In-Plane Correlations in a Polymer-Supported Lipid Membrane Measured by Off-Specular Neutron Scattering

Michael S. Jablin,[1] Mikhail Zhernenkov,[1] Boris P. Toperverg,[2] Manish Dubey,[1] Hillary L. Smith,[1] Ajay Vidyasagar,[3] Ryan Toomey,[3] Alan J. Hurd,[1] and Jaroslaw Majewski[1,*]

[1]*Lujan Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA*
[2]*Department of Physics, Ruhr-University Bochum, D-44780 Bochum, Germany, and Petersburg Nuclear Physics Institute, Gatchina, 188300 St. Petersburg, Russia*
[3]*Department of Chemical and Biomedical Engineering, University of South Florida, Tampa, Florida 33620, USA*

(Received 10 May 2010; published 28 March 2011)

Polymer-supported single lipid bilayers are models to study configurations of cell membranes. We used off-specular neutron scattering to quantify in-plane height-height correlations of interfacial fluctuations of such a lipid bilayer. As temperature decreased from 37 °C to 25 °C, the polymer swells and the polymer-supported lipid membrane deviates from its initially nearly planar structure. A correlation length characteristic of capillary waves changes from 30 μm at 37 °C to 11 μm at 25 °C, while the membrane bending rigidity remains roughly constant in this temperature range.

DOI: 10.1103/PhysRevLett.106.138101 PACS numbers: 87.16.dj, 61.05.fj, 87.16.dt

Lipid membranes play a critical role in living systems. They define the outer boundary of cells, host transmembrane proteins, mediate transport, facilitate intercellular communication, and respond to changes in the surrounding environment. Because they participate in a multitude of tasks, lipid membranes are necessarily complex. To facilitate physical and chemical characterization of specific biomembrane attributes, many model lipid membrane systems have been created such as supported single lipid membranes in a liquid environment [1]. Supported systems separate a bilayer from a solid substrate, easing incorporation of proteins, and therefore are an attractive model system to study protein-membrane interactions [2]. While the goal of model systems is to facilitate accurate characterization of biomembrane phenomena, even supported surrogate membranes frequently are constrained to a planar (or nearly planar) geometry. Therefore, they are unable to mimic the natural deformability and curvature of cellular membranes which both strongly influence and are influenced by the dynamics and localization of proteins, phase-separated domains, and various biological agents [3]. Thus, in addition to allowing the incorporation of proteins, a model system must permit the membrane structural freedom in order to accurately reproduce cellular membrane morphology. Neutron reflectometry (NR) is a unique tool that can determine both in- and out-of-plane structure of layered systems without disturbing them [4]. Over the past several decades, NR has developed as a standard tool to characterize the out-of-plane structure of lipid membranes in many different environments by analyzing the specular reflection (SR) [5]. Off-specular scattering (OSS) is frequently ignored despite the fact that it is often simultaneously collected with the SR and may be used to elucidate in-plane structure [6].

Previous analysis of the SR from a polymer-cushioned membrane describes a system that allows both efficient separation of the membrane from a solid support and *in situ* control of its out-of-plane deviations [7]. This polymer-membrane system provides a unique opportunity to controllably modify a model membrane's morphology *in situ*, facilitating future investigations of membrane mechanics and revealing how membrane curvature and topography are affected by membrane composition and various external cues. An initial investigation is presented here.

The polymer cushion was composed of poly(*N*-isopropylacrylamide) copolymerized with 3 mol % of methacroylbenzophenone (MaBP) to form poly(NIPAAm-co-MaBP) [8]. A bilayer of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was deposited via the Langmuir-Blodgett-Schaefer technique at a surface pressure of 40 mN/m at 37 °C onto the polymer cushion [7]. NR experiments were performed on the reflectometer (SPEAR) at the Los Alamos Neutron Science Center [9]. SR and OSS from the polymer-membrane system were measured simultaneously at several temperatures between 25 °C and 37 °C to assess the membrane's three-dimensional structure. A D_2O subphase was used to provide contrast between the hydrogenated membrane and the hydrated polymer. Although it has been shown that dynamic and static fluctuations can be distinguished using inelastic neutron scattering techniques [10], with our NR measurements (no energy transfer discrimination and time averaged over ~1 h), it is not possible to directly probe the dynamics of fluctuations or assess fast membrane kinetics. The SR data were compared to the model reflectivity using a standard routine [7] which provides the scattering length density profile.

Scattering information from the polymer-membrane system was collected, and OSS was analyzed quantitatively to determine the in-plane morphology of the membrane. For the SR, the momentum transfer vector Q (the difference between the outgoing k_f and incoming k_i wave vectors) is normal to the sample surface, $Q = Q_z$, whereas for OSS, Q also contains a component parallel to this surface, $Q = Q_z + Q_\parallel$. This component probes the in-plane structure of the system (Fig. 1). OSS can be described accurately within the framework of the distorted wave Born approximation (DWBA) formulated by Sinha *et al.* for x-ray scattering from rough interfaces [11], and further developed by Toperverg *et al.* [12–15] and Zabel *et al.* [16] for neutron scattering from various lateral structures. DWBA attributes OSS to local deviations of the scattering length density (SLD) from its mean value laterally averaged over the coherence length of the incident neutrons. Unlike the commonly used Born approximation (BA), the incoming and scattered wave distortions caused by reflection from and refraction into the mean SLD potential are accounted for exactly by DWBA. Therefore, while predictions of DWBA and BA are consistent at high Q values, only DWBA accurately models scattering near the total reflection region [15], where most of our OSS signal is recorded. Near this region, OSS is enhanced substantially due to constructive interference between incoming and outgoing waves (transmitted and reflected, respectively) from the mean SLD potential. This enhancement constitutes the Yoneda effect well described by DWBA [17]. At very small incident angles, $<1°$, the lateral projection of the neutron's coherence length is extended significantly, providing access to in-plane height-height correlation lengths from about 100 nm up to the submillimeter scale. Note that, apart from the main liquid to gel phase transition at 41 °C (not probed in this study), as temperature is decreased, the phase of a DPPC membrane transitions from ripples ($P_{\beta'}$) to lamellar-tilted chains ($L_{\beta'}$) at 33 °C. Characteristic in-plane length scales in the range of a few tens of nm have been observed for both the aforementioned phases [18], and, therefore, these phenomena are not probed by the OSS. Apart from these phase changes, membrane morphology can also be affected by thermal fluctuations which are known to excite capillary waves and bending modes on liquid surfaces [19,20].

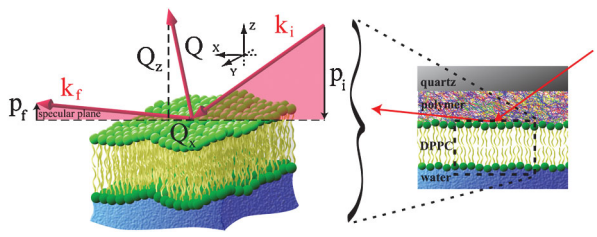

FIG. 1 (color). Geometry for an OSS event. Neutrons penetrate the quartz substrate and polymer cushion to reach the buried membrane (right). The thickness of the bilayer (38 Å) as compared to the polymer (200–900 Å) has been greatly exaggerated for the sake of clarity. If the incident and outgoing angles are unequal, then the momentum transfer vector Q has components perpendicular Q_z and parallel Q_x to the scattering interface.

OSS intensity maps were simulated by applying an in-plane height-height correlation function in conjunction with DWBA. These theoretical intensity maps were compared to the experimental data to determine the in-plane structure of the membrane. A reasonable description of the OSS data was achieved assuming that the system is dominated by thermally excited capillary waves and bending modes, which are reflected in a height-height correlation function with two length scales (ξ_1 and ξ_2). Our previous measurements (not shown here) did not detect any significant OSS from the pure polymer in a D_2O subphase, and, therefore, we assume negligible in-plane fluctuations due to the polymer in the present study. The membrane can be regarded as a single entity, and, therefore, conformal fluctuations on both membrane surfaces were assumed. We also assume no lipid domains in the membrane since this is a single component system studied below the liquid-gel transition temperature. In the case of weak fluctuations, the bending modes are decoupled from capillary waves such that the in-plane height-height correlation function can be approximated as

$$\langle |u(Q_\parallel)|^2 \rangle = \frac{\langle |u(0)|^2 \rangle}{1 + (Q_\parallel \xi_1)^2 + (Q_\parallel \xi_2)^4}, \qquad (1)$$

where $Q_\parallel = \sqrt{Q_x^2 + Q_y^2}$, ξ_1 and ξ_2 are in-plane correlation lengths. Using equipartition of energy, one finds $2\pi\xi_1^2\sigma^2 = \langle |u(0)|^2 \rangle \ln(\xi_1/\xi_2)^2$, where σ is the root mean square (rms) roughness [9,17]. The length $\xi_1 \propto \sqrt{\gamma/K}$ is determined by γ, the membrane surface tension, and K which is related to the second derivative of the substrate-polymer-membrane interaction potential. The second correlation length, ξ_2, is proportional to $\sqrt[4]{\kappa/K}$, where κ is the membrane bending rigidity. The ξ_2 term dominates when γ approaches zero (membrane equilibrium) and also may play a role at high values of $Q_\parallel$ changing the decay rate of correlations. Because our resolution in the direction normal to the reflection plane (Fig. 1) is totally relaxed, Eq. (1) should be integrated with respect to Q_y, and, therefore, the form factor of OSS is a function of only Q_x. Such integration substantially changes the asymptotic behavior of the form factor which at high Q_x decays slower than Eq. (1). Resolution effects within the reflection plane were accurately taken into account in order to normalize off-specular scattering to the specular reflection. Using DWBA and the previously determined transverse structure of the membrane [7], we simulated OSS intensity maps and performed quantitative comparisons of the experimental data and simulations. Upon reduction of temperature, OSS showed the most pronounced changes in the Yoneda wings. Therefore,

the measured intensity distribution is compared with the DWBA simulations in p_f vs p_i space (p_f and p_i are the perpendicular components of k_f and k_i, respectively, Fig. 1) where the Yoneda wings are almost parallel to the axes, simplifying the analysis. This approach allows determination of the in-plane height-height correlation lengths at each interface of the system. The polymer-bilayer and bilayer-subphase interfacial roughnesses are significantly larger than the roughness on any of the other interfaces. Therefore, the total OSS signal can be attributed primarily to the bilayer interfaces. In-plane parameters (ξ_1 and ξ_2) for the two bilayer interfaces are similar and conformal because of strong interleaflet coupling [21].

First, the in- and out-of-plane structure of the DPPC-polymer system was determined at 37 °C. The measured SR and OSS are presented in Fig. 2(a). Figure 2(b) shows the SR data, the corresponding fit, and its real-space interpretation. Using DWBA, the scattering map was simulated [Fig. 2(c)] and compared to the measured scattering. In particular, the Yoneda wings bounded by the dashed red lines in Figs. 2(a) and 2(c) are compared quantitatively [Fig. 2(d)]. It was determined that the membrane had in-plane height-height correlation lengths of 30 and ~1 μm (ξ_1 and ξ_2, respectively) and rms interfacial roughness of 35 Å at 37 °C. ξ_1 is related to membrane capillary waves. ξ_2 corresponds to a thermally excited bending mode and cannot be determined to better precision because the $(Q_\parallel \xi_2)^4$ term in Eq. (1) is a small fraction of the $(Q_\parallel \xi_1)^2$ term, within the accessible $Q_\parallel$ range [9]. The out-of-plane membrane variation is most likely due to thermally excited capillary waves and bending mode.

Next, the structure of the polymer-membrane system was characterized at 25 °C when the membrane is lifted from the rigid substrate by the swollen polymer. The measured [Fig. 3(a)] and theoretical [Fig. 3(c)] scattering are compared; a cut along a Yoneda wing is presented in Fig. 3(d). Figure 3(b) shows the SR data along with the corresponding fit based on the simplest model of physical relevance. As opposed to the nearly planar geometry of the membrane at 37 °C, ξ_1 decreased to 11 μm, ξ_2 remained approximately 1 μm, and the membrane rms interfacial roughness increased to ~105 Å at 25 °C. The more distorted geometry of the membrane is represented by the broadening and decreasing depth of the dip in SLD corresponding to the membrane [compare the insets of Figs. 2(b) and 3(b)]. ξ_2 is proportional to the power 0.25 of the bending rigidity of the membrane which is similar within the measured temperature range between 25 °C and 37 °C [22]. The increased rms roughness and decreased ξ_1 are a manifestation of the relieved mechanical constraint due to the cushion swelling. This results in the reduction of membrane surface tension when nearly aqueous phases are present on both sides of the membrane. The polymer volume fraction and bending rigidity in the proximity of the membrane are suppressed upon swelling, and, therefore, the polymer-membrane interaction only weakly contributes to membrane dynamics.

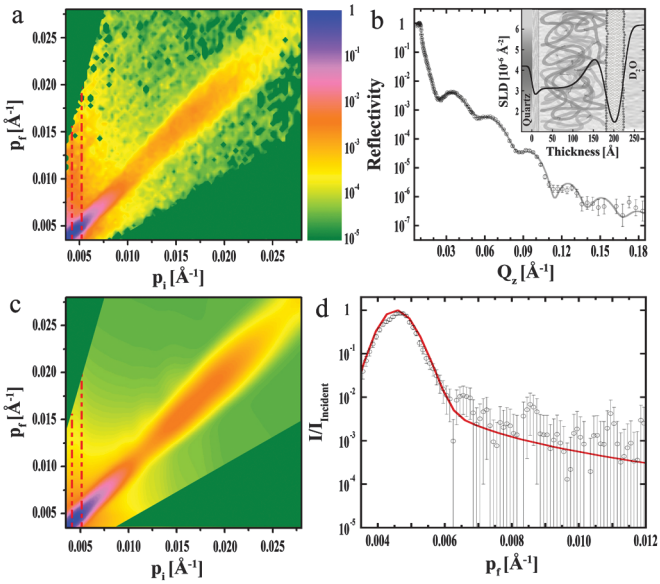

FIG. 2 (color). Analysis of the neutron scattering from the polymer-DPPC system at 37 °C. Panel (a) shows the measured SR (along the diagonal from lower left to upper right) and surrounding OSS (intensity is normalized by the incident beam). Panel (b) depicts the SR data (open circles), fit (gray line), error bars (1 standard deviation), and corresponding real-space interpretation (SLD distribution shown in inset). Panel (c) depicts the theoretical scattering map, convoluted with the experimental resolution function and accounting for experimental background. The intensity scale is common to (a) and (c). No scattering was collected in the upper left or lower right corners. Panel (d) compares the measured (open circles) and theoretical (red line) OSS along the Yoneda wing [intensity integrated along p_i in the region inside the dashed red lines (a) and (c)]. Error bars indicate 1 standard deviation.

The measured membrane in-plane height-height correlation lengths cannot be explained by conformation of the membrane to corrugations in the surface of the underlying polymer. When a constrained polymer swells, it experiences a biaxial compression which can induce surface undulations. It has been theorized that the in-plane wavelength of these corrugations is on the order of the polymer film thickness [23]. In our study the polymer thickness is ~900 Å at 25 °C, and, therefore, the expected corrugation wavelength is of order 1000 Å, inconsistent with the measured membrane in-plane height-height correlation lengths of 11 and 1 μm.

A polymer-supported model membrane facilitates controlled *in situ* manipulation of membrane morphology. Analysis of both the SR and OSS from the system allowed quantification of the membrane in- and out-of-plane structure as the polymer swelled. At 37 °C when the polymer cushion was collapsed, only weak OSS was detected, suggesting that the membrane was nearly planar [in-plane

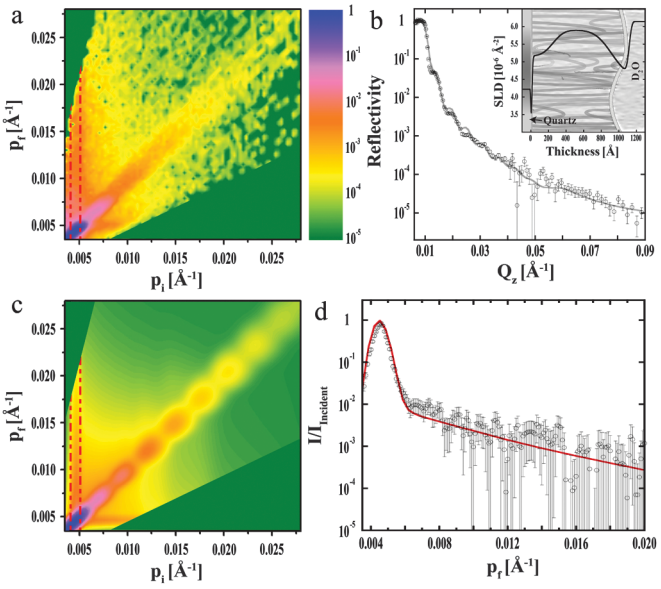

FIG. 3 (color). Analysis of the neutron scattering from the polymer-membrane system at 25 °C. Panels (a) and (c) depict the measured and theoretical scattering maps, respectively, with a common intensity scale. Panel (b) illustrates the SR data (open circles), fit (gray line), error bars (1 standard deviation), and corresponding real-space interpretation (SLD distribution shown in inset). Panel (d) compares the measured (open circles) and theoretical (red line) OSS along a Yoneda wing. Error bars indicate 1 standard deviation.

height-height correlation lengths of 30 μm (ξ_1) and $\sim$1 μm (ξ_2), rms roughness of $\sim$35 Å]. Cooling the system to 25 °C caused a dramatic enhancement of the OSS signal, unambiguously indicating the development of a short in-plane undulation of the membrane ($\xi_1 = 11$ μm, $\xi_2 \sim 1$ μm, rms roughness of about 105 Å). ξ_1 corresponds to thermally excited capillary waves and suggests that the membrane is flat at 37 °C and significantly more distorted at 25 °C [24]. ξ_2 was independent of temperature and is attributed to membrane bending mode fluctuations.

Analysis of OSS can provide insight into a wide range of in-plane phenomena *in situ* to understand the complex behavior of cellular membranes: protein transport and docking, lipid segregation into ordered domains, and modification of bulk membrane elastic properties due to membrane constituents and external stimuli. The presented polymer-membrane system successfully mimics the complexity of cellular membrane morphology and offers numerous exciting future opportunities to investigate how membrane composition and various external biological agents such as toxins, viruses, and other pathogens, affect in- and out-of-plane membrane structure.

This work benefited from the use of the Lujan Neutron Scattering Center at LANSCE funded by the DOE Office of Basic Energy Sciences and Los Alamos National Laboratory under DOE Contract No. DE-AC52-06NA25396. This work was partially supported by the Camille and Henry Dreyfus Foundation and a National Science Foundation CAREER Grant DMR-0645574. We thank Professor Efim Kats (ILL, Grenoble, France) for enlightening discussions.

*jarek@lanl.gov

[1] E. Sackmann, Science **271**, 43 (1996).
[2] D. J. McGillivray *et al.*, Biophys. J. **96**, 1547 (2009).
[3] J. T. Groves, Annu. Rev. Phys. Chem. **58**, 697 (2007).
[4] V. Lauter-Pasyuk *et al.*, Phys. Rev. Lett. **89**, 167203 (2002).
[5] S. Krueger, Curr. Opin. Colloid Interface Sci. **6**, 111 (2001).
[6] R. Pynn, Phys. Rev. B **45**, 602 (1992).
[7] H. L. Smith *et al.*, Phys. Rev. Lett. **102**, 228102 (2009).
[8] A. Vidyasagar, J. Majewski, and R. Toomey, Macromolecules **41**, 919 (2008).
[9] See supplemental material at http://link.aps.org/supplemental/10.1103/PhysRevLett.106.138101 for a description of SPEAR, data collection and presentation, estimation of the Q_x range, and further explanation of Eq. (1).
[10] M. C. Rheinstadter, W. Haussler, and T. Salditt, Phys. Rev. Lett. **97**, 048103 (2006).
[11] S. K. Sinha *et al.*, Phys. Rev. B **38**, 2297 (1988).
[12] B. P. Toperverg, Physica (Amsterdam) **297B**, 160 (2001).
[13] B. P. Toperverg, Appl. Phys. A **74**, s1560 (2002).
[14] B. P. Toperverg *et al.*, Physica (Amsterdam) **297B**, 169 (2001).
[15] B. P. Toperverg, O. Scharpf, and I. S. Anderson, Physica (Amsterdam) **276-278B**, 954 (2000).
[16] H. Zabel, K. Theis-Brohl, and B. P. Toperverg, *Handbook of Magnetism and Advanced Magnetic Materials* (Wiley, New York, 2007).
[17] M. Tolan, *X-Ray Scattering from Soft-Matter Thin Films* (Springer, Heidelberg, 1999).
[18] S. Karmakar, V. A. Raghunathan, and S. Mayor, J. Phys. Condens. Matter **17**, S1177 (2005).
[19] R. Laudon, *Surface Excitations* (North-Holland, Amsterdam, 1984).
[20] J. C. Earnshaw, *Fluid Interfacial Phenomena* (John Wiley & Sons Ltd., New York, 1986).
[21] E. B. Watkins *et al.*, Phys. Rev. Lett. **102**, 238101 (2009).
[22] D. H. Boal, *Mechanics of the Cell* (Cambridge University Press, Cambridge, England, 2002).
[23] T. Tanaka *et al.*, Nature (London) **325**, 796 (1987).
[24] Using κ for a DPPC membrane [22] and ξ_1 and ξ_2 at each temperature, surface tension can be estimated roughly to be of order 10^{-5} J/m^2 at 25 °C and 10^{-4} J/m^2 at 37 °C.