Biologically Addressable Monolayer Structures Formed by Templates of Sulfur-Bearing Molecules

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ABSTRACT We demonstrate that the combined application of Langmuir-Blodgett and self-assembly techniques allows the fabrication of patterns with contrasting surface properties on gold substrates. The process is monitored using fluorescence microscopy and surface plasmon spectroscopy and microscopy. These structures are suitable for the investigation of biochemical processes at surfaces and in ultrathin films. Two examples of such processes are shown. In the first example, the structures are addressed through the binding of a monoclonal antibody to a peptide. This demonstrates the formation of self-assembled monolayers by cysteine-bearing peptides on gold, and the directed binding of proteins to the structured layers. A high contrast between specific and unspecific binding of proteins is observed by the patterned presentation of antigens. Such films possess considerable potential for the design of multichannel sensor devices. In the second example, a structured phospholipid layer is produced by controlled self-assembly from vesicle solution. The structures created—areas of phospholipid bilayer, surrounded by a matrix of phospholipid monolayer—allow formation of a supported bilayer which is robust and strongly bound to the gold support, with small areas of free-standing bilayer which very closely resemble a phospholipid cell membrane.

INTRODUCTION

The controlled construction of supramolecular organic structures has, in recent years, become a topic of intense research (Ulman, 1991; Whitesides et al., 1991). These structures provide an increased understanding of low-dimensional systems and of organic surfaces at a molecular level and have potential applications in such fields as biosensors and molecular electronics. An especially fruitful approach has been to focus on the basic processes of self-organization and molecular recognition which take place in and at biological cell membranes (Ahlers et al., 1990). Self-assembly (SA) and Langmuir-Blodgett (LB) techniques allow preparation of ultrathin films which serve as models for such biological lipid membranes on solid supports (Müller et al., 1993; Stelzle et al., 1993). Although these layers are very well characterized, their potential for the reconstitution of functional peptides and proteins is far from being fully exploited (Thompson et al., 1993; Watts et al., 1986).

This paper deals with the formation of two-dimensional micro-scale structures on solid surfaces. The preparation of these structures makes use of the combined application of the LB technique and the formation of self-assembled monolayers (SAMs) of sulfur-bearing molecules (Duschl et al., 1994). This combination allows the generation of well-defined surface patterns with different functionalities. Our motivation for creating these structures is to study recognition processes that occur at organic surfaces and how these are influenced by the manner of presentation of the recognition partners on the surface. Here we present two applications of these structures for such studies. The two-dimensional structuring of the surface has a different function in each of the examples. In the first application, the structure is selectively functionalized with an antigen to give patterned binding of an antibody. This patterned binding allows us to compare the reactions which occur on different surfaces under identical conditions, which not only provides an internal control on the processes studied, but also allows simultaneous investigation of several processes. In the second example, we form structured planar lipid bilayers which should be especially suitable for the incorporation of integral membrane proteins. Here the structuring allows us to fulfill a set of conditions which are almost contradictory for a simple monolayer. Both examples have potential for application as biosensors: the first example as a multichannel biosensor, and the second for biosensors based on membrane proteins.

The generation of a laterally structured thiol layer is shown schematically in Fig. 1. A mixture of palmitic acid and of a sulfur-bearing lipid (Duschl et al., 1994) is spread on the water/air interface of a Langmuir trough to form a monolayer. This monolayer phase-separates on compression to give regularly distributed domains containing predominantly fatty acid. The size of the domains depends only on the self-organizing properties of the mixture and its manipulation on the water surface. The film is then transferred to a gold substrate where the thiolipids bind covalently to the support. The fatty acid is only physisorbed to the gold surface and can be washed away, using a suitable solvent, to leave domains of bare gold. In these regions a second covalently bound monolayer, with surface properties which contrast with those of the thiolipid, can be formed through a subsequent self-assembly step. The entire process can be followed

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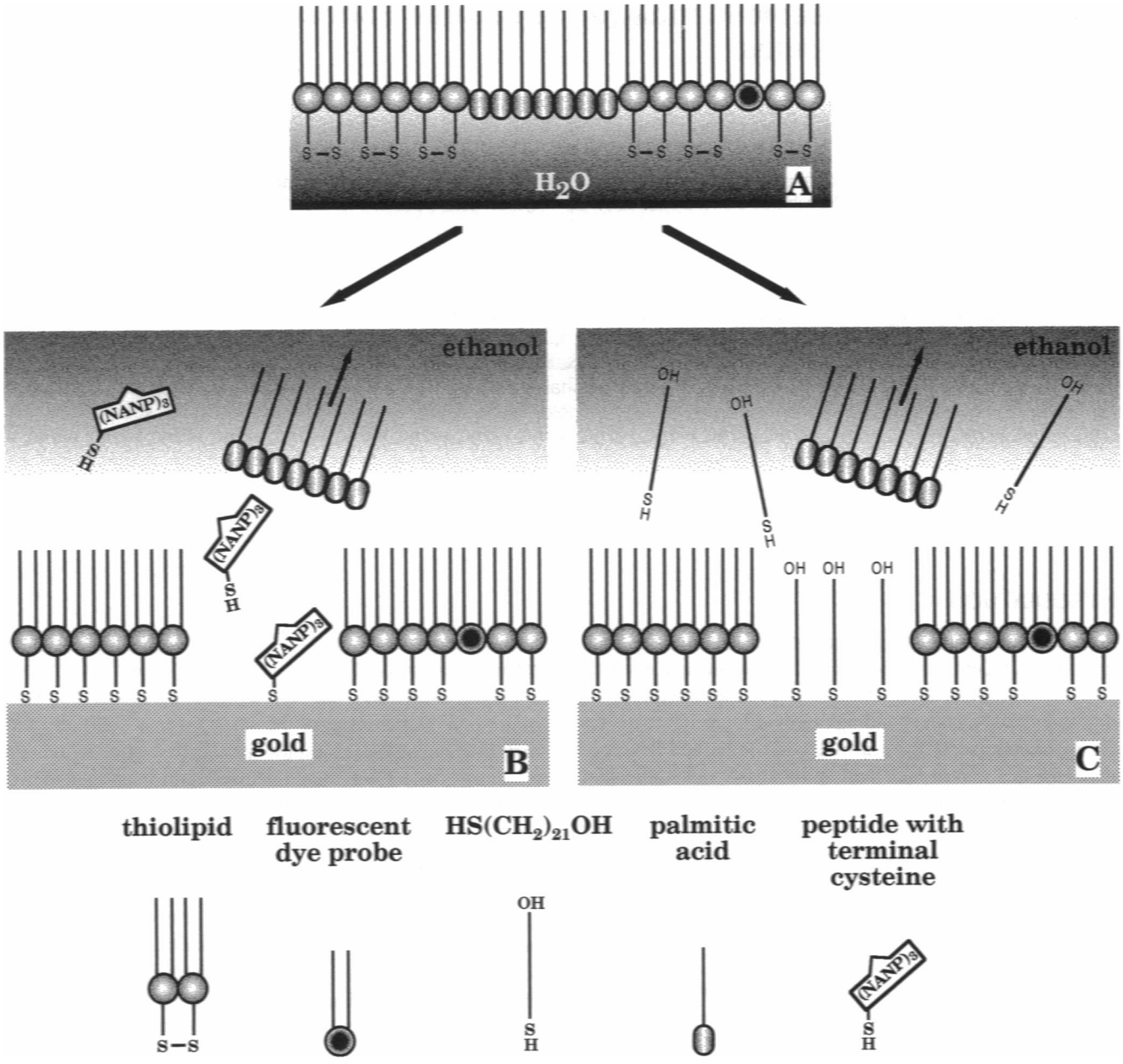


FIGURE 1 (*A*) Langmuir film consisting of a mixture of palmitic acid and thiolipid, at the water/air interface. In *B* and *C* the Langmuir monolayer has been transferred to a gold surface using the LB technique. After LB transfer the gold surface is washed in an appropriate solvent. The physisorbed palmitic acid domains are removed from the surface, while the covalently bound thiolipid is not removed. Organic thiol molecules present in the solvent chemisorb to the newly exposed gold areas to form self-assembled monolayers. *B* shows the formation of a patterned layer consisting of a thiolipid monolayer and a mixed peptide-thioglucose monolayer (thioglucose not drawn for reasons of clarity). In the other example (*C*), hydroxythiols form a monolayer within the washed domains to form a substrate with patterned wetting properties. (Not to scale; molecular details not accurate)

using two experimental techniques: fluorescence microscopy at the air/water interface (Möhwald, 1988) and surface plasmon microscopy at the gold surface (Rothenhäusler and Knoll, 1988).

For the creation of a structured antigen-bearing surface, the peptide CY(NANP)$_3$ which carries an N-terminal cysteine is used in the self-assembly step. Cysteine contains a thiol group, which allows the peptide to self-assemble to form a monolayer on the gold surface. A monoclonal antibody, raised against the NANP amino acid sequence in the peptide, was used in experiments, and showed preferential binding to the peptide-covered parts of the surface.

The NANP sequence was chosen because of its relevance for the immune response against malaria parasites. The major, species and stage-specific antigen of the human malaria parasite, *Plasmodium falciparum*, is the circumsporozite (CS) protein. It uniformly surrounds the external coat of the parasite (Godson, 1985) and consists of a major tandem repetitive amino acid sequence, Asn-Ala-Asn-Pro (NANP)

which is flanked by nonrepetitive sequences (Nussenzweig and Nussenzweig, 1989). The (NANP) repeats appear to be highly conserved in all the geographical isolates of *P. falciparum*. Furthermore, these sequence repeats seem to present the main target of the antibody response induced by sporozoites after natural or experimental infections. Therefore, by covering the gold substrate with the $CY(NANP)_3$ peptide, we intended to mimic the surface of the human malaria parasite in order to develop a device for sensing the presence of anti-(NANP) antibodies.

In the second example, we investigate a new concept for the incorporation of integral membrane proteins or peptides into planar lipid bilayers. For successful incorporation of the proteins, a compromise has to be found between a native environment for these molecules (fluid membrane, decoupling from the rigid substrate, accommodation of extramembranous parts) to keep them in an active form, and an environment which holds the molecules in a configuration suitable for the application of analytical techniques. These constraints are particularly stringent for the very powerful modern techniques such as scanning force (Radmacher et al., 1992), optical near-field (Betzig and Trautman, 1992) and scanning tunneling microscopy (Frommer, 1992). In an approach to this problem, we have created structured supported lipid bilayers. In our experiments, the domains of bare gold were filled with a monolayer of a hydroxyl thiol ($HS(CH_2)_{21}OH$). On immersion of the substrate in a vesicle solution of the lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), a phospholipid monolayer is spontaneously formed on the hydrophobic thiolipid areas (Lang et al., 1992, 1994; Spinke et al., 1992), while a bilayer forms on the hydrophilic regions (Fig. 1 *C* and Fig. 8 *B*). These areas of lipid bilayer are the key to this approach. By reducing the length of the hydrophilic thiol, we intend to create areas of free-standing supported bilayer in the thiolipid matrix (see Fig. 8). These free-standing bilayers should be an excellent environment for membrane proteins or peptides, while the thiolipid areas attach the entire layer to the gold surface in an extremely stable manner.

MATERIALS AND METHODS

The synthesis and characterization of the amphiphilic thiolipid bis[8-(1,2-dipalmitoyl-*sn*-glycero-3-phosphoryl)-3,6-dioxaoctyl] disulfide are described in (Lang et al., 1994). Palmitic acid (puriss.) and 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC) (puriss.) were purchased from Fluka (Buchs, Switzerland). *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dimyristoyl-*sn*-glycero-3-phosphoethanol-amine (NBD-PE) was purchased from Molecular Probes (Eugene, OR) and 1-thio-β-D-glucose from Sigma (Buchs, Switzerland). 21-Mercaptoheneicosanol $HS(CH_2)_{21}OH$ was the generous gift of A. Ulman. The peptide $CY(NANP)_3$ synthesized according to Atherton et al. (Atherton et al., 1979) using the F-moc, T-Bu strategy. The peptide was then purified by gel filtration and purity was determined by analytical HPLC (>90% pure) and amino acid composition. The monoclonal antibody H_3 was a generous gift from H. Matile (Hoffmann-La Roche, Basel, Switzerland). It was raised in mice against the synthetic peptide $(NANP)_{40}$ as described elsewhere (Giudice et al., 1991). All solvents used for preparation were of UV quality. The proteins were kept in phosphate buffer at pH 7. Hydroxythiols were dissolved in ethanol at a concentration of 0.5 mg/ml. A molar ratio of 1:2 of peptide and thioglucose (2 mM and 4 mM, respectively) in a 1:1 mixture of water (Millipore, Switzerland) and ethanol was used for the preparation of the peptide-based self-assembly layers.

Substrates for film transfer were prepared by evaporating gold films onto optical glass slides. The slides (Schott glass SF10, Mainz, Germany) were cleaned thoroughly and placed in a vacuum chamber (pressure $<2 \times 10^{-6}$ mbar) where a 500-Å thick gold film was evaporated. The substrates were allowed to cool under vacuum for about 30 min, after which the vacuum chamber was vented with argon. If the time between evaporation and venting was longer than 30 min, the slides were treated with an argon discharge plasma for 1 min before they were removed from the vacuum chamber.

The mixed monolayers were prepared on a commercial Langmuir trough (RK II, Riegler & Kirstein, Mainz, Germany (Riegler, 1988)), mounted on the three-directional stage of a fluorescence microscope (Zeiss Axiotron, Zürich, Switzerland). The lipids were dissolved in chloroform together with 1 mol% NBD-PE and spread on doubly deionized water (Millipore, Volketswil, Switzerland, ρ = 18 MΩcm). The lateral distribution of different phases (liquid, solid, etc.) of the monolayer at the air/water interface was studied using fluorescence microscopy. The different solubilities of the fluorescent probe (NBD-PE) in the different phases allows optimization of the size and the distribution of the patterns in the monolayers.

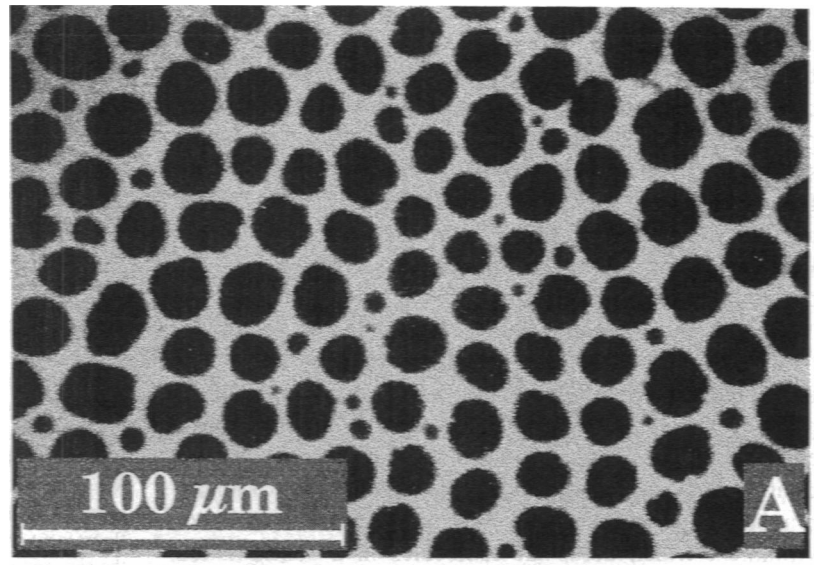
The LB transfer of the mixed monolayers to the gold substrates was executed at a speed of ~5 μm/s during the up-stroke. No difference in the film properties was found between slides which were dipped into the subphase before the molecules were spread on the water surface and those which were dipped into the subphase after spreading. After the film transfer, the samples were rinsed in ethanol and hexane to remove the palmitic acid. The samples were dried in an argon stream and were then immediately incubated for at least 12 h in the solution containing the respective sulfur-bearing molecules for the formation of self-assembly layers.

Transferred films were characterized using surface plasmon spectroscopy and surface plasmon microscopy (Knoll, 1991; Raether, 1977). These techniques allow both the determination of the optical thickness of the films and the visualization of small lateral thickness or refractive index variations. We used the Kretschmann coupling scheme (Kretschmann, 1972) with either an equilateral or a 90° high index prism (Schott glass SF10, n = 1.723). A glass slide with a 50-nm-thick gold film was optically coupled to the base of the prism. A laser beam (He-Ne, λ = 632.8 nm) was internally reflected from the base of the prism, and the reflected light intensity measured as a function of angle of incidence. A sharp resonance is observed when coupling of normal light to surface plasmons takes place. Deposition of a thin organic film causes a shift of the angular position of the resonance minimum. This shift allows determination of the optical thickness (= geometrical thickness (d) * refractive index (n)) of thin dielectric coatings on the gold surface. For the calculation of d, we assume a refractive index, n, of 1.45 (Pethig, 1979). This value corresponds to the molar refractive indices for many proteins. For measurements in aqueous solution, the choice of refractive index has a large effect on the geometrical thickness, d, obtained. Thus, the thicknesses quoted in the text are only estimates of the geometrical thickness of the films. However, they are a good and convenient measure of the mass adsorbed to the surface. All thicknesses quoted in this paper were measured at least three times. The deviations of the average values were not more than 3 Å.

Introduction of a simple lens (focal length 25 mm) and a CCD video camera (Hamamatsu C3077, Herrsching, Germany) into the reflected beam, allows microscopy of the film (Rothenhäusler and Knoll, 1988), with the surface plasmon resonance serving as a contrast mechanism.

Thiolipid

We recently introduced a new class of synthetic lipids which are amphiphilic and which can covalently bind to the surface of a zero-valent metal (gold, silver, copper) (Lang et al., 1994). They consist of two lipid molecules, each attached to a hydrophilic spacer of variable length, and joined by a disulfide bond at the end of the two spacers. Such molecules allow the formation of stable, covalently bound monolayers using either the SA or LB technique. The resultant monolayers can be converted by a further lipid monolayer



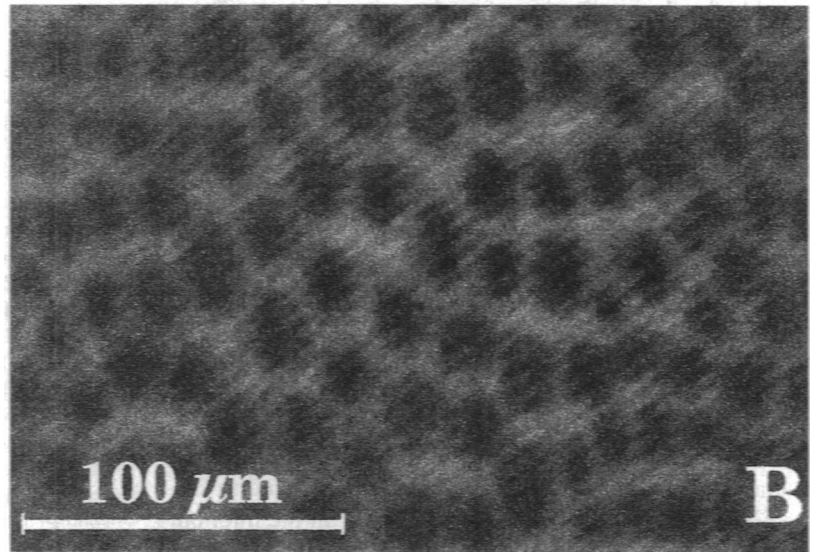


FIGURE 2 (*A*) Fluorescence micrograph of a mixed monolayer of thiolipid and palmitic acid with 1 mol % NBD-PE. The size and the shape of the domains are identical to those of a pure palmitic acid monolayer, indicating de-mixing of the components. (*B*) Surface plasmon micrograph of a corresponding film on a gold substrate which was washed with organic solvents after Langmuir transfer. The angle of incidence (32.1°) was tuned to excite surface plasmon waves on the bare gold surface. The domains, therefore, appear dark.

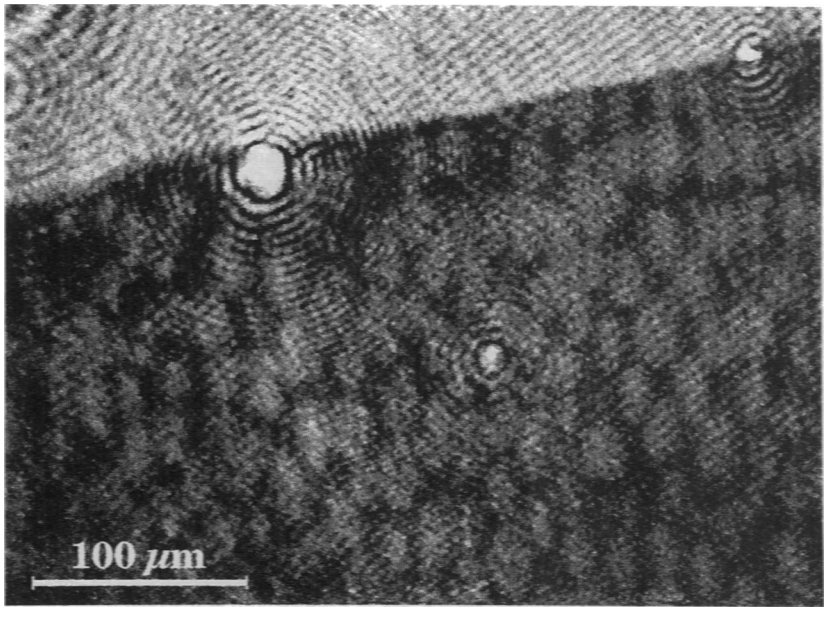


FIGURE 3 Surface plasmon micrograph of a monolayer template containing thiolipid (dark regions) and a mixture of the $CY(NANP)_3$-peptide and thioglucose (bright domains). The line crossing the image indicates the upper limit of the LB transfer. Above this line, a homogeneous layer of peptide and thioglucose has been formed.

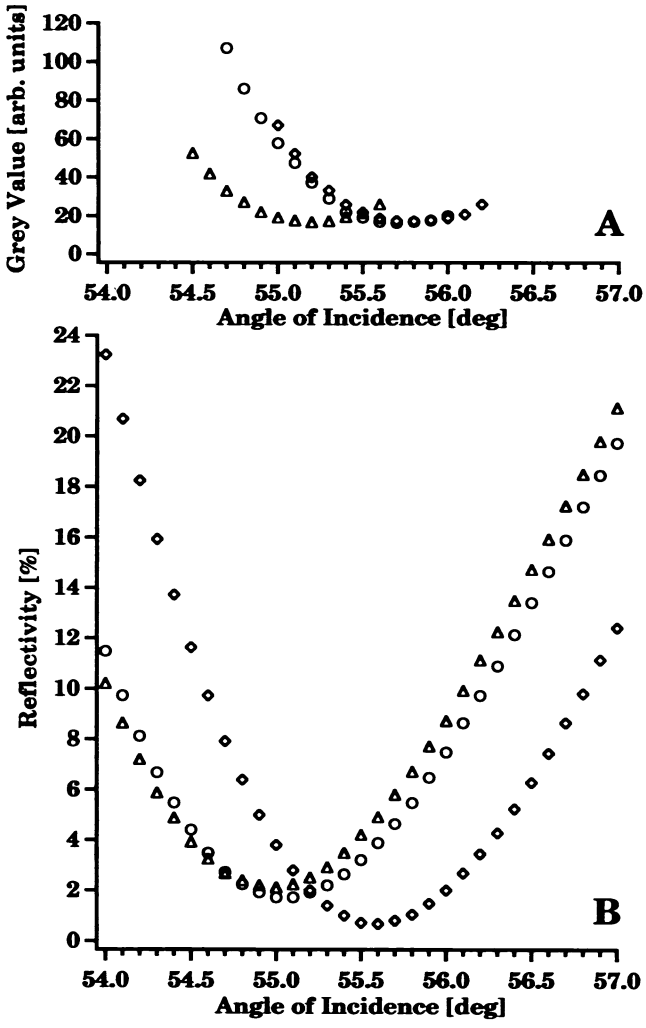


FIGURE 4 Reflected-intensity versus angle-of-incidence scans of surface plasmon resonance curves at various stages of the protein adsorption experiments. The scans in *A* were taken from the thiolipid-covered regions through a quantitative analysis of the gray values of a series of surface plasmon micrographs. The scans in *B* were taken in a normal reflectivity scan mode from the region homogeneously covered by the $CY(NANP)_3$-peptide (Fig. 3). (△) Curves taken from the covalently bound monolayers (thiolipid or $CY(NANP)_3$-peptide/thioglucose respectively). (○) Curves taken after incubation of the sample in a BSA solution. (◇) Curves taken after incubating the substrate in the solution containing the antibody H_3. The differences in the response can be clearly seen: in *A* a large shift occurs, due to BSA adsorption on the thiolipid film, while only a minor shift takes place on the peptide regions (*B*). The opposite behavior is observed in the antibody solution. Specific binding of the antibody takes place on the peptide-covered regions (indicated by a large shift of the corresponding resonance curve) while virtually no shift is seen for the BSA-covered areas. The results obtained from the peptide-covered domains correspond qualitatively to those obtained from the homogeneous peptide monolayer (see text).

deposition to form a supported lipid bilayer (Lang et al., 1992, 1994; Spinke et al., 1992). These bilayers are good candidates for the successful reconstitution of transmembrane proteins and offer advantages over conventional supported bilayers. First, the hydrophilic spacer of the thiolipids decouples the monolayer from the rigid substrate; in addition, the spacer is hydrated, providing a hydrophilic environment on both sides of the membrane. These spacers, when sufficiently long, should allow the accommodation of extramembranous parts of transmembrane proteins.

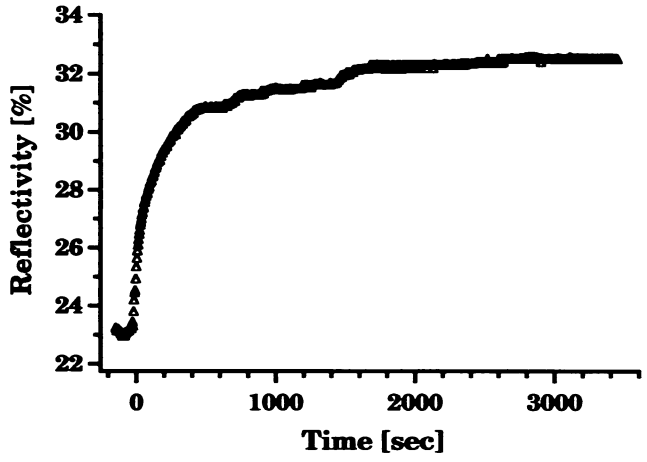


FIGURE 5 Reflected intensity versus time showing the time course of the adsorption of the monoclonal antibody H_3 on a mixed monolayer of $CY(NANP)_3$-peptide and thioglucose on gold (measured on the upper part of the sample shown in Fig. 3). The reflected intensity was measured slightly off the resonance angle. On adsorption to the surface, the resonance curve is shifted to higher angles, causing an increase in the reflected intensity (Spinke et al., 1993).

RESULTS

Patterned antibody binding

Fig. 2 shows the formation of a patterned surface for the differential binding of proteins. The process has been described in more detail elsewhere (Duschl et al., 1994). Fig. 2 *A* is a fluorescence micrograph of a monolayer of 4:1 palmitic acid/thiolipid on the water surface. The dark areas are domains of solid palmitic acid, while the bright areas are regions of thiolipid in fluid-analogous phase. Fig. 2 *B* is a surface plasmon micrograph of the film after LB transfer to the gold surface, washing to remove the palmitic acid, and SA in the bare gold patches. The two patterns clearly correspond: the bright areas in the second image are the covalently bound thiolipid, while the dark areas are the thinner film formed by the subsequent SA. In this case a mixture of thioglucose and the peptide $CY(NANP)_3$ were used in the self-assembly step. $CY(NANP)_3$ is a synthetic peptide, which binds to gold through a terminal cysteine. Thioglucose was chosen because it binds covalently through the thiol group, leaving the sugar moiety exposed. In nature, sugar-bearing surfaces are highly resistant to nonspecific binding of proteins, and it was hoped that, by mimicking this approach, nonspecific binding of proteins to the $CY(NANP)_3$ regions would be reduced (Prime and Whitesides, 1991).

By taking a series of surface plasmon micrographs (Fig. 3 and Fig. 6) and analyzing the gray values (i.e., the intensities) of areas of the micrographs, it was possible to obtain the local resonance angles of the structured surface. This allowed the relative thicknesses of the thiolipid film and the peptide/thioglucose film to be determined, demonstrating that the $CY(NANP)_3$/thioglucose layer is 13 Å thinner than the thiolipid layer. Independent measurements of the thiolipid layer show that it is 22.5 Å thick, which gives an absolute thickness

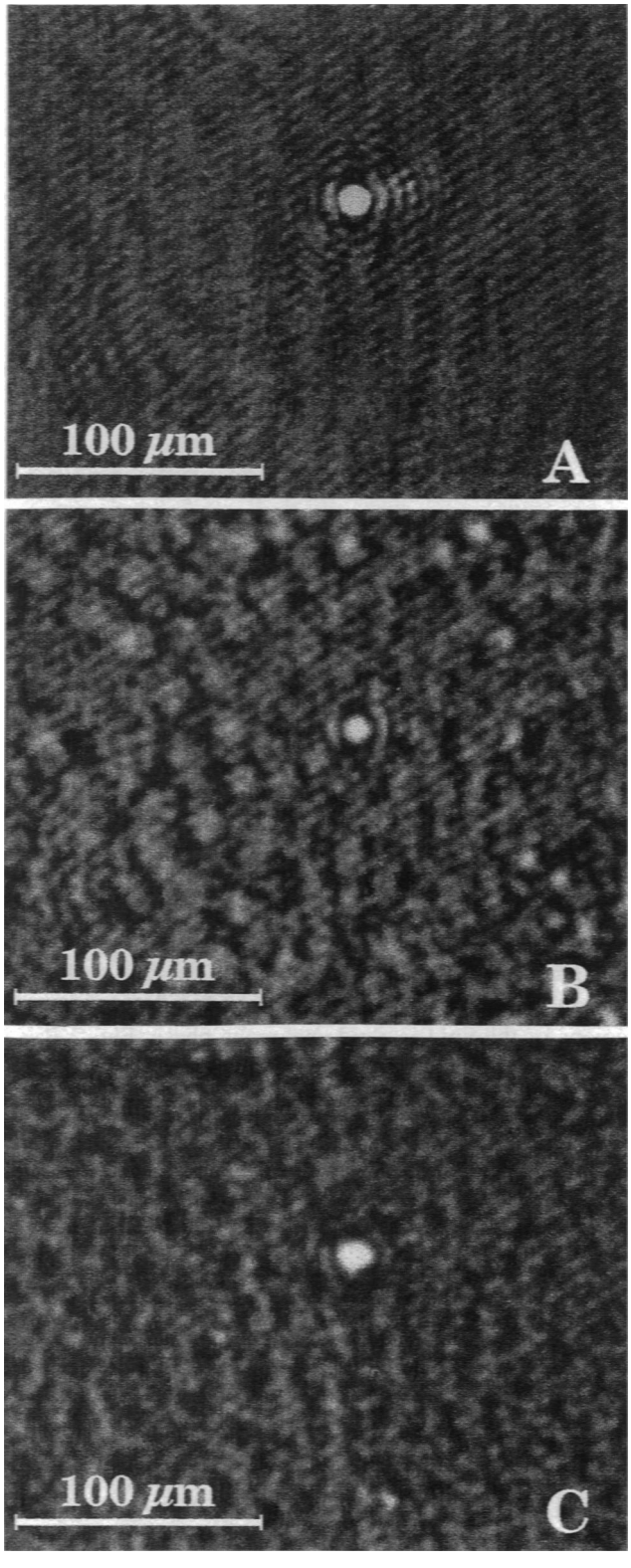


FIGURE 6 Surface plasmon micrographs of a laterally structured layer of thiolipid and a long-chain hydroxythiol at a water-gold interface before (*A*) and after (*B* and *C*) immersion in a POPC vesicle solution. All micrographs show exactly the same area of the layer (note bright feature which is probably caused by a dust particle). No contrast can be seen in *A* due to the match in thickness of the thiolipid and the hydroxyl thiol layer. In *B* and *C*, a contrast is observed due to the different spreading behavior of the POPC vesicles on the hydrophobic and hydrophilic surfaces. Micrograph *B* was taken at an angle of 55.2° and *C* was taken at 56.2°.

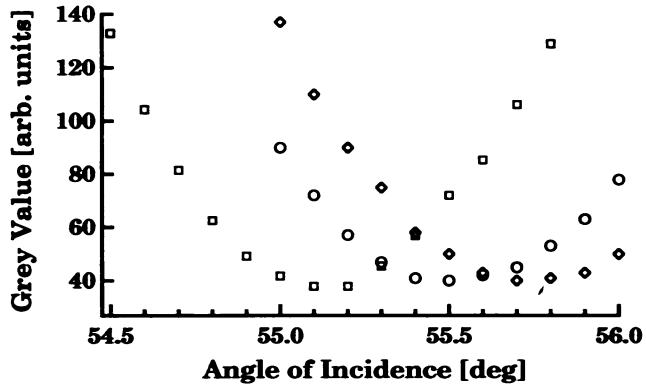


FIGURE 7 Results of the gray value analysis of surface plasmon micrographs taken under the same conditions as in Fig. 6, *B* and *C*. The shift of the resonance curve of the thiolipid-covered areas gives a thickness increase of 22 Å (n = 1.45) after incubation in the vesicle solution indicating formation of a POPC monolayer (from □ to ○). On the hydroxyl group-exposing areas the thickness measurement gives 42 Å for the POPC layer, which corresponds to the formation of a bilayer (from □ to ◇). The curve □ is the resonance initially measured on the laterally structured thiolipid/hydroxythiol film (Fig. 6 *A*).

of 9.5 Å for the peptide/thioglucose layer. This procedure was repeated after every protein incubation to determine the respective thickness changes due to adsorption of proteins. In addition, comparison was made with an unstructured peptide/thioglucose layer formed by self-assembly on an area of the same gold surface that was not covered with a monolayer during LB transfer.

The structured surface was incubated in a 1.5×10^{-6} M solution of bovine serum albumin (BSA). In Fig. 4, the shifts of the resonance for the thiolipid and peptide-covered areas are shown. A considerable shift (0.5°) was measured for the thiolipid covered regions, indicating adsorption of a 32-Å thick film of BSA (see Fig. 4 *A*). However, on the peptide-covered areas much less adsorption occurred; for the plain peptide film a thickness change of only 3 Å was measured (see Fig. 4 *B*), whereas for the peptide film in the structured regions a thickness increase of 19.5 Å was observed. This is in accord with the known properties of BSA, a protein which exhibits high nonspecific binding, and is often used to saturate sites of nonspecific protein binding. It is known that hydrophobic surfaces cause a high degree of nonspecific protein binding (Andrade and Hlady, 1986), and it is on the highly hydrophobic thiolipid-covered regions that most binding takes place. In contrast, very little nonspecific binding is observed for the homogeneous peptide/thioglucose film. The higher binding in the domains of peptide/thioglucose almost certainly indicates a less homogeneous film with many defects where nonspecific binding takes place.

Even more pronounced was the contrast between the BSA-covered thiolipid film and the peptide film when the sample was subsequently incubated in a 1.3×10^{-6} M aqueous solution of a monoclonal antibody H_3 against the $(NANP)_3$ region of the chemisorbed peptide. The on-line time-dependent binding process on the homogeneous peptide layer is shown in Fig. 5. The reflectivity of the sample is

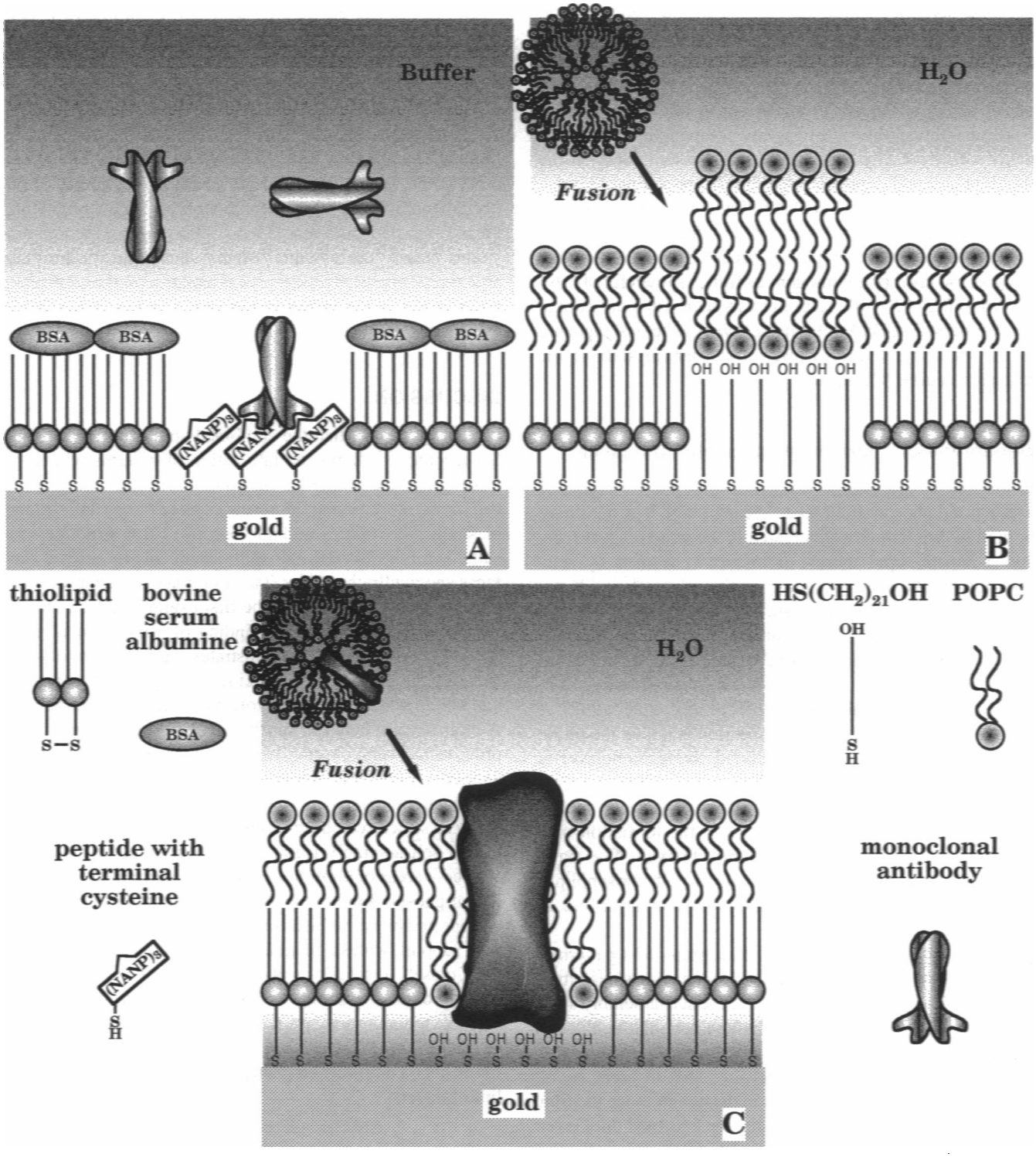


FIGURE 8 Schematic representation of our interpretation of the final states of the two described experiments (*A* and *B*). (*A*) Layer system containing the immobilized peptide after successive immersion in a BSA solution and an aqueous solution of the monoclonal antibody H_3. The BSA binds preferentially (10:1) to the hydrophobic thiolipid parts of the monolayer while the antibody addresses only the peptide-covered surface parts. (*B*) Patterned surface with contrasting wetting properties formed in the second example. Incubation of such a substrate in an aqueous POPC vesicle dispersion leads to the formation of a POPC monolayer on the hydrophobic regions and a POPC bilayer in the hydrophilic domains. Edge effects between the POPC mono- and bilayers have not been taken into account in the schematic representation. (*C*) Principle of reconstitution of membrane-spanning peptides and proteins in supported lipid bilayers is depicted on the basis of our results. (Not to scale; not including any molecular details)

approximately proportional to the quantity of antibody bound (Spinke et al., 1993). A detailed measurement shows thickness increases of 20 Å and of 16.5 Å for the patterned and the homogeneous peptide layers, respectively. A thickness change of only 3 Å was observed for the thiolipid-covered areas.

The different binding properties of the patterned organic film can be nicely demonstrated by comparison of the resonance curves in Fig. 4. *A* shows the measurements on the thiolipid-covered region: a large shift of the resonance curve due to BSA binding (*circles*) is followed by a much smaller shift when the sample was incubated with the antibody (*diamonds*). Exactly the opposite occurred on the peptide/thioglucose layer (the homogenous film is shown for clarity) shown in Fig. 4 *B:* little response was seen when the sample was immersed in BSA solution (*circles*), whereas exposure to the antibody solution caused a substantial shift of the resonance curve (*diamonds*).

Generation of a structured phospholipid layer

In the following example we demonstrate the formation of a structured phospholipid layer: areas of high hydrophilicity or high hydrophobicity are created in the structured surface, and a phospholipid mono- or bilayer is formed by self-assembly on the different areas. Several groups have shown that if a highly hydrophobic surface consisting of long hydrocarbon chains is brought into contact with an aqueous solution containing phospholipid vesicles, the vesicles spontaneously fuse to the surface and form a lipid layer (Kalb et al., 1992; Lang et al., 1992, 1994; Spinke et al., 1992). If the temperature of the vesicle dispersion is above the phase transition temperature, a well-defined single monolayer is formed. In contrast, we show that if a surface presents densely arranged hydroxyl groups the exposure of such a surface to a vesicle dispersion leads to the formation of a well-defined supported phospholipid bilayer. We have exploited these effects to form a structured phospholipid layer containing well-defined regions of monolayer and of bilayer.

In Fig. 6 *A* a surface plasmon micrograph of a patterned thiolate film on gold is shown. It was prepared as described in the previous section. However, for the self-assembly step, a hydroxyl thiol was used instead of the peptide/thioglucose mixture. This results in a film with a strong contrast in hydrophilicity/hydrophobicity between the different areas. However, no contrast can be seen in the image, since the film thicknesses of the thiolipid layer and the hydroxyl thiol layer are almost identical. This film served as a template for the formation of the phospholipid layer.

Fig. 6, *B* and *C*, consists of two surface plasmon micrographs of exactly the same area of the sample as shown in Fig. 6 *A* (note the white spot which is probably caused by a dust particle), but after immersing the substrate in an aqueous vesicle solution. In *B*, the angle of incidence has been tuned so that surface plasmon modes are excited in the regions surrounding the domains (θ = 55.2°; no light is reflected from these regions, therefore the domains appear bright), whereas in *C* the angle of incidence is slightly greater (θ = 56.2°) in order to excite the surface plasmon modes within the domains (no light is reflected from the domains and they appear dark). From a detailed analysis of the gray values of a sequence of micrographs the thickness changes on immersing the substrate in the vesicles have been determined (Fig. 7). The domains became 42 Å thicker, while the surrounding regions became 22 Å thicker. These values correspond very well to the formation of bilayers within the domains (expected thickness 46 Å) and of a monolayer for the remaining areas (expected thickness 23 Å). This experiment demonstrates clearly the potential of this technique for forming structured supported lipid layers as a matrix for the controlled reconstitution of membrane spanning peptides and proteins as schematically shown in Fig. 8.

DISCUSSION

It has been shown that the combined application of LB and SA techniques allows the fabrication of patterns with contrasting surface properties on gold substrates. The process can be monitored using fluorescence microscopy and surface plasmon spectroscopy and microscopy. These structures are suitable for the investigation of biochemical processes at surfaces and in ultrathin films. Two examples of such processes have been demonstrated. In the first example, the structures were addressed through the binding of a monoclonal antibody to a peptide. This demonstrates the formation of self-assembled monolayers by cysteine-bearing peptides on gold, and the directed binding of proteins to the structured layers. A high contrast between specific and nonspecific binding of proteins has been observed by the patterned presentation of antigens. Such films possess considerable potential for the design of multichannel sensor devices. In the second example, a structured phospholipid layer was produced by controlled self-assembly from vesicle solution. The structures created—areas of phospholipid bilayer, surrounded by a matrix of phospholipid monolayer—should allow formation of a supported bilayer which is robust and strongly bound to the gold support, with small areas of free-standing bilayer which very closely resemble a phospholipid cell membrane. By using protein-containing vesicles as a shuttle it should be possible to incorporate proteins in the free standing domains of the bilayer. We believe that this is an important concept for the reconstitution of transmembrane proteins in supported lipid bilayers.

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