Patterning Solid-Supported Lipid Bilayer Membranes by Lithographic Polymerization of a Diacetylene Lipid**

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Solid-supported lipid bilayers are membrane systems, in which a single lipid bilayer is adsorbed on the solid surface either by physical interactions or by chemical bonds. These systems have been studied extensively as a simple model of the biological membrane.[1] The bilayer membrane can also incorporate proteins and provides possibilities to prepare biosensors based on electrical and optical detection.[2] In order to mimick the sophisticated functions that real biological membranes perform, one must inevitably increase the complexity of the artificial systems. For this purpose, patterning of the membranes is a feasible approach because various components can be integrated into the membrane system with defined spatial control. The patterning of lipid bilayers has been attempted in the past either by the modification of the substrate[3] or by the microcontact printing method.[4]

Herein, we report a novel approach for creating patterned bilayers on solid supports. The basic idea is to imprint a pattern within the lipid bilayer by photochemical polymerization of the lipids. Two-dimensional polymerization of lipids has been studied extensively in liposomes (lipid vesicles), principally with the aim of stabilizing liposomes for drug delivery purposes.[5] On the other hand, there have been several lines of research that exploited polymerizable thiol monolayers or fatty acid multilayers as potential photoresist materials for photolithography.[6] One can, therefore, expect two effects from the lithographic polymerization of bilayers, namely mechanical stabilization of fluid bilayers and confinement of them in a designed region.

The general procedure is schematically illustrated in Figure 1. Firstly, a bilayer of polymerizable lipid is deposited onto a solid support (Figure 1 A). The bilayer is subsequently polymerized by UV light, whereby a pattern can be imposed by using a mask (Figure 1 B). The bilayer has to be kept in an aqueous solution up to this step so that the integrity of the membrane is preserved. Once the bilayer is polymerized, it becomes insoluble in organic solvents or detergent solutions. By treatment with organic solvents, monomeric lipids may be selectively removed, while the polymerized part remains on the substrate (Figure 1 C). The lipid-free areas (holes) thus created can be filled with biologically relevant lipid bilayers by the vesicle fusion technique (Figure 1 D). In the case of hydrophilic surfaces, such as oxidized silicon, a lipid bilayer adsorbs spontaneously from small unilamellar lipid vesicles by the fusion and reorganization processes.[7] The lipid bilayer is trapped in the vicinity of the surface by colloidal interactions with an estimated separation of about 10 Å[8] and the thin water layer acts as a lubricant for the membrane. Therefore, the newly incorporated bilayers are laterally fluid and should have much of the properties that native cell membranes possess.[1]

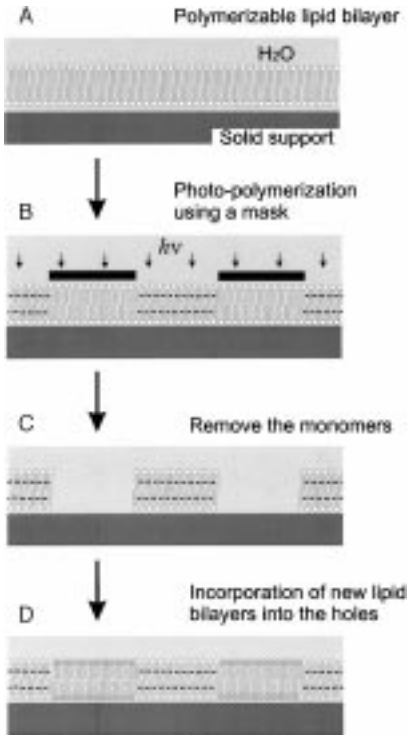
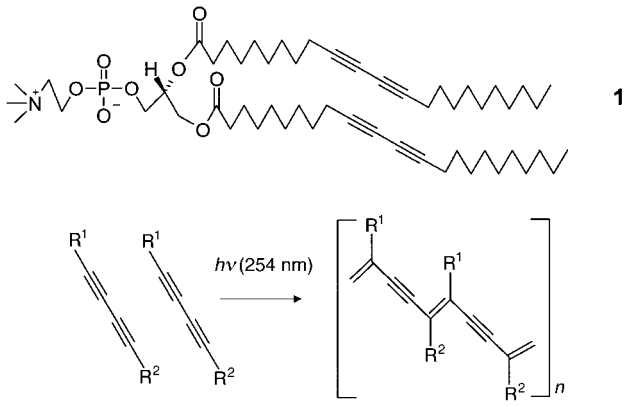


Figure 1. Schematic illustration of the bilayer patterning and introduction of new lipid bilayers.

We have used a diacetylene phosphatidylcholine **1** as the polymerizable lipid (Scheme 1); its polymerization in liposomes has been characterized in detail.[9] The polymerization



Scheme 1. The chemical structure of the diacetylene phospholipid **1** and its polymerization scheme.

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of **1** occurs only if the molecules are in a highly ordered state (topochemical polymerization) and the two alkyl chains react only with the same alkyl chain of adjacent molecules (α to α and β to β).[10] Therefore, the crosslinking of two alkyl chains within a single molecule is avoided. On account of the two polymerizable chains per molecule, **1** forms crosslinked network upon polymerization and becomes insoluble in organic solvents.[11] Such crosslinked polymers have been reported to reduce the lateral mobility of monomeric lipids drastically.[12] The formation of a highly conjugated polymer backbone induces strong absorption and fluorescence bands in the UV/Vis range (Figure 2) and we could observe the patterned polymeric bilayers by the fluorescence microscope (Figure 3).

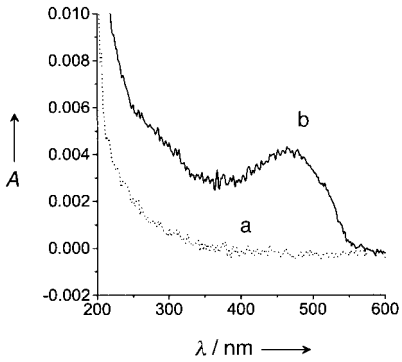

Figure 2. The UV/Vis absorption spectra of a bilayer on quartz before (a) and after (b) the photopolymerization.

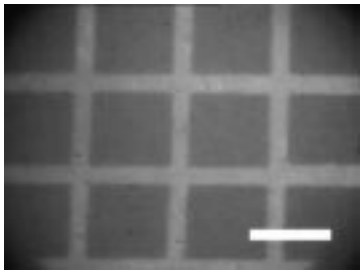

Figure 3. Fluorescence micrograph of the polymerized bilayer. The brightly fluorescent area (grid) was irradiated with UV light. The dark, square-shaped areas (wells) were protected by the mask and the lipid molecules remained momomeric. The scale bar corresponds to 200 μm.

In order to prove that new lipid bilayers can be introduced into the wells that had been created by removal of the monomeric bilayers by ethanol, we have fused small unilamellar vesicles of egg yolk phosphatidylcholine (egg PC) doped with 1 mol % of the fluorescently labeled lipid NBD-PE. Observation by the fluorescence microscope revealed that the wells were intensely fluorescent due to the incorporated lipid bilayers (Figure 4). The bilayers were fluid, as proven by fluorescence recovery after photobleaching (FRAP) experiments on a small spot (diameter 4 μm). Furthermore, if a larger area (diameter about 200 μm) in a single well was bleached by continuous illumination of UV light through a pinhole, the entire well became dark with time (Figure 5). The NBD-PE molecules in the same well obviously diffused laterally and were bleached when they came

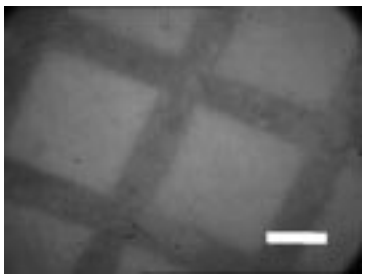

Figure 4. Fluorescence micrograph of the polymerized **1** and egg PC/NBD-PE bilayers incorporated in the wells. The contrast is inverted compared with Figure 3 because NBD-PE molecules fluoresce more strongly than the polymerized bilayer. The scale bar corresponds to 100 μm.

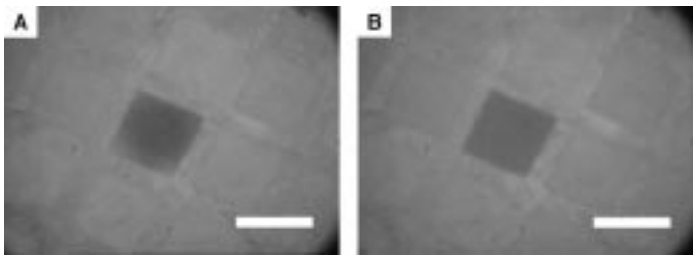

Figure 5. The egg PC/NBD-PE bilayer in a single well, bleached by UV light for 1 min, immediately (A) and 20 minutes (B) after the illumination. The illuminated spot was round (about 200 μm diameter). One can observe a slight trace of fluorescence at the edges of the bleached well in (A) that is not present in (B) due to the lateral diffusion of the lipids. The scale bars correspond to 200 μm.

into the illuminated spot. The neighboring wells separated by the polymerized bilayers retained the fluorescence. This result suggests that the polymerized bilayers act as an effective barrier that confines the lipid molecules. If one treated the sample with ethanol, the egg PC bilayers were completely removed, to leave the bare grid structure of the polymerized bilayer. The polymerized bilayer could be used repeatedly as a 2D master for the incorporation of new lipid bilayers. The bilayers were again fluid and confined in each single well.

Compared with other approaches that have been demonstrated to be effective to create patterned fluid lipid bilayers, the current method has a unique feature, in that the pattern is imprinted in the bilayer membrane as polymerized domains. This fact allows the patterned two-dimensional structure to be independent of the substrate and one could possibly construct a patterned bilayer separated from the substrate by a thin layer of soft polymeric cushion.[13] Such polymer layers have been used as a spacer in order to accommodate membrane proteins in solid-supported bilayers in a functionally active form.[14] It should be also possible to covalently attach the polymerized bilayer to the underlying polymer cushion by using a chemically reactive head group. The polymeric lipid bilayer might contribute to the mechanical stabilization of the membrane system, similar to cytoskeleton – transmembrane protein conjunctions.[15]

The patterning of bilayers by lithographic polymerization described here should provide new opportunities both for the basic studies of model membrane systems and for the biomedical applications. The potential applications include incorporation of membrane proteins in order to monitor their

activities,[16] separation of membrane proteins by 2D electrophoresis,[17] creation of addressable arrays of modified lipid bilayer membranes for combinatorial chemistry,[18] and controlled cell culture on designed membrane surfaces.[19]

Experimental Section

Materials: Diacetylene lipid **1** and egg PC were purchased from Avanti Polar Lipids, Alabaster, AL, USA. NBD-PE (*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine) was purchased from Molecular Probes, Eugene, OR, USA.

Lithographic polymerization of bilayers: Bilayers of **1** were deposited onto glass substrates from the air/water interface by the Langmuir–Blodgett (LB) and Langmuir–Schaefer (LS) methods (surface pressure $\pi = 35\ mN\,m^{-1}$). Prior to the photopolymerization, oxygen was removed from the aqueous solution by an argon gas purge. After the patterned polymerization, monomeric **1** was removed by immersing the sample in ethanol and subsequently rinsing it extensively with Milli-Q water. The fluorescence microscope observation has been made using excitation and observation wavelength of 490 nm and 530 nm, respectively.

Incorporation of new lipid bilayers: Vesicle suspensions of egg PC/NBD-PE (1 mM in a 0.05 M phosphate buffer with 0.1 M NaCl (pH 7.0)) were extruded through a filter with pores of diameter ~50 nm. A small volume of filtered suspension (100 to 200 μL) was placed onto the patterned **1** bilayer samples and sandwiched with another slide glass with a thin cover glass in between in order to avoid scratching the patterned surfaces. The sample was rinsed with the same buffer solution after 5 minutes.

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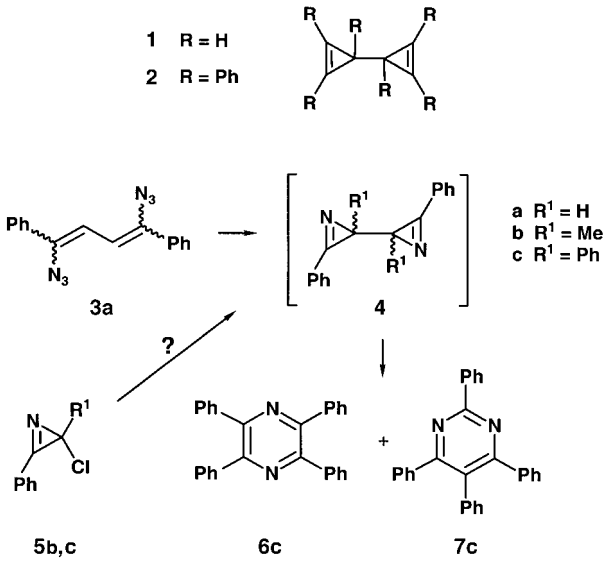
Synthesis of 1,4-Diazidobuta-1,3-dienes by Electrocyclic Ring Opening: Precursors for Bi-2*H*-azirin-2-yls and Their Valence Isomerization to Diazabenzenes**

Klaus Banert* and Frank Köhler

Dedicated to Professor Horst Kunz on the occasion of his 60th birthday

Bicycloprop-2-enyl **1** was isolated first in 1989 as the last remaining valence isomer of benzene (Scheme 1).[1] This compound, which was calculated to be the highest in energy of the $(CH)_6$ species,[2] polymerizes above $-10\,^{\circ}C$,[1] while other bicycloprop-2-enyls, for example **2**,[3] undergo a valence isomerization to give benzene derivatives on heating. Several



Scheme 1. Previous unsuccessful attempts to identify the bi-2*H*-azirin-2-yls **4**.

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