Invited Feature Article

Formation of Solid-Supported Lipid Bilayers: An Integrated View

Ralf P. Richter,*,† Rémi Bérat, and Alain R. Brisson

Laboratoire d'Imagerie Moléculaire et Nanobiotechnologie, IECB, UMR-CNRS 5471, Université Bordeaux I, 2 Rue Robert Escarpit, 33607 Pessac Cedex, France

Received October 4, 2005. In Final Form: November 22, 2005

Supported lipid bilayers (SLBs) are popular models of cell membranes with potential bio-technological applications. A qualitative understanding of the process of SLB formation after exposure of small lipid vesicles to a hydrophilic support is now emerging. Recent studies have revealed a stunning variety of effects that can take place during this self-organization process. The ensemble of results in our group has revealed unprecedented insight into intermediates of the SLB-formation process and has helped to identify a number of parameters that are determinant for the lipid deposition on solid supports. The pathway of lipid deposition can be tuned by electrostatic interactions and by the presence of calcium. We emphasize the importance of the solid support in the SLB-formation process. Our results suggest that the molecular-level interaction between lipids and the solid support needs to be considered explicitly, to understand the rupture of vesicles and the formation of SLBs as well as to predict the properties of the resulting SLB. The impact of the SLB-formation process on the quality and the physical properties of the resulting SLB as well as implications for other types of surface-confined lipid bilayers are discussed.

Introduction

Biological membranes play key roles in cell life, controlling the transfer of information and the transport of ions and molecules between the inside and outside cellular worlds and participating in various intra- and extracellular processes. These highly complex and dynamic assemblies, only a few nanometers thick, consist of two main components: a two-dimensional space made of lipid molecules held together by hydrophobic interactions and self-assembled as a continuous bilayer and proteins embedded within the membrane or transiently associated with it.

Our current knowledge of the molecular processes occurring at biological membranes is based on studies performed both on integrated and on reconstituted systems using models of biological membranes. The deposition of model membranes on solid supports has become very popular,[1−3] both for studying basic membrane processes and for possible biotechnological applications.[4−16] The growing interest in confining lipid membranes on surfaces has been nourished by the emergence of a multitude of surface-sensitive characterization techniques,[5,17−19] advanced surface patterning methods,[5,20−24] and liquid handling systems (microfluidics).[10]

During the past decade the conceptual base of surface-confined membrane systems has grown considerably. A large number of systems has been described, including solid-supported lipid bilayers,[9,11,15,25,26] polymer-cushioned lipid bilayers,[27−29] hybrid bilayers,[30,31] tethered lipid bilayers,[32] suspended lipid bilayers,[33,34]

* Corresponding author. E-mail: ralf.richter@urz.uni-heidelberg.de.

† Current address: Department of Biophysical Chemistry, Institute of Physical Chemistry, Heidelberg University, Im Neuenheimer Feld 253, 69 120 Heidelberg, Germany.

(1) Sackmann, E. *Science* **1996**, *271*, 43−48.
(2) Boxer, S. G. *Curr. Opin. Chem. Biol.* **2000**, *4*, 704−709.
(3) Knoll, W.; Frank, C. W.; Heibel, C.; Naumann, R.; Offenhäusser, A.; Rühe, J.; Schmidt, E. K.; Shen, W. W.; Sinner, A. *Rev. Mol. Biotechnol.* **2000**, *74*, 137−158.
(4) Bieri, C.; Ernst, O. P.; Heyse, S.; Hofmann, K. P.; Vogel, H. *Nat. Biotechnol.* **1999**, *17*, 1105−1108.
(5) Kung, L. A.; Kam, L.; Hovis, J. S.; Boxer, S. G. *Langmuir* **2000**, *16*, 6773−6776.
(6) Sapuri, A. R.; Baksh, M. M.; Groves, J. T. *Langmuir* **2003**, *19*, 1606−1619.
(7) Sackmann, E.; Tanaka, M. *Trends Biotechnol.* **2000**, *18*, 58−64.
(8) Knoll, W.; Park, H.; Sinner, E. K.; Yao, D.; Yu, F. *Surf. Sci.* **2004**, *570*, 30−42.
(9) Reviakine, I.; Brisson, A. *Langmuir* **2001**, *17*, 8293−8299.
(10) Kam, L.; Boxer, S. G. *Langmuir* **2003**, *19*, 1624−1631.
(11) Larsson, C.; Rodahl, M.; Höök, F. *Anal. Chem.* **2003**, *75*, 5080−5087.
(12) Milhiet, P. E.; Giocondi, M.-C.; Baghdadi, O.; Ronzon, F.; Roux, B.; le Grimellec, C. *EMBO Rep.* **2002**, *3*, 485−490.
(13) Yip, C. M.; Darabie, A. A.; McLaurin, J. *J. Mol. Biol.* **2002**, *318*, 97−107.
(14) Salafsky, J.; Groves, J. T.; Boxer, S. G. *Biochemistry* **1996**, *35*, 5, 14773−14781.
(15) Reviakine, I.; Bergsma-Schutter, W.; Brisson, A. *J. Struct. Biol.* **1998**, *121*, 356−361.
(16) Watts, T. H.; Brian, A. A.; Kappler, J. W.; Marrack, P.; McConnell, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 7564−7568.
(17) Shao, Z.; Mou, J.; Czajkowsky, D. M.; Yang, J.; Yuan, J.-Y. *Adv. Phys.* **1996**, *45*, 1−86.
(18) Knoll, W. *Annu. Rev. Phys. Chem.* **1998**, *49*, 569−638.
(19) Höök, F.; Larsson, C.; Fant, C. In *Encyclopedia of Surface and Colloid Science*; Marcel Dekker: New York, 2002; pp 774−791.
(20) Srinivasan, M. P.; Ratto, T. V.; Stroeve, P.; Longo, M. L. *Langmuir* **2001**, *17*, 7951−7954.
(21) Michel, R.; Reviakine, I.; Sutherland, D.; Fokas, C.; Csucs, G.; Danuser, G.; Spencer, N. D.; Textor, M. *Langmuir* **2002**, *18*, 8580−8586.
(22) Svedhem, S.; Pfeiffer, I.; Larsson, C.; Wingren, C.; Borrebaeck, C.; Höök, F. *ChemBioChem* **2003**, *4*, 339−343.
(23) Morigaki, K.; Kiyosue, K.; Taguchi, T. *Langmuir* **2004**, *20*, 7729−7735.
(24) Jackson, B. L.; Groves, J. T. *J. Am. Chem. Soc.* **2004**, *126*, 13878−13879.
(25) Brian, A. A.; McConnell, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6159−6163.
(26) Lenz, P.; Ajo-Franklin, C. M.; Boxer, S. G. *Langmuir* **2004**, *20*, 11092−11099.
(27) Goennenwein, S.; Tanaka, M.; Hu, B.; Moroder, L.; Sackmann, E. *Biophys. J.* **2003**, *85*, 646−655.
(28) Wagner, M. L.; Tamm, L. K. *Biophys. J.* **2001**, *81*, 266−275.
(29) Munro, J. C.; Frank, C. W. *Langmuir* **2004**, *20*, 10567−10575.
(30) Silin, V. I.; Wieder, H.; Woodward, J. T.; Valincius, G.; Offenhausser, A.; Plant, A. L. *J. Am. Chem. Soc.* **2002**, *124*, 14676−14683.
(31) Terrettaz, S.; Mayer, M.; Vogel, H. *Langmuir* **2003**, *19*, 5567−5569.
(32) Purrucker, O.; Förtig, A.; Jordan, R.; Tanaka, M. *ChemPhysChem* **2004**, *5*, 327−335.
(33) Römer, W.; Lam, Y. H.; Fischer, D.; Watts, A.; Fischer, W. B.; Göring, P.; Wehrspohn, R. B.; Gösele, U.; Steinem, C. *J. Am. Chem. Soc.* **2004**, *126*, 16267−16274.
(34) Römer, W.; Steinem, C. *Biophys. J.* **2004**, *86*, 955−965.

10.1021/la052687c CCC: $33.50
Published on Web 01/14/2006

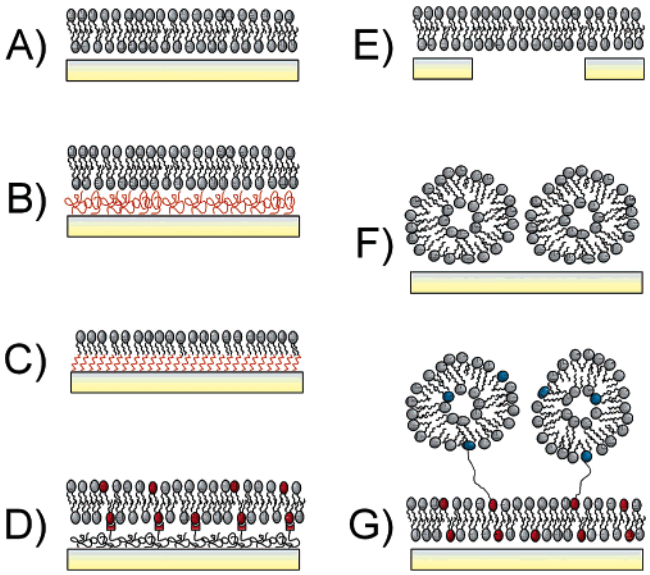

Figure 1. Surface-confined membrane models: (A) solid-supported lipid bilayer; (B) polymer-cushioned lipid bilayer; (C) hybrid bilayer, consisting of a self-assembled monolayer (e.g., thiols on Au or silanes on glass or silica) and a lipid monolayer; (D) tethered lipid bilayer; (E) freely suspended lipid bilayer; (F−G) supported vesicular layers.

or supported vesicular layers[22,35] (Figure 1). In parallel, a multitude of methods has been proposed to create such biomimetic edifices, including Langmuir-type approaches (Langmuir−Blodgett or Langmuir-Schäfer deposition)[36−39] and the spreading of vesicles on various preconditioned supports.[20,38,40−44]

The spreading of small lipid vesicles on hydrophilic solid supports, pioneered by McConnell et al.,[25] presents an attractive and simple route to form supported lipid bilayers (SLBs). The one-step procedure allows creating SLBs of different lipid mixtures.[45,46] The fact that such SLBs form a fluid two-dimensional space allowing free diffusion in translation and rotation of lipid molecules and lipid-associated proteins makes them well suited to analyze lipid domain formation,[13,47−54] intermembrane interactions,[55−57] or membrane processes such as protein adsorption,[58,59] protein self-assembly,[13,48,60] protein localization at lipid phase boundaries,[12] or protein function.[37]

The self-organization steps involved in this method—vesicle adsorption, rupture, and spreading into planar membranes—present fundamental interest in colloidal and interfacial science. Both theoretical[61−63] and experimental work[41,45,64−70] during the past decade have considerably improved the general understanding of this process, and a detailed image of the structural intermediates and the driving forces is now emerging. Figure 2 shows four archetypes of lipid deposition processes, as followed by quartz crystal microbalance with dissipation monitoring (QCM-D). The QCM-D technique has proven very valuable to screen the overall properties of the lipid deposition,[41] thanks to the dissipation parameter that allows distinguishing between intact, adsorbed vesicles (high dissipation) and bilayer patches (low dissipation). As shown in the schemes in Figure 2, vesicles either do not adsorb (Figure 2A), adsorb and remain intact, giving rise to a supported vesicular layer (SVL) (Figure 2B), or form an SLB (Figure 2, panels C and D). Notably, SLB formation can occur via two scenarios with distinctly different kinetics. In one case, vesicles rupture quickly upon interaction with the solid support (Figure 2D), whereas in the other, a large amount of intact vesicles is adsorbed at an intermediate state of the process (Figure 2C).

Recent technical developments, combining QCM-D and atomic force microscopy (AFM), have allowed us to characterize the intermediate states leading to SLB formation in unprecedented detail. Here we present an overview of work performed in our group that sheds light on the mechanisms and critical parameters involved in the formation of SLBs as well as on the properties and the quality of the resulting SLB.

Mechanism of SLB Formation

To satisfactorily describe the mechanism of SLB formation, two critical steps in this process need to be understood: (i) the adhesion and rupture of vesicles on the support and (ii) the evolution of the supported bilayer patches thus formed into a complete SLB. Figure 3 provides an overview of mechanisms of vesicle rupture that have been reported or suggested in the literature.

Stability of Adsorbed Vesicles. A simple rationale to evaluate the binding and the stability of surface-bound vesicles was provided by the theory of Seifert and Lipowsky.[62,71] In their continuum approach, where the bilayer is treated as a thin two-dimensional sheet embedded in three-dimensional space, the balance between the gain in adhesion energy (as given by the

(35) Yoshina-Ishii, C.; Boxer, S. G. *J. Am. Chem. Soc.* **2003**, *125*, 3696−3697.
(36) Swart, R. M. In *Langmuir−Blodgett Films*; Roberts, G., Ed.; Plenum Press: New York, 1990; pp 273−316.
(37) Grandbois, M.; Clausen-Schaumann, H.; Gaub, H. E. *Biophys. J.* **1998**, *74*, 2398−2404.
(38) Wong, J. Y.; Majewski, J.; Seitz, M.; Park, C. K.; Israelachvili, J.; Smith, G. S. *Biophys. J.* **1999**, *77*, 1445−1457.
(39) Ross, M.; Steinem, C.; Galla, H.-J.; Janshoff, A. *Langmuir* **2001**, *17*, 2437−2445.
(40) Steinem, C.; Janshoff, A.; Ulrich, W.-P.; Sieber, M.; Galla, H.-J. *Biochim. Biophys. Acta* **1996**, *1279*, 169−180.
(41) Keller, C. A.; Kasemo, B. *Biophys. J.* **1998**, *75*, 1397−1402.
(42) Starr, T. E.; Thompson, N. L. *Langmuir* **2000**, *16*, 10301−10308.
(43) Zhao, J.; Tamm, L. K. *Langmuir* **2003**, *19*, 1838−1846.
(44) Baumgart, T.; Offenhäusser, A. *Langmuir* **2003**, *19*, 1730−1737.
(45) Richter, R. P.; Mukhopadhyay, A.; Brisson, A. *Biophys. J.* **2003**, *85*, 3035−3047.
(46) Richter, R. P.; Brisson, A. *Biophys. J.* **2005**, *88*, 3422−3433.
(47) Mou, J.; Yang, J.; Shao, Z. *J. Mol. Biol.* **1995**, *248*, 507−512.
(48) Reviakine, I.; Simon, A.; Brisson, A. *Langmuir* **2000**, *16*, 1473−1477.
(49) Schneider, J.; Dufrêne, Y. F.; Barger, W. R., Jr.; Lee, G. U. *Biophys. J.* **2000**, *79*, 1107−1118.
(50) Rinia, H. A.; Kruijff, B. d. *FEBS Lett.* **2001**, *504*, 194−199.
(51) Lawrence, J. C.; Saslowsky, D. E.; Edwardson, J. M.; Henderson, R. M. *Biophys. J.* **2003**, *84*, 1827−1832.
(52) Tokumasu, F.; Jin, A. J.; Feigenson, G. W.; Dvorak, J. A. *Biophys. J.* **2003**, *84*, 2609−2618.
(53) Giocondi, M.-C.; Milhiet, P. E.; Dosset, P.; le Grimellec, C. *Biophys. J.* **2004**, *86*, 861−869.
(54) Janshoff, A.; Bong, D. T.; Steinem, C.; Johnson, J. E.; Ghadiri, M. R. *Biochemistry* **1999**, *38*, 5328−5336.
(55) Kaizuka, Y.; Groves, J. T. *Biophys. J.* **2004**, *86*, 905−912.
(56) Parthasarathy, R.; Groves, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12798−12803.
(57) Fix, M.; Melia, T. J.; Jaiswal, J. K.; Rappoport, J. Z.; You, D.; Söllner, T. H.; Rothman, J. E.; Simon, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7311−7316.
(58) Corsel, J. W.; Willems, G. M.; Kop, J. M. M.; Cuypers, P. A.; Hermens, W. T. *J. Colloid Interface Sci.* **1986**, *111*, 544−554.
(59) Andree, H. A. M.; Stuart, M. C. A.; Hermens, W. T.; Reutelingsperger, C. P. M.; Hemker, H. C.; Frederik, P. M.; Willems, G. M. *J. Biol. Chem.* **1992**, *267*, 17907−17912.
(60) Czaikowsky, D. M.; Shao, Z. *FEBS Lett.* **1998**, *430*, 51−54.
(61) Seifert, U.; Lipowsky, R. *Phys. Rev. A* **1990**, *42*, 4768−4771.
(62) Seifert, U. *Adv. Phys.* **1997**, *46*, 13−137.
(63) Zhdanov, V. P.; Keller, C. A.; Glasmästar, K.; Kasemo, B. *J. Chem. Phys.* **2000**, *112*, 900−909.
(64) Rädler, J.; Strey, H.; Sackmann, E. *Langmuir* **1995**, *11*, 4539−4548.
(65) Keller, C. A.; Glasmästar, K.; Zhdanov, V. P.; Kasemo, B. *Phys. Rev. Lett.* **2000**, *84*, 5443−5446.
(66) Jass, J.; Tjärnhage, T.; Puu, G. *Biophys. J.* **2000**, *79*, 3153−3163.
(67) Reviakine, I.; Brisson, A. *Langmuir* **2000**, *16*, 1806−1815.
(68) Johnson, J. M.; Taekijp, H.; Chu, S.; Boxer, S. G. *Biophys. J.* **2002**, *83*, 3371−3379.
(69) Reimhult, E.; Höök, F.; Kasemo, B. *Langmuir* **2003**, *19*, 1681−1691.
(70) Benes, M.; Billy, D.; Benda, A.; Speijer, H.; Hof, M.; Hermens, W. T. *Langmuir* **2004**, *20*, 10129−10137.
(71) Lipowsky, R.; Seifert, U. *Mol. Crys. Liq. Cryst.* **1991**, *202*, 17−25.

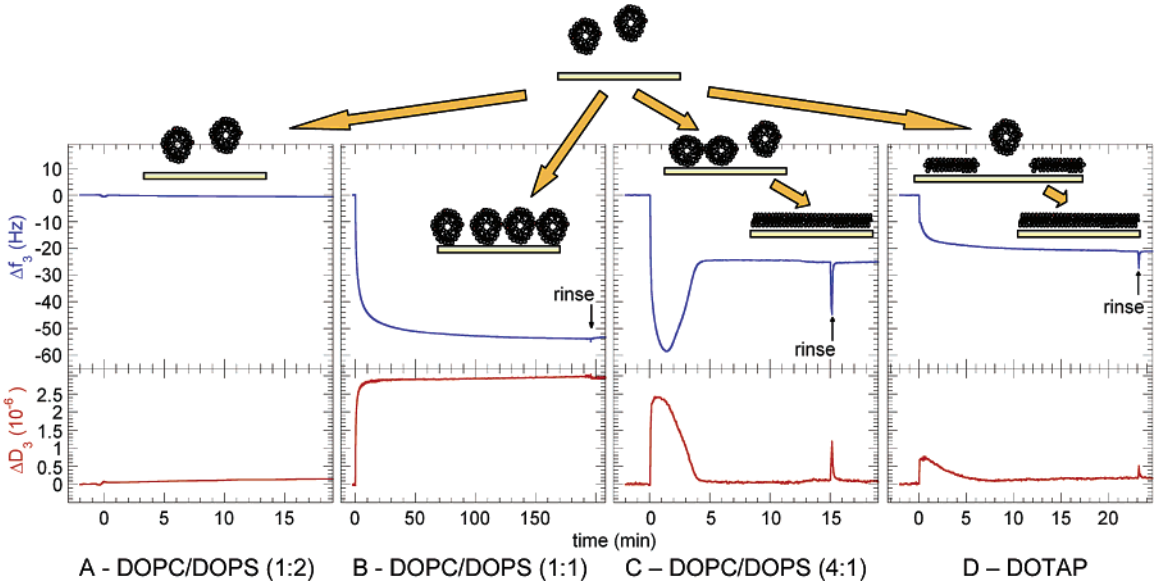

Figure 2. Lipid deposition pathways measured by QCM-D on silica. (A) Vesicles do not adsorb. (B) Vesicles adsorb and remain intact, forming a supported vesicular layer (SVL). (C) Vesicles adsorb and remain initially intact. At high vesicular coverage an SLB is formed. (D) Vesicles adsorb and rupture instantaneously, to form an SLB. The dissipation, ΔD, allows distinguishing between the morphological state of the adsorbed lipids: intact vesicles exhibit high dissipation while bilayer (patches) show low dissipation. The legends indicate the lipids used—dioleoyltrimethylammonium-propane (DOTAP), dioleoylphosphatidylcholine (DOPC), and dioleoylphosphatidylserine (DOPS)—with their molar mixing ratios.[45]

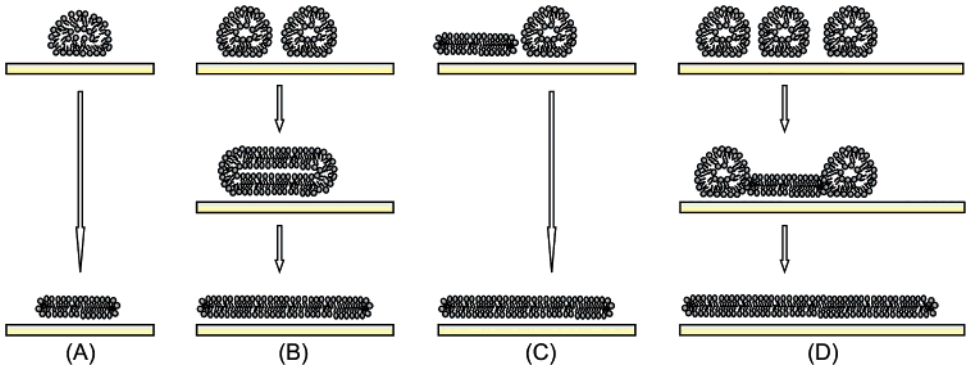

Figure 3. Mechanisms of vesicle rupture: (A) an isolated adsorbed vesicle ruptures spontaneously, driven by its support-induced deformation; (B) neighboring adsorbed vesicles fuse and eventually rupture; (C) the active edge of a supported bilayer patch induces the rupture of a neighboring vesicle; (D) the cooperative action of several neighboring vesicles leads to the rupture of a first vesicle (at the critical vesicular coverage). The active edge thereby exposed triggers the rupture of adjacent vesicles.

adhesion area) and the cost in the vesicles' curvature energy (as given by the bilayer's bending rigidity) is determinant for the adsorption, deformation, and rupture of vesicles. Initial data in our group provided support for this model for egg-PC on mica.[67] The examples given in Figure 2, panels A, B, and D, exemplify the scenarios were vesicles do not adsorb, adsorb intact, and rupture spontaneously, respectively.

However, recent experimental data have provided evidence that this continuum approach does not convey the whole answer to the question of vesicular stability under conditions commonly employed for SLB formation. Cooperative effects of neighboring vesicles as well as the dynamic distribution of different lipid species in a vesicle have to be taken into account for a better description of the rupture propensity of surface-bound vesicles.

Critical Vesicular Coverage. An intriguing effect of the cooperative action of surface-bound vesicles was first reported by Kasemo and co-workers. By combining measurements by QCM-D and surface plasmon resonance (SPR)[65] together with computer simulations,[63] the group could show (i) that isolated vesicles of egg-PC remain intact when bound to a silica support and (ii) that a certain surface density of vesicles (henceforward denoted the critical vesicular coverage) is required to initiate the decomposition of surface-bound vesicles into bilayer patches. Zhdanov and Kasemo[72] proposed that the support-induced stress (or deformation) of an adsorbed vesicle is further enhanced by the adsorption of vesicles in its vicinity. When a certain confinement of neighboring vesicles, corresponding to the critical coverage, is reached, the stress on the vesicle becomes sufficient to induce its rupture (Figure 3D).

Figure 2C exemplifies the response obtained by QCM-D, when the critical vesicular coverage is involved. As techniques such as QCM-D and SPR give average information about the adsorbed material, a small fraction of prematurely ruptured vesicles may potentially go undetected.[73] Our images by atomic force microscopy (AFM) provide direct evidence that silica wafers can indeed be covered with vesicles that remain stable for days, being devoid of bilayer patches over areas of several square micrometers (Figure 4).[45] These images also demonstrate the

(72) Zhdanov, V. P.; Kasemo, B. *Langmuir* **2001**, *17*, 3518−3521.
(73) Reimhult, E.; Höök, F.; Kasemo, B. *J. Chem. Phys.* **2002**, *117*, 7401−7404.

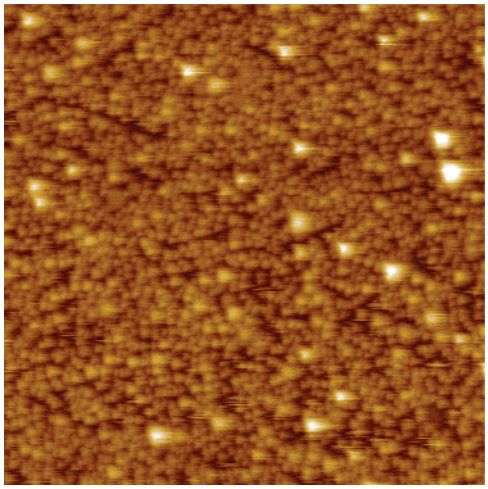

Figure 4. Imaging an intermediate of the SLB-formation process by AFM. Vesicles made of DOPC/DOPS (4:1) were exposed to a silica wafer. Spherical objects, identified as vesicles, densely populate the surface. No bilayer patches are visible, indicating that the critical vesicular coverage is not attained. Image size (*z* scale): 2 μm (50 nm). Adapted from ref 45. Copyright 2003 Biophysical Society.

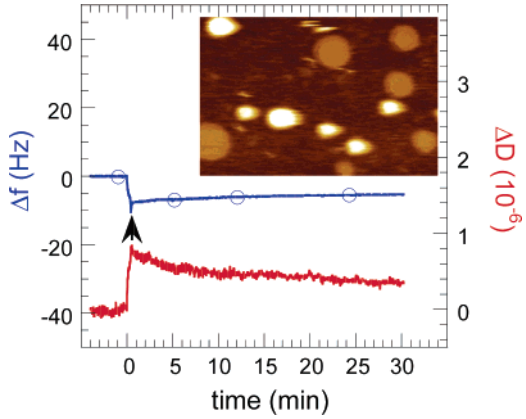

Figure 5. Tracing vesicle rupture kinetics by QCM-D. Vesicles initially adsorb intact but rupture into bilayer patches over the time range of 30 min and more, as indicated by the decrease in dissipation (- - -) and the increase in frequency (-○-), after rinsing away vesicles in solution (arrow). The AFM image (inset, image size: 750 nm × 500 nm), taken after the QCM-D measurement, confirms the coexistence of vesicles and bilayer patches. Adapted from ref 46. Copyright 2005 Biophysical Society.

strength of the AFM to resolve the local morphology of the lipid assemblies down to nanometer resolution.

Long-Term Stability of Adsorbed Vesicles. We observed a peculiar effect for vesicles containing a mixture of DOPC and DOPS when exposed to mica in a calcium-containing solution: when adsorbing vesicles at low surface density (i.e., the interaction of neighboring vesicles is negligible), they initially remained intact but ruptured individually over a time range of minutes to hours (Figure 5).[74] This strongly contrasted our common observation on silica supports that isolated vesicles either rupture immediately (i.e., within less than a second) after adsorption or remain intact for days.

What is the origin of such particular rupture kinetics? It appears reasonable that a support-induced reorganization of the two lipid species within the adsorbed vesicle may lead to dynamic changes in the vesicle−support interaction and in the stability of the vesicles. The observed time range for rupture is, however, much

(74) Richter, R. P.; Brisson, A. *Biophys. J.* **2005**, *88*, 3422−3433.

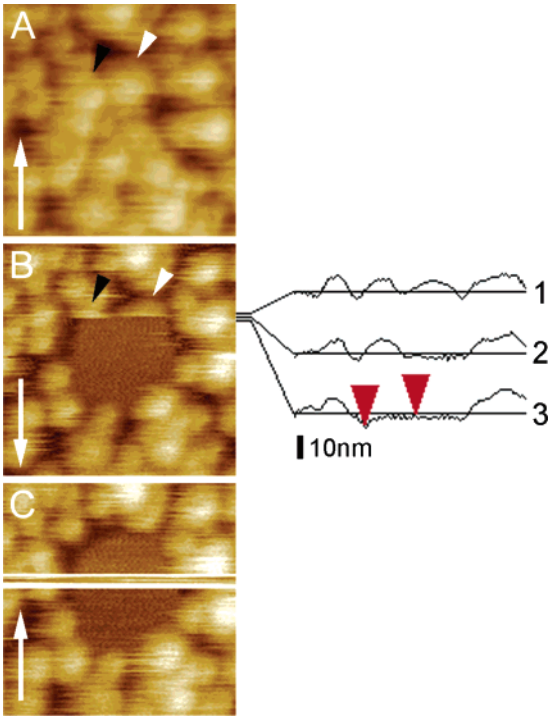

Figure 6. Tracking the propagation of bilayer patch formation by AFM. (A) All vesicles are intact. (B) Two vesicle segments are resolved (arrowheads) followed by an extended bilayer domain, indicating the rupture of vesicles. The cross sections of three successive scan lines (right) reveals that the two vesicles do not rupture simultaneoulsy: The right vesicle (white arrowhead) ruptures first (between scan line 1 and 2), likely induced by the AFM-tip. At scan line 3, the left vesicle (black arrowhead) is ruptured, likely induced by the "active edge" of the bilayer patch that is formed from the right vesicle. (C) The rupture of a single vesicle induces the transformation of several adjacent vesicles into a stable bilayer patch. A small gap (a few nanometers) separates the patch edge from neighboring intact vesicles. A part of the image is distorted because the tip was accidentally retracted from the surface. Image size: 250 nm. The slow scan direction is indicated with white arrows. Adapted from ref 45. Copyright 2003 Biophysical Society.

slower than the time needed for lipids within a single lipid leaflet to reorganize.[75] We therefore propose that the translocation of lipids between the two leaflets of the vesicle is the parameter responsible for the slow vesicle rupture. The suggested rupture mechanism correlates with our observation that mica induces an asymmetric inter-leaflet lipid distribution in SLBs,[76] an issue that will be discussed in detail later on.

Growth and Coalescence of Supported Lipid Bilayers. Once a vesicle has ruptured, the resulting bilayer patch exposes an edge.[77,78] These edges are energetically unfavorable and, at least from a thermodynamic perspective, expected to promote the interaction with adjacent lipid material, such as the rupture of surface-bound vesicles (Figure 3C) or vesicles from solution. Provided the density of adsorbed vesicles is sufficiently high, such a process can propagate in a cascade of rupture events across several neighboring vesicles and leads to the formation of extended bilayer patches.[45,63] The intermediate steps in this process can be traced by AFM, as illustrated in Figure 6, and suggest that the propagation speed is in the range of seconds.[45] Furthermore, adjacent bilayer patches usually coalesce in order

(75) Bernard, A.-L.; Guedeau-Boudeville, M.-A.; Jullien, L.; de Meglio, J.-M. *Langmuir* **2000**, *16*, 6809−6820.
(76) Richter, R. P.; Maury, N.; Brisson, A. *Langmuir* **2005**, *21*, 299−304.
(77) Kasson, P. M.; Pande, V. S. *Biophys. J.* **2004**, *86*, 3744−3749.
(78) Jiang, F. Y.; Bouret, Y.; Kindt, J. H. *Biophys. J.* **2004**, *87*, 182−192.

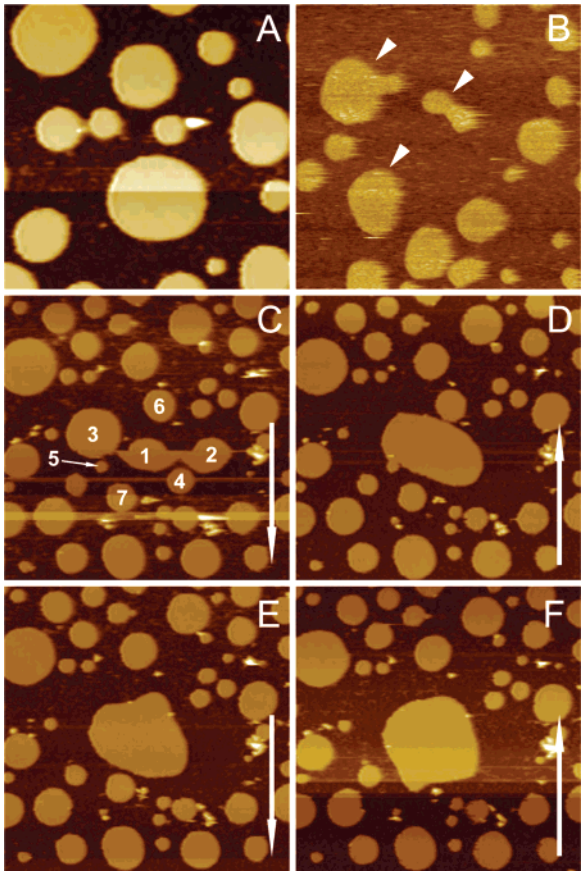

Figure 7. (A) Incomplete SLB made from DOPC/DOPS (4:1) vesicles on mica: bilayer patches are predominantly circular, indicating that they are laterally mobile. Image size: 1 μm. (B) Incomplete SLB made from DOTAP-vesicles on silica. Some bilayer patches (arrowheads) exhibit stable, strongly noncircular shapes. Image size: 1 μm. (C−F) Sequential images of patch coalescence induced by dynamic changes in the shape of a bilayer patch. After the merger of patches 1−3 (C), induced by the AFM-tip, the coalescence with patches 4 (D), 5−6 (E), and 7 (F) is generated by movements of the reshaping patch. Image size: 1.75 μm. Adapted from ref 46. Copyright 2005 Biophysical Society.

to minimize their edge length.[45,46,67] Taken together, these effects increase the size of individual bilayer patches and the overall bilayer coverage and will, in the ideal case, lead to a complete SLB.[45,46]

Some of the vesicles imaged in Figure 6 remain intact even though they are situated as close as a few nanometers to the edge of a bilayer patch.[45] This suggests that the edge almost needs to contact a vesicle to induce its rupture and illustrates that the efficiency of edge-induced processes relies strongly on the spatial arrangement of vesicles and bilayer patches.

Lateral Mobility of Vesicles and Bilayer Patches. Lipid assemblies as a whole can be laterally mobile and undergo collective shape changes, an effect not to be confused with the lateral diffusion of individual lipid molecules. The shape of bilayer patches on the solid support provides a first indication about their mobility. Laterally mobile patches tend to reshape into circular patches to minimize their line tension, an effect that we observed on mica surfaces (Figure 7A).[46] In contrast, bilayer patches on silica frequently retained a strongly noncircular shape (Figure 7B), providing evidence for the lack of mobility.[45]

It is instructive to compare our observations on the mobility of lipid assemblies on mica and on silica with data previously reported by Rädler et al.[64,79,80] With reflection interference contrast microscopy (RICM), they observed that lipid bilayers, continuously formed from a deposited blob of concentrated DOPC in water, easily slide over surfaces of both types of support. The kinetics of the sliding motion on mica could be described quantitatively by the shear flow of a thin water film that is sandwiched between the solid support and the bilayer[81,82] and rather high spreading coefficients of up to 40 μm^2/s were obtained.[79]

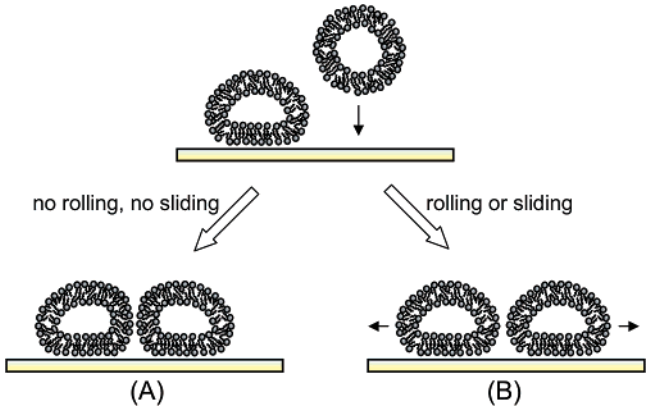

Figure 8. Possible scenarios of the mutual interaction of neighboring vesicles. The surface-induced flattening of a newly adsorbing vesicle induces the deformation of a neighboring one. If sliding and rolling are inhibited (A), the deformation represents a persisting stress for the vesicles, and facilitates their rupture. Sliding or rolling along the surface can release the stress (B). Thus, if sliding or rolling is enabled, neighboring vesicles can only induce added stress (and thereby rupture) when a high overall packing of vesicles on the surface is attained.

In light of the results by Rädler and co-workers, it was surprising that we found lipid assemblies to be immobile on silica. Also, the shape changes observed by us and others[83] on mica seem considerably slower than postulated from the action of a lubricating water film.[83] Variations in the employed experimental conditions, in particular the presence of divalent ions[45,74,83] versus pure water,[64,79] may well be at the origin of the observed differences. However, the large range of variations in mobility remains intriguing and points toward a current lack in understanding the coupling between the bilayer and the solid support.

What kind of effects can be induced by the lateral mobility of lipid assemblies? The series of AFM images in Figure 7C−F demonstrates how dynamic changes of the patch shape can enhance the coalescence of neighboring bilayer patches.[46] Similarly, vesicles are expected to rupture, induced by the active edge of an approaching bilayer patch.

The mobility of surface-bound vesicles also has important implications for the nature of the critical vesicular coverage. Mobile vesicles can avoid stress from neighboring vesicles by displacement along the surface (Figure 8B). Consequently, stress due to intervesicle interactions can build up only when the *overall* vesicular coverage is high enough to force the vesicles to interact. The critical vesicular coverage is thus directly determined by the overall density of adsorbed vesicles. Given that intervesicle interactions are commonly short-ranged, this implies that the critical coverage must be elevated.[72] In contrast, the shape relaxation of immobile vesicles is constrained to the local

(79) Nissen, J.; Gritsch, S.; Wiegand, G.; Rädler, J. O. *Eur. Phys. J. B* **1999**, *10*, 335−344.
(80) Nissen, J.; Jacobs, K.; Rädler, J. O. *Phys. Rev. Lett.* **2001**, *86*, 1904−1907.
(81) Bayerl, T. M.; Bloom, M. *Biophys. J.* **1990**, *58*, 357−362.
(82) Johnson, S. J.; Bayerl, T. M.; McDermott, D. C.; Adam, W. A.; Rennie, A. R.; Thomas, R. K.; Sackmann, E. *Biophys. J.* **1991**, *59*, 289−294.
(83) Muresan, A. S.; Lee, K. Y. C. *J. Phys. Chem. B* **2001**, *105*, 852−855.

environment (Figure 8A), and hence, the critical coverage originates in a *local* effect that involves a limited number of neighboring vesicles. In the minimal configuration, two immobilized neighboring vesicles may be sufficient to induce rupture. Due to the statistical distribution of vesicles over the surface, the local effect, however, translates into an apparent critical coverage of the ensemble. In this case the critical vesicular coverage can thus span from very low to very high values, as we could demonstrate for silica surfaces.[45]

Parameters that Govern SLB Formation

Which pathway of vesicle deposition will be taken is essentially determined by the interplay of bilayer-support, interbilayer, and intrabilayer interactions. In principle, the relative contribution of these interactions will be susceptible to the nature of the support (its surface charge, chemical composition and roughness), the lipid vesicles (their composition, charge, size, and physical state), as well as the aqueous environment (its composition, pH and ionic strength). In the following, we will outline some essential experimental parameters that appear to control SLB formation.

Electrostatic Interactions. Several studies have pointed out the influence of the charge of support and lipids as well as the ionic strength of the solution on the adsorption of vesicles.[64,84−86] In systematic studies on silica[45] and mica,[46] we provided evidence that all four pathways of vesicle deposition outlined in Figure 2 can actually be generated by varying one experimental parameter only: the vesicle charge. These studies demonstrate that the SLB-formation process will be strongly influenced by electrostatic interactions. Consequently, adjustments in the pH or in the ionic strength are expected to constitute relatively simple means to optimize the formation of SLBs for a given surface and a given lipid composition.[85]

Calcium Ions. The influence of divalent ions in general and calcium in particular appears notoriously surprising. The ions do not only participate in the screening of charges, thereby modifying the electrostatic interactions, but they also directly interact, in often subtle ways, with surfaces and lipids.[48,87] As a general trend, calcium was found to promote the adsorption and rupture of vesicles and SLB formation.[45,67,84,88] Effects are particularly strong on mica.[46,67,70] Often minor concentrations (mM and below) of the ion are sufficient to generate significant effects.

Solid Support. The role of the solid support in the process of SLB formation cannot be underestimated. It is probably the most complex and still the most enigmatic parameter.

Work on different supports has pointed out that hydrophilicity is a necessary[26] but not a sufficient condition to promote the rupture of vesicles and subsequent SLB formation. A number of reports has actually revealed difficulties to form SLBs on surfaces such as gold,[41] $SrTiO_2$,[42] TiO_2,[42,73] or platinum,[69] leaving mica and silicon-based materials, such as glass, Si_3N_4, or silica, as the most common surfaces used for the preparation of SLBs. Progress on TiO_2 has though recently been reported,[89] again confirming the importance of electrostatic interactions and calcium.

Although surface roughness in general was reported to have considerable effects on the spreading of bilayers on solid supports,[64,85] we experienced that SLB formation is only little affected by roughness in the nanometer range.[45,90,91] It is remarkable that SLBs can even be formed on silica films exhibiting extreme roughness and porosity at the nanoscale, such as aerogels or xerogels,[92] even though the kinetics of SLB formation and the quality of the final bilayer seem substantially affected under such conditions. An obvious question is to what extent the SLB follows the support's corrugations. An answer to this question has recently been given by imaging, by transmission electron cryo-microscopy, of lipid vesicles adsorbed to and lipid bilayers surrounding silica nanoparticles. Membrane-coated nanoparticles (Figure 9) illustrate that the lipid membrane follows very intimately the topography of the underlying support.[91] The attraction between the solid support and the lipid membrane is obviously strong enough to overcome the bilayer's bending energy, inhibiting the formation of solvent-rich pockets between SLB and support.

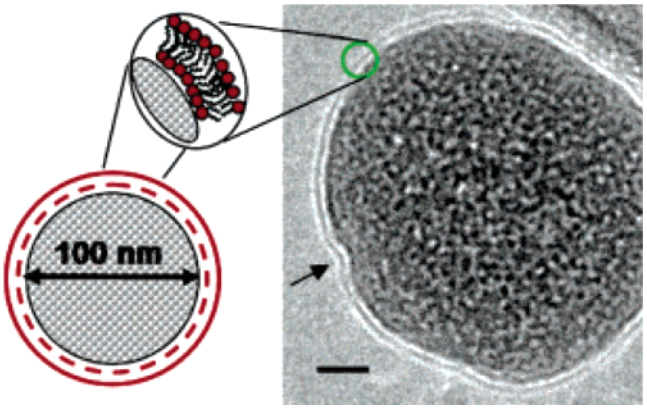

Figure 9. Transmission electron cryo-microscopy image of a silica-nanoparticle covered by a nanoSLB. The silica nanoparticle appears as a sphere of uneven density with a rough surface. The surrounding ring of electron-dense material (green circle) corresponds to the outer lipid layer of an SLB covering the particle surface. The SLB tightly follows the particle's corrugations (arrow) and does not leave solvent-rich pockets. Scale bar: 20 nm. Adapted from ref 91 with permission. Copyright 2005 American Chemical Society.

Although relatively little acknowledged in the literature, the surface preparation may considerably influence the kinetics of lipid deposition and the nature of the lipid assembly that is ultimately formed.[45] The hydroxylation state of silica surfaces, for example, can vary considerably, as a function of the manufacturing procedure, exposure to high temperature or to basic solutions[93] and thus influence the charge[94] and other physicochemical properties of the support. Apart from effects on the physicochemical state of the surface as a whole, surface manufacturing and preparation are susceptible to creating lateral heterogeneities in the surface properties. Some responses in the SLB formation have indeed been attributed to surface defects ("hot spots").[68] The AFM images in Figure 10 present some examples of lipid deposits on several glass surfaces provided by different manufacturers. Even though all surfaces are essentially silica-like, substantial variability in the morphology of the lipid deposits can be observed.

The interaction between lipids and solid support can also strongly affect the properties[95,96] and the quality of the final SLB. Some aspects will be discussed below.

(84) Nollert, P.; Kiefer, H.; Jähnig, F. *Biophys. J.* **1995**, *69*, 1447−1455.
(85) Cremer, P. S.; Boxer, S. G. *J. Phys. Chem. B* **1999**, *103*, 2554−2559.
(86) Hennesthal, C.; Steinem, C. *J. Am. Chem. Soc.* **2000**, *122*, 8085−8086.
(87) Wilschut, J.; Hoekstra, D. *Trends Biochem. Sci.* **1984**, 479−483.
(88) Ekeroth, J.; Konradsson, P.; Höök, F. *Langmuir* **2002**, *18*, 7923−7929.
(89) Rossetti, F. F.; Bally, M.; Michel, R.; Textor, M.; Reviakine, I. *Langmuir* **2005**, *21*, 6443−6450.
(90) Richter, R. P.; Brisson, A. *Langmuir* **2003**, *19*, 1632−1640.
(91) Mornet, S.; Lambert, O.; Duguet, E.; Brisson, A. *Nano Lett.* **2005**, *5*, 281−285.
(92) Weng, K. C.; Stålgren, J. J. R.; Duval, D. J.; Risbud, S. H.; Frank, C. W. *Langmuir* **2004**, *20*, 7232−7239.
(93) Iler, R. K. *The Chemistry of Silica. Solubility, Polymerization, Colloid and Surface Properties, and Biochemistry*; Wiley-Interscience: New York, 1979.
(94) Toikka, G.; Hayes, R. A. *J. Colloid Interface Sci.* **1997**, *191*, 102−109.

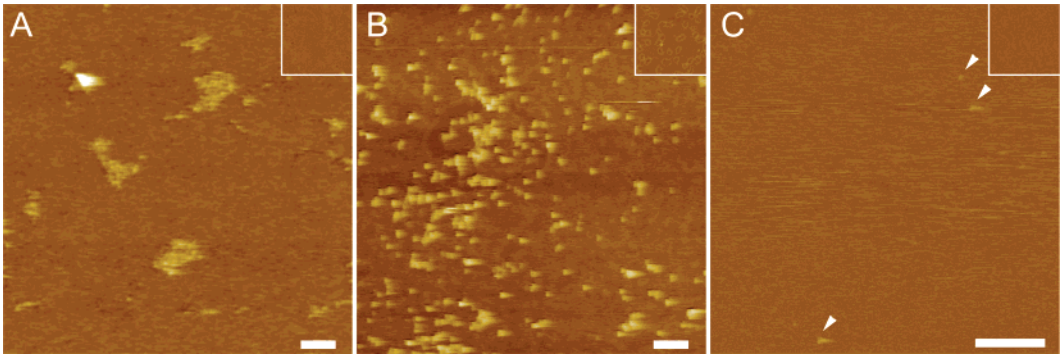

Figure 10. Quality of the final SLB on different supports as imaged by AFM. (A) Glass cover slip (Deckgläser Nr. 1, Marienfeld GmbH, Lauda-Königshofen, Germany): islands of many adsorbed vesicles remain stable for hours within the SLB. (B) Glass slide (Objektträger mit Mattrand, Knittel Gläser GmbH, Braunschweig, Germany): many individual vesicles remain intact within the SLB. (C) Silicon wafer: the SLB is homogeneous over areas of several μm^2. Only a few defects, trapped vesicles, are visible (arrowheads). Surfaces, after cleaning with SDS and UV/ozone,[45] were incubated with DOPC/DOPS (4:1)-vesicles at 2 mM $CaCl_2$. Scale bar: 400 nm. The insets show respective surfaces prior to vesicle exposure at the same magnification. Imaging was performed as described in ref 46.

Table 1. Amount of DOPS in the Bulk-Facing Leaflet of SLBs Made of DOPC and DOPS

nominal DOPS content (%)[a]	DOPS-content in the bulk-facing leaflet (%)		
	on SiO_2[b]	on mica[c]	on TiO_2[d]
0	0	0	0
10	10	3 ± 1	<3
20	20	7 ± 1	<3
30	30		6 ± 1
33	33	13 ± 2	
50		20 ± 2	17 ± 2
67		>55	>33
80		>60	>60

[a] As determined by the mixing ratio of DOPS and DOPC in the vesicles. [b] From ref 97. [c] From ref 76. [d] From Figure 11.

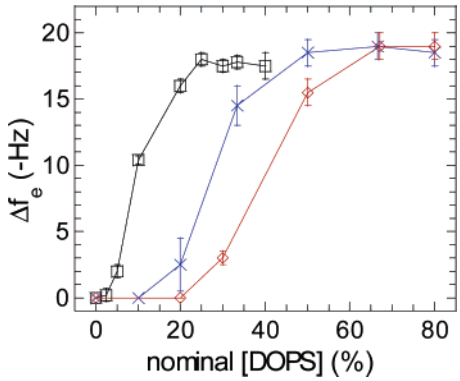

Figure 11. Adsorbed amounts of annexin A5, a protein that binds to DOPS in a calcium-dependent manner, to SLBs made of different ratios of DOPS and DOPC on silica (-□-), mica (-×-) and titanium oxide (-◇-). Annexin A5 was incubated at 2 mM $CaCl_2$. The responses, given by the shifts, Δf_e, in QCM-D frequency, indicate the amounts that remain bound after removing excess annexin A5 from solution. The sigmoidal curves are shifted toward higher nominal DOPS contents for mica and titanium oxide, indicating that less DOPS is accessible in the SLB's bulk-facing leaflets on these supports.

Interleaflet Distribution of Lipids in the SLBs

Let us consider SLBs that are formed from vesicles containing a mixture of different lipid species. How are lipids distributed between the two SLB leaflets? This question, even though highly relevant for many applications, has until recently received rather little consideration. The interleaflet distribution is commonly assumed to be symmetrical.

We have investigated the adsorption behavior of prothrombin and annexin A5, two proteins that bind specifically to DOPS, to quantify the amount of DOPS in the bulk-facing lipid leaflet of SLBs containing both DOPC and DOPS. The ensemble of our results[76,97] provides evidence for a substantial degree of asymmetry in the interleaflet distribution of DOPS on mica. For example, an SLB that is formed from vesicles containing 20% DOPS exhibits a DOPS content in the bulk-facing leaflet of only 7% (Table 1). In contrast, we found the distribution of DOPS on silica to be symmetrical, within experimental error. The asymmetry on mica was suggested to originate from a specific calcium-mediated interaction between the support and DOPS.[46,76]

Such an interaction is not restricted to mica. Recent studies on titanium oxide provide evidence for a similar, yet even stronger asymmetry in the distribution of DOPS (Figure 11, Table 1). These results suggest that an asymmetrical lipid distribution of lipids in SLBs may be more prominent than commonly appreciated.

Integrity of the Final SLBs

Direct or indirect evidence for the presence of defects in SLBs has frequently been reported. Defects were attributed to the choice of the employed lipids[45] and their mixture,[9] the preparation of the liposomes, or the preparation of the solid support. The AFM images on glass samples in Figure 10, panels A and B, illustrate that the formation of ideal SLBs cannot be taken for granted and that the integrity of the final SLB needs to be validated.

The importance of defects in an SLB will depend on the envisaged application. The action of lipases (i.e., lipid digesting enzymes), for example, was proposed to be triggered by the presence of point-defects in the membrane.[37] A few such defects, even though they cover much less than one percent of the surface, may thus considerably affect the lipase activity. On the other hand, membranes that contain discontinuities that cover a few percent of the surface may be acceptable for other applications, such as protein adsorption studies.

Methods to Characterize Defects. Whatever the application, appropriate characterization methods are required to determine the density and the nature of the defects in the SLB. In this

(95) Hetzer, M.; Heinz, S.; Grage, S.; Bayerl, T. M. *Langmuir* **1998**, *14*, 982−984.
(96) Feng, Z. V.; Spurlin, T. A.; Gewirth, A. A. *Biophys. J.* **2005**, *88*, 2154−2164.
(97) Richter, R. P.; Lai Kee Him, J.; Tessier, B.; Tessier, C.; Brisson, A. *Biophys. J.* **2005**, *89*, 3372−3385.

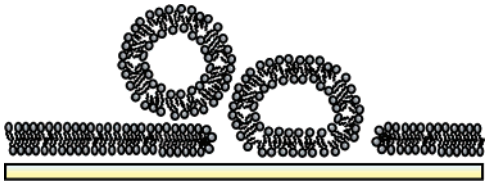

Figure 12. "Trapped" vesicle. The surface-bound vesicle is located sufficiently far away to remain unaffected by the bilayer edges though close enough to prevent the edge-induced rupture of other vesicles from solution. We propose that such an arrangement inhibits the further propagation of bilayer growth, leaving trapped vesicles as defects.

context, bulk methods such as QCM-D or ellipsometry can provide an overall characterization of the state of the SLB and the intermediates in the SLB-formation process. However, local defects that cover less than a few percent of the surface are difficult to detect. A similar statement holds true for fluorescence recovery after photobleaching (FRAP): the immobile fraction of molecules can rarely be determined to better than 1% and the detection of defects by fluorescence microscopy is limited by the optical resolution which exceeds the size of vesicles commonly used to form SLBs. Only AFM appears capable of directly visualizing defects such as single holes or intact vesicles with a resolution in the range of a few nanometers. We note, however, that a careful control of the imaging conditions is required in order to obtain reliable results, as AFM images may erroneously present ideal bilayers, due to imaging artifacts.[74,90]

Apart from the effects that are due to the preparation of lipids and support, one may wonder whether some of the above-discussed mechanisms, which underlie the SLB formation, could inherently be insufficient to generate a complete defect-free SLB. The AFM image in Figure 10C demonstrates that SLBs of high quality can be created on solid supports via the pathway in which vesicle rupture is triggered by the critical vesicular coverage. The same is considered likely for the pathway in which vesicles rupture individually, though conclusive evidence is yet lacking as our measurements were obstructed by contamination of the lipid sample.

Investigations by Rädler et al. gave rise to the idea that sliding-promoting (or "self-healing") surfaces should be ideal for the formation of defect-free SLBs.[1,64] However, we found SLBs of high quality under conditions where bilayer patches and vesicles were virtually pinned to the surface.[45] This indicates that the mobility of lipid assemblies is not strictly necessary to form close to ideal SLBs.

Notwithstanding the indications that close to ideal SLBs can be formed via the pathway of critical vesicular coverage, the spatial arrangement of surface bound vesicles and bilayer patches may in some cases inhibit further propagation of bilayer growth. For example, a vesicle that is located sufficiently distant from an edge to remain undisturbed may still prevent the encounter of other vesicles from the solution with the edge (Figure 12). Such a vesicle, trapped in a bilayer hole, will thus stop bilayer growth. From simple geometrical considerations, such an effect would be expected to be more pronounced for larger vesicles. Indeed, a considerable amount of residual vesicles has been reported in the case of larger vesicles on silica.[73] However, SLB formation from the smallest available vesicles seems to be largely devoid of this effect.

One application for which the quality of supported lipid membranes has been a matter of recurrent discussion may be mentioned here: the action of a few defects in the membrane potentially creates short circuits that disturb the measurement of ion transport through membranes or membrane-incorporated proteins by electrosensing methods.[31,98] Despite frequently reported problems with the electrical properties of surface-confined membranes, no study has to the best of the authors' knowledge been undertaken to characterize the nature of the defects in detail. To date it remains therefore unclear how far the supported lipid bilayers used in the relevant studies correspond to the quality of the bilayers that we have reported here. Combined approaches with AFM, QCM-D, and electrosensing methods may provide valuable insight, to what extent SLBs can constitute suitable membrane-mimics for the investigation of the channel properties of membrane proteins.

Conclusions and Perspectives

We have described recent advancements in understanding the process of self-organization that leads from small vesicles in aqueous solution to a solid-supported lipid bilayer. Systematic studies have allowed a number of mechanisms underlying SLB formation to be elucidated. Important insight in the involved interactions on the mesoscopic level has been gained, and parameters that are critical for the SLB-formation process have been identified.

AFM and QCM-D, the main techniques employed in our studies, provide topographical information at the nanometer level and quantitative physicochemical characterization of surface-confined lipid assemblies, respectively. The combination of both techniques on identical supports has allowed for a considerable improvement of our qualitative understanding of the SLB formation and opens up for a more quantitative assessment of this process.

AFM, QCM-D, other methods, such as ellipsometry, SPR, fluorescence, electrosensing methods, and combinations thereof, constitute now an established toolbox for the detailed characterization of SLB formation. These tools may help to elucidate a number of apparently simple, but still debated questions, including the orientation of the lipid layers after vesicle rupture as well as the role of vesicle fusion (Figure 3B) in the SLB-formation process. AFM has emerged as a unique tool to investigate defects in SLBs down to the nanometer level. A detailed and yet quick characterization of the quality of SLBs, however, remains a challenge.

The experimental approaches described here and the present understanding of the mechanisms involved in SLB formation on solid supports can easily be extended to more complex systems, such as the formation of SLBs from protein-containing liposomes as well as polymer-cushioned, tethered, or pore-spanning lipid bilayers. It is hoped that, thanks to the recent maturation in understanding the SLB-formation process, formerly rather "artistic" approaches to SLB formation will be replaced by a well-controlled technology, thereby extending the applicability of surface-confined lipid membranes.

We have pointed out the important role of the solid support in the SLB-formation process. The interaction between lipids and support appears complex and a good understanding on the molecular level is still lacking. It is intriguing that the solid support does not only affect the properties of the SLBs but also the two-dimensional organization of proteins bound to it.[90,97] Future work will need to elucidate the nature of the thin solvent layer that separates the lipid bilayer from the solid support[81,82] and its effect on the diffusion of lipid molecules in each of the two bilayer leaflets.

(98) Naumann, R.; Schiller, S. M.; Giess, F.; Grohe, B.; Hartmann, K. B.; Kärcher, I.; Köper, I.; Lübben, J.; Vasilev, K.; Knoll, W. *Langmuir* **2003**, *19*, 5435−5443.

Acknowledgment. The authors acknowledge the contributions of their collaborators in Bordeaux and A.R.B.'s former group in Groningen. We thank Aleš Benda (Heyrovský Institute, Prague, Czech Republic) for discussions and providing the glass slides (Figure 10). This research was supported by the Conseil Régional d'Aquitaine (France), the Fonds Européen de Développement Régional, and European Community Grant FP6-NMP4-CT2003-505868 "Nanocues".

LA052687C