Published in final edited form as:

Langmuir. 2011 April 5; 27(7): 3792–3797. doi:10.1021/la1050942.

Scattering Studies of Hydrophobic Monomers in Liposomal Bilayers: an Expanding Shell Model of Monomer Distribution

Andrew G. Richter[†], Sergey A. Dergunov[‡], Bill Ganus[‡], Zachary Thomas[‡], Sai Venkatesh Pingali[#], Volker Urban[#], Yun Liu^{§,⊥}, Lionel Porcar[¶], and Eugene Pinkhassik^{*,‡}

[‡]Institute for Nanomaterials Development and Innovation at the University of Memphis (INDIUM) and Department of Chemistry, The University of Memphis, 213 Smith Chemistry Building, Memphis, Tennessee 38152-3550

[†]Department of Physics and Astronomy, Valparaiso University, Valparaiso, Indiana 46383

[#]Center for Structural Molecular Biology, Oak Ridge National Laboratory, P.O. Box 2008 MS-6430, Oak Ridge, Tennessee 37831-6430

[§]NIST Center for Neutron Research, National Institute of Standards and Technology, 100 Bureau Drive, MS 6100, Gaithersburg, MD 20899-6100

[⊥]Department of Chemical Engineering, University of Delaware, Newark, DE 19716

[¶]Institut Laue-Langevin, BP 156 6, rue Jules Horowitz, 38042 Grenoble Cedex 9, France

Abstract

Hydrophobic monomers partially phase-separate from saturated lipids when loaded into lipid bilayers in amounts exceeding 1:1 monomer:lipid molar ratio. This conclusion is based on agreement between two independent methods of examining the structure of monomer-loaded bilayers. Complete phase separation of monomers from lipids would result in increase in bilayer thickness and slight increase in the diameter of liposomes. Homogeneous distribution of monomers within the bilayer would not change the bilayer thickness and would lead to the increase in the liposome diameter. The increase in bilayer thickness, measured by the combination of small angle neutron scattering (SANS) and small angle X-ray scattering (SAXS), was approximately one half of what was predicted for complete phase separation. The increase in liposome diameter, measured by dynamic light scattering (DLS), was in the middle between values predicted for homogeneous distribution and complete phase separation. Combined SANS, SAXS, and DLS data suggest that at 1.2 monomer:lipid ratio, approximately one half of monomers are located in an interstitial layer sandwiched between lipid sheets. These results expand our understanding of using self-assembled bilayers as scaffolds for directed covalent assembly of organic nanomaterials. In particular, partial phase separation of monomers from lipids corroborates successful creation of nanometer-thin polymer materials with uniform imprinted nanopores. Pore-forming templates do not need to span the lipid bilayer to create a pore in the bilayer-templated films.

Introduction

Lipid bilayers are attractive self-assembled scaffolds for directed assembly of functional nanomaterials.¹⁻¹⁵ We used them for the synthesis of nanorattles,¹ rigid organic nanodisks,² and nanothin membranes containing nanopores with programmed size and chemical environment.³⁻⁶ Other groups also used liposomes to form polymer nanocapsules.⁷⁻¹⁴ In

*Corresponding author epnhssk@memphis.edu.

these applications, hydrophobic monomers are polymerized in the hydrophobic bilayer interior. Bilayers act as two-dimensional solvents and limit the thickness of polymerized materials. Detailed knowledge of interactions between hydrophobic monomers and lipid bilayers is important for shaping supramolecular assemblies and possibly controlling the structure and properties of new nanomaterials. Understanding of monomer distribution within the bilayer would help in further development of methods for directed assembly of organic nanostructures using self-assembled scaffolds.

Most of the previous studies of interactions between hydrophobic molecules and lipid bilayers focused on biologically relevant molecules such as steroids, vitamins or anesthetics.¹⁶⁻¹⁸ Very few systematic studies of interactions between monomers and bilayers have been published. Recent and growing use of bilayers as organized scaffolds for directed assembly of nanostructures highlighted the need for detailed understanding of interactions between lipids and building blocks accommodated within the bilayer. In contrast with small amounts of bioactive molecules that are dissolved within biological membranes, monomers are routinely loaded into bilayers in large amounts. In recent studies, we showed that loading of styrene derivatives into bilayers by exposing aqueous solution of liposomes to neat monomers results in monomer:lipid ratios of up to 1.2.^{19,20} When monomers were mixed with lipids prior to the formation of liposomes, 3:1 monomer:lipid ratio was achieved.⁶

We have previously reported controlled loading of monomers into lipid bilayers.¹⁹ We found that the loading of monomers has a timescale of several hours and the initial loading rate is inversely proportional to the aqueous solubility of monomers. Interestingly, while the monomer/lipid molar ratio in fully loaded liposomes varied among different monomers, the total volume of monomers was constant for each lipid. The curvature of the bilayer affected neither kinetics of monomer loading nor that total capacity of the bilayer.²⁰ We are thus gaining the control over critical parameters necessary to produce nanocapsules consistently for practical applications, such as drug delivery or creation of nanoreactors and nanosensors. However, many questions remain to be answered for complete understanding of interactions between lipids and monomers. One particularly interesting question is where do monomers localize within the bilayer? One can imagine two opposite possibilities (Figure 1). Monomers could completely phase separate from lipids and form an interstitial layer (Figure 1A) or, conversely, distribute randomly throughout the bilayer (Figure 1B). The real monomer distribution may also be anywhere between these extremes.

One could hypothesize that the homogeneous distribution of monomers in the bilayer (Figure 1B) may result in the formation of a sponge-like structure upon polymerization. On the other hand, phase-separated monomers (Figure 1A) may form a dense polymer film.

Here we demonstrate partial phase separation between saturated phospholipids and styrene monomers. We used multipronged approach to reach this conclusion. Bilayer thickness was measured by small angle X-ray scattering (SAXS) and small angle neutron scattering (SANS). SAXS data were reported previously.¹⁹ The increase in liposome diameter was measured with dynamic light scattering (DLS), and the results were interpreted using an expanding shell model. These methods were chosen because of the limitations of traditional techniques for studying the depth of bilayer penetration, such as quenching of fluorescence of lipid-bound chromophores, in applications involving high concentrations of small molecules within the bilayer.

We used unilamellar liposomes with narrow size distribution that can be reliably prepared by conventional techniques.²¹ We chose styrene as the model monomer for this work because styrene-based monomers were used in the synthesis of nanocapsules and because deuterated styrene is readily available for SANS experiments.

Experimental Details

Chemicals²¹

All solvents used were HPLC grade. The phospholipid (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was purchased from Avanti Polar Lipids, Inc. as a dry powder. Styrene-*d*⁸ was purchased from Cambridge Isotope Lab.

Unilamellar liposome preparation

Unilamellar liposomes were prepared by extrusion method.²² DMPC (20 mg) was hydrated with 1 mL of D₂O to produce a suspension of multilamellar liposomes. The solution was then extruded through a polycarbonate membrane with 0.1 μm pores (GE Whatman) using an Avanti Mini-Extruder (Avanti Polar Lipids).

Liposome loading with monomers

1 mL of liposomes was transferred into a 5 mL glass vial containing a 5×2 mm diameter PTFE coated stir bar. 50 μL of styrene-*d*⁸ was added, and the vial was sealed with the cap (polypropylene with pulp-backed metal foil liner). Excess styrene was clearly visible floating atop the aqueous liposome solution throughout every experiment. Each sample was stirred gently (approximately 60-90 rpm) to prevent the formation of a monomer emulsion in the liposome solution. Loading with monomers was carried out for at least 12 hours at 4-6 °C. We showed previously that liposomes become fully loaded with styrene in less than 10 hours.¹⁹

High performance liquid chromatography (HPLC)

50 μL of sample was taken from the bottom of the vial and mixed with 950 μL of methanol to lyse liposomes. 20 μL of sample or standard was injected into split-mode injector. Analytical HPLC was performed with a Waters 600 pump and Waters 2487 dual wavelength UV-vis detector. The detection wavelengths used were 250 and 270 nm. The column was a Nova-Pak C18, 3.9mm diameter × 150mm length. HPLC grade methanol was used as the mobile phase. The flow rate was 2 mL/min. Samples or concentration standards were run at least 5 times for each measurement, and the data were averaged. Standard samples of known concentrations of styrene-*d*⁸ were prepared by both direct addition of styrene-*d*⁸ to methanol and by serial dilution for lower concentrations to obtain a calibration curve for quantifying HPLC data.

Dynamic light scattering (DLS)

DLS measurements were performed on a Malvern Nano-ZS zetasizer (Malvern Instruments Ltd., Worcestershire, U.K.). 50 μL samples taken from the reaction vials with a pipette and were placed into disposable cuvettes without dilution (70 μL, 8.5mm center height Brand UV-Cuvette micro). The DLS scans were run at 25°C. Each data point was an average of 10 scans. The refractive index of the solution was measured to be 1.3360 before and after loading of styrene.

Small angle neutron scattering (SANS)

SANS measurements were performed with the CG-3 Bio-SANS instrument²³ at the High Flux Isotope Reactor (HFIR) facility of Oak Ridge National Laboratory and at the NG-7 SANS instrument at the National Institute of Standards and Technology's Center for Neutron Research (NCNR).²⁴ Quartz cells of 1 mm thickness were used to hold the liquid samples. At NCNR the shortest detector distance, 1.0 m, was employed to collect data over the range of scattering vectors $0.045 \text{ \AA}^{-1} < Q < 0.55 \text{ \AA}^{-1}$ at a fixed neutron wavelength (λ) of 6 Å. $Q = (4\pi/\lambda)\sin\theta$ and 2θ is the scattering angle. At HFIR, a detector distance of 1.7 m was

used at the same neutron wavelength to achieve a Q range of $0.013 \text{ \AA}^{-1}$ to $0.37 \text{ \AA}^{-1}$. The wavelength spread, $\Delta\lambda/\lambda$, at NCNR is 12% and at HFIR 15%. Both beamlines use Ordela area detectors. The scattering intensity profiles $I(Q)$ versus Q , were obtained by azimuthally averaging the processed 2D images, which were normalized to incident beam monitor counts, and corrected for detector dark current, pixel sensitivity and empty beam scattering background.²⁵

In order to maximize contrast, fully hydrogenated DMPC liposomes were formed in 100% D_2O and were loaded with deuterated monomers as described above. At NCNR, the concentration of lipids was 20 mg/ml corresponding to a volume fraction of 2%; at HFIR, the concentration was 2 mg/ml. The samples were held at 30°C in order to stay above the gel transition temperature (23°C for neat DMPC).²⁶ Data was collected for 90 minutes at NCNR and 30 minutes at HFIR. Background scans of 100% D_2O , integrated over the same times, were subtracted from the data.

The data was quantitatively analyzed by performing modified Guinier analysis.^{27,28} At larger Q , corresponding to smaller length scales, the fact that the lipid bilayer is curved becomes unimportant, instead appearing as a sheet with some thickness d . For $QR_g < 1$, where R_g is related to the thickness as $d = R_g \sqrt{12}$, the intensity of scattering from a sheet goes as

$$I \propto \frac{1}{Q^2} \exp(-Q^2 R_g^2) \quad (1)$$

Therefore, plotting $\ln(IQ^2)$ versus Q^2 will give a line with slope $-R_g^2$ from which the thickness can be calculated.

Results and Discussion

We used two independent methods, described below, to arrive at the conclusion of partial phase separation of monomers from the lipids (Figure 2). One approach is based on examining the change of bilayer thickness caused by accommodating monomers. In the case of phase separation, the thickness of the bilayer is expected to increase proportionally to the amount of monomers. Experimentally determined styrene:DMPC ratio of 1.2:1 translates to 0.73 nm increase in bilayer thickness. A combination of SAXS and SANS measurements shows that the thickness increased by 0.3-0.4 nm, suggesting that roughly one half of monomers are phase separated from lipids, and the rest are homogeneously distributed within the bilayer.

Another approach is based on applying an expanding shell model to two scenarios of monomer distribution shown on Figure 1. Homogeneous distribution of monomers would result in the increase of liposome diameter from 113 nm to 124 nm as shown below. Complete phase separation of styrene from DMPC would increase liposome diameter by 1.5 nm. Experimentally measured diameter increase from 113 to 119 nm suggests partial phase separation of monomers, in agreement with combined SAXS and SANS data.

Bilayer thickness and SANS

In the case of complete phase separation and neglecting the bilayer curvature, the expected thickness increase can be calculated from equation (2):

$$\Delta d = 2 \frac{n \times MW}{S_l \times \rho \times N_A} \quad (2)$$

Where Δd is the difference between the thickness of empty and loaded bilayers, n is the monomer/lipid molar ratio, MW is the molecular weight of monomers (112 for styrene- d^8), S_l is the surface area of a lipid molecule (0.61 nm² for DMPC above its phase transition temperature),²⁹ ρ is the density of monomers (0.98 g/ml for styrene- d^8), and N_A is the Avogadro's constant.

The styrene:DMPC ratio was determined experimentally using HPLC analysis of liposomes loaded with monomers.²⁰ In these experiments, neat monomers were added in excess to the aqueous solution of liposomes and allowed to diffuse into the bilayer. After 12 hour, an aliquot of the aqueous solution was separated from neat monomers, mixed with methanol, and analyzed with HPLC. The amount of styrene soluble in water was subtracted from the total amount of styrene found in the aliquot. The amount of water-soluble styrene was more than an order of magnitude lower than the total amount of styrene. This method yielded the amount of styrene associated with the bilayer. Using $n=1.2$ as the experimentally determined styrene:DMPC ratio, we calculated Δd to be 0.73 nm.

We used a combination of small angle X-ray scattering (SAXS) and small angle neutron scattering (SANS) to determine the change in bilayer structure induced by the hydrophobic monomers. SAXS offered information on the distance between polar heads of the phospholipids with coordinated water molecules (d_h , Figure 3).¹⁹ Due to insufficient contrast between hydrocarbon monomers and hydrocarbon fatty acid chains of lipids, electron density profile of the loaded bilayer, obtained from SAXS, could not offer insight into the localization of the monomers within the bilayer. SANS was used to measure the thickness of the hydrophobic region of lipid tails (d_t , Figure 3). To maximize the contrast with hydrophobic hydrocarbon region and minimize incoherent scattering, we used 100% D₂O as a solvent. Although calculated scattering length density (SLD) of phospholipid heads is not equal to the SLD of 100% D₂O, multiple D₂O molecules, coordinated within the polar head group region, helped minimize the contrast with the solvent, resulting in prominent signal from the lipid tails. An alternative to measuring the bilayer thickness could be direct measurements of the layer of monomers if the lipids were fully contrast-matched with a D₂O/ H₂O mixture. We were unable to obtain useable data with this approach. On the one hand, due to significant differences in SLD of polar heads and non-polar tails, achieving a perfect match is extremely challenging. On the other hand, increasing amount of hydrogens in the solvent increases incoherent scattering in the high Q region, drastically complicating accurate background subtraction and providing data with high noise level and no discernable features in the lengthscale of expected thickness of monomer layer (between 0.7 and 3 nm).

SAXS and SANS measurements were performed on empty liposomes and liposomes loaded with styrene in approximately 1.2:1 monomer/lipid ratio. SAXS data were reported previously.¹⁹ The monomer content was determined by HPLC.²⁰ Changes in the distance between polar head groups ($\Delta d_h = d'_h - d_h$) and the thickness of the hydrophobic (tail) region of the bilayer ($\Delta d_t = d'_t - d_t$) were very similar. Similarity of Δd_h and Δd_t suggests that accommodation of monomers did not affect the conformation of lipids.

The data at high Q for the loaded and unloaded samples are shown in Figure 4. Simple models of the SLD profile of the DMPC bilayer did not lead to satisfactory fits to the data for either the loaded or unloaded samples. Due to paucity of strong features in the scattering

curve, rather than using a more complicated model of the SLD profile, the data was instead analyzed using Eq. (1) to determine the change in the overall thickness of the lipid bilayer upon loading.

Figure 5 shows the results of this modified Guinier analysis for the NCNR data. The data are adequately linear over the region of $0.045 \text{ \AA}^{-1} \leq Q \leq 0.087 \text{ \AA}^{-1}$, giving slopes of $-88.9 \pm 2.0 \text{ \AA}$ for the unloaded sample and $-105.1 \pm 2.0 \text{ \AA}$ for the loaded sample. The thicknesses corresponding to these slopes are $3.27 \pm 0.04 \text{ nm}$ and $3.55 \pm 0.03 \text{ nm}$, respectively, indicating that upon loading the bilayer swells by $0.28 \pm 0.05 \text{ nm}$. Data from HFIR (not shown) looks very similar and gives a bilayer thickness increase of $0.26 \pm 0.03 \text{ nm}$.

It should be noted that the particular values for the thicknesses are somewhat dependent on the Q range that is used for the linear fitting and the amount of incoherent scattering from the sample that is subtracted from the data; however, by treating the unloaded and loaded data in exactly same way, the thickness change upon loading is independent of these choices. The change in thickness found using SANS is similar to, though less than, that found using SAXS in a previous experiment, which was determined to be 0.42 nm .¹⁹ Using SANS, Uhríkova, et al, found that loading DOPC liposomes with n-decane caused a swelling of $0.24 \pm 0.13 \text{ nm}$,²⁸ comparing favorably with our SANS results.

Expanding shell model and DLS

The results of SANS studies were corroborated with DLS measurements. Accommodation of monomers within the bilayer causes increase in liposome size. This increase can be described in terms of an expanding shell model. Let us examine two distinct cases of shell expansion corresponding to either complete phase separation or completely homogeneous distribution of monomers (Figure 6).

In the case of complete phase separation, the thickness of the shell should increase as described by Equation 2. The approximate diameter increase for DMPC liposomes loaded with styrene in 1.2 monomer:lipid ratio is twice greater than the bilayer thickness increase, or approximately 1.4 nm . In these calculations we neglect the curvature of the bilayer. For most common unilamellar liposomes with diameter in the $50\text{--}200 \text{ nm}$ range, the diameter is at least 20-fold greater than the shell thickness; therefore, a simplified equation is sufficiently accurate, with $<5\%$ error in overestimating the diameter increase.

If no interstitial layer is formed, the thickness of the bilayer should not change. Since the volume of the bilayer is now increased due to monomer accommodation, the radius of the shell should increase accordingly. Equation 3 describes the change in diameter assuming constant bilayer thickness:

$$D' = 2 \sqrt{\frac{V_{\text{mon}}}{8l\pi} + (Rl)^2} + l \quad (3)$$

Where D' is the diameter of the expanded liposome, V_{mon} is the volume of monomers in each liposome, R is the radius of empty liposome, and l is one half of the bilayer thickness or the length of a phospholipid molecule (1.9 nm for DMPC).

Using volume of loaded monomers, obtained from HPLC analysis, and starting with liposomes with the diameter of 113 nm , the diameter of the expanded shell should be 124 nm in case of homogeneous distribution of monomers (Table 1). The experimental value was determined to be 119 nm by dynamic light scattering (DLS) as shown on Figure 7.

These data suggest partial phase separation of monomers from lipids, in agreement with SANS data discussed above.

In these experiments, neat styrene was added to the aqueous liposome solution, and the sample was monitored by DLS (Figure 7a). Simultaneously, styrene content in the bilayer was measured by HPLC as described previously (Figure 7b).²⁰ In control experiments, a solution of blank DMP liposomes was monitored by DLS to show no change in liposome size over time (Figure 7a). Increase in liposome size correlated with the increase of styrene content in the bilayers. Such increase is made possible by the diffusion of water molecules through the bilayer. As monomers populate the bilayer interior, the hydrophobic barrier for water diffusion should increase, reaching the point when no noticeable diffusion happens at ambient conditions. At this stage, accommodation of further monomers in the bilayer would become unfavorable. These considerations may explain why bilayers of saturated lipids have limited capacity toward accommodating monomers through diffusion while allowing greater amounts of monomers to be placed within the bilayer when lipids and monomers are hydrated together.

Combining SANS, SAXS, DLS and HPLC data, we conclude that the most likely structure of the lipid bilayer containing styrene monomers is that shown on Figure 2. At styrene:DMPC ratio of 1.2, approximately one half of monomers are phase separated from the lipids. It is likely that at higher monomer:lipid ratios even greater amount of monomers are located in an interstitial layer. The simplicity of the DLS monitoring is attractive for studying interactions between amphiphilic scaffolds and a broad range of building blocks. We believe the expanding shell model would be a useful tool in further development of scaffold-templated synthesis of nanostructures.

This distribution of monomers within the bilayer corroborates successful imprinting of nanopores using templating molecules that do not span the membrane.^{3,4,6} As we have previously shown, hydrophobic molecules, such as glucose pentaesters, can be dissolved in the bilayer interior together with monomers (Figure 8).

Following the polymerization and template removal, we can isolate nanometer-thin crosslinked polymer materials with uniform imprinted nanopores. The bulk of monomers and crosslinkers are concentrated in the interstitial layer in the middle of the bilayer. It is reasonable to expect that large hydrophobic molecules would be accommodated within the same interstitial layer where they would cause minimal changes of the bilayer structure. It appears that the preferred location for growing polymer network is in the interstitial layer between lipid leaflets. A likely scenario is the formation of a dense polymer film in the middle of the bilayer. As a consequence, even molecules that do not span the membrane are likely to be suitable for incorporation in the bilayer-templated polymer materials. Such compounds may be pore-forming templates, as shown in recent papers, or molecules with recognition sites or catalytic centers.

Conclusions

We examined localization of hydrophobic monomers inside the phospholipid bilayer using complimentary techniques. Combined SAXS and SANS data suggest that upon absorption of hydrophobic monomers, bilayers expanded without reorganization of lipid configuration. In the case of styrene loaded into DMPC liposomes, approximately 1.2 monomer/lipid ratio was achieved, and about one half of styrene molecules were phase separated from lipids to form an interstitial layer between lipid leaflets, while the other half of styrene molecules were distributed throughout the bilayer. DLS data were analyzed using an expanding shell model. Time-resolved loading of monomers into bilayers showed expansion of liposomes.

The rate of diameter increase coincided with the rate of loading monomers into bilayers. These data corroborated the partial phase separation of monomers from lipids. DLS studies combined with expanding shell model interpretation may be a convenient tool for studying accommodation of a broad range of molecules at high concentration within the bilayers. Formation of an interstitial layer of monomers has broad implications on the synthesis of bilayer-templated polymer materials. A diverse set of molecules is likely to be incorporated into nanometer-thin materials following, giving rise to a new generation of functional nanomaterials.

Acknowledgments

This work was supported by NSF (CHE-1012951 and CHE-0349315) and NIH (1R01HL079147-01) grants, FedEx Institute of Technology Innovation Award and CIBA foundation gift.

References

1. Shmakov S, Pinkhassik E. Chem Commun. 2010; 46:7346–7348.
2. Tekobo S, Pinkhassik E. Chem Commun. 2009;1112–1114.
3. Danila DC, Banner LT, Karimova EJ, Tsurkan L, Wang X, Pinkhassik E. Angew Chem Int Ed. 2008; 47:7036–7039.
4. Dergunov SA, Pinkhassik E. Angew Chem Int Ed. 2008; 47:8264–8267.
5. Dergunov SA, Miksa B, Ganus B, Lindner E, Pinkhassik E. Chem Commun. 2010; 46:1485–1487.
6. Dergunov SA, Kesterson K, Li W, Zhang, Pinkhassik E. Macromolecules. 2010; 43:7785–7792. [PubMed: 21423872]
7. Hotz J, Meier W. Langmuir. 1998; 14:1031–1036.
8. Poulain N, Nakache E, Pina A, Levesque G. J Polym Sci Part A: Polym Chem. 1996; 34:729–737.
9. Nardin C, Hirt T, Leukel J, Meier W. Langmuir. 2000; 16:1035–1041.
10. Kurja J, Noelte RJM, Maxwell IA, German AI. Polymer. 1993; 34
11. McKelvey CA, Kaler EW, Zasadzinski JA, Coldren B, Jung HT. Langmuir. 2000; 16:8285–8290.
12. Jung M, Huber DHW, Bomans PHH, Frederic PM, Meuldijk J, Herk AMv, Fischer H, German AI. Langmuir. 1997; 13:6877–6880.
13. Gomes, JFPdS; Sonnen, AF-P.; Kronenberger, A.; Fritz, J.; Coelho, MAN.; Fournier, D.; Noel, CF.; Mauzac, M.; Winterhalter, M. Langmuir. 2006; 22:7755–7759. [PubMed: 16922560]
14. Wolfgang M. Chem Soc Rev. 2000; 29:295–303.
15. Choi M, Na K, Kim J, Sakamoto Y, Terasaki O, Ryoo R. Nature. 2009; 461:246–249. [PubMed: 19741706]
16. Chong PLG, Zhu WW, Venegas B. Biochim Biophys Acta-Biomembr. 2009; 1788:2–11.
17. Atkinson J, Epand RF, Epand RM. Free Radical Bio Med. 2008; 44:739–764. [PubMed: 18160049]
18. Trudell JR, Bertaccini E. Brit J Anaesth. 2002; 89:32–40. [PubMed: 12173239]
19. Banner LT, Danila DC, Sharpe K, Durkin M, Clayton B, Anderson B, Richter A, Pinkhassik E. Langmuir. 2008; 24:11464–11473. [PubMed: 18781782]
20. Dergunov SA, Schaub SC, Richter AG, Pinkhassik E. Langmuir. 2010; 26:6276–6280. [PubMed: 20030340]
21. Certain commercial equipment, instruments, or materials are identified in this document. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the products identified are necessarily the best available for the purpose.
22. Torchilin, V.; Weissig, V. Liposomes - A Practical Approach. 2. Vol. 264. Oxford University Press; Oxford, N.Y.: 2003.
23. Lynn GW, Heller W, Urban V, Wignall GD, Weiss K, Myles DAA. Physica B: Condensed Matter. 2006:385–386. 880–882.

24. Glinka CJ, Barker JG, Hammouda B, Krueger S, Moyer JJ, Orts WJ. *J Appl Cryst.* 1998; 31:430–445.
25. Kline SR. *J Appl Cryst.* 2006; 39:895–900.
26. Cevc, G.; Marsh, D. *Phospholipid Bilayers: Physical Principles and Models.* John Wiley & Sons; New York: 1987.
27. Roe, R-J. *Methods of X-Ray and Neutron Scattering in Polymer Science.* New York: Oxford University Press; 2000.
28. Uhrikova D, Balgavy P, Kucerka N, Islamov A, Gordeliy V, Kuklin A. *Biophys Chem.* 2000; 88:165–170. [PubMed: 11152273]
29. Kucerka N, Liu Y, Chu N, Petrache HI, Tristram-Nagle S, Nagle JF. *Biophys J.* 2005; 88:2626–2637. [PubMed: 15665131]

Figure 1. Possible distribution of hydrophobic monomers within the bilayer. Two extreme possibilities are shown: complete phase separation between monomers and lipids and homogeneous distribution of monomers throughout the bilayer.

Figure 2.

Most probable depiction of styrene in DMPC bilayer: approximately half of monomers are phase-separated from lipids in an interstitial layer, while the rest are homogeneously distributed within the bilayer.

Figure 3.

Change in bilayer thickness induced by loading styrene. Water molecules coordinated with polar heads of phospholipids are shown in light blue; dark blue ovals represent monomers. D_h and d'_h are the distances between polar head groups in empty and monomer-loaded liposomes, respectively. D_t and d'_t denote the thickness of the hydrophobic (tail) region in empty and monomer-loaded liposomes, respectively.

$$\Delta d_h = 0.42 \pm 0.15 \text{ nm (SAXS)}^{19}$$

$$\Delta d_t = 0.28 \pm 0.05 \text{ nm (SANS)}$$

Figure 4.

Plots of SANS data, modified as $(I - I_{bkg})Q^4$, for unloaded and loaded DMPC samples at 30° C. Plotting the data in this way emphasizes the features at high Q . Loading the liposomes with monomers causes a small shift to lower Q of all the features, indicating that the bilayer swells when loaded with hydrophobic monomers.

Figure 5. Modified Guinier plots of unloaded and loaded DMPC SANS data from NCNR. The solid lines are linear fits to the data, giving slopes of $-88.9 \pm 2.0 \text{ \AA}$, and $-105.1 \pm 2.0 \text{ \AA}$, corresponding to bilayer thicknesses of $3.27 \pm 0.04 \text{ nm}$ and $3.55 \pm 0.03 \text{ nm}$, respectively, indicating that the bilayer swells by $0.28 \pm 0.05 \text{ nm}$ upon loading.

Figure 6. Expanding shell model. Two extreme possibilities are shown: monomers are completely phase separated to form an interstitial layer (left) or homogeneously distributed within the bilayer (right).

Figure 7.

A) Diameter of liposomes measured by DLS. Loading of styrene-d⁸ caused increase in liposome diameter, while blank liposomes remained unchanged. B) Styrene-d⁸/DMPC molar ratio measured by HPLC.

Figure 8.
Directed assembly of crosslinked nanometer-thin polymer film with uniform imprinted nanopores. Lipid bilayer is used as a temporary self-assembled scaffold.

Table 1

Diameter of blank liposomes, nm ^a	Diameter of fully loaded liposomes, nm ^a	Styrene/DMPC molar ratio ^b	Calculated diameter for phase separation, nm ^c	Calculated diameter for homogeneous mixing, nm ^d
113	119	1.2	114	124

^a measured by DLS;

^b measured by HPLC;

^c calculated from Equation 2 using experimental values for diameter and monomer/lipid ratio;

^d calculated with Equations 3 using experimental values for diameter and monomer/lipid ratio.