.9 isopentyl groups per glucose unit: the average degree of polymerization is 86 glucose units and the molecular weight per rod is 372.55



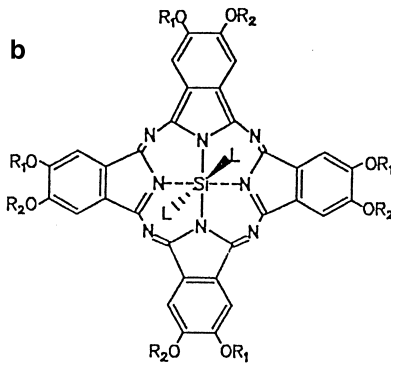


Fig. 3 **a, b** Pressure-area isotherms of phtalocyaninatopolysilane (PCPS) monolayers. On the abscissa the area per repeat unit is given. The basic unit is phtalocyanine interconnected by Si-O-Si-bonds shown in (**b**). The hairs are composed of $R_1 = CH_3$ and $R_2 = C_8H_{17}$. Mixing of chain lengths prevents crystallization

as soft cushions for supported membranes we applied the following techniques and/or tests. The continuity of the first few films on glass-substrates was studied by RICM. On gold covered glass we measured the incremental increase in film thickness after each deposition of a new monolayer of the hairy rods by NIH-SPR and the quartz crystal microbalance (QCM). On Si/SiO_2-wafers such measurements were achieved by ellipsometry. A further important test of the quality of the polymer films was based on the lateral diffusion measurements within lipid monolayers or bilayers deposited onto the hairy rod multilayers.

3.2.1 Hairy rod multilayers on Si/SiO_2-wafers and glass-substrates

Multilayers of IPC and PCPS were transferred onto hydrophilic and hydrophobic surfaces. The thickness of the transferred layers was continuously measured by ellipsometry (on Si/SiO_2). As a second method to characterize the quality of the transfer process we measured the area deposited per transfer step.

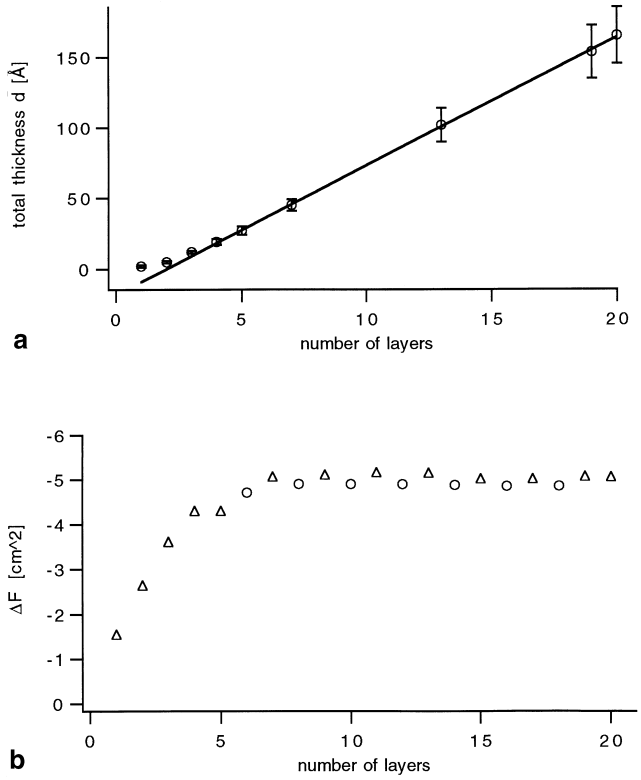


Fig. 4 **a** Plot of thickness (measured by ellipsometry) of isopentyl cellulose layers deposited onto hydrophobic Si/SiO_2-wafers as function of number of transferred monolayers. For the calculation of the thickness a refractive index of the film of $n = 1.44$ was assumed. The transfer pressure was 20 mN/m. The first 6 and the 20th layer were deposited by pulling the substrate out of the wafer while layers 6–19 were deposited by alternatingly pulling-out and dipping-in of the substrate. **b** Plot of incremental decrease of area after each transfer step. Note that the area transferred is systematically smaller for the dipping-in process (o) than for the pulling-out-step (Δ)

A) *IPC*

In a first experiment we found that one monolayer of IPC could be deposited onto the hydrophilic substrate by pulling it out of the aqueous phase through a monolayer kept at 15 mN/m (just below the horizontal deflection regime seen in Fig. 2). However, this layer detached again from the substrate, if it was transferred back into the subphase through the monolayer. Moreover, the mean monolayer thickness was only 6±1 Å compared to the value of 9 Å expected for a closely packed monolayer. Multilayers of IPC could, however, be deposited successfully on Si/SiO_2 surfaces hydrophobized by a monolayer of octadecylsilane. In Fig. 4 we show the sequential increase in layer thickness in the course of the transfer of 20 monolayers of IPC. The layer thickness was obtained by assuming a refractive index of the polymer of $n = 1.44$. The incremental increase of the thickness Δd per transferred monolayer is much smaller for the first three layers and then approaches a constant value of $\Delta d = 9.1$ Å. This correlates well with the monolayer area transferred with each step, which is much smaller for the first three layers than for subsequent layers. Below (Fig. 6) we provide evidence that this incomplete transfer is due to the formation of voids within the monolayer rather than to a reorganization of the transferred monolayer leading to local collapses. The formation of the voids is due to the fact that the first monolayer was transferred by pulling the substrate out of the subphase. The experiment thus shows that an initial imperfection of the hairy rod films can be compensated by transfer of a large enough number (≥6) of monolayers.

B) *PCPS-layers*

In a first experiment we found that a monolayer of PCPS could be deposited onto a hydrophilic surface by pulling the substrate out of the aqueous phase with the monolayer

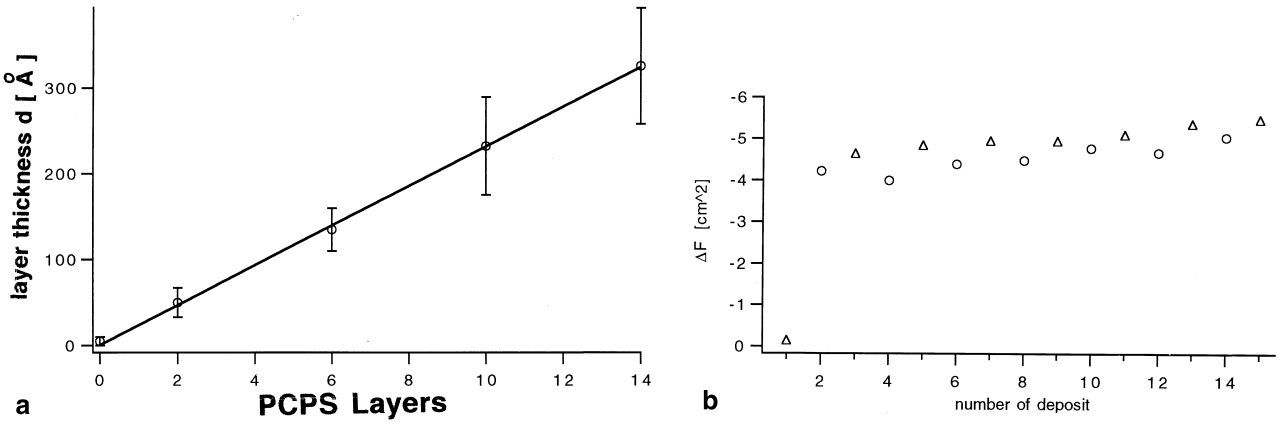


Fig. 5 **a** Plot of increase of thickness (measured by ellipsometry) of PCPS multilayer on Si/SiO_2-wafers with number of transfer step. The error bars give the variations of the thickness measured at different sites on the surface. The lateral pressure was kept at 20 mN/m (at T = 20 °C). **b** Plot of area transferred by each step. Note that the area deposited during the dipping-in steps (o) is systematically smaller than the value transferred during the pulling-out-steps (Δ)

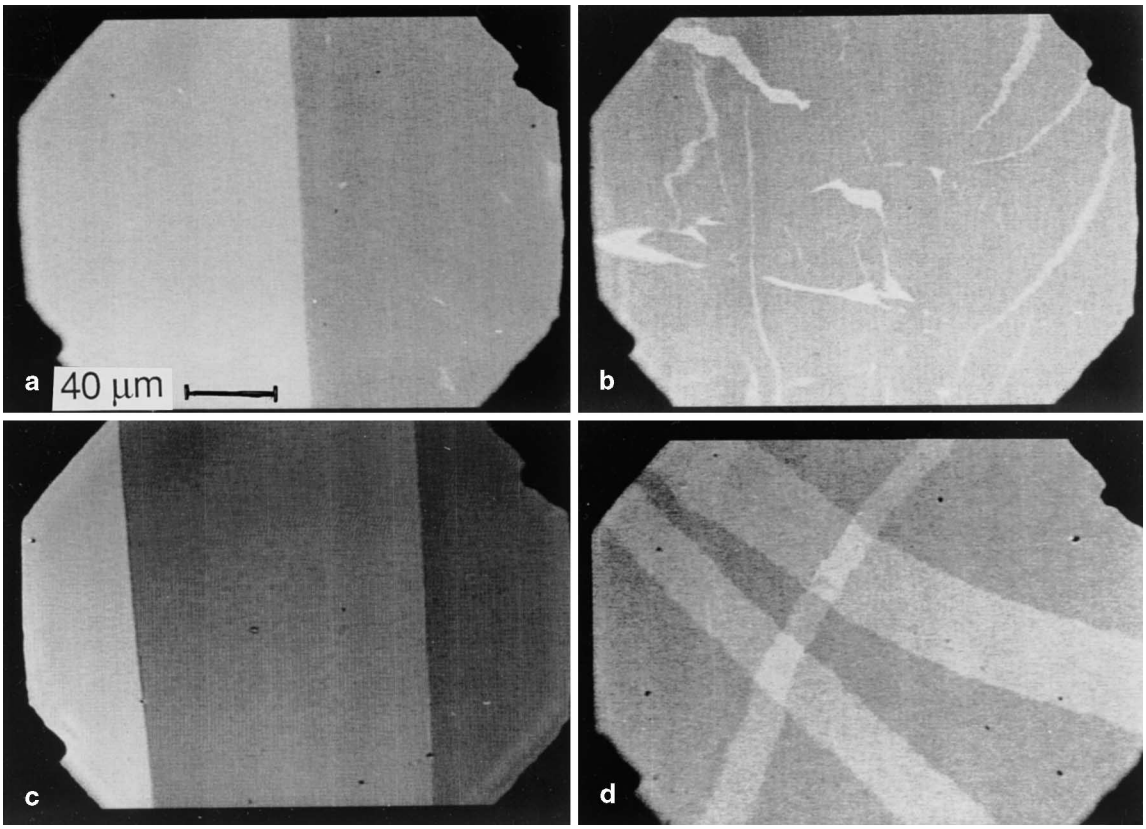


Fig. 6 a–d RICM images of PCPS layers exhibiting steps deposited onto OTS-covered glass substrates. **a** Transition between bare glass surface (left) and PCPS-bilayer covered area (*right*). Transfer pressure 19.5 mN/m. **b** PCPS-bilayer covered region of glass substrate showing uncovered clefts in bilayer. The brighter clefts (arrows) are voids penetrating both layers while the darker clefts indicate defects in only one monolayer. **c** Transition region covered by 0, 4 and 6 PCPS-layers, respectively, from left to right. **d** Highly defective 6-fold layer of PCPS deposited at 17.5 nN/m onto hydrophobic substrate. The net transferred area corresponded to only 60% of the surface of the substrate

kept at 20 mN/m. The area deposited corresponds well with the total area of the substrate. We found a layer thickness of 23 Å by assuming a complex refractive index of $n = 1.5 - 0.08$ i. The layer thickness agrees well with the repeat distance of a monolayer (d = 23 Å) measured by x-ray diffraction, showing that the monolayer is nearly perfect. However, the layer is unstable below water and therefore multilayers can not be deposited.

PCPS-multilayers can, however, again be deposited on hydrophobized surfaces. As shown in Fig. 5 even the first monolayer is already perfect. A remarkable finding is again (as in the case of the isopentyl cellulose) that the area deposited during the dipping-in steps is systematically smaller than during the pulling-out step.

As shown previously reflection interference contrast microscopy (RICM) is a very sensitive technique for the two-dimensional mapping of the thickness of supported organic films on glass (Rädler and Sackmann 1993). It allows measurements of the thickness to an accuray of ≤1 nm (or better if the method of contrast enhancement by coverage of the glass substrate with MgF_2-$\lambda/8$-layers is applied) while the lateral resolution is about 0.3 µm.

Figure 6 shows RICM images of PCPS-multilayers on OTS-covered cover glasses deposited under different conditions:

– Fig. 6 a and b shows an example of two layers of PCPS deposited onto one half of the substrate at a transfer pressure of 19.5 mN/m. In Fig. 6 a the transition between the free and covered area is shown while Fig. 6 b exhibits defects of the PCPS-bilayer consisting of non-covered clefts in the PCPS-film.
– Fig. 6 c shows the transition regions of a substrate covered by 0, 4 and 6 PCPS-layers, respectively, (from left to right).
– Fig. 6 d shows a RICM image of a 6-fold PCPS-layer deposited at 17.8 mN/m. It is an example of an unstable multilayer with incomplete transfer of PCPS-monolayers.

3.2.2 NIR-SPR and QCM-Experiments on gold covered substrates

The immobilization of proteins on gold covered substrates under non-denaturing conditions is a prerequisite for the design of high sensitivity sensors based on detection of ligand binding by affinity sensors e. g. devices using surface acoustic waves or surface plasmon resonance as detection modes. Another major, still widely unsolved, problem is

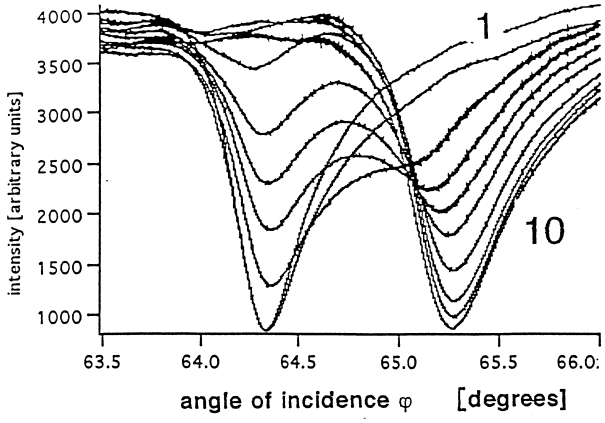


Fig. 7 NIR-surface plasmon resonance spectra of transition region between hairy rod multilayer and bare gold surface. Plots of reflectivity as function of angle of incidence (cf. Fig. 1). These were recorded after each step of a stepwise motion of the incident laser beam over the edge separating the free gold surface an the area covered by 8 layers of PCPS. Curve 1 and 10 were obtained by focusing on the bare gold surface and the PCPS covered surface, respectively. Curve 4 corresponds to the situation were the incident beam is focused onto the edge

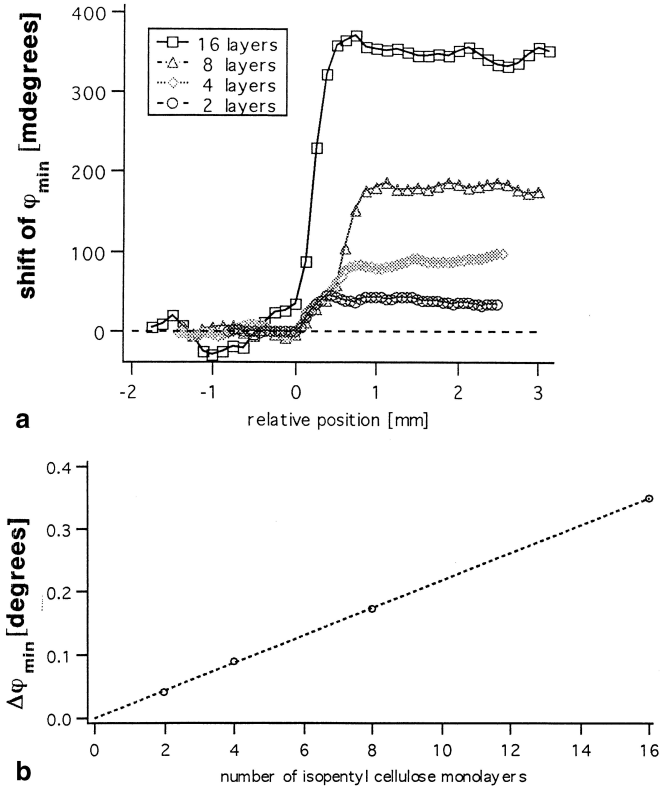


Fig. 8 **a** Shift of average angle of minimum reflectivity observed by scanning the laser beam once over the edge separating the bare gold surface and the half of the substrate covered by isopentyl cellulose layers. Curves are shown for various numbers of monolayers ($n = 2, 4, 8, 16$). In this case only a broad band was observed in contrast to the double resonance curves Fig. 7. **b** Shift of angle of minimum reflectivity as a function of the number of monolayers of isopentyl cellulose. The finding of a constant incremental shift per each two monolayers deposited demonstrates the high quality of the transfer process. These data were obtained by averaging over large areas and the accuracy of the thickness measurement is much higher than in the case of **a**

to prevent non-specific binding of ligands (e. g. antigen). The passivation of gold surfaces by multilayers of hairy rods which can subsequently be functionalized is a promising strategy to achieve this goal. We therefore studied the multilayers of hairy rods on 50 nm thick gold films on Si-wafers. The advantage of Si-wafers is that they can be prepared with much smoother surfaces than glass-substrates. Our recent extension of surface plasmon spectroscopy (cf. Hickel and Knoll (1990) for references of SPR in the visible and at 1150 nm (Rothenhäusler and Knoll 1987)) into the near infrared wavelength region of (1.3 – 1.7 μm) provides a new powerful method for the structural characterization of organic layers on Si/gold-substrates since Si is only sufficiently transparent at 1.3 μm.

In the present experiment layers of IPC or PCPS were deposited on one half of the wafer while the second half was left free. The reflectivity curves were then recorded repeatedly while the incident beam was moved over the edge separating the covered and non-covered area. As shown in Fig. 7 for the case of PCPS two bands with minima at 64.4° and 65.3° appear simultaneously and their relative intensity depends on the position of the beam. In this way one can simultaneously compare the angle of minimum reflectivity of the covered (that is active) area and the uncovered reference state.

As shown in Fig. 8 a for the case of isopentyl-cellulose, information on the sharpness of the edge can be obtained in cases where no double bands are observable by recording the shift of the minimum angle of reflectivity as a function of position while the beam is scanned over the edge.

In Fig. 8 b the shift $\Delta\varphi_{\min}$ of the angle of minimum reflectivity is plotted as a function of the number of deposited monolayers for the case of isopentyl cellulose. Clearly, the shift increases by the same increment with each deposited double layer and the straight line fitted to the measured data passes through the origin of the $\Delta\varphi$-versus-n-plot.

A remarkable difference between isopentyl-cellulose and PCPS is found for the shift of the angle of minimum reflectivity per monolayer. For the IPC-system mentioned, one finds $\Delta\varphi_{\min} = 22 \cdot 10^{-3}$ degree per monolayer or $2.4 \cdot 10^{-3}$ degree/Å (monolayer thickness 9 Å) while for PCPS $\Delta\varphi_{\min} = 0.22$ degree/monolayer or 10^{-2} degree/Å. Moreover, the width of the reflectivity curve is considerably larger for PCPS then for cellulose which strongly suggests that the former compound absorbs in the NIR. Spectroscopic measurements of the refractive index of PCPS from the visible up to 1200 nm do not show an exceptionally high refractive index (Schaub et al. 1995). A possible explanation is that 1300 nm is near an absorption line (possibly CH-vibration overtones) leading to increases in the real part of the refractive index.

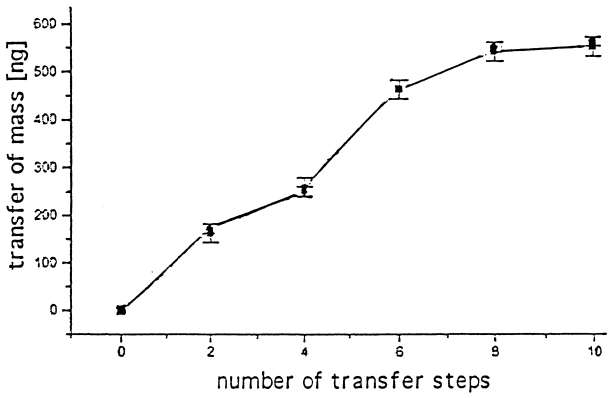


Fig. 9 Comparison of mass transfer per step obtained by the quartz crystal balance (QCB; data points marked by dots) and the LB-technique for the case of PCPS. The gravimetrical data are obtained from frequency shifts in the oscillator circuits according to the well known Sauerbrey equation (Guilbault 1984). For the film balance data an area per monomer of 95 Å and a molecular weight of each monomer of 1197 g/mol was assumed. The film was transferred at constant pressure of 20 mN/m. For this figure we used a preparation where incomplete transfer has occurred to better demonstrate the equivalency of mass transferred and area consumed in LB deposition of thin films of PCPS

Figure 7 suggests a simple differential technique for the comparison of ligand binding to differently functionalized surfaces on a substrate (cf. also Hickel and Knoll, 1990). By focusing the incident laser beam onto the interface between two differently functionalized areas of the substrate one should be able to observe the shifts of the reflection band caused by ligand binding simultaneously.

We have used the QCM to validate the proportionality of transferred surface area as observed with the Langmuir Blodgett technique and the amount of molecules transferred. The QCM is a powerful technique to measure thin fims gravimetrically (Guilbault 1984) and can be employed for the observation of very small mass changes, e. g. to determine desorption of amphiphiles from LB-films (Johannesmann and Knoll, 1990) and to measure precisely the formation of self-assembled monolayers of small molecules (cf. Kößlinger et al. 1992). Although our quartz crystal was cut to vibrate transversally and estimations could have been performed in fluid surroundings as well, we used the QCM in the gas-phase in order to achieve a higher accuracy. The results of the QCM experiments shown in Fig. 9 demonstrate the equivalence of the transferred area

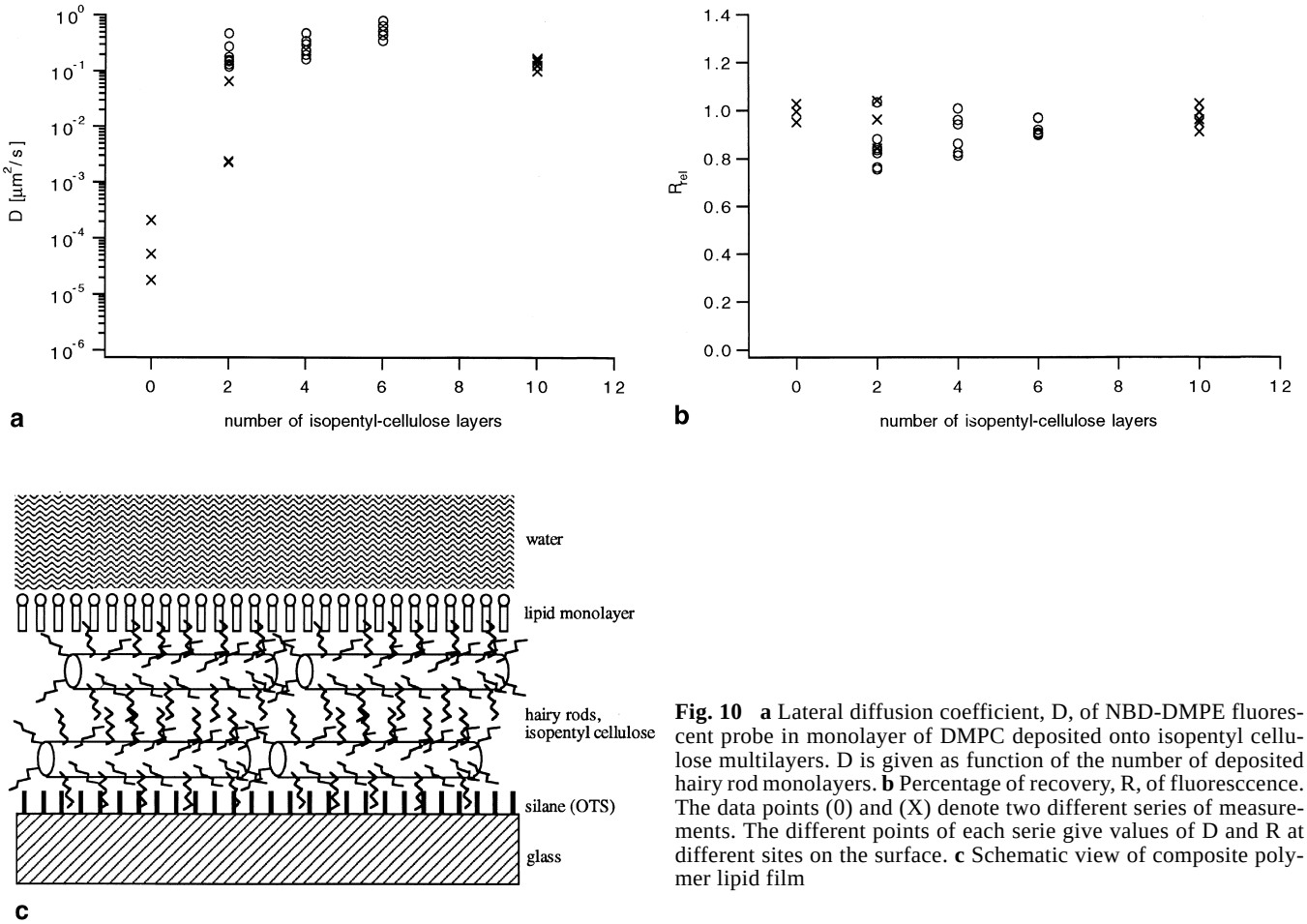


Fig. 10 **a** Lateral diffusion coefficient, D, of NBD-DMPE fluorescent probe in monolayer of DMPC deposited onto isopentyl cellulose multilayers. D is given as function of the number of deposited hairy rod monolayers. **b** Percentage of recovery, R, of fluoresccence. The data points (0) and (X) denote two different series of measurements. The different points of each serie give values of D and R at different sites on the surface. **c** Schematic view of composite polymer lipid film

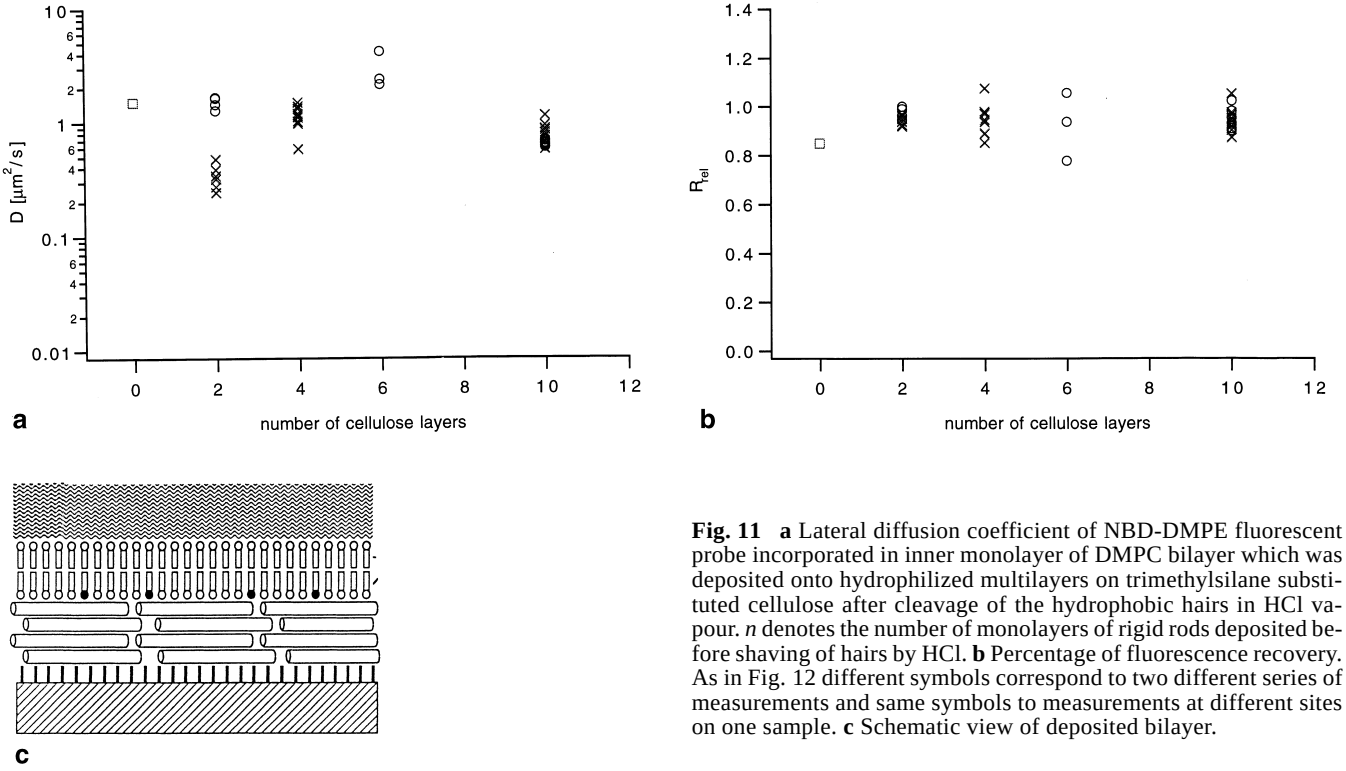


Fig. 11 **a** Lateral diffusion coefficient of NBD-DMPE fluorescent probe incorporated in inner monolayer of DMPC bilayer which was deposited onto hydrophilized multilayers on trimethylsilane substituted cellulose after cleavage of the hydrophobic hairs in HCl vapour. *n* denotes the number of monolayers of rigid rods deposited before shaving of hairs by HCl. **b** Percentage of fluorescence recovery. As in Fig. 12 different symbols correspond to two different series of measurements and same symbols to measurements at different sites on one sample. **c** Schematic view of deposited bilayer.

during LB-transfer of the monolayers and the gravimetrically observed transfer of mass.

3.3 Lipid bilayers and monolayers on hairy rod cushions

3.3.1 Shaving of hairy rod multilayers

For the transfer of lipid bilayers onto hydrophilic polymer layers we chose trimethylsilyl-cellulose (TMSC) since it was shown in separate monolayer experiments that for this compound the cleavage of the hydrophobic substituents by HCl proceeded smoothly and rapidly, thereby reconstituting the original cellulose (IPC).

First multilayers of TMSC were deposited onto glass substrates which were hydrophobized by deposition of an octadecyltrichlorosilane (OTS) monolayer. In order to avoid cleavage of some of the trimethylsilyl groups, a basic buffer (10 mM KH PO_4 adjusted to pH 8 by NaOH) was used as subphase. During the first two transfers only 80–97% of the substrate surface was covered while for the subsequent (3rd to 6th) layers the transferred area corresponded to 100% of the area of the substrate surface.

Cleavage of the trimethylsilyl groups was performed by exposing the TMSC-covered substrate to the atmosphere above concentrated HCl for about 10 min. The deposition of the lipid bilayers was achieved as described previously (Kühner et al. 1994). In brief: The first lipid monolayer was transferred by pulling the substrate out of the monolayer covered subphase and the second by dipping it again through the surface into the aqueous phase (this time it was kept in a horizontal position). Finally, the substrate was covered below water with a microscope slide exhibiting an indentation in the center in order to keep the supported bilayer stable for the subsequent lateral diffusion measurement.

Lipid monolayers were deposited onto hydrophobic multilayers of IPC. The latter were deposited onto glass previously made hydrophobic by exposition to a solution of octadecyltrichlorosilane as described above. The lipid monolayer was deposited by lowering the substrate through the monolayer covered trough while keeping the substrate in a horizontal position. The substrate was again covered below water with a microscope slide with an indentation in order to keep the monolayer in contact with water after transfer of the sample to the microscope stage for lateral diffusion measurement.

3.3.2 Lateral diffusion measurements

The lateral diffusion measurements were performed immediately after preparation of the fluorescent lipid probe. Figure 10 shows measurements of the lateral diffusion coefficient of the fluorescent lipid probe and the percentage of fluorescence recovery, R, in a DMPC-monolayer as a function of the number of layers of isopentyl cellulose. R is a measure for the continuity of the lipid monolayer on the length scale of the bleaching spot diameter (6.5 μm). For n >1 D is about 1 μm^2/sec which is expected for a fluid lipid layer in frictional contact with a polymer film (cf. Kühner et al. 1994 and Discussion Section). The much

lower value of D for $n = 0$ (DMPC in contact with OTS-monolayer) is in agreement with previous results. The nearly complete fluorescence recovery (R = 1) clearly shows that the monolayer is homogeneous.

Figure 11 shows the lateral diffusion measurements for the DMPC bilayer deposited on the multilayers of IPC after removal of the hydrophobic side chains (hairs). The fluorescent probe was incorporated into the monolayer facing the polymer. The diffusion coefficient agrees rather well with that of a bilayer separated from a substrate by an ultrathin water film and is, by about one order of magnitude, smaller than the lipid mobility in a free bilayer (cf. Merkel et al. 1989).

4. Discussion

The formation of insoluble monolayers of hairy rods at the water/air interface is rather surprising in view of the high degree of hydrophobicity of the molecules. Compared to normal amphiphiles both IPC and PCPS molecules do not exhibit hydrophilic surfaces. On the other hand, a polar asymmetry of the monolayers with respect to the long axes of the rods is suggested by the finding that the area transferred during the dipping-in and pulling-out steps is slightly different, showing that the repeat-unit of the supported multilayers is a bilayer rather than a monolayer. More direct evidence for this was provided by X-ray diffraction studies of multilayers of PCPS and of n-pentenyl-isopentyl cellulose by Schaub et al. (1995). In particular, these authors showed (for PCPS) the appearance of a Bragg peak for the multilayers, which implies a double layer of 44 Å thickness.

Finally, evidence for the polar structure of the monolayers is provided by our finding that the first monolayer transferred onto glass substrates is only stable in air if it is deposited in such a way that the side initially facing water is in contact with the glass surface whereas the films are unstable if the glass surface is rendered hydrophobic before the transfer.

An important practical message of the present work is that the continuity of newly transferred polymer monolayers and their stability below water depends critically on the polarity of the two surfaces facing each other. Their hydrophobicities have to be matched. The same holds for the lipid monolayers deposited onto the polymer cushions, that is in order to deposit bilayers the IPC layers have to be made hydrophilic.

Consider the finding that continuous monolayers of hairy rods can be deposited onto hydrophilic surfaces by pulling the substrate out of the subphase. Since the monolayer is facing the hydrophilic surface with its hydrated surface, a thin lubricating water film can form between substrate and monolayer which enables the rather defect free transfer of the lipid monolayer from the water to the substrate surface. The situation is similar to the transfer of lipid layers on solid surfaces (Merkel et al. 1989). However, owing to the weak adhesion to the substrate, the film floats away when the substrate is dipped into water again. In contrast, the transfer of the monolayer onto hydrophobic surfaces (OTS covered glass, Fig. 7) achieved by pulling the substrate out of the aqueous subphase yields strong adhesion but is associated with the dehydration of the film (if it is transferred again into air) resulting in a very incomplete transfer.

In thus follows that well defined continuous polymer multilayers can only be deposited onto hydrophobic surfaces (e. g. silanized glass substrates) by alternating transfer of the substrates into and out of the subphase. It is a consequence of the fact that the basic building unit of the hairy rod multilayers is the bilayer and not the monolayer. It should be noted, however, that annealing of the multilayer assemblies leads in many cases to a relaxation of the double layer periodicity, resulting in a hexagonal structure of densely packed rods (Wegner 1993; Schaub et al. 1995).

The present work showed that rod-like polymers of the type studied here are interesting models of elongated molecules exhibiting amphiphilicity with respect to the long axis. They are thus of interest as model systems to study fundamental physical properties of this class of amphiphiles. Moreover, they are expected to form two dimensional liquid crystals (Schwiegk et al. 1992).

Of great practical interest are the potential applications of hairy rods for the biofunctionalization of solids. The major goal of the present work was to show that they are very well suited as ultrathin polymer cushions for the preparation of supported membranes (e. g. on semiconductors). As shown by the lateral diffusion measurements the lipid molecules can freely move over macroscopic distances in monolayers on hydrophobic surfaces and bilayers on hydrophilic surfaces. Consequently, it is expected that fluid bilayers can build up a large internal pressure as in free bilayers, which is essential for the formation of self-healing, defect free membranes (cf. Sackmann 1996). This is a prerequisite for the deposition of membranes exhibiting very high electrical resistance which is an essential step in the design of biosensors based on electrical detection.

Self assembled multilayers of the type studied here offer many other advantages. As shown by the Mainz group (Mathauer et al. 1995) they can be partially cross-linked photochemically and enables the preparation of ultrathin polymer cushions exhibiting a mesh-like lateral structure. Such films are expected to allow the deposition of bilayers with membrane spanning proteins since the latter could penetrate with their head groups into the voids between the links of the meshwork. It is clear that these polymer films (e. g. the top layers) could also be biofunctionalized by the attachment of biotins or active esters (cf. Sackmann 1996 for a review) which would provide another way for the immobilization of proteins under non-denaturing conditions.

Acknowledgements First of all we would like to thank Gunter Elender and Martin Kühner for their help with the ellipsometric and lateral diffusion measurements. The work was supported by the BMBF and the Fonds der Chemischen Industrie. Helpful discussions with Robert Tampé and Lutz Schmitt are gratefully acknowledged. One of the authors (E.S.) is most grateful for the hospitality encountered during his sojourn at the Institute for Theoretical Physics under

the directorship of J. Langer. This research was supported in part by the National Science Foundation under Grant No. PHY 89-04035.

References

Brink G, Sigl H, Sackmann E (1995) Near-infrared surface plasmon resonance in silicon-based sensor: new opportunities in sensitive detection of biomolecules from aqueous solutions by applying microstep for discriminating specific and non-specific binding. Sensors Actuators B 24–25: 756–761

Brozoska JB, Azouz IB, Rondelez F (1994) Silanization of solid substrates: a step towards reproducibility. Langmuir 10: 4367–4373

Dietrich C, Tampé R (1995) Charge determination of fluorescence labeled amphiphilic molecules in polymer-supported lipid monolayers. BBA 1238: 183–191

Elender G, Sackmann E (1994) Wetting and dewetting of Si/SiO_2-wafers by free and lipid-monolayer covered aqueous solutions under controlled humidity. J Phys II France 4: 455–479

Frey W, Sackmann E (1992) Solitary Waves in Asymmetric Soap Films. Langmuir 8: 3150–3154

Guilbault GG (1984) Applications of piesoelectric quartz crystal microbalances; Chap 7; Methods and phenomena. Elsevier Publishing Comp, New York

Hickel W, Knoll W (1990) Surface plasmon optical characterisation of lipid monolayers at 5 μm lateral resolution. J Appl Phys 67: 3572–3575

Johannesmann F, Knoll W (1990) Photodesorption of Langmuir-Blodgett multilayer assemblies. Prog Colloid Poly Sci 83: 146–154

Kalacher A, Sauer T, Vogel V, Plate NA, Wegner G (1990) Thin Solid Films 188: 341–345

Kößlinger C, Drost S, Aberl F, Wolf H, Koch S, Woias P (1992) Biosensors and Bioelectronics 7: 397–404

Kühner M, Tampé R, Sackmann E (1994) Lipid monolayer and bilayer supported on polymer films: composite polymer-lipid films on solid substrates. Biophys J 67: 217–226

Mathauer K, Schmidt A, Knoll W, Wegner G (1995) Synthesis and Langmuir-Blodgett multilayer-forming properties of photocross-linkable polyglutamate derivatives. Macromolecules 28: 1214–1220

Merkel R, Sackmann E, Evans EA (1989) Molecular friction and epitatic coupling between monolayers in supported bilayers. J Phys Fr 50: 1535–1555

Rädler J, Sackmann E (1993) Imaging optical thicknesses and separation distances of phospholipid vesicles at solid surfaces. J Phys II Fr 3: 727–748

Rothenhäusler B, Knoll W (1987) On the influence of the propagation length of plasmon surface polaritons in the visible energy range for the optical characterization of heterogeneous thin films. Surface Science 191: 585–594

Sackmann E (1996) Supported membranes: scientific and practical applications. Science 271: 43–48

Schaub M, Fakirov G, Schmidt A, Lieser G, Wenz G, Wegner G, Albouy PA, Wu H, Foster MD, Majrkzak C, Satija S (1995) Ultrathin layers and supramolecular architecture of isopentylcellulose. Macromolecules 28: 1221–1228

Schwiegk S, Vahlenkamp Th, Yuanze Yu, Wegner G (1992) Origin of orientation phenomena observed in layered langmuir-blodgett structures of hairy rod polymers. Macromolecules 25: 2513–2525

Seufert M, Fakisor Ch, Wegner G (1995) Ultrathin membranes of molecularly reinforced liquids on porous substrates. Adv Materials 7: 52–54

Tamm LK, McConnell HM (1985) Supported phospholipid bilayers. Biophys J 47: 105–113

Wegner G (1993) Control of molecular and supramolecular architecture of polymers, polymer-systems and nanocomposites. Mol Cryst Lig Cryst 235: 1–34