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Polymer Stabilized Lipid Membranes: Langmuir Monolayers

A.P. Siegel and C.A. Naumann

Abstract Polymer-tethered membranes combine fascinating structural, dynamic, and viscoelastic properties. Many important insights into these peculiar supramolecular systems can be obtained from studies on polymer-tethered monolayers. This chapter discusses recent experimental findings on polymer-tethered monolayers at the air–water interface. In particular, Langmuir monolayers which are comprised of pure lipopolymers and of binary phospholipid–lipopolymer mixtures are considered. Thermodynamic data as well as structural data based on a host of experimental techniques including X-ray and neutron reflectrometry, infrared reflection absorption spectroscopy, and sum frequency generation spectroscopy provide information on how lipopolymers organize at the air–water interface. This information is followed by a review of the viscoelastic properties of these systems, including the remarkable gelation transition that can be observed in lipopolymers and mixed phospholipid–lipopolymer monolayers. The diffusion properties are also discussed at length, and show that lipid diffusivity is critically dependent on the strength of inter-polymer interactions of lipopolymers.

Keywords Diffusion, Langmuir monolayer, Lipopolymer, Phospholipid, Viscoelasticity

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1 Introduction

Recent advances in the understanding of assembly and disassembly of biomolecules have led to the design of polymer-tethered membranes. One particularly attractive design of polymer-tethered membranes is based on phospholipid–lipopolymer mixtures. In phospholipid–lipopolymer mixed monolayers, the tethering concentration can be adjusted accurately through the molar concentration of lipopolymers. Importantly, by changing the lipopolymer–lipid mixing ratio, polymer-tethered membranes can be obtained with a wide range of fascinating structural and dynamic properties. Because many of these intriguing properties of polymer-tethered membranes can be observed on Langmuir monolayers, the current contribution summarizes recent advances in the design and characterization of lipopolymer-based polymer-tethered monolayers at the air–water interface.

Lipopolymers and phospholipids are amphiphiles with distinct structural properties. While the hydrophobic moieties show great similarities, the hydrophilic headgroups are structurally distinct. Most importantly, unlike phospholipids, the hydrophilic moiety of lipopolymers consists of a comparably bulky polymer chain, which is end-tethered through a hydrophilic linker to the two-pronged lipid tail of the molecule. The lipid/polymer hybrid character of lipopolymers results in unique molecular properties, which also critically determine the properties in lipopolymer–lipid mixed monolayers. Because the study of lipopolymers at the air–water interface provides important clues about properties of lipopolymer–lipid mixed monolayers, the first half of this chapter (Sect. 2) summarizes reported experimental results obtained from Langmuir monolayers of lipopolymers. Section 2.1 discusses film balance and neutron reflectometry experiments on lipopolymer monolayers, which have provided important structural information. Insight into the fascinating viscoelastic properties of lipopolymer monolayers is given in Sect. 2.2, where recent interfacial rheology experiments are described. Section 2.3 addresses the lateral diffusion properties of lipopolymers at the air–water interface, which offer valuable information about the diffusion properties of polymer-tethered membranes. The second half of this chapter (Sect. 3) focuses on experimental findings obtained from lipopolymer–phospholipid mixed monolayers at the air–water interface. Section 3.1 contains an overview over structural properties of such mixed Langmuir monolayers. Section 3.2 discusses corresponding viscoelastic properties. Finally, Sect. 3.3 summarizes the key data from lipid lateral diffusion studies in lipopolymer–phospholipid mixed monolayers.

2 Lipopolymer Langmuir Monolayers

2.1 Structural Properties

The structural properties of lipopolymers at the air–water interface have been traditionally explored using film balance techniques and neutron/X-ray reflectometry. The film balance method is an attractive tool to study the assembly of lipopolymers at the air–water interface as a function of molecular surface density (area per molecule). In this case, Langmuir monolayers of lipopolymers are constructed by simply adding these amphiphiles to the air–water interface. Here one or two movable barriers are employed to compress or expand the monolayer. The resulting changes in surface density of amphiphiles at the air–water interface are monitored using a film pressure sensor. This method provides valuable thermodynamic information because the pressure–area $(\pi - A)$ isotherm of a Langmuir monolayer can be determined. Complementary, neutron/X-ray reflectometry allows insight into the scattering length density profile of the monolayer perpendicular to the air–water interface with high resolution.

Baekmark et al. first investigated the pressure–area isotherms of lipopolymers at the air–water interface using lipopolymers with poly(ethylene glycol) (PEG) covalently linked to a phospholipid 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) [1]. Figure 1 contains structural information of widely studied lipopolymers together with corresponding structures of some phospholipids. The three main types of polymeric moieties of lipopolymers are poly(ethylene glycol) (PEG) (named by their approximate weight) linked to phospholipids and poly(2-methyl-2-oxazoline)$_n$ (PMOx$_n$), and poly(2-ethyl-2-oxazoline)$_n$ (PEOx$_n$) linked to di-octadecanoyl-glycerol (DiC$_{18}$). Figure 2 illustrates a typical pressure–area-isotherm of the lipopolymer DSPE–PEG2000.

Figure 2 shows that the $\pi - A$ isotherm for DSPE–PEG2000 is characterized by two plateaus. In this figure, the plateaus, or transitions, are labeled π_{low} and π_{high}. By following scaling arguments of polymer physics, Baekmark et al. originally interpreted these plateau regions as "pancake to mushroom" transitions for π_{low} and "mushroom-to-brush" transitions for π_{high} [1]. Interestingly, monolayer experiments of polystyrene–poly(ethylene oxide) diblock copolymers reveal identical low-pressure transition behavior but no transition at higher film pressure [3]. In that case, it was argued that in the low-pressure regime, the PEG chains desorb from the air–water interface in a temperature-independent fashion, which also agrees with the desorption properties of pure PEG at the air–water interface [4, 5].

Several experimental results have been reported which show that the high-pressure transition is qualitatively different to the low pressure counterpart in that it exhibits properties of a first order phase transition. For example, it was shown that the pressure of the high-pressure transition, π_{high}, is dependent on temperature, thus meeting an important criterion of a first order transition [2, 6]. Figure 3 displays a close-up of the high-pressure transition region of $\pi - A$ isotherms for DSPE–PEG2000 taken at different temperatures, showing very clearly that the high

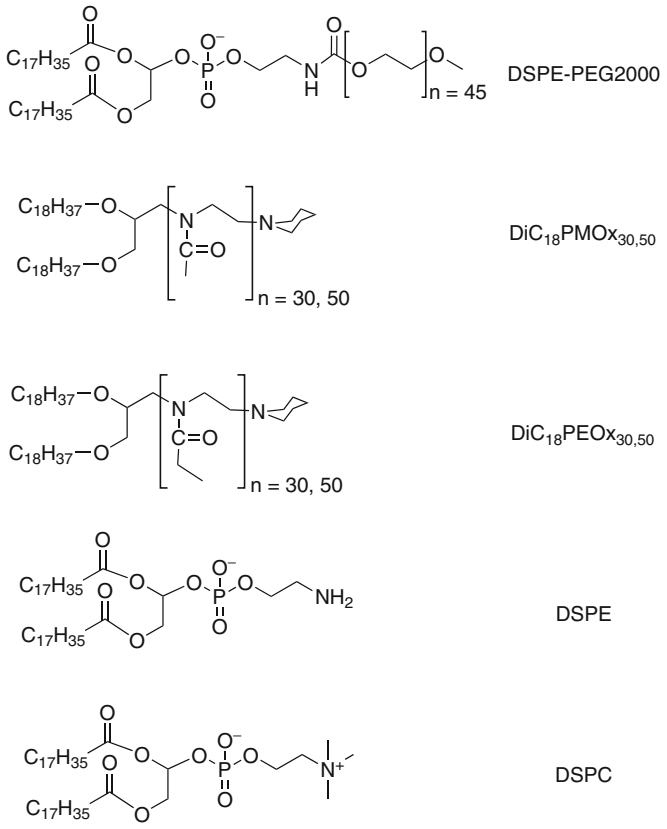

Fig. 1 Commonly investigated lipopolymers and lipids: 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-[poly(ethylene glycol)$_{45}$] (DSPE-PEG2000), 1,2-dioctadecanoyl-*sn*-glycero-[poly((2-methyl-2-oxazoline)$_n$)] ($DiC_{18}PMOx_{30,50}$), 1,2-dioctadecanoyl-*sn*-glycero-[poly((2-ethyl-2-oxazoline)$_n$)] ($DiC_{18}PEOx_{30,50}$), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC)

pressure transition is temperature dependent. The temperature dependence of the high-pressure transition region has also been shown on lipopolymer systems involving $DiC_{18}PMOx$ and $DiC_{18}PEOx$ [6].

To obtain more insight into the nature of the high-pressure transition, a series of film balance experiments were conducted, where the impact of the lipid and polymer moieties on this transition were investigated systematically. For example, $\pi - A$ isotherms were measured for PEG lipopolymers with saturated lipid tails of varying lengths [2]. Interestingly enough, the C_{16} chain DPPE–PEG2000 displayed a $10\,\mathrm{mN\,m^{-1}}$ higher π_{high} relative to the C_{18} chain DSPE–PEG2000, and the C_{14} chain DMPE–PEG2000 never displayed π_{high} at all, thus indicating a sensitive relationship between acyl chain length of the lipid moiety and π_{high}. In order to explore further the importance of the lipid tail to the high-pressure transition,

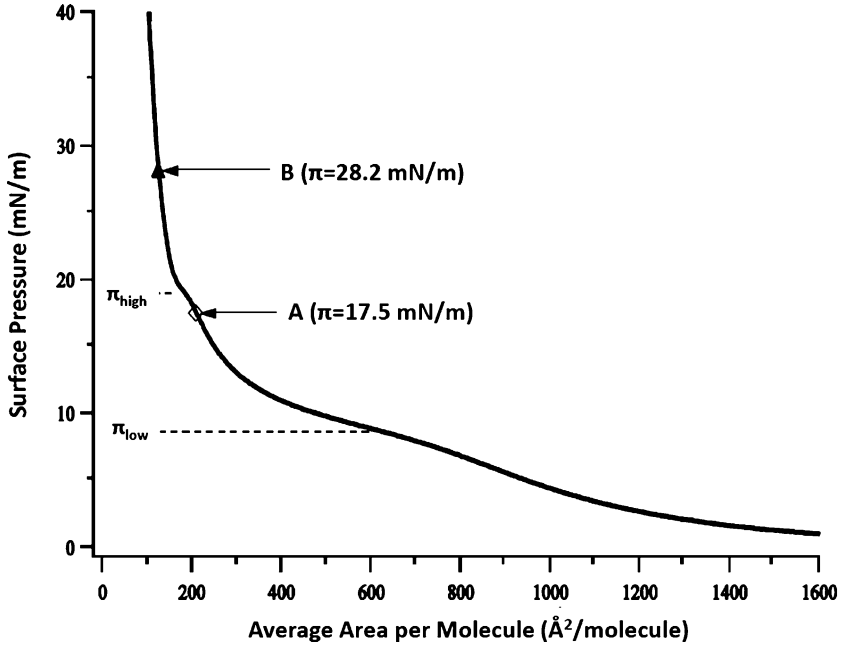

Fig. 2 Pressure–area isotherm of DSPE–PEG2000 at room temperature showing two plateaus indicative of a low-pressure transition (π_{low}) and a high-pressure transition (π_{high}). The points A and B represent film pressures where interfacial rheology experiments were conducted [2] (reproduced with permission from the American Chemical Society)

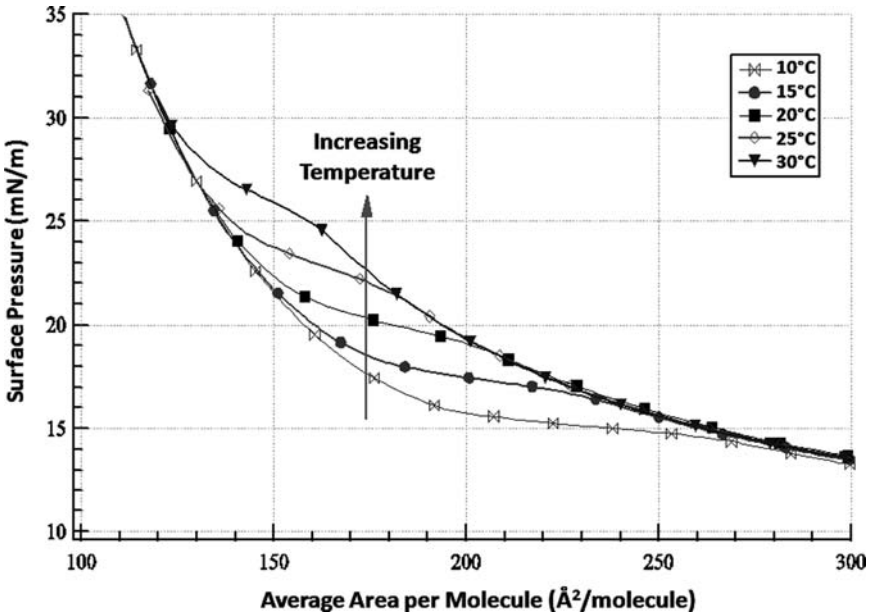

Fig. 3 Close-up of pressure–area isotherms of DSPE–PEG2000 near the high pressure transition at different temperatures [2] (reproduced with permission from the American Chemical Society)

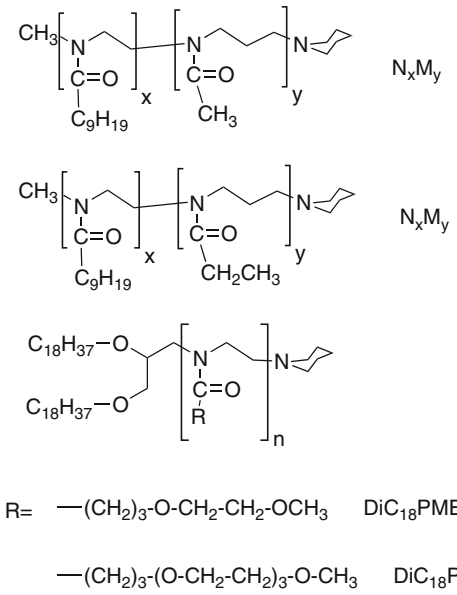

Fig. 4 Molecular structure of diblock copolymers and side-chain modified lipopolymers whose pressure–area isotherms are presented in Figs. 6 and 7, respectively. Diblock copolymers are poly(2-*n*-nonyl)-poly(2-methyl or 2-ethyl-2-oxazoline) (N_xE_y and N_xM_y), where x and y denote the block sizes of the hydrophobic nonyl and hydrophilic oxazoline blocks. Side-chain modified lipopolymers, which contain short oligo-EG sidechains in each monomer of the lipopolymer to create a bottle-brush-like structure, are di-octadecanoyl-glycerol 2-(3′-methoxymonoethylene glycol)propyl-2-oxazoline ($DiC_{18}MEGOx_n$) and di-octadecanoyl-glycerol 2-(3′-methoxytriethyleneglycol)propyl-2-oxazoline ($DiC_{18}TEGOx_n$) (adapted from [7, 8])

pressure isotherms were undertaken with the partially unsaturated 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine–PEG2000 (DOPE–PEG2000), and compared to DSPE–PEG2000 [7]. No high pressure transition was found in the pressure–area isotherm of the unsaturated-lipid lipopolymer Langmuir monolayer. This finding is interesting because DOPE is known to have a substantially lower gel–liquid phase transition temperature than the saturated DPPE and DSPE. Another interesting study compared the pressure–area isotherms of lipopolymers and diblock copolymers, where the diblock copolymer, while containing a lipophilic moiety, did not contain the geometry of two acyl chains attached to a glycerol backbone [7]. The copolymers used, poly(2-*n*-nonyl)-poly(2-methyl or 2-ethyl-2-oxazoline) (N_xM_y or N_xE_y), are shown in Fig. 4. By contrast to lipopolymers, pressure–area isotherms of diblock copolymers in general, and of this type in particular, do not display any high pressure phase transition, thus indicating the crucial role of the saturated lipid moiety for the high-pressure transition to occur. In addition, the ethyloxazoline copolymers, N_xE_y, also show the low pressure transition which is attributed to polymers desorbing from the surface.

Triblock copolymers consisting of 135–800 monomers of PEG end capped with $C_{12}H_{25}$ or $C_{16}H_{33}$ lipid moieties have also been investigated [9]. Upon compression, $\pi - A$ isotherms of $C_{12}H_{25}{-}PEG_{135}{-}C_{12}H_{25}$, for example, exhibit not only the first transition, π_{low}, but apparently also a second transition at π_{high}. With a molecular weight of about 6,000, $C_{12}H_{25}{-}PEG_{135}{-}C_{12}H_{25}$ is fairly similar to DSPE–PEG5000, the results of which are reported above, except the lipid tails are on either end of the polymer from each other, instead of both together on one end. However, unlike lipopolymers, these molecules are not stable above the high pressure transition; if left on a trough for up to 12 h the pressure gradually decreases, indicating desorption of the triblock into the subphase [9]. Moreover, while the $\pi - A$ isotherms show the second plateau on compression of the monolayer, no similar plateaus are seen on expansion; rather the isotherm shows that some fraction of molecules are desorbed at the higher pressure. Finally, when the rate of compression was varied, the apparent π_{high} varied, with the fastest compressing monolayers undergoing the transition at the highest pressure. The low pressure transition, in contrast, displayed no changing behavior on compression and expansion or on varying rates of compression. Changing the rate of compression for lipopolymers, by contrast, does not change the pressure at which a plateau is reached, although compressing lipopolymers quickly may change the initial reading of the area per lipopolymer at which the plateau is reached until the system equilibrates [6].

Collecting all the experimental evidence obtained so far on the high pressure transition in $\pi - A$ isotherms leads to indications that this is a first order phase transition strongly related to the existence of dual lipid tails. Furthermore, the less pronounced the plateau of the high-pressure transition, the lower the gel–liquid phase transition temperature of the corresponding lipid (without attached polymer chain). This correlation suggests that there is a critical relationship between high-pressure transition and the lipids' ability to exhibit acyl chain condensations. And, in fact, in 1999 the high-pressure transition was described as an acyl chain condensation and not, as had been earlier suggested, as a mushroom-brush transition [6, 10]. The experimental evidence provided for this conclusion was based on infrared reflection absorption spectroscopy (IRRAS) data taken on lipopolymers at the air–water interface below, at, and above π_{high} (and at temperatures found to optimize the signal to noise ratio). In this experimental set-up, an infrared beam is reflected off the monolayer at the water surface and the absorbances of these reflections are recorded. After subtracting for the absorbance of a pure water surface, the data are Fourier-transformed into normalized infrared spectra, showing, of particular interest, the symmetric and asymmetric CH_2 stretches (the 2,900 cm^{-1} range) and also the C–O–C stretches (around 1,150 cm^{-1}). IRRAS data were obtained on DSPE–PEG2000, PMOx and PEOx systems [6] and on DSPE–PEG5000 and partially deuterated DSPE–PEG5000 [10]. The IRRAS data showed two trends. First, the maximum reflection-absorbance for both the symmetric and asymmetric CH_2 vibrations shifted to smaller wavenumbers as the monolayers were compressed, and this shift was most dramatic during the compression associated with π_{high}. The decrease seen was 4–7 times stronger than would be expected from simply compressing the monolayer, and was seen rather as an indicator that the CH_2 groups become more

ordered during the transition. As noted by Baekmark et al., the absolute values for these CH_2 stretches are quite similar to IRRAS data on liquid condensed phospholipid monolayers [6]. The IRRAS spectra on DSPE–PEG5000 with the lipid moiety containing either hydrogen or deuterium were particularly informative because by subtracting the two spectra it was possible to show that this decrease in the maximum absorbance trend seen on all the lipopolymer systems studied was due to the CH_2 stretches of the lipid, and not the CH_2 stretches in the polymers [10]. Second, the C–O–C stretches for the PEG IRRAS spectra above and below the transition pressure contained a broad band shape, indicative of an amorphous, and not ordered state. Consequently, these authors concluded from their data that the high pressure transition involves a dramatic ordering in the conformation of the acyl chains without an accompanying ordering of the polymeric moiety.

While the PEG and polyoxazoline lipopolymers all showed increased acyl chain order upon compression, many differences appear in the manner of their transitions, suggesting a fascinating interplay between polymer and lipid moieties in the assembly of lipopolymers at the air–water interface. To explore the influence of the polymer moiety on $\pi - A$ isotherms, several film balance experiments have been conducted where the polymer moiety of lipopolymers was modified systematically. Figure 5 shows a close-up around π_{high} of a study on DSPE–PEG lipopolymers of different chain length, and thus of different molecular weight [11]. The $\pi - A$ isotherms suggest a qualitative difference between the short-chain DSPE–PEG750 and DSPE–PEG1000 on one hand and DSPE–PEG2000, DSPE–PEG3000, and DSPE–PEG5000 on the other. For example, there is a notably larger shift in the area per molecule and transition pressure when comparing DSPE–PEG1000 (22

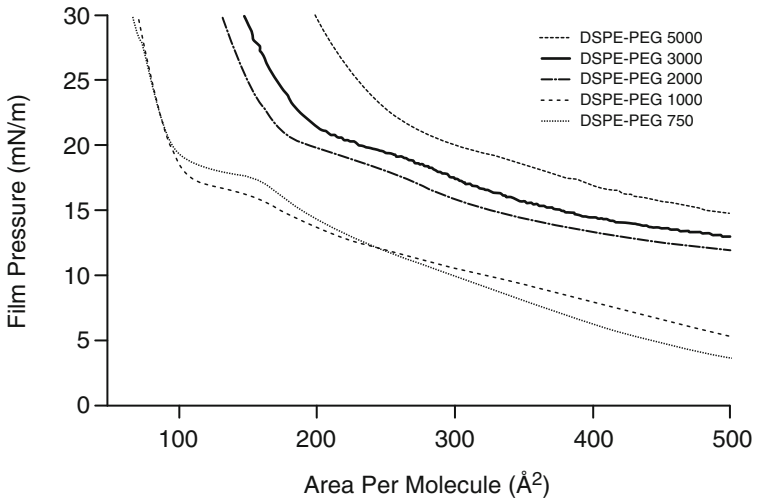

Fig. 5 Pressure–area isotherms of DSPE–PEG750, DSPE–PEG1000, DSPE–PEG2000, DSPE–PEG3000 and DSPE–PEG5000 around the high pressure transition [11] (reproduced with permission from the American Chemical Society)

monomers) and DSPE–PEG2000 (45 monomers), vs DSPE–PEG2000 and DSPE–PEG3000 (67 monomers). Based on these data, it was proposed that the PEG chains of the three longer chain lipopolymers are in a coiled, but slightly elongated conformation close to the high pressure transition, whereas those of the two shorter chain lipopolymers resemble a rodlike, fibrillar structure [11].

There is also experimental evidence that the nature of the polymer moiety may have a significant effect on the high-pressure transition as well. Comparisons of film balance experiments on $DiC_{18}PMOx_{35}$, $DiC_{18}PEOx_{31}$, and DSPE–PEG2000, which all have the same length lipid moiety, show that the high-pressure transition varied significantly between the polyoxazoline and PEG systems, with the polyoxazoline systems undergoing the transition at a much higher surface pressure. In addition, film balance experiments were performed comparing $DiC_{18}PEOx_{31}$ and dioctadecylamine [poly(ethyloxazoline)$_{35}$] $(DODA - PEOx_{35})$, which is nearly the same as $DiC_{18}PEOx_{31}$ other than the fact that the 18 C chains are connected to the polymer through an amine group instead of a glycerol group. The high-pressure transition varied significantly, with the amine system undergoing a transition nearly 10 mN m^{-1} again higher and about 15% more compressed than the $DiC_{18}PEOx_{31}$ system, so that $\pi_{high}{}^{PEG2000} < \pi_{high}{}^{PMOx35} < \pi_{high}{}^{PEOx31} < \pi_{high}{}^{DODA35}$ [12]. Overall, these data indicate that the location of the high-pressure transition depends on the subtle interplay of several factors, including the polymer structure and molecular weight and the nature of the hydrophobic anchor.

Using a synthetic approach to understanding the nature of the acyl chain condensation, a number of novel oxazoline lipopolymers were synthesized with the same lipid backbone, two 18 carbon chains attached to glycerol, but with polymers characterized by having different sidechains including a methoxymonoethylene glycol and an isopropylmethoxymonoethylene glycol on the ethyl end of the ethyloxazoline polymer ($DiC_{18}PMOGOx_{21}$ and $DiC_{18}PTEGOx_{18}$) [8]. These polymers are collected in Fig. 4. The thinking was that bulky side chains on the polymer would force physical distance between each lipopolymer, and thus inhibit the ability of the lipid moiety of each lipopolymer to condense with the lipid moiety of a neighboring lipopolymer, and be another way to explore the importance of lipid–lipid interactions on the high pressure transition region. This proved to be correct, as Fig. 6 shows that the oligo–EO substituted lipopolymers ($DiC_{18}PMOGOx_{21}$ and $DiC_{18}PTEGOx_{18}$) did not exhibit the high pressure transition at all before film collapse, and thus did not undergo the acyl chain condensation.

Additional film balance experiments on diblock and triblock copolymers have been shown to be helpful in evaluating the properties of the low pressure transition, π_{low}, at the air–water interface. Many $\pi - A$ isotherms of diblock and triblock copolymers have been published: one excellent example is Gonçalves Da Silva's polystyrene–polyethylene glycol diblock copolymers published in 1996 which showed not only the absence of a high pressure transition in these nonlipid amphiphiles, but also that the low pressure transition occurred at a constant area/monomer of PEG regardless of the size of the PEG polymer [3]. This is also more evidence that the pressure relates to submersion, monomer by monomer, of the PEG from the surface. Reviewing film balance studies on polyoxazoline-containing

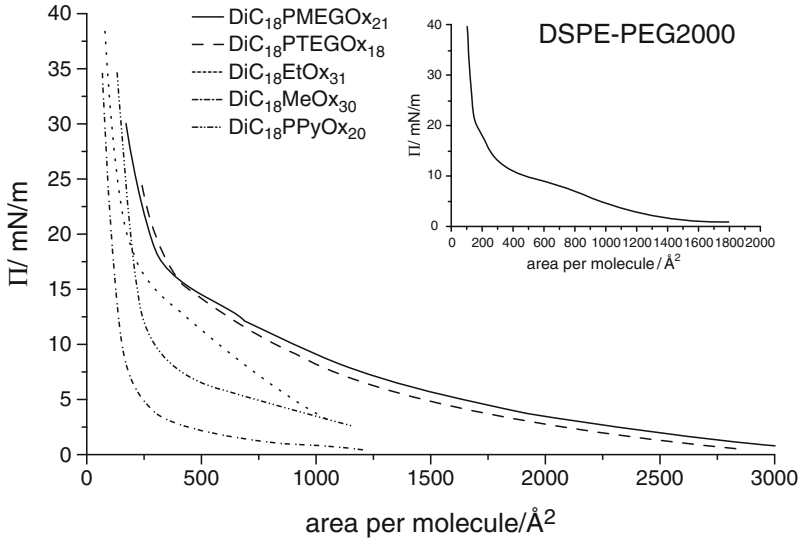

Fig. 6 $\pi - A$ isotherms of $DiC_{18}PPyOx_{20}$, $DiC_{18}PMOx_{30}$, $DiC_{18}PEOx_{31}$, $DiC_{18}PTEGOx_{18}$ and $DiC_{18}PMEGOx_{21}$ at room temperature, with the isotherms of DSPE–PEG2000 included for comparison (*inset*) [8] (reproduced with permission from Wiley)

lipopolymers, and diblock, and triblock copolymers confirmed that the low pressure transition can be found in the presence of amphiphilic PEOx, but not with the more hydrophilic PMOx (Fig. 7) [7]. It is recalled that the PEG lipopolymers, like the PEOx lipopolymers, display a strong degree of amphiphilicity at the air–water interface and also undergo the low pressure transition.

To obtain more information about the structural properties of lipopolymers at the air–water interface, several groups have pursued X-ray and neutron scattering experiments. Using X-ray and neutron reflectometry, Wurlitzer et al. confirmed acyl chain condensation above π_{high} but also found that the surface of the monolayer was rougher, less planar [13]. In particular, just below the surface there was a range of 15 Å where lipid tails, ether linkers between the lipid tails and the glycerol backbone, and PMOx monomers can be found. The first eight carbons of the PMOx chain were deuterated in order to better show the location of the polymer within the subphase, and they showed these first eight carbons in the same location as the ether linkers but the hydrogenated PMOx carbons, further down the chain, also had great density at this same height, just below the surface. The acyl chains and ether linkers do not penetrate further down than this and the deuterated carbons only extend another 10–15 Å further, but the hydrogenated PMOx carbons extend down to below 100 Å from the interface. Wurlitzer et al. hypothesized that the energy associated with the elastic effect of forcing the polymers closer together led to a partial immersion of the hydrophobic acyl anchors into the aqueous medium [13, 14].

Grazing incidence X-ray diffraction and specular X-ray diffraction studies by Ahrens et al. agree that some form of lipid condensation takes place at the

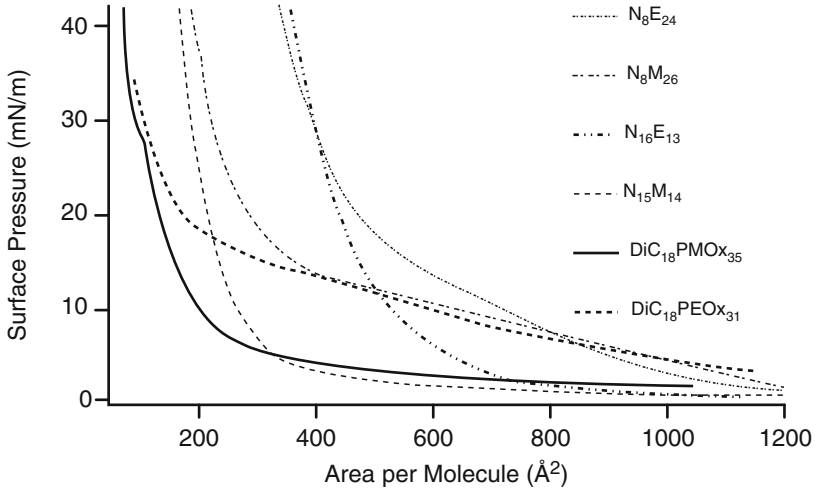

Fig. 7 $\pi - A$ isotherms of diblock copolymers with isotherms of lipopolymers $DiC_{18}PMOx_{35}$ and $DiC_{18}PEOx_{31}$ included for reference. Note the ethyloxazoline systems display low pressure transitions which the methyloxazolines do not, and the diblocks do not display high pressure transitions [7] (reproduced with permission from the American Chemical Society)

high-pressure transition, and also provide evidence of a possible superstructure [15, 16]. Looking at DSPE–PEG2000, Ahrens et al. found tilt angles of the acyl chains between 14° and 18° with respect to the surface normal for pressures above π_{high}, with tilt angles decreasing upon compression. The packing density for the samples based on the calculated lattice constants, however, was not in agreement with the bulk density of the lipids, but in fact showed the lipid tails much more closely packed (but not quite as densely packed as phospholipids in the absence of polymers) [15,16]. This finding corresponds to the IRRAS data from Baekmark and Wiesenthal, which found CH_2 stretches very similar to stretches for phospholipids packed closely together, although they could not be homogeneously so condensed because the average area per lipopolymer was much too large for a continuously condensed lipid surface. Ahrens, et al. suggested this was possible using a theory of surface micellization, whereby some aggregate of lipopolymers collects and the lipid tails within each aggregate condense together during the high-pressure transition. For evidence, grazing incidence X-ray diffraction showed that lattice constants *increased* upon compression above π_{high}, creating superstructures spaced 134–160 Å apart [16]. These investigators transferred the monolayers onto mica and found surface stripes of about the same periodicity using atomic force microscopy. However, it is difficult to compare fixed, dry monolayers which are necessarily subject to substrate interactions, with fluid monolayers on a water surface. Another group looking at X-ray grazing-incidence diffraction and reflectivity of lower molecular weight DSPE–PEG chains found little coherence for DSPE–PEG90 and DSPE–PEG350, but for DSPE–PEG750 found clusters of about 43 lipopolymers

within which the acyl chains were perpendicular to the surface, and showed good hexagonal packing [17].

Israelachvili considered the possibility of Langmuir monolayers of any sort of amphiphiles forming surface micelles in 1994 [18]. In his model, there is a critical micellar area (CMA or A_c), below which few micelles form and the concentration of the system is nearly equal to the concentration of discrete molecules, but above which, the concentration of micelles increases while the concentration of discrete molecules is constant. Below A_c, the total average area per molecule, A, will be the same as the area per molecule of the discrete molecules, defined A_1. If A_0 is defined as the hard-disk excluded area of a molecule in a micelle, and N is the number of molecules in a micelle, then the $\pi - A$ isotherm for a system forming surface micelles can be written as

$$\pi = \frac{kT}{N}\left[\frac{1}{A - A_0} + \frac{(N-1)}{(A_1 - A_0)}\right]. \tag{1}$$

The significant finding from this is that, for a hypothetical system, surface micellar formation for N even as small as 25 molecules leads to a plateau on a $\pi - A$ isotherm, and conversely, a plateau on a $\pi - A$ isotherm may indicate surface micellar formation. Israelachvili considers the case of fluid alkane chains connected to repelling hydrophilic head groups which are all in the plane of the monolayer, noting that micellar formation would enable the headgroups to increase the distance between them, and lower the interaction energy per molecule. Counterbalancing this, there is a maximum aggregation size related to the fully extended length of the hydrocarbon chain, l_c, above which micelles are not energetically favored, since the headgroups would presumably be repelled by the interior of a micelle even more than by nearby other headgroups. The shape of such micelles, Israelachvili goes on to suggest, would be either small circles or ribbons with a half width less than l_c.

Langmuir monolayers of diblock and triblock copolymers have been thoroughly studied, and through analyses of $\pi - A$ isotherms and neutron reflectometry data, it has been shown that many combinations of copolymers form surface micelles. Based on the density at different heights below the air–water interface of polystyrene and PEG in block copolymers, Dewhurst concluded that the polystyrene moieties aggregate into a cluster, with PEG forming a cushion underneath and a corona around the polystyrene center, akin to flower-like micelles [19]. Naturally, the nature of lipopolymers, with their acyl chain condensation, would lead to a different geometry than diblock copolymer micellization. However, trends observed by Deschenes et al. lead to the prediction that the size of the micelles is controlled by the ratio of hydrophobic to hydrophilic block area, with higher hydrophobic areas aggregating into planar morphologies, and lower ratio hydrophobic areas (different than, but similar to lipopolymers) forming cylinders, wormlike or dendritic structures [20]. The experimental findings from di- and triblock copolymers are interesting because there is some experimental evidence that lipopolymers may assemble into micellar structures at the air–water interface. For example, as already noted, Ahrens and Helm reported the formation of stripe-like structures on lipopolymer monolayers

after the monolayers had been transferred to mica [16]. Similarly, neutron reflectometry data, in combination with film balance and interfacial rheology results, have been interpreted in terms of a surface micellization of lipopolymers [7].

Up to this point, the information about the monolayers has looked at the structural properties as if the lipopolymer monolayers were static and fixed above a body of water. However, a truly remarkable aspect of these monolayers is their fascinating fluidity and visoelastic properties, and the range of distinct fluid and viscoelastic behavior they exhibit under different conditions and with different lipopolymers. These properties can be studied by analyzing the viscosity and elasticity of the monolayer, as discussed in Sect. 2.2, as well as by investigating the lateral diffusion of individual lipopolymers within the monolayer, as discussed in Sect. 2.3.

2.2 *Viscoelastic Properties of Lipopolymers in Langmuir Monolayers*

To obtain information about the viscoelasticity of lipopolymers at the air–water interface, Langmuir monolayers of lipopolymers were studied using interfacial rheology. Initial experiments were conducted using a custom-built interfacial needle shear rheometer, as described before [2, 21] and illustrated in Fig. 8. In this experimental setup, a trough is constructed with a Langmuir monolayer as noted in previous experiments, but in addition, a magnetic rod is stabilized at the air–water interface and subjected to an oscillatory magnetic field gradient, which is provided by a pair of Helmholtz coils surrounding the trough [21]. The position of the rod is tracked using an inverted microscope and a linear photodiode array. From the rod's position (strain) relative to the applied current in the coils (stress), it is possible to determine δ, the phase lag between the strain and the stress, as well as the amplitude ratio, AR, which is defined as the ratio of strain to stress. If it is assumed that the contribution from the underlying subphase is negligible compared with the interface, which is true in practice, these parameters define the dynamic surface modulus $G_s{}^*$, from which can be determined the storage modulus, $G_s{}'$ and the loss modulus, $G_s{}''$. These pioneering experiments on PEG lipopolymers revealed a remarkable change of viscoelastic properties in the range of the high-pressure transition [2]. As illustrated in Fig. 9, below this transition, the monolayer is fluid and the loss modulus, G'' (a measure of the viscosity of the film), is larger than the storage modulus, G' (corresponding to the elasticity of the film). In contrast, above the high pressure transition, the monolayer becomes elastic with $G' > G''$, thus suggesting the formation of a physical gel. Originally, this physical gel formation was interpreted in terms of two types of associative interactions: microcondensation of acyl chains to form small clusters, and water molecules acting as intercalators mediating the interaction between PEG chains via hydrogen bonding [2].

In a following study, it was confirmed that the gelation transition was not limited to PEG lipopolymers because comparable viscoelastic properties were observed on monolayers of polyoxazoline lipopolymers as well [12]. This called into question

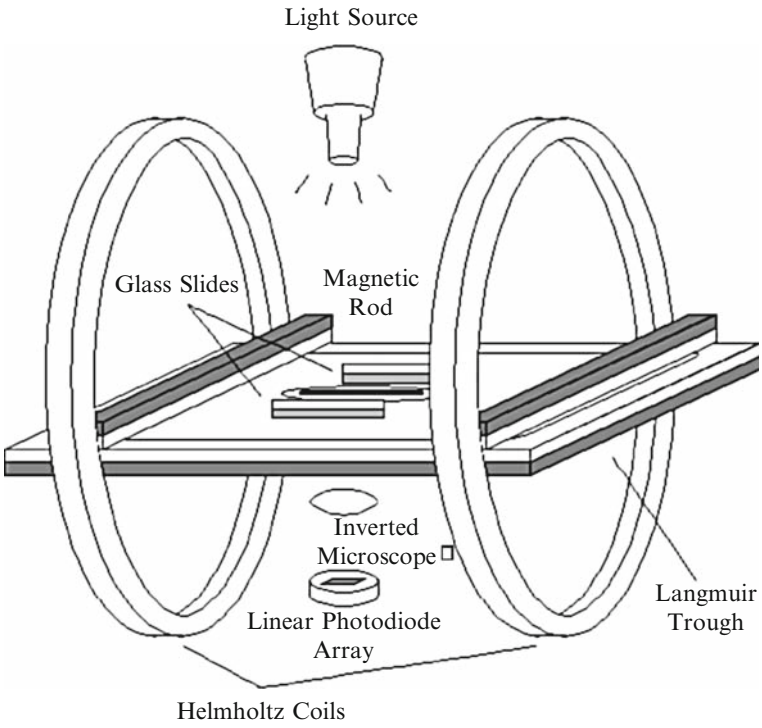

Fig. 8 Design of an interfacial stress rheometer. Here a magnetized rod is subjected to an oscillatory force generated by the Helmholtz coils. The motion of the rod is detected using a microscope and photodiode array. Differences between the applied force and resulting phase and magnitude of the displacement give information on the viscoelastic properties of the monolayer. Both the storage modulus G' and the loss modulus G'' can be determined [2, 21] (reproduced with permission from the American Chemical Society)

the initial model whereby intercalated water molecules via hydrogen bonding were the basis for the elasticity of the monolayer. To uncover the nature of the viscoelastic transition, additional interfacial rheology experiments were conducted where the polymer and lipid moieties of lipopolymers were altered systematically [7, 11]. These studies were conducted using an oscillating ring rheometer. For example, the molecular weight of the PEG moiety of PEG lipopolymers was changed (MW: 750, 1,000, 2,000, 3,000, 5,000) [11]. As illustrated in Fig. 10, these experiments showed that the gelation transition shifts to smaller areas per molecule and that there is a qualitative difference between higher MW species (MW: 2,000, 3,000, 5,000) and lower MW species (MW: 750, 1,000). In the first case, G' exhibits a power law-like behavior above the gelation transition. In the second case, a breakdown of the gel is observed after an initial power law-like behavior [11]. Interestingly, the strength of the gel (prior to collapse) was found to follow the trend G′(DSPE–PEG750)< G′(DSPE–PEG1000)< G′(DSPE–PEG2000)> G′(DSPE–PEG3000)> G′(DSPE–PEG5000). This result showed that the strength of the physical gel can

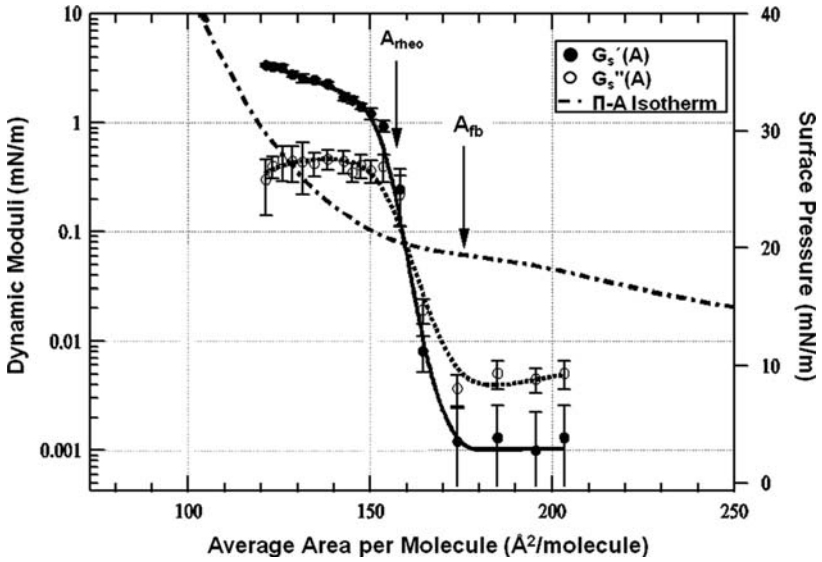

Fig. 9 Dynamic moduli vs area isotherm for DSPE–PEG2000, with $\pi - A$ isotherm also shown, pointing out that the viscoelastic transition point, where storage modulus $G_s' =$ loss modulus G_s'' is only slightly above the plateau of the high-pressure transition [2] (reproduced with permission from the American Chemical Society)

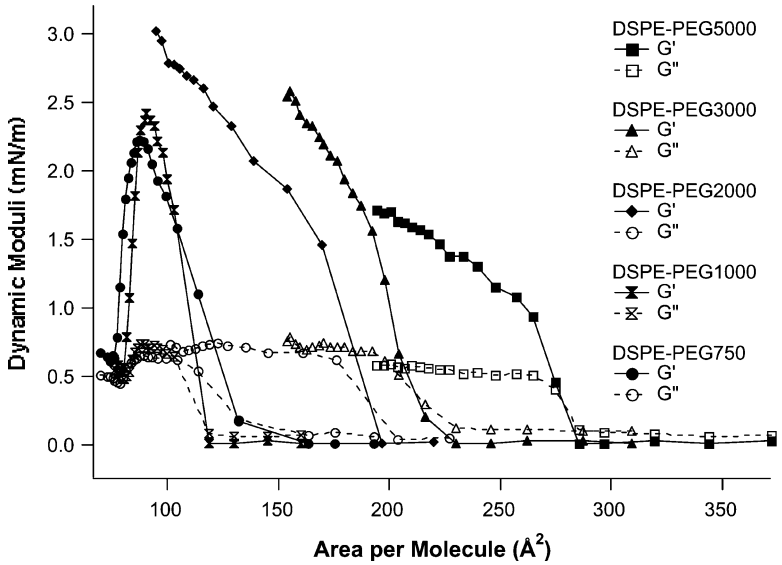

Fig. 10 Storage modulus, G_s', and loss modulus, G_s'', of DSPE–PEG750, DSPE–PEG1000, DSPE–PEG2000, DSPE–PEG3000, and DSPE–PEG5000 plotted vs area per molecule. All lipopolymers show a viscoelastic transition [11] (reproduced with permission from the American Chemical Society)

be regulated by changing the PEG molecular weight of lipopolymers. When PEG chains of PEG lipopolymers are shorter or longer than PEG2000, the strength of the polymer gel is weakened. These data are significant because they emphasize that polymer and lipid moieties of lipopolymers are equally important in the regulation of the high-pressure and gelation transitions. Furthermore, these results showed that both types of transitions are critically dependent on the area mismatch between lipid and polymer moieties of lipopolymers.

To explore the role of molecular structure of amphiphiles on the physical gelation transition, additional interfacial rheology experiments were conducted using polyoxazoline-based diblock copolymers and PEG lipopolymers with lipid anchors of various acyl chain lengths [7]. Figure 11 illustrates that only lipopolymers, and not diblock copolymers, exhibit a gelation transition. Interestingly, when $DiC_{18}PEOx_{31}$ and $DiC_{18}PMOx_{35}$ transitions are compared by area per molecule, as opposed to film pressure, they exhibit the gelation transition at the same area per molecule, about 90 $Å^2$.

Also, in contrast to DPPE–PEG2000 and DSPE–PEG2000, which have acyl chains of C_{16} and C_{18}, respectively, no rheological transition was observed for lipopolymers with relatively short acyl chains (C_{14}), DMPE–PEG2000. It should be recalled that no high pressure film balance transition was found for DMPE–PEG2000 either, thus suggesting a direct relationship between high-pressure and gelation transitions [7]. High pressure transitions and rheological transitions are not limited to PEG and polyethyloxazoline systems: DiC_{18} linked to glycerol which is also attached to a sugar-based polymeric moiety, namely three end-linked lactose units, also displayed the transition from a fluid to an elastic film [22]. Finally,

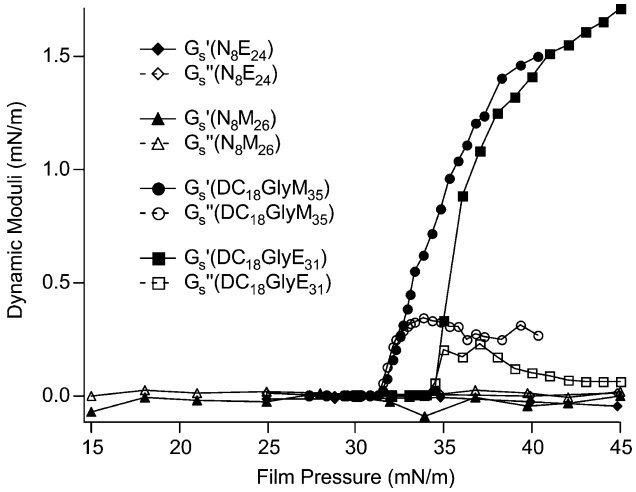

Fig. 11 Storage modulus, G_s', and loss modulus, G_s'', of N_8E_{24}, N_8M_{26}, $DiC_{18}PMOx_{35}$, and $DiC_{18}PEOx_{31}$ plotted vs film pressure (which increases as area per molecule decreases). Lipopolymers do show a viscoelastic transition but diblock copopolymers do not [7] (reproduced with permission from the American Chemical Society)

there is no rheological transition pressure for unsaturated acyl chains, just as there was high-pressure film balance transition [7]. Overall, these experiments confirmed that a high-pressure film balance transition is necessary for a rheological (gelation) transition to occur.

In another experiment it was shown that, while necessary, a high pressure film balance transition is not sufficient to cause this gelation to occur. The lipopolymer composed of lipids and polyethyloxazoline connected through an amine headgroup, $\mathrm{DODA-PEOx_{35}}$ underwent a high pressure film balance transition. However, it showed a loss modulus consistently higher than the storage modulus at all surface areas measured, and thus never displayed a rheological gelation transition [12]. Saturated phospholipids without polymer chains also never display rheological transitions, even though they obviously undergo acyl chain condensation [7]. In summary, the strength of the network, as characterized by its elasticity, is dependent on the strength of molecular interactions within the lipid moiety, but the lipid must be covalently connected to a polymer for gelation to occur. In particular, the strongest rheological transition occurs for DSPE–PEG2000; shortening or desaturating the lipid chain minimizes the rheological transition (and diblocks at the air–water interface without the dual acyl chains do not undergo the rheological transition to gels at all); changing the connecting head group can disrupt the rheological transition; and substituting PMOx for PEOx in otherwise identical systems does not affect the rheological transition, but both exhibit a transition at more concentrated areas per molecule than PEG lipopolymers. Cataloging the various lipopolymeric rheological transitions to an elastic monolayer does not, however, by itself, bring an understanding of the underlying phenomenon causing this behavior.

Polymers are known to become elastic upon interdigitation and entanglement, which might explain the elasticity of the monolayers above the viscoelastic transition. However, such a process is highly unlikely in a lipopolymer monolayer at the air–water interface given the short lengths of the polymeric chains involved. An alternative possible explanation is that hydrogen bond bridges between the head groups during lateral compression to higher pressures store the elastic energy, as proposed in the earlier work of Naumann and Schneider [2,22]. However, it has been shown that there is no attractive interaction potential between PEG chains [23, 24]. In addition, studies of PEG star copolymers in different pH solutions showed that it is unlikely there are hydrogen bond bridges between the PEG moieties, at least during lateral compression [25].

To understand further the nature of the rheological transition, a series of experiments were performed by our lab monitoring the time evolution of viscoelastic properties in PEG lipopolymer monolayers at film pressures near the gelation transition (previously unpublished data). In particular, the DSPE–PEG series with PEG molecular weights from 750 to 5,000 were measured at particular film pressures slightly less than and slightly more than the rheological transition pressure (accuracy of dynamic moduli is around $\pm 5\%$). The storage and loss modulus of a monolayer of pure DSPE–PEG2000 just below the rheological transition pressure, at $20.1\,\mathrm{mN\,m^{-1}}$, started out with the loss modulus higher than the storage modulus (non-gel state) at time $= 0$, but after 30 min, these values switched, and by 1 h,

the storage modulus was significantly higher and remained so for the duration of the experiment (4 h) (Fig. 12b). Similar behavior was observed when storage and loss of DSPE–PEG2000 were tracked at slightly higher pressures as well (data not shown). Thus, for DSPE–PEG2000, the longer the system was tracked by a rheological probe, the higher the storage modulus.

As illustrated in Fig. 12, however, the behavior of DSPE–PEG2000 was notably different than the behavior exhibited by either the longer chained lipopolymers or the shorter chained lipopolymers. DSPE–PEG750 had a very different response. When dynamic moduli were monitored over time at a pressure of 19.5 mN m^{-1}, corresponding to slightly *above* the expected rheological transition pressures, the storage modulus started out higher, in a gel state, but over 2–3 h, the storage modulus decayed until the loss modulus was higher, or liquefaction occurred. It is recalled that for these length chains, at pressures more than 10 mN m^{-1} above the rheological transition pressure, the gel state also collapsed and the monolayer liquefied [11]. When the time study was performed below the transition pressure, the low-weight monolayers stayed in the non gel state, and did not achieve the gel state within the time period studied, unlike the behavior of DSPE–PEG2000 (data not shown). Looking at the other end of the PEG spectrum, the DSPE–PEG5000 at 20.5 mN m^{-1}, just below the rheological transition pressure, stayed in the non gel state for the full 4 h, but at 22.0 mN m^{-1}, the DSPE–PEG5000 started out well into the gel-state with storage more than twice as high as loss modulus, but over a time period of less than 2 h, the monolayer liquefied and the situation was reversed. In summary, DSPE–PEG2000, over time, quickly developed into the gel state from just below the rheological transition pressure, but shorter and longer lipopolymers not only did not develop into the gel state from just below the rheological transition pressure, but decayed from the gel state to a liquid state at pressures just above the rheological transition pressure.

The results obtained from the study of the time evolution of viscoelastic properties are exciting because they show that the behavior of the DSPE–PEG2000 is reminiscent of the rheological behavior of star polymers [26, 27]. In those systems, concentrations of star polymers in good solvent swell upon heating and form jammed clusters which cause the solution to become elastic. This condition is thermally reversible. The conditions necessary are dense star solutions, a high number (>64) of arms for the star polymer, and intermediate (that is, better than Θ but not necessarily athermal) or good solvent [26]. Jamming of polymeric micelles of diblock copolymers, again in 3D have also been observed [28, 29]. Renou et al. noted that the transition which can be obtained by varying the temperature can also be obtained by increasing the volume fraction [29]. Here the diblock micelles first form upon increasing concentration, and then upon further compression act as dynamic (as opposed to covalently linked) star polymers and jam together while retaining their soft boundaries, thus leading to elastic behavior. At some increased concentration, these micelles form a crystalline structure.

The similarity between viscoelastic properties of lipopolymer monolayers and star polymers suggests that the gelation transition in lipopolymer monolayers might be caused by a jamming transition of micelles as well. Such a model is attractive because the ability to form surface micelles should be strongly connected to the

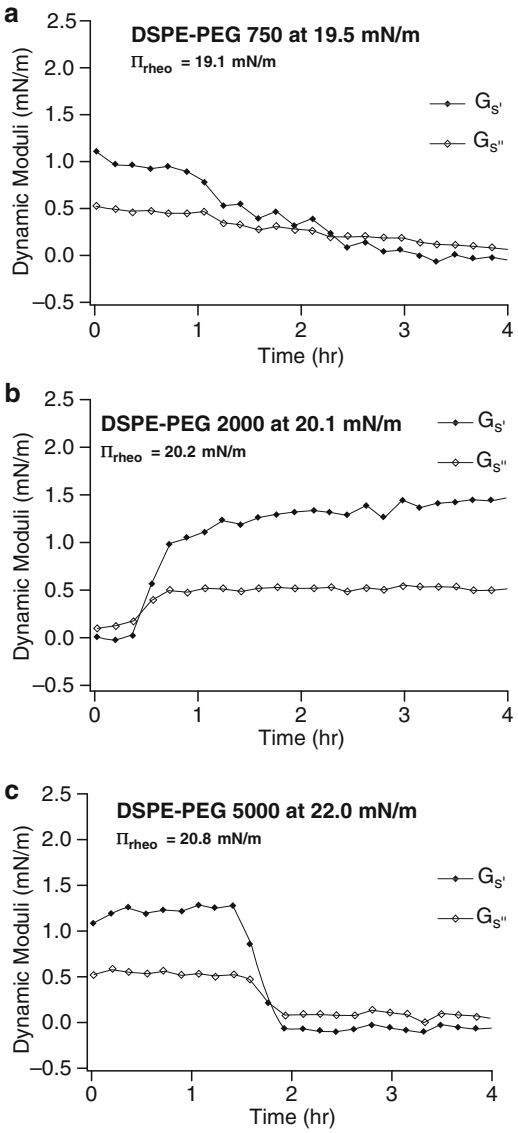

Fig. 12 a–c Time evolution of viscoelastic properties near the gel point for DSPE–PEG750, DSPE–PEG2000, and DSPE–PEG5000. **a** DSPE–PEG750, short-chain lipopolymer, starts out above the viscoelastic transition point at 19.5 mN m^{-1} but after 1 h, a gradual breakdown of the gel can be observed. After 2.5 h the loss modulus becomes higher than the storage modulus. **b** DSPE–PEG2000 starts out below the viscoelastic transition point, at 20.1 mN m^{-1}, and within 30 min has undergone gelation leading to a notably higher storage modulus and a slightly higher loss modulus. **c** DSPE–PEG5000, a long-chain lipopolymer, starts out above the viscoelastic transition point at 22.0 mN m^{-1} but within 2 h, the viscoelastic gel has broken down leading to similar results as **a**

ability of lipopolymers to exhibit a gelation transition. The data obtained from star polymers and diblocks indicate that the gelation transition requires the jamming of such surface micelles. Unlike other models, the jamming model is able to explain the importance of a long saturated lipid tail that can undergo acyl chain condensation in order to obtain a gelation transition. Within this model, it can also be rationalized that the length of the polymer chains will affect the ability of the lipopolymers to form jammed surface micelles. Polymers which are shorter will aggregate into surface micelles with shorter, less soft polymer shells less able to accommodate and form jams, so that increasing compression can cause the elastic monolayer to collapse. Monolayers of longer polymers, such as DSPE–PEG5000, may form surface micelles with insufficient aggregation numbers, which may lead to increased micelle interpenetration or deformability, thus enabling surface micelles to avoid lateral stress more easily, and thus present themselves as less elastic and more likely to rearrange and break down over time. It will be interesting to compare the viscoelastic behavior of mixtures of lipopolymers and phospholipids, since if they form micelles, there would be fewer polymeric moieties for the same number of condensed acyl chains in a mixture. This will be discussed in depth below in Sect. 3.2.

2.3 Diffusion Properties of Lipopolymers in Langmuir Monolayers

Another method of investigating monolayers is to study the diffusion of lipopolymers within the monolayer as a function of surface density (area per molecule). The manner in which the lipopolymers diffuse can shed light on how they organize and their usefulness in mixed lipopolymer phospholipid bilayers. Diffusion analysis can be accomplished through wide-field single molecule fluorescence microscopy. It must be remembered that determining diffusion data from fluid monolayers at the air–water interface is experimentally quite challenging, since the possibility of water flow affects the diffusion measurements. Unless properly accounted for, surface flow can introduce large margins of error. The specifics of a single molecule imaging set-up for monolayer experiments at the air–water interface have been reported elsewhere [30, 31], but in essence, lipopolymers are labeled with tetramethyl rhodamine isothiocyanite (TRITC) through thiourea coupling, and added to a lipopolymer monolayer at a mol concentration of 1×10^{-8} mol%. Then, after the lipopolymers are assembled on the monolayer at the desired area per molecule, an excitation source coupled to an intensified CCD camera with a synchronized shutter creates instantaneous micrographs of the position of the fluorescent particles, and the data are transferred to image recording and single molecule tracking software. From this, the positional change of single fluorescently-labeled molecules is analyzed for each successive frame using a constant time lag. By tracking two to four molecules per frame, it is possible to determine relative positional changes and obtain flow-corrected square displacements, r^2. When enough of such data are collected, these can be averaged to determine the mean squared displacement, and if the data fit a normal diffusion curve, a diffusion coefficient, D, can be determined.

By using the described single molecule imaging approach, the diffusion properties of $DiC_{18}PMOx_{30}$ and $DiC_{18}PMOx_{50}$ were determined at eight different surface concentrations, from fairly dilute up to just below the high-pressure transition concentration, at which level the diffusion decreases nearly to zero [31]. PMOx systems were chosen since they do not undergo a low pressure transition, and two different length polymers were utilized for the purpose of comparing diffusion coefficients of lipopolymers with different length polymers. The lateral diffusion was found to be Brownian at all concentrations studied, and the diffusion coefficient, D is plotted vs area per molecule, A, for both $DiC_{18}PMOx_{30}$ and $DiC_{18}PMOx_{50}$ in Fig. 13.

Interestingly, the lipopolymers exhibit two different diffusion regimes, labeled as Regions I and II. In Region I, in the case of weak interpolymer interactions, D is independent of A, but the plateau or Region I value depends on the number of polymeric units, N. In Region II, D scales proportionally with A, and is also dependent on N.

The diffusion properties in Region I are well described by the Rouse model, which predicts the self-diffusion coefficient will scale as 1/N, the number of monomeric units. Applied to the two lipopolymers of interest, the Rouse model predicts the ratio

$$\frac{D_{\text{diC18PMOx30}}}{D_{\text{diC18PMOx50}}} = \frac{50}{30} = 1.67. \tag{2}$$

This Rouse ratio is in excellent agreement with our diffusion data in Region I, which provide

$$\frac{D_{\text{I,30}}}{D_{\text{I,50}}} = \frac{9.7}{5.7} = 1.7. \tag{3}$$

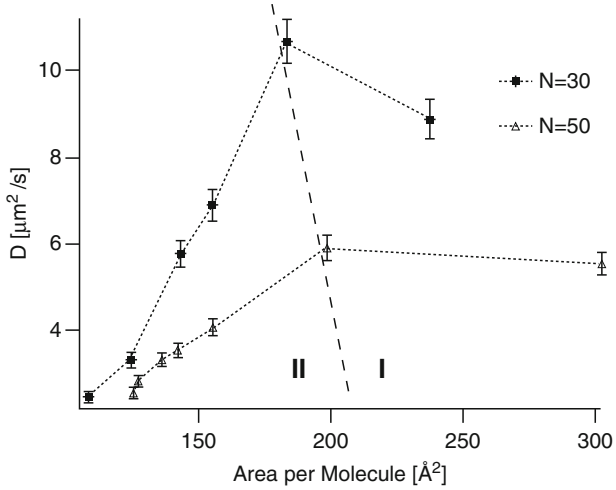

Fig. 13 Single molecule tracking data of dye-labeled PMOx lipopolymers as a function of area per molecule. The plots of the lateral diffusion coefficient, D, vs area per molecule for $DiC_{18}PMOx_{30}$ and $DiC_{18}PMOx_{50}$ show two different diffusion regions (labeled I and II). Unlike in Region II, D follows Rouse scaling in Region I [31] (reproduced with permission from the American Chemical Society)

Obviously, the diffusion data in Region II do not obey Rouse scaling because the diffusion coefficient is now dependent on lipopolymer surface concentration. Due to the higher surface density of lipopolymers in this region, more significant interpolymer interactions can be expected. The diffusion properties of polymers in bulk at elevated concentrations are characterized by chain entanglement and reptation. Here the self diffusion of reptating chains can be expressed by $D \sim c^{-\alpha}N^{\beta}$, with $\alpha = 1.75$ and $\beta = 2$ [32, 54, 55]. The data shown in Fig. 13 for the lipopolymers do not fit these coefficients well; the best fit for α for $diC_{18}PMOx_{30}$ is 4.9, and for $diC_{18}PMOx_{50}$ it is 2.4, and the best fit for β is 1.6 [31]. This disagreement is not surprising because it is hard to visualize lipopolymers with their lipid tails constrained to a surface involved in a two- or three-dimensional reptation and because the chains are too short to exhibit significant entanglement.

Another model for understanding the diffusion of lipopolymers at the air–water interface in Region II is the free area model, useful for describing the motion of phospholipids on a Langmuir monolayer and many systems where the diffusing particles can be approximated by hard spheres, disks or cylinders [38]. In this model, a particle can diffuse in any direction that is free, or in other words, in any direction that is empty of another particle. As would be expected, more crowded or concentrated systems diffuse more slowly. Assuming the particles are at a constant temperature and that other energetic considerations can be described within a constant, D_o, this type of diffusion can be expressed as

$$D = D_o \exp\left(-\frac{\gamma A_{\min}}{A_{\text{free}}}\right), \tag{4}$$

where γ is a scaling constant to be found, $A_{\min}$ is the minimum free area per lipopolymer required for diffusion, A_{free} is the average free area per lipopolymer given by $A_{\text{free}} = A_{\text{lipo}} - A_{\min}$, and A_{lipo} is the area per molecule, as graphed in Fig. 13 [31]. If $A_{\min}$ is estimated by extrapolating the D vs A_{lipo} plot to $D = 0$, both lipopolymer curves depicted above show an excellent agreement with this model in Region II when $\ln(D/D_o)$ is plotted against $(A_{\min}/A_{\text{free}})$, and this graph is shown as Fig. 14.

In addition, the slopes of the lines are well within the expected range for the free area model of $0.5 \leq \gamma \leq 1$, with values of 0.77 and 0.66. The good agreement between diffusion data and free-area model indicates that the lipopolymer lateral diffusion is dependent on the strength of interpolymer interactions and that the polymer moieties behave like rigid spheres or cylinders during the diffusion process (nondraining behavior). Figure 14 provides a few interesting implications for lipopolymer–lipid mixtures. First, lipopolymers characterized by significant interpolymer interactions can simply be considered diffusion obstacles for phospholipids, as confirmed in polymer-tethered monolayers and bilayers [39, 40]. Second, if the lipopolymers behave as hard cylinders in fluid conditions under appropriate conditions such as explored in this section, it is reasonable to expect that they can be modeled as hard cylinders in mixed phospholipid–lipopolymer monolayers, explanation of which will be the subject of the second half of this chapter.

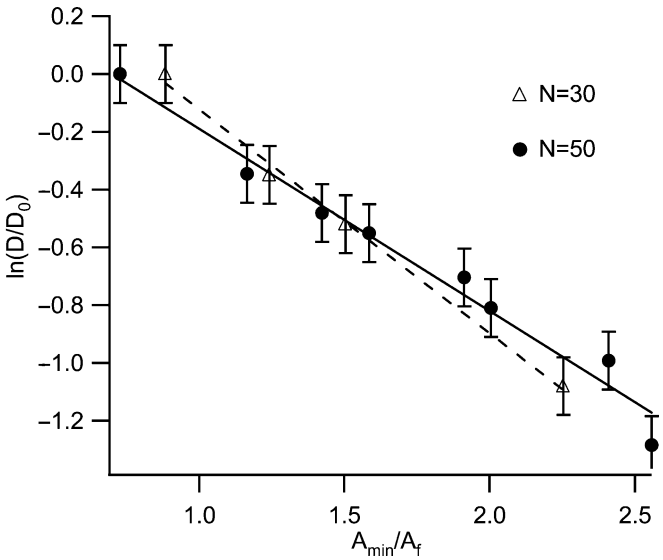

Fig. 14 Plot of $\ln(D/D_0)$ vs $A_{\min}/A_f$, for $n = 30, 50$ in diffusion Region II. The *dashed and solid lines* represent the best linear fits for $n = 30$ and $n = 50$, respectively. The excellent agreement between data points and fits shows that D of end-tethered PMOx chains in diffusion region II is well described by the free area model [31] (reproduced with permission from the American Chemical Society)

It is worth noting that the observed diffusion behavior of lipopolymers at the air–water interface shows some similarity to corresponding results on diblock copolymers, which are arranged in 2D. Lower molecular weight diblocks were found to follow Rouse scaling, whereas their higher molecular weight counterparts were better described by processes of activated reptation and block retraction [33–37]. Furthermore, temperature-dependent studies on diblocks organized in polymerosomes also showed that the self diffusion can be interpreted by a free volume theory [33].

3 Lipopolymer–Phospholipid Monolayer

3.1 Structural Properties

Although the first section of this chapter was concerned with structural and dynamic information on monolayers of lipopolymers, before investigating lipopolymer–phospholipid mixtures, it is reasonable to consider the structural information that exists concerning pure phospholipid monolayers at the air–water interface. Film balance experiments, X-ray and neutron reflectometry, and molecular dynamics simulations have provided insight into the structural properties of these biologically

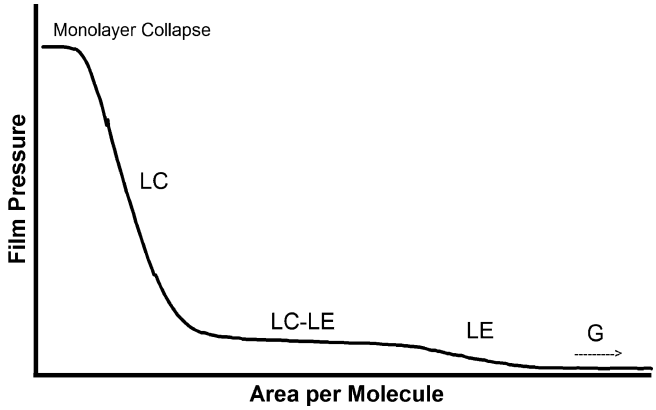

Fig. 15 Conceptual $\pi - A$ isotherm for DPPC showing the different phases: G for gas, LE for liquid expanded, LC–LE for the transition region where both liquid expanded and liquid condensed exist, and LC for the liquid condensed region

important amphiphiles. Figure 15 illustrates a $\pi - A$ isotherm of the saturated phospholipid DPPC, which exemplifies the typical phase properties of saturated lipids in a monolayer at the air–water interface (data from our laboratory).

At high area per molecule, the monolayer is first incomplete and is described as being in a gassy state, but after completion exists in the liquid-expanded (LE) state. Upon further compression, a plateau is reached, in the range of 50–70 $Å^2$, followed by a sharp increase in surface pressure after the phospholipids are all in the liquid-condensed (LC) state. Molecular dynamics simulations suggest that the head groups do not change orientations or order when transitioning from LE to LC [41]. In particular, the phosphate–nitrogen tilt angle is roughly parallel to the surface of the water and is not affected by compression of the monolayer through the phases from LE to LC; and the methyl groups on the choline prefer to sit at the air–water interface in both phases. In contrast, the lipid tails change from disordered in the LE phase to hexagonal packing in the LC phase [41], and thus the plateau represents a conformational change very similar to the acyl chain condensation described for lipopolymers by [6]. This is interesting because it shows the great similarity between the phase transitions of the phospholipids and the lipopolymers: in both, the systems start out widely spread, then upon compression, both undergo acyl chain condensation. Therefore, it is reasonable to project that mixtures of phospholipids and lipopolymers will also undergo similar processes. On the other hand, at high lipopolymer molar concentrations, significant repulsive interpolymer interactions are likely to occur, which should cause high lateral stress in the mixed monolayer with possible consequences for structural and dynamic properties. Here it cannot be excluded that lipopolymers with a very hydrophilic polymer moiety, such as polymethyloxazoline, and those with an amphiphilic polymer moiety, such as PEG and polyethyloxazoline, cause different structural and dynamic properties. The current section will provide an overview over the existing knowledge on mixed lipopolymer–phospholipid Langmuir monolayers.

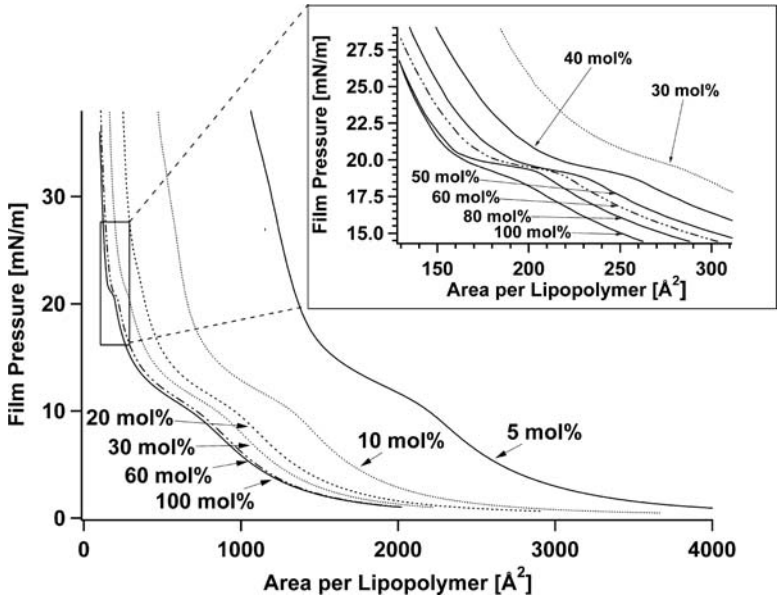

Fig. 16 $\pi - A$ isotherms of different DMPC–DSPE–PEG2000 mixtures for lipopolymer molar concentrations of 5–100 mol%. At ≥ 30 mol%, the $\pi - A$ isotherms show the high-film transition at ~ 19 mN m^{-1} (*see inset*). At lower mol%, the transition becomes much less noticeable and shifts to higher film pressures [42] (reproduced with permission from the American Chemical Society)

Several film balance studies have been reported on lipopolymer–phospholipid mixed monolayers at the air–water interface. Figure 16 displays the $\pi - A$ isotherms of a binary DMPC/DSPE–PEG2000 mixed monolayers ranging from 5 to 100 mol% DSPE–PEG2000 [42]. The isotherm of the lowest concentration of lipopolymer, 5 mol%, is to the right of all the other isotherms, since that mixture contains a large fraction of phospholipid (95 mol%) which is not taken into consideration in this pressure–"area of lipopolymer" isotherm. The analysis of these data provides several interesting results. First, for concentrations larger than 30 mol% lipopolymer, the isotherm of the mixture is nearly identical to the isotherm of the pure lipopolymer. Second, all of the isotherms, even as low as 5 mol% DSPE–PEG2000, show the same low film pressure plateau around 9 mN m^{-1}. As discussed before, this plateau is related to the desorption of the PEG polymers from the air–water interface, which possibly is assisted by the presence of choline headgroups of DMPC. Third, the high pressure transition is still visible at nearly the same pressure for lipopolymer concentrations of 30 mol% and higher. It has been pointed out that the observed disappearance of the high-film pressure transition at lower lipopolymer molar concentrations could be related to the inability to force the polymer chains into a more stretched configuration. Under such circumstances, lipopolymers are expected to be too far away from each other to undergo acyl chain condensation [42]. These data are interesting because they suggest that phospholipids act as templating molecules

for lipopolymers in a binary phospholipid–lipopolymer mixed monolayer. Such an interpretation is in good agreements with epifluorescence microscopy studies on this binary mixture, which found no evidence for large-scale phase separations between DMPC and DSPE–PEG2000 at any lipopolymer molar concentration studied [42]. This result is particularly notable if one considers that binary mixtures of phospholipids with a comparable mismatch in acyl chain length are known to exhibit pronounced phase separations [43, 44].

It is also instructive to analyze the behavior of mixtures where both phospholipids and lipopolymers contain the same sized 18 carbon lipid tails, such as mixtures of DSPC and DSPE–PEG2000. $\pi - A$ isotherms of these mixtures are qualitatively similar to the isotherms of DMPC/DSPE–PEG2000 mixtures. The high pressure transition can be seen with mixtures as low as 10 mol% lipopolymer, but at this concentration it occurs at a higher surface pressure [45]. By plotting the area per molecule vs mole fraction of DSPE–PEG2000 for a constant surface pressure at 6.1 mN m^{-1} (below the first transition point) and at 14.8 mN m^{-1} (above the first transition point), Xu et al. also obtained several other interesting results, as shown in Fig. 17. They found that at the low surface pressure, 6.1 mN m^{-1}, an exactly linear relationship existed between area per molecule and mole fraction, indicating there is additivity in molecular area with increasing PEG–lipid. In other words, both PEG–lipid and lipid compete equally for space at the air–water interface in that regime. At the higher pressure, however, increasing the fraction of PEG–lipids up to about 5 mol% does not increase the average area per molecule proportionately. The authors concluded that in this regime, at very low concentrations, the area per molecule is dominated by the headgroup area of the phospholipid at the interface, but at around 5 mol% lipopolymer the area per molecule value for a given pressure begins to become dominated by the area occupied by the lipopolymer in the water subphase. Moreover, this effect is most marked during a transition which starts about 5 mol% and continues to around 20 mol%. Above 20 mol% lipopolymer, the area per molecule is again a straight line proportional to the concentration of DSPE–PEG2000, as can be seen in Fig. 17. This elegant experiment shows that for low pressures, phospholipids and lipopolymers mix homogeneously at the air–water interface, and lipopolymers act essentially the same as phospholipids, but at higher pressures, the polymer moiety plays a significant role in determining the surface pressure. Xu et al. also looked at $\pi - A$ isotherms of pure PEG-2000, unconnected to a lipid anchor, and determined that it submerges from the air–water interface at pressures a little less than 5 mN m^{-1}, lending support to the concept that the first transition is the submersion of the polymers from the surface.

Xu et al. also considered the hydration of the polymer moiety of PEG lipopolymers at different surface pressures and concentrations [45]. From $\pi - A$ isotherms of pure PEG, they determined that each PEG monomer is fully hydrated with about three water molecules. Upon increasing the concentration of PEG lipopolymers at the air–water interface, they determined that the water is gradually squeezed out. This finding leaves the possibility that the high-pressure plateau of lipopolymers is at least partially accompanied by a dehydration process in the polymer moiety. Thus, the energetic factors contributing to the second transition and the acyl chain conden-

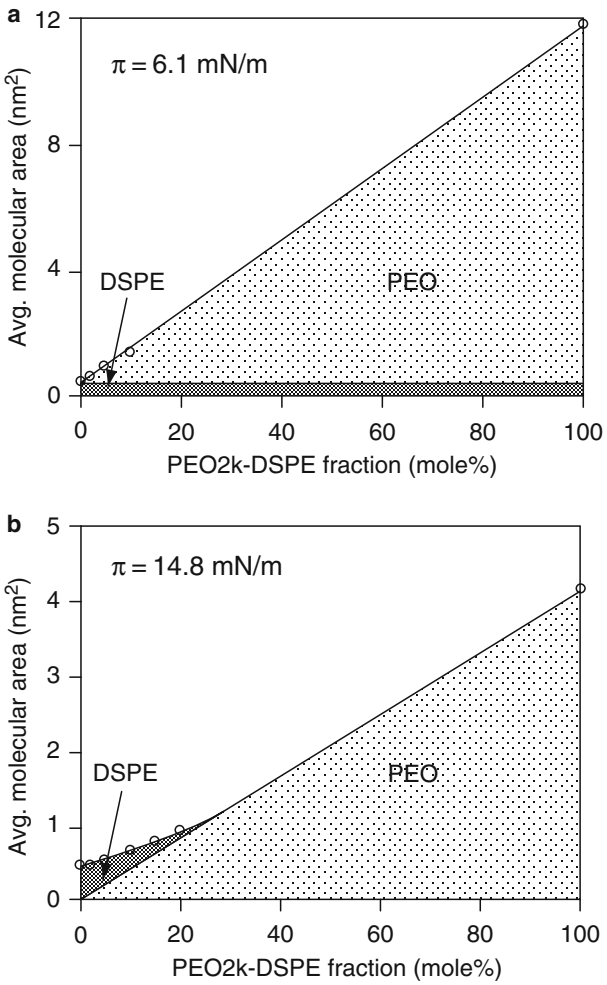

Fig. 17 a, b Area occupied per PEG2000 molecule grafted to DSPE as a function of mol% of DSPE–PEG2000 in the lipid mixture at surface pressures of **a** 6.1 mN m^{-1} and **b** 14.8 mN m^{-1} [45] (reproduced with permission from the American Chemical Society)

sation include not only the enthalpic gain of the lipids becoming aligned but also the entropic loss of the dehydration of the PEG chains and the entropic loss due to the lipid ordering. This dehydration has been reported as a suggestion that water acts as a poor solvent for lipopolymers at higher pressures [6]. Finally, through comparison of pure PEG $\pi - A$ isotherms with mixed monolayer systems, Xu et al. considered whether it was appropriate to label the high pressure transition a mushroom to brush transition. If a brush is said to be present when there are no remaining monolayers at the air–water interface, and the surface area per monomer is determined through the $\pi - A$ isotherm of the PEG2000 in the pancake conformation, then it is a simple

matter to calculate the area per molecule when the last PEG monomer will desorb from the surface. Xu et al. calculated that the transition to brush occurs at areas slightly smaller than the first transition, but much larger than the second, high pressure transition. The mushroom-brush nomenclature, however, may not be the best terminology if the systems are, as discussed in Sect. 2.3, in jammed micelles or other aggregates.

$\pi - A$ isotherms of mixtures have also been taken at different temperatures as well, in an effort to understand the stability of these monolayers and the entropic factors associated with the mixing [46]. There, a two-dimensional Clausius–Clapeyron equation was used to find the heat of mixing, and from this, the entropy associated with the low pressure transition. Unfortunately, these authors did not extend their analysis to mixtures at the high pressure transition to compare their findings with the predictions of Xu et al. Majewski et al. earlier published $\pi - A$ isotherms of DSPE with 0–9% DSPE–PEG2000 with nearly identical results to those reported by Xu et al. [47]. For example, 9 mol% lipopolymer also displayed a high pressure transition. Their work is particularly interesting because the film balance experiments were accompanied by complementary neutron reflectometry studies which will be discussed below.

Different experimental methods have been used to obtain information on how the phospholipids and lipopolymers pack together in a binary mixture. Using neutron reflectometry, Majewski et al. determined the scattering length density profile of the monolayer perpendicular to the air–water interface for mixtures of DSPE with 0–9 mol% DSPE–PEG2000 at high surface pressures, around 40–45 mN m^{-1} well above π_{high} [47]. Reflectivity curves and corresponding scattering length density profiles from this study are depicted in Fig. 18.

Majewski et al. found that for the system of pure DSPE at this high pressure of 42 mN m^{-1}, the lipid tails obtain their greatest density around 25 Å below the surface (the air–lipid interface), the head group is evident by a change in density around 40 Å below the surface, and this is followed by a return to the density of water at around 50 Å below the surface. The reflectometry curves in the presence of 1.3 and 4.4 mol% DSPE–PEG2000 are qualitatively similar to that of pure DSPE. In particular the 1.3 mol% PEG trace shows great similarity, with only a slightly greater depth for the location of the head group to 45 Å below the surface, and the trace has largely returned to the density of water around 55 Å below the surface. For the 4.5 mol% PEG trace, the acyl chain peak is less pronounced, but the depletion layer signifying the headgroup is still very prominent. In addition, there is a contribution to the density from the polymeric chain beyond 55 Å even down to 110 Å below the surface. The situation for the 9 mol% PEG trace, however, is quite different. The acyl chain peak is much less pronounced, with a peak perhaps one third the height of the pure DSPE system. Next, unlike the other mixtures, there is no corresponding dip in the scattering length density signifying the headgroup, but the trace instead displays a slow trailing off of density. This is interpreted as showing a roughening of the acyl chains over a larger depth, as well as PEG existing in the area of the headgroup. The contribution from PEG beneath the headgroup reaches a minimum around 65 Å below the surface and then slowly returns to the density of water at

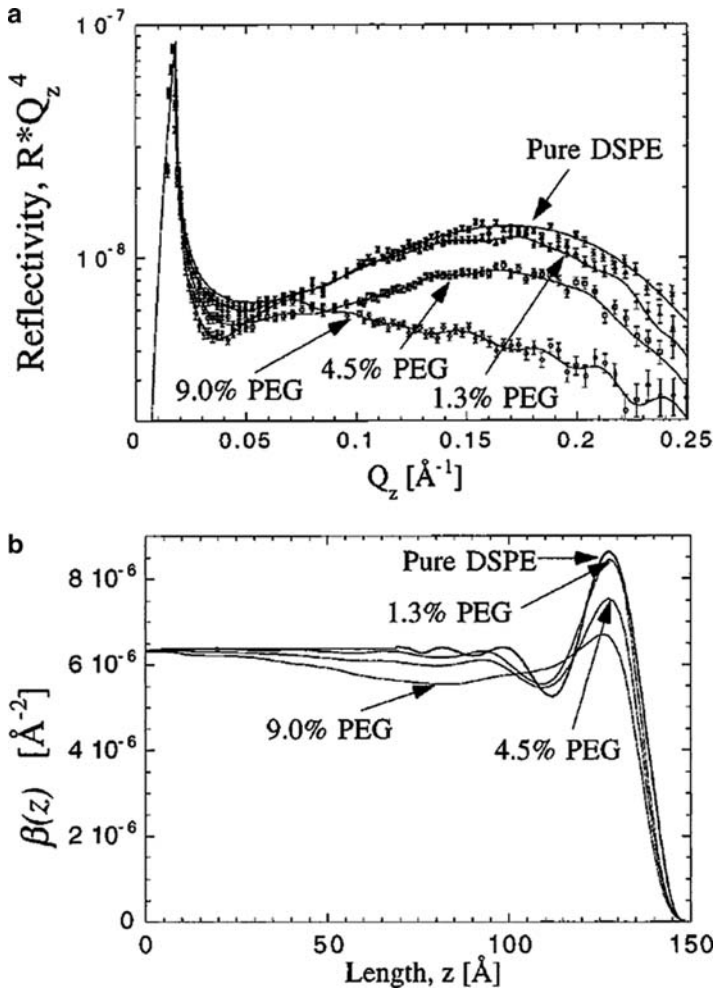

Fig. 18 **a** Neutron reflectometry data for lipid/PEG–lipid monolayers on a pure D_2O subphase. The four reflectivity curves correspond to a pure DSPE monolayer and to mixtures of DSPE and DSPE–PEG2000. In this set of data, all of the DSPE and DSPE–PEG2000 lipid hydrocarbon chains were fully deuterated (*case 1*). *Full lines* represent free form fits to the individual measurements. **b**. Corresponding scattering length densities $(\beta(z))$ obtained from the fits shown in **a** [47] (reproduced with permission from the American Chemical Society)

around 145 Å below the surface. Majewski interprets the 4.5 and 9 mol% systems as evidence of mushroom and brush conformations. In the first, here called mushroom conformation, the monolayer acts essentially as a phospholipid monolayer, but with some density beneath the headgroups. In the second, here called brush conformation, there is roughening of the lipid layer as seen by the lowering and spreading out of the acyl chain peak, and there is no expected depletion layer as the polymeric brushes are crowded in among the headgroups, and forced to stretch out further from the surface than in the lower mol% system [47]. The film balance data

support the notion that DSPE–PEG2000 has different polymer conformations at 4.5 and 9 mol% lipopolymer at 42 mN m^{-1}. The 4.5 mol% mixture does not show the high pressure transition and thus the PEG chains of the lipopolymers appear to be in roughly the same conformation as a pure lipopolymer at a lower pressure, for example, at about 15 mN m^{-1}. By contrast, the 9 mol% system appears very similar to pure lipopolymer systems above the high-pressure transition, at about 30 mN m^{-1}.

Gutberlet et al. obtained neutron and X-ray reflections from surface monolayers of phospholipid–lipopolymer mixtures of DMPC and diC_{18}−$PMOx_{30}$ at three surface pressures, 4, 17, and 30 mN m^{-1} and systems of 0, 25, and 50 mol% diC_{18}−$PMOx_{30}$. They found a linear increase in layer thickness with increasing film pressure for all three lipopolymer molar concentrations and concluded that the polyoxazoline layer thickness develops rather continuously as a function of the lateral pressure, at least up to 30 mN m^{-1} [48]. Unfortunately, Gutberlet et al. did not publish a $\pi - A$ isotherm for their mixtures. The pure PMOx system starts to transition at around 29 mN m^{-1} at 20°C [6], and introducing lipids to lipopolymers either does not change the pressure at which the high pressure transition occurs, or it increases the high pressure transition for low concentrations of lipopolymers [12] so it may well be that these data are looking at monolayers which, although at different pressures, are all in the same conformation, which would explain the linearity of change in layer thickness.

Another technique that has been used to characterize a lipopolymer–phospholipid monolayer at the air–water interface is sum frequency generation (SFG) spectroscopy [49]. SFG is useful for analyzing monolayers at the air–water interface because the conformation of the molecules at the surface can be analyzed and compared to the IR peaks of functional groups on molecules with well known conformations. For example, the OH stretches will display information on how the water interacts with the mixture: a $3,200\,cm^{-1}$ band is seen when water is hydrogen bonded to other molecules in a coordinated fashion; a $3,400\,cm^{-1}$ is observed when water is loosely coordinated or hydrogen bonded with other molecules at the surface; and a band around $3{,}700\,cm^{-1}$ is found for pure liquid water at the air–water interface. In addition, the CH_2 and CH_3 stretches can give information on the conformation and tilt angle of the lipid tails. Ohe et al. took data on monolayers of DSPE with varying concentrations of DSPE–PEG2000 from 0 up to 16.7 mol%, at 5, 15, and 35 mN m^{-1}, corresponding to the states below, between and above the two transition pressures [49].

These authors showed that pure DSPE displayed very low contributions of OH bands at any of the three surface pressures, corresponding to little water at the surface (as would be expected, since the top layer is all acyl chains), but there were small bands at 3,200 and 3,400, though none at 3,700. On increasing the mole fraction of DSPE–PEG2000 in the monolayer, however, both the 3,200 and $3,400\,cm^{-1}$ bands became more pronounced at all surface pressures, increasing with mol fraction. This reconfirms that the PEG headgroups at the surface are surrounded by a hydration shell and thus there are tightly coordinated waters hydrogen bonded to the PEG. In fact, the ice-like band, $3,200\,cm^{-1}$, becomes more pronounced on increasing pressures or increasing DSPE–PEG2000 mol% concentration. Interest-

ingly, for concentrations of 1.3 and 4.5% DSPE–PEG2000 at all surface pressures, as well as for all concentrations at the low 5 mN m^{-1} pressure, the relative proportion of ice-like band and liquid-like bands are roughly equal, with the ice-like band having a somewhat greater amplitude than the liquid-like band. Comparing SFG data at 5 and 15 mN m^{-1} shows that increasing lipopolymer concentrations beyond 4.5 mol% does little to increase the 3,400 cm^{-1} liquid-like stretch, but it does increase the ice-like 3,200 cm^{-1} band up to 16.7 mol%. At 35 mN m^{-1}, there is very little change in either OH stretch from 4.5 to 16.7 mol%, and even pure DSPE–PEG2000 shows little change in the magnitude of the OH stretches compared to 4.5 mol%. Ohe et al. interpreted this data to show that, at higher pressures (above 5 mN m^{-1}) and higher concentrations (above 4 mol%), there was no corresponding increase in water as would be expected from water hydrogen bonded to the PEG, but instead the PEG in those systems must be increasingly dehydrated relative to the PEG in the lower pressure or lower concentration monolayers. Thus, energetic factors must be responsible for squeezing the water out of the monolayer. This analysis agrees in principle with the findings of Xu et al. with regard to dehydration, which were obtained from film balance studies [45].

The CH_2 and CH_3 stretches were also analyzed at the same variations of pressures and concentrations [49]. Typically, CH_3 can display a band at 2,950 cm^{-1} which is the overlap of an asymmetric stretching at 2,960 cm^{-1} and Fermi resonance bands at 2,940 cm^{-1} and a stretch at 2,870 cm^{-1} corresponding to a CH_3 symmetric stretching band. In addition, there is a CH_2 symmetric band at 2,850 cm^{-1} which corresponds to a system with gauche isomers. The gauche isomer is slightly energetically less stable, but is found in liquid or noncondensed systems. For pure DSPE–PEG2000, at 15 mN m^{-1} there is a slight band showing evidence of gauche isomers. At 35 mN m^{-1}, this band has disappeared, and there is no evidence of gauche isomers. This is in agreement with the findings in [6]. Interestingly, in the mixed monolayers, Ohe et al. found no evidence of the 2,850 cm^{-1} band, that is, no gauche isomers are seen, at any concentrations or pressures [49]. By comparing the line amplitude of the symmetric and asymmetric stretches of the terminal methyl groups in the different mixtures, it is also possible to draw conclusions about the tilt angle of the terminal methyl groups. At the low surface pressure of 5 mN m^{-1}, tilt angle increases with increasing concentration of DSPE–PEG2000 to nearly 90° at greater than 10 mol% DSPE–PEG2000. This result can be well understood: There is a decrease in the density of lipid tails and therefore terminal methyl groups with increasing concentration of lipopolymer and this causes the lipid tails to be less upright, and thus the tilt angle becomes larger. The situation is different for the 15 and 35 mN m^{-1} pressures. For 35 mN m^{-1}, initially the tilt angle increases slightly with increasing mol fraction of lipopolymer, but by 10 mol%, the tilt angle has reached a plateau of around 47° and it stays there up to 16 mol%. Ohe interprets these data for the 35 mN m^{-1} system to show that the PEG groups are completely submerged at this pressure and the acyl groups of the DSPE–PEG2000 interact much as the DSPE itself, so a change in relative concentration does not change the tilt angle of the terminal methyl groups of either substituent. For the intermediate, 15 mN m^{-1} system, the tilt angle increases more

strongly with increasing mole fraction of DSPE–PEG2000 to about 10 mol%, where it has increased to 58° or so. Thereafter, it appears to level off somewhat, or perhaps increase slightly. This, Ohe interprets, arises because the PEG at this pressure are not completely submerged and thus the acyl chains are in an intermediate state. These authors label the 15 mN m^{-1} conformation "mushroom" and the 35 mN m^{-1} conformation "brush." It is clear from Ohe's work that the methyl groups on the end of the acyl chains behave differently in the different mixtures at the different pressures, and there is a change which occurs around 10 mol% lipopolymer where the acyl chains act in a different manner than below that concentration. Ohe also goes on to explain Majewski's result regarding the decrease in the mole fraction of acyl chains at increasing mol% of DSPE–PEG2000 as being due to the tilting of the acyl chains, at least in the region up to 10 mol% lipopolymer. This work again confirms that low concentrations of lipopolymers at high pressures in mixtures act like pure lipopolymers at low pressures and concentrations, but this must be read carefully in light of others' findings on tilt angles of lipopolymeric systems [15].

3.2 Viscoelastic Properties of Lipid–Lipopolymer Mixtures

To this point, the structural data have indicated that mixed monolayers at low pressures act like low pressure phospholipid monolayers, and mixed monolayers at medium and high pressures act like lipopolymer monolayers in different conformations, depending on the concentration of lipopolymer and the surface pressure studied. Film balance and interfacial rheology experiments on pure lipopolymer monolayers also suggest that the gelation transition occurs at or slightly above the high-pressure transition observed in $\pi - A$ isotherms. Corresponding experiments on mixed phospholipid–lipopolymer monolayers will show that the gelation transition may also occur further away from the plateau of the high-pressure transition. Before looking at the viscoelastic properties of mixtures, the viscoelastic properties of phospholipids and lipopolymers should be recalled. Monolayers of phospholipids, even in liquid condensed phases, never become elastic, which is to say the storage modulus is never greater than the loss modulus, but both do increase significantly if the monolayer is compressed to a small enough area per molecule [2]. Monolayers of lipopolymers are fluid below a rheological transition pressure which is nearly the same as the high transition pressure found via a plateau in $\pi - A$ isotherms, and elastic above the rheological transition pressure. As discussed before, the observed elasticity is probably due to the formation of small, two dimensional micellar structures which jam into each other at the air–water interface. As illustrated in Fig. 19, the rheological response of mixtures of DSPE–PEG2000 and DMPC show a very interesting trend [42]. For lipopolymer concentrations of 60 mol% or higher, the rheological response is nearly identical to that of pure lipopolymer, when looked at as a function of area per lipopolymer. The response is liquid below a transition pressure, and elastic above the pressure, and continues to be elastic at all higher pressures. In fact, the rheological transition point is the same, about 165 $Å^2$, independent of

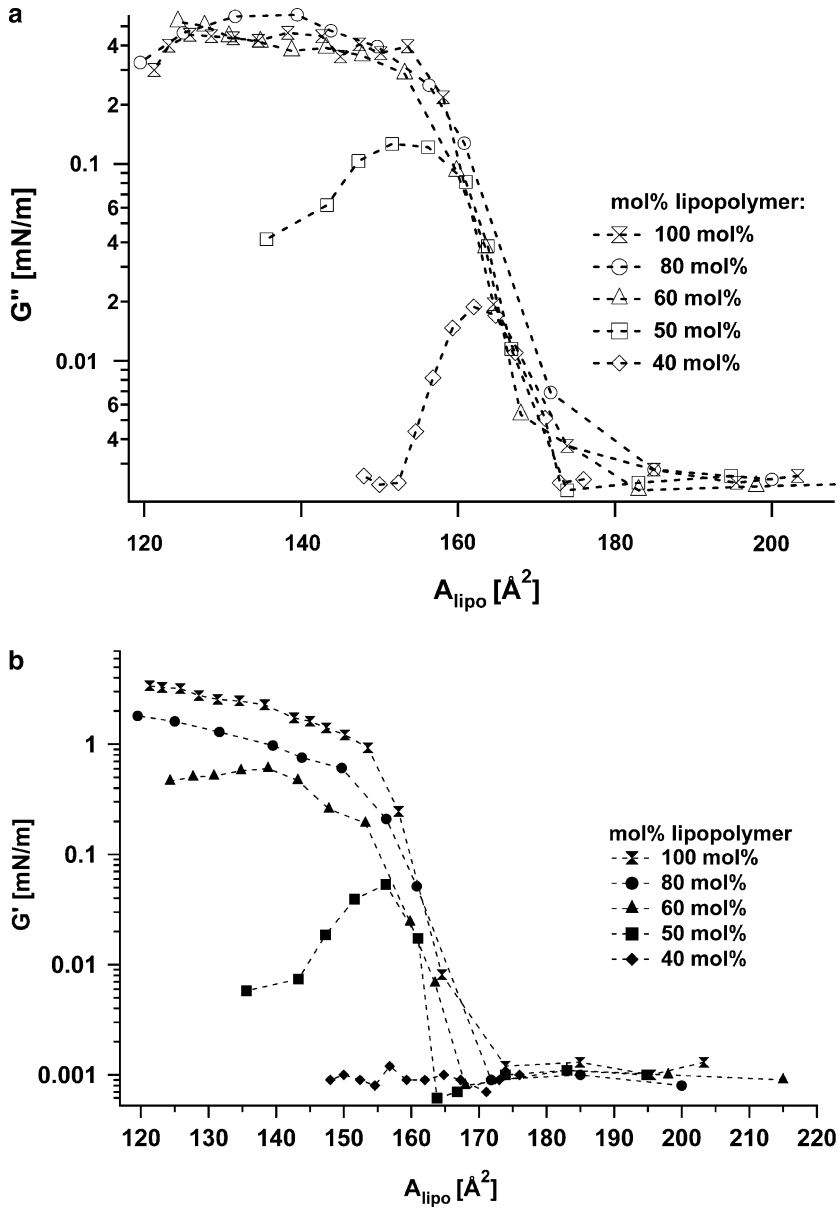

Fig. 19 a, b Viscoelastic response of the DSPE–PEG2000 monolayer as a function of amount of DMPC incorporated. Loss modulus **a** and storage modulus **b** are shown vs A_{lipo}, and are essentially independent of amount of phospholipids incorporated for mol% lipopolymer >40%. No viscoelastic transition occurs for mol% lipopolymer <40% [42] (reproduced with permission from the American Chemical Society)

the amount of phospholipids incorporated, with the only difference being that the higher mol% have a stronger elastic modulus response. This should be contrasted with the film balance studies, where high pressure transitions were seen as low as 9 mol% for DSPE/DSPE–PEG2000 systems. At 40–50 mol% DSPE–PEG2000 with DMPC, there is a rheological transition at about $A_{\text{rheo}} = 165$ Å^2 for the loss modulus, but not for the storage modulus at 40 mol%, and the increase in the storage modulus at 50 mol% never exceeds the loss modulus so the monolayer does not become elastic at any area. This is interesting because unlike the structural studies which show similar behavior down to 9 mol% lipopolymer, here a significant difference is found even at 50 mol% lipopolymer. Clearly, there are conditions where no gelation transition can be observed, even though a high-pressure transition is found.

To evaluate further the viscoelastic properties in lipid–lipopolymer mixed monolayers, the frequency dependence of the magnitude of the dynamic modulus $|G_s^*(\omega)|$ was determined as well, where $G_s^*(\omega) = G_s'(\omega) + i\ G_s''(\omega)$ [42]. Here two different situations were considered. First, $|G_s^*(\omega)|$ at different frequencies was monitored at 50 mol% DSPE–PEG2000 as a function of area per lipopolymer (Fig. 20a) Second, $|G_s^*(\omega)|$ at different frequencies was determined for different lipopolymer molar concentrations at a constant area per lipopolymer (Fig. 20b). Overall, the data in Figs. 19 and 20 indicate that increasing amounts of phospholipids weaken the ability to form gels and reduce the strength of such physical networks, thus supporting the notion of phospholipids acting as templating molecules in the mixed phospholipid–lipopolymer monolayer.[1] These data also show that, in many significant ways, dilute mixtures of lipopolymers at high surface pressures act like pure lipopolymers at low surface pressures.

As illustrated in Fig. 21, Naumann et al. also explored the reversibility of the gelation transition [42]. Here the loss modulus was tracked in a DMPC/DSPE–PEG2000 mixed monolayer with 40 mol% DSPE–PEG2000 during compression and subsequent expansion of the monolayer. Both curves are almost identical, thus suggesting a thoroughly reversible process. Interestingly, as can be seen by referring back to Sect. 2.2., the viscoelastic response for 40 mol% DSPE–PEG2000 is also remarkably similar to the behavior of the loss modulus at different A_{lipo} for monolayers of pure $DiC_{18}PEOx_{31}$, DSPE–PEG750, and DSPE–PEG1000, which underwent a comparable collapse.

To obtain more insight into the relationship between high-pressure film balance and gelation transitions, Naumann et al. also determined the location of the gelation transition in the $\pi - A$ isotherms of the DMPC/DSPE–PEG2000 mixed monolayer at various lipopolymer molar concentrations [42]. As shown in Fig. 22, unlike for pure lipopolymer systems, viscoelastic and high-pressure film balance transitions typically do not overlap. Furthermore, below 80 mol% lipopolymer, the gelation transition is clearly outside the plateau region of the corresponding $\pi - A$ isotherm

[1] Interestingly enough, the storage modulus of diblock copolymers is weakened by increasing the mol fraction of one of the substituents of the diblock, unfunctionalized PEG chains [47]. This is similar to the current situation if a DSPE-PEG2000 lipopolymer is considered a short diblock copolymer and the mixed-in phospholipid is considered one of the substituents of the diblock.

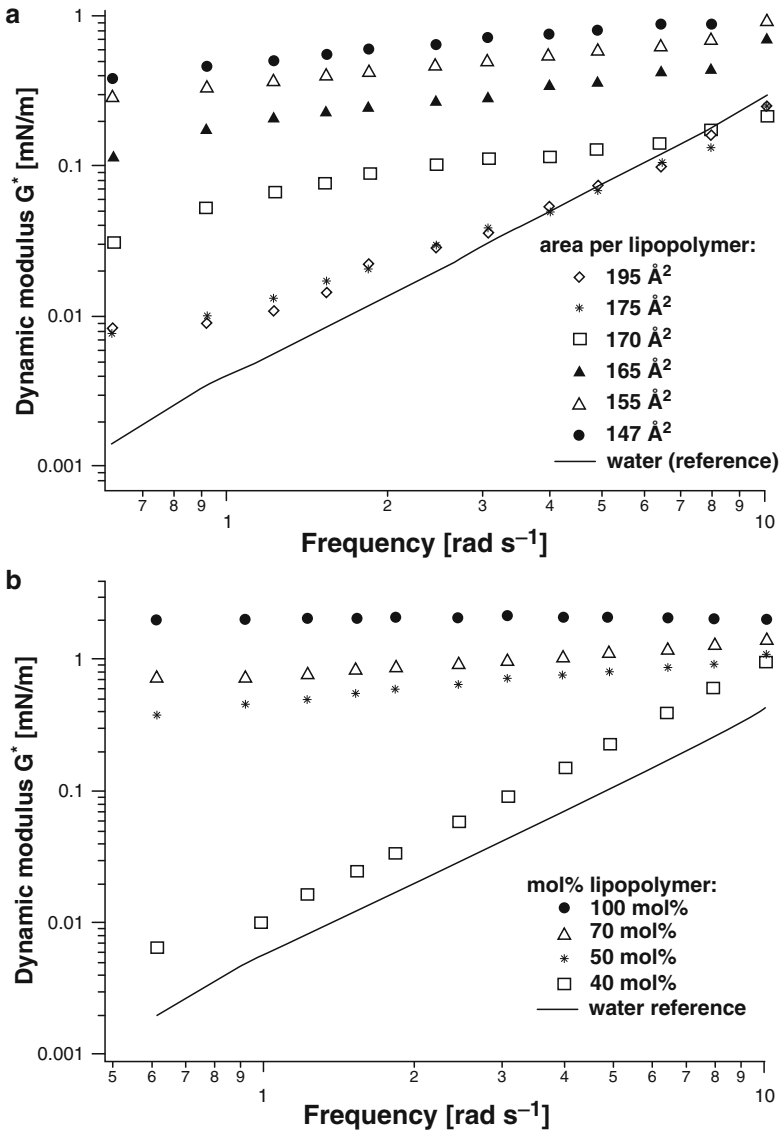

Fig. 20 a, b Frequency dependence of the magnitude of the dynamic modulus **a** for different A_{lipo} at a constant mol% = 50 mol% lipopolymer and **b** for different mol% at a constant $A_{\text{lipo}} = 150$ Å^2. At high A_{lipo} and low mol% no network forms but at low A_{lipo} and high mol% there is clear evidence of gel formation. *Solid line* represents response of the needle at the clean water surface [42] (reproduced with permission from the American Chemical Society)

and does not cause any change in the slope of the isotherms. This result clearly indicates that the high film pressure and gelation transitions describe two related, but different, transition phenomena. It also supports the notion that the high-pressure

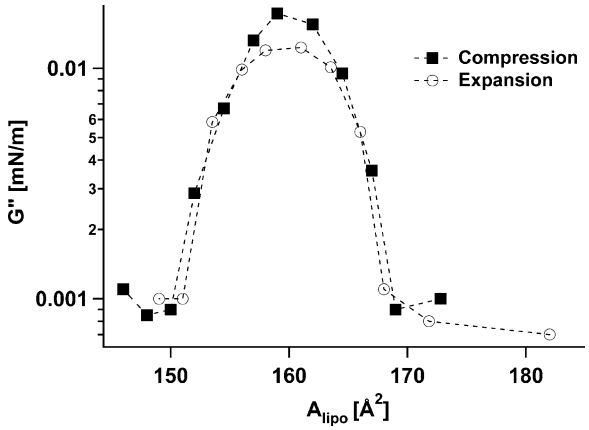

Fig. 21 Loss modulus vs A_{lipo} plotted for 40% lipopolymer during compression and expansion of bilayer shows reversibility of the viscoelastic transition. Also, the gel exhibits a collapse at smaller A_{lipo} (and higher pressures) than pure lipopolymer [42] (reproduced with permission from the American Chemical Society)

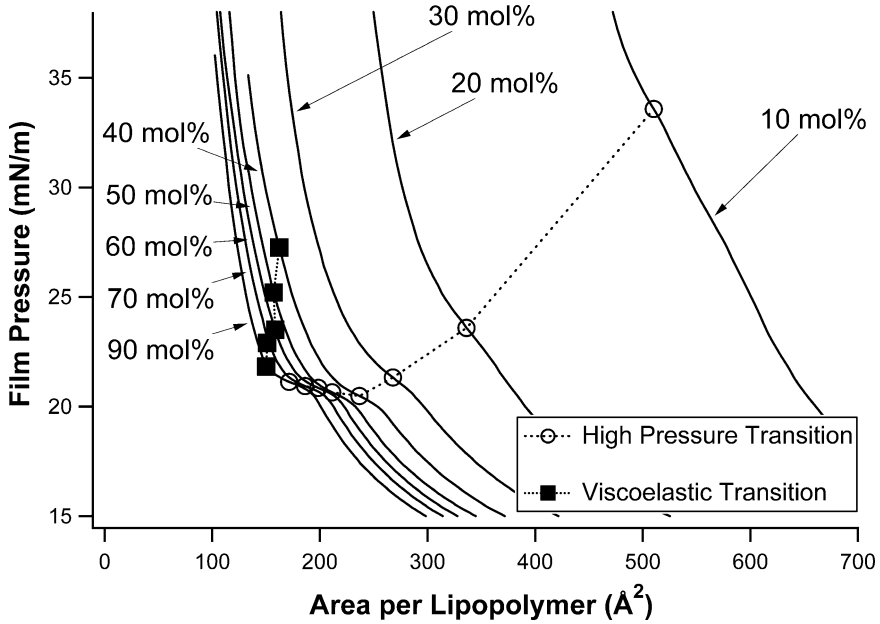

Fig. 22 $\pi - A$ isotherms of mixtures of DMPC/DSPE–PEG2000 from 10 to 100 mol% DSPE–PEG2000, where the film balance transitions are plotted for each isotherm and the viscoelastic transitions are plotted for 40–100 mol%. The transitions only coincide at 100% DSPE–PEG2000. The different trends underlie the fact that the high pressure transition and viscoelastic transition signify different physical phenomena [42] (modified, with permission from the American Chemical Society)

transition is associated with the formation of surface micelles and that the gelation transition requires not only formation but also the jamming of such micelles. Figure 22 also shows that π_{high} is well pronounced and largely constant between 30 and 100 mol% lipopolymer, but becomes less obvious and increases with decreasing lipopolymer concentrations below 30 mol%. This changing behavior between high and low lipopolymer molar concentrations can be understood in terms of the changing location of the main lateral pressure in the lipopolymer–phopsholipid mixed monolayer at high film pressure. At elevated lipopolymer molar concentrations, the main lateral pressure is localized in the polymer moiety of lipopolymers. In contrast, with decreasing lipopolymer content at medium to low lipopolymer molar concentrations, the lateral stress builds up increasingly in the lipid moieties of lipids and lipopolymers.

As an interesting side note, the viscoelastic properties of phospholipid monolayers mixed with hydrophobically modified PEG polymers (HMPEG) have also been studied [56]. A hydrophobically modified PEG polymer is a PEG polymer linked to an *n*-butyl group linked to an 18 carbon straight chain thence linked to another long PEG polymer, all through the use of peptide bonds. In this study, the PEG polymers were three or six times as long as PEG2000 and each molecule contained three to five C_{18} groups interspersed between PEG polymers. These should behave somewhat like lipopolymers, but these HMPEG are covalently linked to each other, and are investigated for the degree of protection hydrophobically modified PEG polymers can afford to liposomes to enable them to evade immune recognition and protect against complement binding. Pressure–area isotherms of mixtures of these HMPEG with phospholipids show a plateau around 10 mN m^{-1} and then an increase up to the film breaking at pressures greater than 50 mN m^{-1} with no high pressure plateau. Similarly, analysis of the storage and loss modulus show that these systems do not exhibit the elastic behavior or rheological transitions such as those found with lipopolymer monolayers (Auguste et al. 2008).

3.3 Diffusion Properties of Lipid–Lipopolymer Mixtures

The diffusion properties of mixtures of phospholipids and lipopolymers should be discussed in light of the diffusion properties of pure phospholipids and pure lipopolymers. Wide-field single molecule fluorescence microscopy studies on phospholipid (DMPC and DMPG) monolayers at the air–water interface showed that the lateral diffusion of phospholipids obeys the two-dimensional free area model [50]. As was noted in Sect. 2.3, pure lipopolymers at appropriate A_{lipo} also obey the free area model in terms of their diffusion characteristics [31]. Previously, the lateral diffusion of phospholipids in mixed phospholipid–lipopolymer mixed monolayers has been determined using fluorescence recovery after photobleaching (FRAP) and wide-field single molecule fluorescence microscopy [39, 51]. The diffusion results from these experiments are summarized in Fig. 23. We found that for lipopolymer molar concentrations up to 10 mol% corresponding to area per lipopolymer

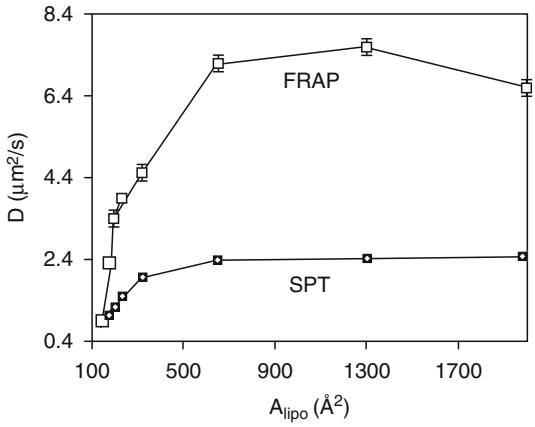

Fig. 23 Lateral diffusion coefficient D as a function of A_{lipo} using FRAP and single molecule fluorescent microscopy methods [39] (reproduced with permission from the American Chemical Society)

of a little over 600 Å^2, with a constant A_{lipid} of 65 mN m^{-1}, the diffusion coefficients were fairly constant. Then, from 10 to 30 mol%, still with A_{lipid} constant, the diffusion coefficients decrease with decreasing A_{lipo} down to 230 Å^2. These data suggest that the lateral diffusion of phospholipids becomes increasingly obstructed in the presence of significant inter polymer interactions between lipopolymers. The observed differences in diffusion coefficients for a given A_{lipo} between FRAP and single molecule imaging in Fig. 23 have been attributed to the different time and length scales of both techniques [39]. In addition, tracking inaccuracies associated with the tracking of photolabile dyes exhibiting on–off blinking should be considered. Despite these discrepancies, both experimental techniques are able to identify the different diffusion regimes described above.

To explore the impact of the high-pressure transition on lipid lateral diffusion, Ke and Naumann also determined the lipid lateral diffusion at constant 30 mol% lipopolymer but decreasing A_{lipo} down to 150 Å^2 [39]. Here, three different diffusion regimes could be identified. In the first regime, the diffusion is independent of the concentration of lipopolymer. This behavior is quite similar to the situation of pure lipopolymers at low pressures, as discussed above. The change-over to the second regime occurs around 600–650 Å^2. This corresponds in terms of the $\pi - A$ isotherms to around the end of the low pressure transition. Naumann et al. considers how squeezed a lipopolymer would be at an $A_{\text{lipo}} = 650$ Å^2, if this represents a polymer squeezed into a tube, following the calculations of de Gennes [52]. For a polymer in a thick tube, the relationship is

$$L = \frac{R_F^2}{d}. \tag{5}$$

This represents the length of the polymer if it is not squeezed, but constrained to a certain diameter d. The Flory radius for a polymer in a mushroom configuration is defined by the number of segments N, (in this case 45) and the length of the monomeric unit, a, (in this case, 3.5 Å) and is written as

$$R_F = aN^{3/5} = 34.3 \text{ Å}. \tag{6}$$

The diameter, d, is calculated from the area per lipopolymer using the familiar relation Area $= \pi r^2$. Substituting 650 Å^2 for Area gives $d = 28.8$ Å, and from (5), $L = 40.9$ Å. Free polymers in solution would have a value of $L = R_F$. According to (6), this solution of 30% lipopolymers where $A_{lipid} = 65$ Å^2 yields $L/R_F = 1.36/1$, or slightly stretched. Thus, it appears that around the density when the polymers start to interact with each other and become stretched, they cause the diffusion of lipids on the monolayer to slow down proportionally [51]. In other words, the lipopolymers start to act like obstacles instead of fellow-phospholipids. The second transition, from the second to the third diffusion regime, appears at around $A_{lipo} = 180$ Å^2. This is a significant concentration because it is around the high pressure transition seen on $\pi - A$ isotherms and the rheological transition pressure of pure lipopolymers. Not surprisingly, the lateral diffusion of lipids is obstructed below this point.

Further analysis of the relationship of the single molecule fluorescent microscopy diffusion coefficients is presented in Fig. 24 [39]. To follow the free area model, a plot of $\ln(D/D_o)$ vs (a_{min}/a_f) or, to simplify matters, a plot of ln(D) vs $1/a_f$ must be a straight line at different A studied. This is uniformly the case for phospholipids such as DMPC, but for lipopolymer mixtures, only the points which were taken at $A_{lipo} > 180$ Å^2 agree with the free area model. This corresponds to points a, b, and c, but not point d.

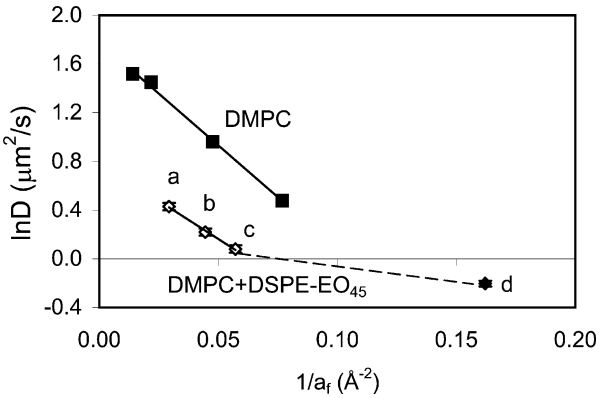

Fig. 24 Plots of ln(D) vs $1/a_f$ for DMPC and the requirements for molecules obeying the free area model, for pure DMPC and 70 mol%DMPC + 30 mol%DSPE–PEG2000. The *straight solid line* indicates that DMPC obeys the free area model. In case of the binary lipid/lipopolymer mixture, the free area model is only valid between points a–c, but not between c–d [39] (reproduced with permission from the American Chemical Society)

4 Conclusion

Overall, existing experimental findings highlight a fascinating relationship between structural, viscoelastic, and diffusion properties in mixed phospholipid–lipopolymer mixed monolayers. The various experimental findings provide strong evidence that these properties can be tuned via the lipopolymer molar concentration. At low lipopolymer molar concentrations, where no polymer–polymer interaction occurs, these peculiar amphiphiles act like their phospholipid cousins. At intermediate lipopolymer molar concentrations, moderate interlipopolymer interactions can be observed, which may have profound effects on membrane organization and dynamics, however, within the context of a fluid monolayer. At elevated lipopolymer concentrations, interlipopolymer interactions become quite strong and may lead to phenomena such as surface micellization/physical gelation and pronounced obstructed diffusion. Clearly, a fundamental understanding of properties on pure lipopolymer monolayers provides important insight into the observed behavior on phospholipid–lipopolymer mixed monolayers. Furthermore, at the air–water interface, phospholipids seem to act as templating molecules, thus providing a tool of regulating lipopolymer–lipopolymer interactions. Although the findings obtained from monolayer systems at the air–water interface cannot generally be applied to polymer-tethered bilayers, they are often quite useful for the characterization and understanding of their bilayer counterparts. Prominent examples are the obstructed diffusion of lipids and membrane proteins and the coupling of obstructed diffusion of phospholipids in polymer-tethered bilayers [40, 53].

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