Published in final edited form as:

Langmuir. 2017 November 07; 33(44): 12624–12634. doi:10.1021/acs.langmuir.7b02279.

Quantifying Binding of Ethylene Oxide - Propylene Oxide Block Copolymers with Lipid Bilayers

Wenjia Zhang¹, Karen J. Haman¹, Joseph M. Metzger², Benjamin J. Hackel¹, Frank S. Bates^{*,1}, and Timothy P. Lodge^{*,1,3}

¹Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, Minnesota 55455, United States

²Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis, Minnesota 55455, United States

³Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, United States

Abstract

Block copolymers composed of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) have been widely used in cell membrane stabilization and permeabilization. To explore the mechanism of interaction between PPO-PEO block copolymers and lipid membranes, we have investigated how polymer structure influences the polymer-lipid bilayer association by varying the overall molecular weight, the hydrophobic and hydrophilic block lengths, and the endgroup structure systematically, using 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) unilamellar liposomes as model membranes. Pulsed-field-gradient NMR (PFG-NMR) was employed to probe polymer diffusion in the absence and presence of liposomes. The echo decay curves of free polymers in the absence of liposomes are single exponentials, indicative of simple translational diffusion, while in the presence of liposomes, the decays were biexponential, with the slower decay corresponding to polymers bound to liposomes. The binding percentage of polymer to the liposome was quantified by fitting the echo decay curves to a biexponential model. The NMR experiments showed that increasing the total molecular weight and hydrophobicity of the polymer can significantly enhance the polymer-lipid bilayer association, as the binding percentage and liposome surface coverage both increase. We hypothesize that the hydrophobic PPO block inserts into the lipid bilayer, due to the fact that little molecular exchange between bound and free polymers occurs on the time scale of the diffusion experiments. Additionally, as polymer

Authors for correspondence: bates001@umn.edu, lodge@umn.edu.

ASSOCIATED CONTENT

Supporting information

MALDI spectra and SEC traces of representative polymers, R_h of pure liposomes and pure F127 characterized by PFG-NMR and DLS, errors of f_{bound} and related parameters N and θ , R_g of each polymer, f_{bound} of 0.2 mg/mL P103, P104, P105 and F108 at no incubation and 1-week incubation with liposome, fitting results of 3 replicates of 1 mg/mL P105 in the presence of liposomes, fittings results with corresponding errors by Kärger model, simulations of the Kärger model at different τ_{bound} , the echo-decay curves of water in the presence of liposome and in the presence of liposome plus 1 mg/mL P105, the echo-decay curves of F108, F68, P104, P84, tPPO₂₉-PEO₆₈, tPPO₁₄-PEO₄₆, tPPO₁₄-PEO₇₈, tPPO₁₄-PEO₁₅₉ and hPPO₁₃-PEO_{43m}, at 1 mg/mL in the presence of liposomes, the echo-decay curves of P103, P104, P105 and F108 at 0.2 mg/mL in the presence of liposomes, the echo-decay curves of F127 at 0.2, 0.5 and 1 mg/mL in the presence of liposomes.

The authors declare the following competing financial interest(s): J.M.M. is on the scientific advisory board of and holds shares in Phrixus Pharmaceuticals Inc., a company developing novel therapeutics for heart failure.

concentration increases, the liposome surface coverage increases and approaches a limit. These results demonstrate that PFG-NMR is a simple yet powerful method to quantify interactions between polymers and lipid bilayers.

TOC Graphic

INTRODUCTION

The cell membrane is a permselective barrier that controls the material transport and energy exchange between extra- and intra-cellular fluids.^{1,2} As a relatively vulnerable interface, many types of injury can disrupt the cell membrane structure, including thermal trauma,³ frostbite,⁴ electric shock,^{5,6} radical mediated radiation,⁷ and barometric trauma.^{8,9} Therapeutically, membrane integrity can be restored by surfactants that interact with the lipid bilayers.² Pluronics, also known as Poloxamers, are one widely used surfactant family. These are commercially available triblock copolymers, comprising a hydrophobic block of poly(propylene oxide) (PPO) flanked by two symmetric hydrophilic blocks of poly(ethylene oxide) (PEO).^{10,11,12} Based on their amphiphilicity and biocompatibility, these triblocks have been studied over several decades for injured cell membrane stabilization.¹³ For example, Pluronic F68 (Poloxamer 188), with relatively large hydrophilic PEO blocks (*ca.* 75 repeat units), has found broad potential application as a membrane stabilizer in enhancing functional recovery of heat-shocked fibroblasts,¹⁴ restoring the cell viability of damaged neurons,¹⁵ preventing skeletal muscle cells from necrosis,¹⁶ and stabilizing cardiac muscle cell membrane with dystrophin deficiency.^{17,18} More hydrophobic polymers in the Pluronic family containing very short PEO blocks, such as L61, or polymers with long hydrophobic PPO blocks, such as F127, have been used to promote membrane permeability and facilitate drug delivery, in contrast to the membrane stabilization effect.^{19,20,21,22,23}

Despite the biomedical effectiveness of Pluronics, the fundamental mechanisms of the interactions between block copolymers and cell membranes are not fully understood. Earlier work using lipid monolayers, unilamellar vesicles, or supported bilayers as model membrane systems has provided some physical insight into polymer-membrane association.^{24,25,26,27,28,29} For example, it has been proposed that the hydrophobic PPO block favors

insertion into the lipid tail region, whereas the hydrophilic PEO block may only weakly adsorb onto the lipid headgroups.²⁵ Small angle X-ray scattering (SAXS) experiments by Firestone *et al.* further demonstrated that the length of the hydrophobic PPO block is a key determinant of the interaction mechanism with lipid bilayers.^{30,31,32} When the PPO block length approximates the lipid bilayer thickness, the polymer could span the lipid bilayer, whereas Pluronics with a shorter PPO blocks only partially insert into the lipid bilayer, with the two PEO blocks remaining on the same side of the membrane. Similar results were obtained by coarse-grained molecular dynamics and Monte Carlo simulations.^{33,34} A previous study of polymer interactions with supported lipid membranes using a microcantilever compared two Pluronics with the same PPO/PEO ratio but different molecular weight, and found that higher molecular weight enhances the association with lipid membranes.²⁹ Recently, on the basis of an *in-vitro* cellular assay it was found that the protection efficacy of PPO-PEO block copolymers on cell membranes is highly affected by the polymer composition as well as the overall molecular weight.³⁵ In this context, the polymer structure plays a critical role in the nature of the interactions between PPO-PEO block copolymers and lipid membranes, which therefore determines the performance of polymers in both membrane stabilization and permeabilization. However, few studies have been able to quantify the associations between polymers and membranes directly.

Various NMR techniques have been employed to explore phospholipid membranes and their interactions with polymers.^{36,37} In this study, we investigate how polymer structure dictates the polymer-lipid membrane association by quantitative measurements of polymer binding via pulsed-field-gradient NMR (PFG-NMR). Liposomes, or more specifically, large unilamellar vesicles (LUVs), were used as the model membrane system. Liposomes have been used extensively to study membrane protection and permeabilization from a fundamental perspective.^{19,20,21,24,25,26} Compared to monolayer systems, liposomes are more appropriate substitutes for cell membranes since the liposomes also provide a phospholipid bilayer. The controlled composition and relative stability of liposomes make them a model system for exploring the polymer-lipid bilayer association. The polymer species involved in this work all belong to the family of PPO-PEO block copolymers, as shown in Scheme 1. In addition to commercial Pluronics (Scheme 1a), diblock analogs with *tert*-butyl endgroups or hydroxyl endgroups on the PPO block were also synthesized as complements to the triblock architecture (Schemes 1b and 1c). Most relevant studies in the literature have been restricted to commercially available triblock copolymers. The inclusion of the diblock architecture expands the array of available polymer structures within this chemical family, and thereby provides greater flexibility to manipulate molecular parameters systematically, such as the overall molecular weight, the hydrophobic and hydrophilic block lengths, and the endgroups. The interactions between polymers and lipid bilayers are quantified by PFG-NMR. After mixing a polymer solution with the liposome solution, a fraction of the polymer molecules associate with the lipid bilayers, while the majority remain as free coils in the solution, as shown schematically in Figure 1. Since the polymers bound to the liposome move much more slowly than free polymers, two distinct polymer diffusivities (*i.e.*, free and bound) in the presence of liposomes can be characterized, and therefore, the amount of polymers attached to the liposomes can be determined.

MATERIALS AND METHODS

Materials

Plurionics F68, F108, F87, F127, P103, P104, P105, L35, P65 and P84 were provided by BASF. Ethylene oxide (EO, 99.5%), propylene oxide (PO, 99%), methoxy-terminated polyethylene glycol (methoxy-PEO, 2000 g/mol), potassium *tert*-butoxide, *n*-butyllithium, potassium, naphthalene, and 18-crown-6 ether for anionic polymerization were purchased from Sigma-Aldrich and used as received, unless otherwise noted. PEO-8 (8000 g/mol) was purchased from EMD Millipore (Billerica, MA). 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC, PC (16:0–18:1 PC)) in chloroform was purchased from Avanti Polar Lipids (Alabaster, AL) and used as received. Silver trifluoroacetate (AgTFA) and α -cyano-4-hydroxycinnamic acid (CHCA, 98%) for MALDI characterization were also purchased from Sigma-Aldrich. Deuterium oxide (D₂O, 99.9 atom % D) and chloroform-*d* (CDCl₃, 99.8 atom % D + 0.05% V/V TMS) were purchased from Cambridge Isotope Laboratories, Inc.

Polymer Synthesis

PPO-PEO diblock copolymers were synthesized by sequential anionic polymerization. Reaction setup and monomer purification followed procedures described elsewhere.^{38,39,40} All reactions occurred in an air- and water-free environment, using tetrahydrofuran (THF) as the solvent. To synthesize diblock copolymers with a *tert*-butyl endgroup (tPPO₁₄-PEO₄₆, tPPO₁₄-PEO₇₈, tPPO₁₄-PEO₁₅₉ and tPPO₂₉-PEO₆₈, where “t” stands for the *tert*-butyl endgroup and the subscripts denote the number average degrees of polymerization), PO monomer was first initiated by potassium *tert*-butoxide at room temperature, followed by successive addition of PO monomers in the presence of 18-crown-6 ether (freeze-dried, 2:1 molar ratio to *tert*-butoxide) to reduce side reactions. The reaction was terminated by excess acidic methanol (1:10 37 w/w% hydrochloric acid:methanol) after 48 h. The PPO block was obtained by filtering out the precipitates of potassium salts and crown ether complexes and evaporating all the THF solvent. The hydroxyl endgroup on the PPO block was then reinitiated by freshly made potassium naphthalenide at 45 °C, followed by 24 h propagation and termination of the PEO block. *Tert*-butyl PPO-PEO diblock copolymers were recovered by filtration and dialysis to remove potassium salts and naphthalene. The polymer was finally freeze-dried from benzene to obtain a fine white powder, and used for further characterization.

To synthesize a PPO-PEO diblock copolymer with a hydroxyl endgroup on the PPO block (hPPO₁₃-PEO_{43m}, where “h” represents the hydroxyl endgroup on the PPO block and “m” represents the methyl endgroup on the PEO block), the commercial monomethoxy-PEO (2000 g/mol) was reinitiated by freshly made potassium naphthalenide at 45 °C, followed by propagation in the presence of 18-crown-6 ether to improve the conversion of the PPO block. The reaction was terminated after 36 h by acidic methanol. The final product was recovered by filtration, dialysis and freeze-drying.

Polymer Characterization

The number-average molecular weight (M_n) and dispersity ($\mathcal{D}$) of each polymer were determined by both matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (AB SCIEX TOF/TOF 5800) and size exclusion chromatography (SEC) (Phenogel columns, Phenomenex) with a multi-angle laser light scattering detector (Wyatt DAWN) and THF as the mobile phase. MALDI samples were prepared by mixing 0.2 mL of a matrix solution (20 mg/mL CHCA in THF), 5 μ L of a salt solution (1 mg/mL AgTFA in THF), and 0.1 mL of a polymer solution (1 mg/mL in THF). Note that MALDI shows a bimodal distribution for commercial triblock copolymers due to diblock contamination (Figure S1). The intensity of the peak with smaller molecular weight is greater than the one with larger molecular weight for F108 and F127, since MALDI preferentially samples lower molecular weights. Thus M_n and $\mathcal{D}$ values of the commercial triblock copolymers as characterized by MALDI are based on larger molecular weight peaks only. SEC samples were prepared as 10 mg/mL polymers in THF. The refractive index increment (dn/dc) for each polymer was estimated as the weight average of PPO ($dn/dc = 0.087$ mL/mg) and PEO ($dn/dc = 0.068$ mL/mg).^{41,42} Polymer compositions (w_{PEO}) were calculated from the molar ratios characterized by ¹H NMR spectroscopy (Bruker AV-500) in CDCl₃. Table 1 summarizes M_n , $\mathcal{D}$, w_{PEO} and the corresponding average number of repeat units of PO (N_{PO}) and EO (N_{EO}).

Liposome Preparation

The procedure to prepare liposomes was adapted and modified from methods detailed elsewhere.^{43,44,45,24} First, POPC phospholipids in chloroform (10 mg/mL) were dried with argon to form a thin film of lipid on the wall of the glass vial. Trace amounts of chloroform were further removed under vacuum for 30 min. The phospholipids were then hydrated with D₂O to 10 mM and heated to 37 °C for 30 min. The lipid solution was vortexed every 5 min during the hydration process until the lipids were fully emulsified. The final liposome solution was obtained by extruding the lipid solution back and forth 27 times through a polycarbonate membrane of 100 nm pore size using an Avanti Mini-Extruder. The liposome solution was characterized by PFG-NMR as well as dynamic light scattering (DLS) to determine the mean hydrodynamic radius R_h (Figure S2).

NMR Measurements

PFG-NMR experiments were performed on a Bruker Avance III 500 MHz NMR instrument with a 5 mm broadband fluorine observe (BBFO) probe. The “ledbpgp2s” stimulated pulse sequence (longitudinal eddy current delay experiment using bipolar gradients acquired in 2D)⁴⁶ was applied to probe the translational diffusivity (D) of the samples, following:

$$\ln\left(\frac{I}{I_0}\right) = \gamma^2 G^2 \delta^2 D \left(\Delta - \frac{\delta}{3}\right) \quad (1)$$

where G is the input gradient strength, varied from 2 to 95% of the maximum strength, I is the observed intensity corresponding to each G , I_0 is the intensity at $G = 0$, γ is the gyromagnetic ratio of ¹H (42.6 MHz/T), δ is the length of the gradient pulse (set to 5 ms), and Δ is the diffusion time (set to 300, 500 and 700 ms for polymer-liposome mixtures and

700 ms for pure polymer solutions). PFG-NMR experiments recorded a series of 1D ^1H spectra and diffusion coefficients were obtained by fitting the signal intensity decay at increasing gradient strengths to eqn 1. Data were analyzed by the Topspin 3.5 software package.

To prepare the samples for PFG-NMR experiments, 0.25 mL of freshly made 10 mM liposome stock solution was mixed with 0.25 mL of polymer stock solution in D_2O at room temperature. Pure polymer and pure liposome solution were prepared as controls by adding 0.25 mL of D_2O to 0.25 mL of stock solutions of polymer and liposome at room temperature. All the samples were measured at 27 °C without incubation. The temperature fluctuation during the measurement is less than ± 0.3 °C, as indicated by the temperature monitor inside the NMR equipment. Note that the incubation time of polymers with liposomes prior to measurement apparently has little effect on polymer binding, as Table S1 shows that the binding percentage f_{bound} does not change appreciably from no incubation to 1-week incubation at room temperature, for all four selected polymers (P103, P104, P105 and F108 at 0.2 mg/mL) with liposomes.

RESULTS AND DISCUSSION

Figure 2 shows the ^1H NMR spectrum of a 5 mM POPC liposome solution in D_2O containing 1 mg/mL P105 as a representative polymer. The proton peaks of PEO (labeled “a”) and choline head groups (labeled “c”) were selected to probe the diffusion of polymer and liposome, respectively. The interactions between polymers and lipid bilayers were investigated by comparing the diffusion of polymers with and without liposomes. Taking P105 as an example in Figure 3a, the logarithmic normalized echo-attenuated intensity $\ln(I/I_0)$ of the polymer in the absence of liposomes (open circles) decreases linearly with the quantity $\gamma^2 G^2 \delta^2 (-\delta/3)$ according to eqn 1, indicating that polymer molecules undergo simple translational diffusion in water and that there is no micelle formation. The self-diffusion coefficients of the free polymers were determined from the slope of the echo decay curves, and are summarized in Table S2. A representative polymer F127, which has the lowest critical micelle concentration (CMC) among all the Pluronics employed in this study, was measured by DLS to determine the mean hydrodynamic radius R_h .⁴⁷ The echo decay curve of F127 along with the DLS measurements (Figure S3) confirmed that F127 are free coils in D_2O at 1 mg/mL at 27 °C.

For polymers in the presence of liposomes, however, the echo decay curves (filled symbols in Figure 3a) are no longer linear, which means that the diffusion of the polymers cannot be described by eqn 1. In the mixed solution, a certain amount of polymer interacts with the lipid bilayers, whereas the remaining polymers are still free coils. The diffusion of the bound polymer is hindered by the liposome, so that it is reasonable to assume that the bound polymers have the same diffusivity as that of the liposome, which is at least one order of magnitude slower than that of free polymers. The difference in polymer mobility gives rise to two decay processes: a rapid decay corresponding to free polymers in the mixture, and a slower decay corresponding to the polymers associated with the lipid membranes. The initial slope of the echo decay curves, D_{free} , coincides with that in the absence of liposomes because the echo-attenuated intensity is dominated by the signal from free polymers at low

$\gamma^2 G^2 \delta^2 (-\delta/3)$. The intensity from rapid diffusion is completely attenuated at high $\gamma^2 G^2 \delta^2 (-\delta/3)$, where the slope represents the diffusion coefficient of the polymers associated with the lipid bilayers, D_{bound} . The three echo decay curves of the polymers in the presence of liposomes measured at fixed δ (5 ms) and various Δ (300, 500 and 700 ms) almost overlap, which implies that the molecular exchange rate between the free polymers and the polymers bound to liposomes is very slow on the time scale of the diffusion experiments. Thus we can quantify the interaction of polymers with lipid bilayers by fitting the echo decay curves with a simple biexponential model, as shown in eqn 2,⁴⁸ assuming that molecular exchange between bound and free states is negligible.

$$\frac{I}{I_0} = f_{\text{bound}} \cdot \exp\left(-\gamma^2 G^2 \delta^2 D_{\text{bound}} \left(\Delta - \frac{\delta}{3}\right)\right) + (1 - f_{\text{bound}}) \cdot \exp\left(-\gamma^2 G^2 \delta^2 D_{\text{free}} \left(\Delta - \frac{\delta}{3}\right)\right) \quad (2)$$

D_{free} was obtained from the slope of the echo decay curves of the polymer in the absence of liposomes, whereas D_{bound} was obtained from the final slope of the curves of the polymer in the presence of liposomes. The diffusion coefficient of the liposome in the mixture was also used to estimate D_{bound} if the interaction between the polymers and lipid membranes is too weak to give a reliable slope of the final plateau. In all cases the extracted values of D_{bound} were within 20% of the diffusion coefficient of free liposomes. The only fitting parameter in eqn 2 is f_{bound} , which represents the molar percentage of the polymer molecules bound to the liposomes. The average number of bound polymer molecules per liposome, N , was further calculated by:

$$N = \frac{c}{M_n} \cdot \frac{f_{\text{bound}}}{\frac{n_{\text{lipid}}}{N_{\text{tot}}}} \quad (3)$$

where c is the mass concentration of polymer and n_{lipid} is the molar concentration of lipid molecules (5 mM) in the mixture solution. The number of POPC lipid molecules per liposome, N_{tot} , was estimated by:⁴⁹

$$N_{\text{tot}} = \frac{4\pi R_{\text{lipo}}^2}{A} + \frac{4\pi(R_{\text{lipo}} - t)^2}{A} \quad (4)$$

where t is the thickness of POPC lipid bilayer ($52 \pm 2 \text{ \AA}$),^{50,51} A is the area per lipid ($68.3 \pm 1.5 \text{ \AA}^2$),⁵² and R_{lipo} is the radius of the liposome, approximated as R_h ($41 \pm 2 \text{ nm}$). R_h was obtained from the self-diffusion coefficient of the liposome via the Stokes-Einstein equation (eqn 5), where D_{lipo} was determined from the PFG-NMR measurements on the pure liposome solution (Figure S2a).

$$D_{\text{lipo}} = \frac{k_B T}{6\pi\eta R_h} \quad (5)$$

The fraction of liposome surface area covered by bound polymer molecules, θ , was further estimated from N by:

$$\theta = N \cdot \frac{\pi R_g^2}{4\pi R_{\text{lipo}}^2} \quad (6)$$

where R_g is the radius of gyration of the polymer, as summarized in Table S3. This analysis assumes that the bound polymer occupies an area corresponding to the coil size in free solution. To visualize the polymer-lipid bilayer association, a schematic of surface coverage of half a liposome by P105 as a representative polymer is shown in Figure 3b. The zoom-in view depicts a possible mechanism of interaction between polymer and lipid bilayer: the hydrophobic PPO block inserts into the lipid bilayer interior, whereas the hydrophilic PEO blocks adsorb onto the head group of the lipid bilayer.²⁵ Three replicates of 1 mg/mL P105 in 5 mM POPC liposome solution were measured and the corresponding fitting results show that they all have approximately 8% of polymers bound to the lipid bilayers (see Table S4). Due to the high reproducibility of the polymer binding, the remaining polymers were measured only once. Table 2 summarizes the fitting results for f_{bound} and the resulting parameters N and θ for the interactions of the liposome with each polymer species at given mass concentration. Estimates of the error in f_{bound} , N and θ for each polymer species can be found in Table S2. D_{bound} and D_{free} are also summarized in Table S2. The analysis of the water signal is shown in Figure S4. The diffusion coefficient is $1.7 \times 10^{-9} \text{ m}^2/\text{s}$ in both cases: water in the presence of liposome and water in the presence of liposome plus polymer (*e.g.*, 1 mg/mL P105). Note that despite the coexistence of free and encapsulated states of water, the decay curve only has a single slope instead of two distinct slopes, because the exchange rate of water molecules is too rapid to allow observations of two different decays. Leson *et al.* also demonstrated that two different decays cannot be observed even at 50 ms.⁵³ In such case, PFG-NMR measures an average diffusivity of the water molecules in free states and encapsulated states. Due to high diffusivity of water, we qualitatively conclude that the membrane has high permeability to water with and without polymer addition. The following subsections will discuss how polymer structure as well as polymer concentration affect the interactions with lipid bilayers.

Effect of Polymer Molecular Weight

The effect of molecular weight was studied by comparing four sets of commercial polymers: F127 vs. F87, P105 vs. P65 and L35, P104 vs. P84, and F108 vs. F68. In each set the polymers have the same w_{PEO} , as reflected by the last digit of the designation. The only varying parameter in each set is the total molecular weight, which is proportional to the first or first two digits of the designation for a given composition. Among all four sets, F127 ($M_n = 13,200 \text{ g/mol}$) vs. F87 ($M_n = 7,700 \text{ g/mol}$) shows the most significant molecular weight effect. As shown in Figure 4a, the echo decay curves of F127 in the presence of liposome give strong signals at large $\gamma^2 G^2 \delta^2 (-\delta/3)$, providing a reliable slope for the final plateau that demonstrates a significant number of F127 molecules have attached to the liposomes. In contrast, the echo decay curves of F87 in Figure 4b show a weaker signal as the intensity is more attenuated, and barely display a final plateau, which indicates that less interaction has

occurred between F87 and the lipid membranes. The parameter f_{bound} in Table 2 enables quantitative comparison of the binding percentage of F127 and F87 to the liposomes. The binding percentage increases from 0.2 % of F87 to 12.6 % of F127, indicating a remarkable increase upon increasing the molecular weight by 70%. The insets of Figures 4a and 4b illustrate the liposome surface coverage by F127 and F87, respectively. Clearly, there are many more F127 molecules on the liposome surface than F87. Also, F127 molecules have a larger radius of gyration than F87, since F127 has higher molecular weight, and thus larger particles with higher binding percentage result in much greater areal density on the liposome surface. Additionally, another pair of hydrophilic polymers F108 ($M_n = 15,900$ g/mol) vs. F68 ($M_n = 8,200$ g/mol) was investigated. The two polymer species with $w_{\text{PEO}} \approx 0.80$ have less association with the lipid bilayers than the ones with $w_{\text{PEO}} \approx 0.70$. In Figure S5a, whereas the echo decay curves of F108 in the polymer-liposome mixture show a hint of a nonlinear relation, the signal is too weak at high $\gamma^2 G^2 \delta^2 (-\delta/3)$. In Figure S5b, the data for F68 are as linear as for free polymers in the absence of liposome, because the signal of the final plateau is completely lost. These two figures illustrate that polymer-lipid bilayer association is reduced when the polymer is very hydrophilic. Nevertheless, F108 vs. F68 shows the same trend as F127 vs. F87, in that increasing molecular weight can enhance the polymer binding to the lipid bilayers despite more uncertainty for F108 vs. F68 due to the reduced signal. PEO-8 was measured as a control (Figure S5e), which has little interaction with the lipid bilayers.

In addition to hydrophilic polymers, two sets of more hydrophobic polymers were tested: one with $w_{\text{PEO}} \approx 0.50$, including P105 ($M_n = 6,500$ g/mol), P65 ($M_n = 3,400$ g/mol) and L35 ($M_n = 1,900$ g/mol), and one with $w_{\text{PEO}} \approx 0.40$, including P104 ($M_n = 5,700$ g/mol) and P84 ($M_n = 4,200$ g/mol). For the former set, the echo decay curves of P65 and L35 (Figures 4c and 4d, respectively) show two distinct slopes despite the noisy data in the final plateau, corresponding to two different diffusivities of the polymers in the presence of liposomes. Compared to F87 and F68, which barely show the sign of the final plateau, the existence of the plateau suggests that P65 and L35 have stronger interactions with lipid membranes. Nevertheless, the signal fluctuations in the final plateau of P65 and L35 indicate that the amount of polymer associated with the membranes is not sufficient to provide a strong signal. In contrast, the echo curves of P105 in Figure 3a have stronger intensity at the final plateau, which suggests that there are more polymer molecules associated with the membranes than those of P65 or L35. Similarly, as shown in Table 2 and Figures S5c and S5d, P104 has stronger binding ability to lipid membranes than P84, since P104 has higher molecular weight. It can be seen that increasing the molecular weight doesn't enhance the polymer-liposome interaction for L35 to P65 as significantly as for P65 to P105. We hypothesize that there is a favorable range of molecular weight, beyond which further increase in molecular weight induces no noticeable increase in the interaction with lipid bilayers. Based on these four sets of polymers, the binding percentage to the liposomes as well as the liposome surface coverage increase more than 5 times by simply doubling the molecular weight. Note that (1) when the polymer is very hydrophilic (e.g., F68 vs. F108), the comparison of molecular weight effect has higher uncertainty due to weaker binding, and (2) when the molecular weight is too small (e.g., L35 vs. P65), the molecular weight sensitivity of the association with lipid bilayers is reduced.

Effect of Composition

The effect of polymer composition is attributable to the hydrophilic-lipophilic balance (HLB), which can be manipulated by varying the PEO and PPO block lengths, as well as the endgroups. The effect of PEO length was studied by selecting the four commercial triblock copolymers P103, P104, P105 and F108, which have a fixed PPO length (as indicated by the same first two digits of their designations). The PEO length increases from P103 to F108 such that the compositions are $w_{\text{PEO}} \approx 0.30, 0.40, 0.50$ and 0.80 , respectively. Note that these four polymers were measured at 0.2 mg/mL instead of 1 mg/mL because P103, the most hydrophobic polymer among these four, forms micelles at 1 mg/mL . The fluctuation of the echo-attenuated intensity of the final plateau in Figure S6 is attributable to the more dilute concentration of the polymers. Since F108 is the only polymer among these four that lost the signal of the final plateau (Figure S6d), it is clear that the more hydrophobic polymers P103, P104 and P105 have stronger interactions with the lipid membranes than that of the hydrophilic F108. Comparing the binding degree as well as the liposome surface coverage of these four polymers listed in Table 2, the most hydrophobic polymer P103 binds the most, whereas the most hydrophilic polymer F108 binds the least, despite its higher molecular weight. This also underscores the fact that molecular weight has a limited effect for very hydrophilic polymers. P104 and P105 have similar levels of association, which could be explained by the trade-off between total molecular weight and HLB as the PEO length increases. The polymer becomes less hydrophobic from P104 to P105, while the total molecular weight also increases with increasing PEO block length. Thus, the hydrophobicity effect is compensated by the molecular weight effect.

Note that there is some suggestion of exchange between bound and unbound states, in the lack of perfect superposition of the curves for different τ_{bound} in both Figures S5 and S6. These curves were fitted to the Kärger model as summarized in Table S5.⁵⁴ Compared to the biexponential model, the Kärger model adds one more fitting parameter, the residence time of bound polymers τ_{bound} , to account for the molecular exchange rate between bound and unbound states.^{53,55,56} The smaller τ_{bound} , the more rapid the exchange. The binding percentage obtained from the Kärger model shows the same trends as that from biexponential model, but the absolute values of the binding fraction are systematically higher than those from the biexponential model. Note that the uncertainty in τ_{bound} is large due to two factors: (1) the signal intensity at high q^2 ($\sim \delta^2$) is noisy, and (2) the differences between the final plateaus are too small to give precise exchange rates, indicating that the residence time of bound polymers is longer than the current applied diffusion time. Simulations of the Kärger model at different τ_{bound} are shown in Figure S7, which demonstrate that τ_{bound} needs to be less than 1 s to render the exchange between free and bound states observable within the applied diffusion time scale. In our case, however, τ_{bound} of most polymers exceeds 1 s . Based on the relatively slow exchange rate between free and bound states, we consider the biexponential model to be a valid simplification to fit the data.

A set of three diblock copolymers tPPO₁₄-PEO₄₆, tPPO₁₄-PEO₇₈, and tPPO₁₄-PEO₁₅₉ was also tested by PFG-NMR (Figure S8). As implied by their nomenclature, they contain a constant PPO block length and an increasing PEO block length, with $w_{\text{PEO}} \approx 0.7, 0.8$ and 0.9 . The signal quality of the echo decay curves illustrates that tPPO₁₄-PEO₄₆ has the

strongest interaction with the lipid membranes, as a final plateau region was no longer observed for the other two polymers. This follows the trend that the binding percentage decreases with increasing PEO block length, as shown in Table 2. However, the binding percentage of these three polymers in general is much lower than that of P103, P104 and P105, which could be attributed to the higher hydrophilicity of tPPO₁₄-PEO₄₆, tPPO₁₄-PEO₇₈, and tPPO₁₄-PEO₁₅₉.

An alternative way to change the HLB is to modify the PPO block length, while keeping the PEO block length fixed. A pair of synthesized diblock copolymers tPPO₂₉-PEO₆₈ and tPPO₁₄-PEO₇₈ were employed to study this. Although there is a small discrepancy in PEO block length, the PPO block length of tPPO₂₉-PEO₆₈ is twice of that of tPPO₁₄-PEO₇₈. The echo decay curves of these two polymers in Figure S9 look quite different: tPPO₂₉-PEO₆₈ reveals two distinct slopes in the probing window, and the strength of the plateau is moderately high (Figure S9a); tPPO₁₄-PEO₇₈, on the other hand, only shows a single exponential decay, reflecting little interaction with the lipid bilayers (Figure S9b). As quantified in Table 2, tPPO₂₉-PEO₆₈ has approximately 10 times higher binding, as well as the number of bound polymers per liposome and surface coverage, than tPPO₁₄-PEO₇₈. This indicates that as the PPO block length increases, the polymer becomes more hydrophobic and more polymer molecules interact with the lipid membrane.

Endgroup chemistry is also an important contributor to the composition effect. Two synthesized diblock copolymers with different endgroups on the PPO block were investigated: hPPO₁₃-PEO_{43m}, with a hydrophilic hydroxyl endgroup, and tPPO₁₄-PEO₄₆ with a hydrophobic *tert*-butyl endgroup. While ideally the endgroup on the other PEO terminus would be the same for the two polymers, we assume that the variation from a normal hydroxyl endgroup of tPPO₁₄-PEO₄₆ to a methyl endgroup of hPPO₁₃-PEO_{43m} has little effect on the hydrophobicity of the polymer, especially given that the PEO block is relatively long. Based on the echo decay curves in Figure S10a, hPPO₁₃-PEO_{43m} barely interacts with the lipid bilayers. Its counterpart tPPO₁₄-PEO₄₆ has stronger associations with the liposomes as the decay curves in Figure S10b are clearly biexponential. Therefore, the more hydrophobic endgroup on the PPO block drives a stronger attraction of the polymers to the lipid bilayers. Note that the endgroup exerts a relatively minor influence compared to molecular weight and block length. A bulkier hydrophobic endgroup might be required to observe a more noticeable difference.

Effect of Polymer Concentration

Finally, the effect of concentration was investigated by adjusting the concentration of F127 from 0.2 to 1 mg/mL. As concentration increases, the liposome surface coverage also increases, while the binding percentage decreases, as summarized in Table 2 and shown in Figure 5. (The echo decay curves of F127 at 0.2, 0.5 and 1 mg/mL in the presence of liposomes can be found in Figure S11). The latter trend can be attributed to approaching the limit of liposome surface coverage at high polymer concentration. As can be seen in Figure 5, the liposome surface coverage appears to approach a maximum limit as the concentration increases. We speculate that at certain high polymer concentration, the liposome surface coverage would saturate because the membrane surface would become too crowded. It is

also conceivable that the liposome would be disrupted by concentrated polymer due to the resulting osmotic stress, prior to achieving maximum surface coverage, especially for those polymers that have weak interactions or insufficient binding with the liposomes. Concentrations of F127 beyond 1 mg/mL were not tested due to micelle formation.

To summarize the effects of all three variables described above, a 3-D plot of liposome surface coverage by the various polymers is shown in Figure 6. The three axes represent the total molecular weight, w_{PEO} , and concentration, corresponding to the three factors investigated. The volume of the spheres is proportional to the fractional surface coverage of the liposomes. Thus, the larger the sphere, the more polymer binding and thus stronger interactions. When looking at the effect of concentration, the spheres become larger along each vertical dashed line as concentration increases. Examining the plane of w_{PEO} vs. the total molecular weight, the upper corner within the thick dashed line contains relatively large spheres, indicating strong interactions of the polymers with the liposomes. Higher molecular weight and higher hydrophobicity of the polymer both enhance the polymer-lipid bilayer association. By extension, when looking at the binding percentage as a function of the PPO block length (Figure 7), an appreciably large PPO block is necessary for strong interactions. The small spheres on the other side of the dashed line in Figure 6 reflect weaker interactions between the polymers and liposomes, either because the hydrophobicity is not sufficient or the molecular weight is not high enough, or both.

Given the fact that the exchange between the free and bound polymers is not significant on the timescale of the diffusion experiments, we infer that the hydrophobic block at least partly inserts into the lipid bilayer, which makes it harder for the bound polymers to escape from the liposome. If the polymer only adsorbed onto the membrane surface, there should be relatively facile molecular exchange due to weak binding. The experiments here would not detect any weak binding, assuming that each polymer only spent a small fraction of time associated with a liposome. Previously Cheng *et al.* investigated the interactions between Pluronics and lipid bilayers through the measurement of local hydration dynamics in the membrane by ^1H Overhauser dynamic nuclear polarization NMR spectroscopy (ODNP). The results showed that the intrabilayer water diffusivity was enhanced by hydrophobic Pluronics, which indicates that the polymer molecules inserted into the bilayer interior and increased the membrane permeability.²⁵ This result is consistent with our inference that Pluronics insert into the lipid bilayers. That polymers with higher molecular weight exhibit stronger interactions with the lipid bilayers is consistent with the observation that polymers with higher molecular weight have a lower CMC,⁵⁷ which reflects the drive for polymer molecules to insert into hydrophobic bilayer or micellar interiors, rather than remaining as free chains. The effect of composition is also intuitively reasonable, as the HLB determines whether it is energetically more favorable for polymers to stay in water or associate with the liposomes. Earlier work on cellular assays by Kim *et al.* and on liposome protection against peroxidation by Wang *et al.* both showed that only hydrophilic dominant PPO-PEO block copolymers can protect the integrity of the lipid membranes.^{24,35} Given that the more hydrophobic polymers have stronger interactions with the lipid bilayers, there is a higher possibility of hydrophobic polymers to disturb or even disrupt the membrane, which may account for the lack of membrane protection. The cellular system has additional differences to the system studied here, including mixed lipid content, cholesterol, and transmembrane

proteins. Besides tuning the block length, adding a hydrophobic endgroup to the PPO block can help anchor the PPO block into the bilayer interior, since it further reinforces the hydrophobicity of the polymer. A previous *in-vivo* study of membrane stabilization by block copolymers by Houang *et al.* demonstrated that the replacement of a hydroxyl endgroup by a *tert*-butyl endgroup on the PPO block of a diblock analog to F68 could significantly enhance the stabilization efficacy of polymers on the dystrophic membrane.⁵⁸ This result also confirmed the importance of the endgroup, despite the fact that it is less significant in our case.

CONCLUSIONS

We have investigated the effect of polymer structure and concentration on the molecular interactions between PPO-PEO block copolymers and POPC unilamellar liposomes as model lipid bilayers. The polymer structure was tuned by modifying the total molecular weight and the composition, including the PPO and PEO block lengths and the endgroup on the PPO block. The binding of the polymers with lipid membranes was quantified by PFG-NMR, which quantified polymer diffusion in the absence and presence of liposomes. The logarithmic normalized echo-attenuated intensity $\ln(I/I_0)$ of the free polymer showed a linear relationship with the parameter $\gamma^2 G^2 \delta^2 (-\delta/3)$, implying simple translational diffusion in the absence of liposomes. In the polymer-liposome mixture, the non-linear relationship reflects interactions with lipid bilayers. The binding percentage of polymers to the liposomes was quantified by applying a biexponential model to the echo decay curves. The fitting results showed that larger molecular weight and higher hydrophobicity of the polymers give rise to higher binding percentage, as well as liposome surface coverage, and thereby stronger associations with lipid bilayers. As polymer concentration increases, the binding percentage decreases while the liposome surface coverage increases. This is attributable to the saturation of the liposome surface coverage at certain high concentration. Additionally, it has been shown that the binding percentage is independent of incubation time, indicating that the polymer-lipid bilayer association reaches equilibrium very quickly. These results demonstrate that PFG-NMR is a straightforward and useful tool to quantify the binding of block copolymers with lipid bilayers, and we believe it will bring new insights into the mechanistic studies of polymer-membrane association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge Letitia J. Yao for helping with NMR experiments. NMR instrumentation was supported by the Office of the Vice President of Research, College of Science and Engineering, and the Department of Chemistry at the University of Minnesota. This study was funded by National Institutes of Health (grant RO1HL122323).

References

- 1Maskarinec SA, Wu G, Lee KYC. Membrane Sealing by Polymers. *Ann. N. Y. Acad. Sci.* 2006; 1066:310–320.

- 2Lee RC, Hannig J, Matthews KL, Myerov A, Chen C-T. Pharmaceutical Therapies for Sealing of Permeabilized Cell Membranes in Electrical Injuries. *Ann. N. Y. Acad. Sci.* 1999; 888:266–273. [PubMed: 10842638]
- 3Agarwal J, Walsh A, Lee RC. Multimodal Strategies for Resuscitating Injured Cells. *Ann. N. Y. Acad. Sci.* 2006; 1066:295–309.
- 4Rubinsky B, Arav A, Devries AL. The Cryoprotective Effect of Antifreeze Glycopeptides from Antarctic Fishes. *Cryobiology.* 1992; 29:69–79. [PubMed: 1606831]
- 5Gaylor DC, Prakah-Asante K, Lee RC. Significance of Cell Size and Tissue Structure in Electrical Trauma. *J. Theor. Biol.* 1988; 133:223–237. [PubMed: 3236893]
- 6Tsong TY, Su Z. Biological Effects of Electric Shock and Heat Denaturation and Oxidation of Molecules, Membranes, and Cellular functions. *Ann. N. Y. Acad. Sci.* 1999; 888:211–232. [PubMed: 10842635]
- 7Palmer JS, Cromie WJ, Lee RC. Surfactant Administration Reduces Testicular Ischemia-Reperfusion Injury. *J. Urol.* 1998; 159:2136–2139. [PubMed: 9598559]
- 8McNeil PL, Steinhardt RA. Loss, Restoration, and Maintenance of Plasma Membrane Integrity. *J. Cell Biol.* 1997; 137:1–4. [PubMed: 9105031]
- 9Fischer TA, McNeil PL, Khakee R, Finn P, Kelly RA, Pfeffer MA, Pfeffer JM. Cardiac Myocyte Membrane Wounding in the Abruptly Pressure-Overloaded Rat Heart under High Wall Stress. Hypertension. 1997; 30:1041–1046. [PubMed: 9369253]
- 10Pacifico CR, Lundsted LG, Vaughn TH. Flake Form Nonionic Detergents. *Soap Sanit. Chem.* 1950; 26:40–43. 73, 90.
- 11Vaughn T, Suter H, Lundsted L, Kramer M. Properties of Some Newly Developed Nonionic Detergents. *J. Am. Oil Chem. Soc.* 1951; 28:294–299.
- 12Lundsted LG. Polyoxyalkylene Compounds. US. 2674619. 1954
- 13Lee RC, River LP, Pan FS, Ji L, Wollmann RL. Surfactant-Induced Sealing of Electroporeabilized Skeletal Muscle Membranes *in vivo*. *Proc. Natl. Acad. Sci.* 1992; 89:4524–4528. [PubMed: 1584787]
- 14Merchant FA, Holmes WH, Capelli-Schellpfeffer M, Lee RC, Toner M. Poloxamer 188 Enhances Functional Recovery of Lethally Heat-Shocked Fibroblasts. *J. Surg. Res.* 1998; 74:131–140. [PubMed: 9587351]
- 15Serbest G, Horwitz J, Barbee K. The Effect of Poloxamer-188 on Neuronal Cell Recovery from Mechanical Injury. *J. Neurotrauma.* 2005; 22:119–132. [PubMed: 15665607]
- 16Greenebaum B, Blossfield K, Hannig J, Carrillo CS, Beckett MA, Weichselbaum RR, Lee RC. Poloxamer 188 Prevents Acute Necrosis of Adult Skeletal Muscle Cells Following High-Dose Irradiation. *Burns.* 2004; 30:539–547. [PubMed: 15302418]
- 17Yasuda S, Townsend D, Michele DE, Favre EG, Day SM, Metzger JM. Dystrophic Heart Failure Blocked by Membrane Sealant Poloxamer. *Nature.* 2005; 436:1025–1029. [PubMed: 16025101]
- 18Houang EM, Haman KJ, Filareto A, Perlingeiro RC, Bates FS, Lowe DA, Metzger JM. Membrane-Stabilizing Copolymers Confer Marked Protection to Dystrophic Skeletal Muscle *in vivo*. *Mol. Ther.-Methods Clin. Dev.* 2015; 2:15042. [PubMed: 26623440]
- 19Demina T, Grozdova I, Krylova O, Zhirnov A, Istratov V, Frey H, Kautz H, Melik-Nubarov N. Relationship between the Structure of Amphiphilic Copolymers and Their Ability to Disturb Lipid Bilayers. *Biochemistry.* 2005; 44:4042–4054. [PubMed: 15751981]
- 20Krylova OO, Melik-Nubarov NS, Badun GA, Ksenofontov AL, Menger FM, Yaroslavov AA. Pluronic L61 Accelerates Flip-Flop and Transbilayer Doxorubicin Permeation. *Chem. Eur. J.* 2003; 9:3930–3936. [PubMed: 12916119]
- 21Krylova OO, Pohl P. Ionophoric Activity of Pluronic Block Copolymers. *Biochemistry.* 2004; 43:3696–3703. [PubMed: 15035640]
- 22Cohn D, Sosnik A, Levy A. Improved Reverse Thermo-Responsive Polymeric Systems. *Biomaterials.* 2003; 24:3707–3714. [PubMed: 12818542]
- 23Sosnik A, Cohn D. Ethoxysilane-Capped PEO–PPO–PEO Triblocks: A New Family of Reverse Thermo-Responsive Polymers. *Biomaterials.* 2004; 25:2851–2858. [PubMed: 14962563]

- 24Wang J-Y, Marks J, Lee KYC. Nature of Interactions between PEO-PPO-PEO Triblock Copolymers and Lipid Membranes: (I) Effect of Polymer Hydrophobicity on Its Ability to Protect Liposomes from Peroxidation. *Biomacromolecules*. 2012; 13:2616–2623. [PubMed: 22808900]
- 25Cheng C-Y, Wang J-Y, Kausik R, Lee KYC, Han S. Nature of interactions between PEO-PPO-PEO triblock copolymers and lipid membranes: (II) role of hydration dynamics revealed by dynamic nuclear polarization. *Biomacromolecules*. 2012; 13:2624–2633. [PubMed: 22808941]
- 26Wang J-Y, Chin J, Marks JD, Lee KYC. Effects of PEO–PPO–PEO Triblock Copolymers on Phospholipid Membrane Integrity under Osmotic Stress. *Langmuir*. 2010; 26:12953–12961. [PubMed: 20666423]
- 27Frey SL, Zhang D, Carignano MA, Szeleifer I, Lee KYC. Effects of Block Copolymer's Architecture on Its Association with Lipid Membranes: Experiments and Simulations. *J. Chem. Phys.* 2007; 127:114904. [PubMed: 17887877]
- 28Leiske DL, Meckes B, Miller CE, Wu C, Walker TW, Lin B, Meron M, Ketelson HA, Toney MF, Fuller GG. Insertion Mechanism of a Poly(ethylene oxide)-poly(butylene oxide) Block Copolymer into a DPPC Monolayer. *Langmuir*. 2011; 27:11444–11450. [PubMed: 21834565]
- 29Wang J, Segatori L, Biswal SL. Probing the Association of Triblock Copolymers with Supported Lipid Membranes Using Microcantilevers. *Soft Matter*. 2014; 10:6417–6424. [PubMed: 24978842]
- 30Firestone MA, Wolf AC, Seifert S. Small-Angle X-ray Scattering Study of the Interaction of Poly(ethylene oxide)-*b*-Poly(propylene oxide)-*b*-Poly(ethylene oxide) Triblock Copolymers with Lipid Bilayers. *Biomacromolecules*. 2003; 4:1539–1549. [PubMed: 14606878]
- 31Firestone MA, Seifert S. Interaction of Nonionic PEO–PPO Diblock Copolymers with Lipid Bilayers. *Biomacromolecules*. 2005; 6:2678–2687. [PubMed: 16153106]
- 32Lee B, Firestone MA. Electron Density Mapping of Triblock Copolymers Associated with Model Biomembranes: Insights into Conformational States and Effect on Bilayer Structure. *Biomacromolecules*. 2008; 9:1541–1550. [PubMed: 18452333]
- 33Hezaveh S, Samanta S, De Nicola A, Milano G, Roccatano D. Understanding the Interaction of Block Copolymers with DMPC Lipid Bilayer Using Coarse-Grained Molecular Dynamics Simulations. *J. Phys. Chem. B*. 2012; 116:14333–14345. [PubMed: 23137298]
- 34Rabbel H, Werner M, Sommer J-U. Interactions of Amphiphilic Triblock Copolymers with Lipid Membranes: Modes of Interaction and Effect on Permeability Examined by Generic Monte Carlo Simulations. *Macromolecules*. 2015; 48:4724–4732.
- 35Kim M, Haman KJ, Houang EM, Zhang W, Yannopoulos D, Metzger JM, Bates FS, Hackel BJ. PEO-PPO Diblock Copolymers Protect Myoblasts from Hypo-Osmotic Stress *in vitro* Dependent on Copolymer Size, Composition, and Architecture. *Biomacromolecules*. 2017; 18:2090–2101. [PubMed: 28535058]
- 36Ferreira TM, Sood R, Bärenwald R, Carlström G, Topgaard D, Saalwächter K, Kinnunen PKJ, Ollila OHS. Acyl Chain Disorder and Azelaoyl Orientation in Lipid Membranes Containing Oxidized Lipids. *Langmuir*. 2016; 32:6524–6533. [PubMed: 27260273]
- 37Bärenwald R, Achilles A, Lange F, Ferreira TM, Saalwächter K. Applications of Solid-State NMR Spectroscopy for the Study of Lipid Membranes with Polyphilic Guest (Macro)Molecules. *Polymers*. 2016; 8:439.
- 38Ding J, Price C, Booth C. Use of Crown Ether in the Anionic Polymerization of Propylene Oxide—1. Rate of Polymerization. *Eur. Polym. J.* 1991; 27:891–894.
- 39Ndoni S, Papadakis CM, Bates FS, Almdal K. Laboratory- Scale Setup for Anionic Polymerization under Inert Atmosphere. *Rev. Sci. Instrum.* 1995; 66:1090–1095.
- 40Hillmyer MA, Bates FS. Synthesis and Characterization of Model Polyalkane–Poly(ethylene oxide) Block Copolymers. *Macromolecules*. 1996; 29:6994–7002.
- 41Van Krevelen DW, , Te Nijenhuis K. Properties of Polymers: Their Correlation with Chemical Structure; Their Numerical Estimation and Prediction from Additive Group Contributions 4. Elsevier; 2009
- 42Brandrup J, , Immergut EH, , Grulke EA. Polymer Handbook 4. Wiley; 1999

- 43Olson F, Hunt CA, Szoka FC, Vail WJ, Papahadjopoulos D. Preparation of Liposomes of Defined Size Distribution by Extrusion through Polycarbonate Membranes. *Biochim. Biophys. Acta - Biomembr.* 1979; 557:9–23.
- 44MacDonald RC, MacDonald RI, Menco BPM, Takeshita K, Subbarao NK, Hu LR. Small-Volume Extrusion Apparatus for Preparation of Large, Unilamellar Vesicles. *Biochim. Biophys. Acta.* 1991; 1061:297–303. [PubMed: 1998698]
- 45Hope MJ, Bally MB, Webb G, Cullis PR. Production of Large Unilamellar Vesicles by a Rapid Extrusion Procedure. Characterization of Size Distribution, Trapped Volume and Ability to Maintain a Membrane Potential. *Biochim. Biophys. Acta - Biomembr.* 1985; 812:55–65.
- 46Wu DH, Chen AD, Johnson CS. An Improved Diffusion-Ordered Spectroscopy Experiment Incorporating Bipolar-Gradient Pulses. *J. Magn. Reson. Ser. A.* 1995; 115:260–264.
- 47Batrakova E, Lee S, Li S, Venne A, Alakhov V, Kabanov A. Fundamental Relationships between the Composition of Pluronic Block Copolymers and Their Hypersensitization Effect in MDR Cancer Cells. *Pharm. Res.* 1999; 16:1373–1379. [PubMed: 10496652]
- 48Momot KI, Kuchel PW. Pulsed Field Gradient Nuclear Magnetic Resonance as a Tool for Studying Drug Delivery Systems. *Concepts Magn. Reson. A.* 2003; 19A:51–64.
- 49Güven A, Ortiz M, Constanti M, O'Sullivan CK. Rapid and Efficient Method for the Size Separation of Homogeneous Fluorescein-Encapsulating Liposomes. *J. Liposome Res.* 2009; 19:148–154. [PubMed: 19515001]
- 50Dave PC, Tiburu EK, Damodaran K, Lorigan GA. Investigating Structural Changes in the Lipid Bilayer upon Insertion of the Transmembrane Domain of the Membrane-Bound Protein Phospholamban Utilizing ^{31}P and ^2H Solid-State NMR Spectroscopy. *Biophys. J.* 2004; 86:1564–1573. [PubMed: 14990483]
- 51Huber T, Rajamoorthi K, Kurze VF, Bayer K, Brown MF. Structure of Docosahexaenoic Acid-Containing Phospholipid Bilayers as Studied by ^2H NMR and Molecular Dynamics Simulations. *J. Am. Chem. Soc.* 2002; 124:298–309. [PubMed: 11782182]
- 52Kuerka N, Tristram-Nagle S, Nagle JF. Structure of Fully Hydrated Fluid Phase Lipid Bilayers with Monounsaturated Chains. *J. Membr. Biol.* 2006; 208:193–202.
- 53Leson A, Hauschild S, Rank A, Neub A, Schubert R, Förster S, Mayer C. Molecular Exchange through Membranes of Poly (2- vinylpyridine- block- ethylene oxide) Vesicles. *Small.* 2007; 3:1074–1083. [PubMed: 17464955]
- 54Kärger J. NMR Self-Diffusion Studies in Heterogeneous Systems. *Adv. Colloid Interface Sci.* 1985; 23:129–148.
- 55So S, Lodge TP. Rate of Molecular Exchange through the Membranes of Ionic Liquid Filled Polymersomes Dispersed in Water. *J. Phys. Chem. C.* 2014; 118:21140–21147.
- 56So S, Yao LJ, Lodge TP. Permeability of Rubbery and Glassy Membranes of Ionic Liquid Filled Polymersome Nanoreactors in Water. *J. Phys. Chem. B.* 2015; 119:15054–15062. [PubMed: 26588106]
- 57Alexandridis P, Holzwarth JF, Hatton TA. Micellization of Poly(ethylene oxide)-Poly(propylene oxide)-Poly(ethylene oxide) Triblock Copolymers in Aqueous Solutions: Thermodynamics of Copolymer Association. *Macromolecules.* 1994; 27:2414–2425.
- 58Houang EM, Haman KJ, Kim M, Zhang W, Lowe DA, Sham YY, Lodge TP, Hackel BJ, Bates FS, Metzger JM. Chemical End Group Modified Diblock Copolymers Elucidate Anchor and Chain Mechanism of Membrane Stabilization. *Mol. Pharm.* 2017; 14:2333–2339. [PubMed: 28538101]

Figure 1. Schematic of interactions between block copolymers and liposomes. After mixing a liposome solution with a polymer solution, a fraction of the polymer molecules associates with the liposomes, whereas the others remain as free chains.

Figure 2.

¹H NMR spectrum of 1 mg/mL P105 in 5 mM POPC liposome in D₂O.

Figure 3.

(a) Experimental and fitted echo decay curves of the protons from PEO of 1 mg/mL P105 in 5 mM POPC liposome solution in D₂O at 27 °C with $\Delta = 300, 500, 700$ ms (filled circles, filled squares, and filled triangles, respectively) and with fixed $\delta = 5$ ms. The data were fit to eqn 2. The open circles denote the proton signal from PEO of 1 mg/mL P105 in the absence of liposomes; these data were fit to eqn 1. (b) Schematic of surface coverage of a half liposome by P105. The pink circle represents the surface area of a half liposome, and the small particles represent the estimated cross sectional area of polymer molecules associated with the lipid bilayer, using R_g in a theta solvent as the estimated polymer radius. The zoomed-in view shows one possible interaction between a polymer and the lipid bilayer.

Figure 4.

Experimental and fitted echo decay curves of the protons from PEO of 1 mg/mL (a) F127, (b) F87, (c) P65, and (d) L35 in 5 mM POPC liposome solution in D₂O at 27 °C with $\Delta = 300, 500, 700$ ms and fixed $\delta = 5$ ms. The data were fitted by eqn 2. The insets show schematics of the surface coverage of half a liposome by polymer.

Figure 5.

Fraction of liposome surface area covered by polymer molecules as a function of polymer concentration of F127. The insets show schematics of surface coverage by polymers at different concentration.

Figure 6. Summary of polymer molecular weight, block length, endgroup and concentration effect on the interactions between polymer and liposome. The volume of the spheres is proportional to θ .

Figure 7.
Binding percentage as a function of the number of repeat units of PO in the polymer.

Scheme 1.

Polymer structures of (a) a Pluronic, (b) a diblock copolymer with a *tert*-butyl endgroup on the PPO block, and (c) a diblock copolymer with a hydroxyl endgroup on the PPO block and a methyl endgroup on the PEO block.

Table 1

Polymer Characterization.

	$M_{n,1}^a$ (g/mol)	$M_{n,2}^b$ (g/mol)	$M_{n,3}^c$ (g/mol)	DP^b	$w_{PEO,1}^a$	$w_{PEO,2}^d$	N_{PO}^e	N_{EO}^e
F68	8,400	8,200	7,900	1.01	1.04	0.80	27	75
F108	14,600	15,900	14,700	1.01	1.04	0.80	49	148
F87	7,700	7,700	8,000	1.01	1.15	0.70	40	61
F127	12,600	13,200	10,800	1.01	1.07	0.70	64	108
P103	4,950	5,200	4,600	1.02	1.14	0.30	60	19
P104	5,900	5,700	5,300	1.02	1.18	0.40	57	27
P105	6,500	6,500	5,700	1.02	1.12	0.50	56	37
L35	1,900	1,900	2,100	1.04	1.13	0.50	17	10
P65	3,400	3,400	3,200	1.03	1.16	0.50	30	19
P84	4,200	4,200	3,600	1.03	1.05	0.40	42	20
hPPO ₁₃ -PEO ₄₃ m	-	2,600	2,700	1.02	1.08	-	13	43
tPPO ₁₄ -PEO ₄₆	-	2,900	3,000	1.03	1.07	-	14	46
tPPO ₁₄ -PEO ₇₈	-	4,300	3,900	1.03	1.08	-	14	78
tPPO ₁₄ -PEO ₁₅₉	-	7,900	7,500	1.01	1.05	-	14	159
tPPO ₂₉ -PEO ₆₈	-	4,700	4,900	1.04	1.10	-	29	68
PEO-8	8,000	8,600	8,700	1.01	1.03	1	0	195

^aSpecified by supplier.

^bDetermined by MALDI mass spectroscopy.

^cDetermined by SEC. Weight-average dn/dc was used with $dn/dc = 0.068$ for PEO and 0.087 for PPO.

^dDetermined from molar ratios by ¹H NMR spectroscopy.

^eCalculated from number-average molecular weight ($M_{n,2}$) and weight fraction of PEO ($w_{PEO,2}$). For triblock Pluronics, N_{EO} represents the number of repeat units of EO in one PEO block.

Table 2

Summary of interactions between polymers and liposomes

	c (mg/mL)	f_{bound}^a (%)	N^b	θ^c
F68	1	0.1	1	0.002
F108	0.2	1.8	2	0.008
F108	1	0.9	6	0.018
F87	1	0.2	2	0.003
F127	0.2	18.2	30	0.075
F127	0.5	16.0	66	0.17
F127	1	12.6	104	0.26
L35	1	0.8	43	0.015
P65	1	0.9	28	0.017
P105	0.2	9.4	32	0.037
P105	1	7.9	132	0.15
P84	1	1.0	26	0.019
P104	0.2	8.5	32	0.032
P104	1	6.3	119	0.12
P103	0.2	12.5	53	0.046
hPPO ₁₃ -PEO ₄₃ m	1	0.2	7	0.004
tPPO ₁₄ -PEO ₄₆	1	0.5	20	0.010
tPPO ₁₄ -PEO ₇₈	1	0.2	4	0.003
tPPO ₁₄ -PEO ₁₅₉	1	0.1	2	0.003
tPPO ₂₉ -PEO ₆₈	1	1.9	43	0.037
PEO-8	1	0.1	1	0.002

^aObtained from fitting results based on eqn 2.^bCalculated from eqn 3.^cCalculated from eqn 6.