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Giant Phospholipid/Block Copolymer Hybrid Vesicles: Mixing Behavior and Domain Formation

Jin Nam, Paul A. Beales,[†] and T. Kyle Vanderlick*

Chemical & Environmental Engineering, Yale University, New Haven, Connecticut 06511, United States.
[†]Present address: Centre for Molecular Nanoscience, School of Chemistry, University of Leeds, Leeds LS2 9JT, United Kingdom.

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Lipids and block copolymers can be individually assembled into unsupported, spherical membranes (liposomes or polymersomes), each having their own particular benefits and limitations. Here we demonstrate the preparation of microscale, hybrid "lipopolymersomes" composed of the common lipid POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine) and the commercially available copolymer PBd-*b*-PEO (polybutadiene-*b*-poly(ethylene oxide)) with the goal of incorporating the advantageous qualities of the unitary systems into mixed-membrane capsules. We investigate the lipopolymersomes using confocal fluorescence microscopy and demonstrate that these hybrid membranes are well mixed on nanoscopic length scales within the permittable compositional windows for hybrid vesicle formation. We measure the intramembrane dynamics and mechanical properties of these hybrid membranes by fluorescence recovery after photobleaching (FRAP) and micropipet aspiration, respectively. For the first time, we demonstrate the demixing of lipid-rich and polymer-rich membrane domains within the same vesicle membrane. This is achieved by the biotinylation of one of the constituent species and cross linking with the protein NeutrAvidin. The resultant domain patterning is dependent upon which component carries the biotin functionality: cross linking of the copolymer species results in domains that ripen into a single, large, copolymer-rich island, and cross linking of the lipids yields many small, "spot-like", lipid-rich domains within a copolymer-rich matrix. We discuss these morphological differences in terms of the fluidity and mechanical properties of the membrane phases and the possible resultant interdomain interactions within the membrane. These heterogeneous hybrid lipopolymersomes could find applications in fields such as targeted delivery, controlled release, and environmental detection assays where these capsules possess the characteristics of biocompatible lipid membranes combined with enhanced mechanical strength and stability from the copolymer matrix.

Introduction

A host of natural phospholipids as well as a carefully crafted set of synthetic block copolymers can each, on their own, be assembled into closed membrane configurations known as liposomes and polymersomes, respectively. Much of what is appealing about either of these thin-shelled structures is found lacking in the other. The biocompatibility of liposomes is, for example, unmatchable in the synthetic polymeric systems. However, the stability associated with the block copolymer membranes is accessible only to these man-made creations. In this letter, we address the challenge of combining the best of both membrane systems by creating hybrid "lipopolymersomes" on the micrometer scale. We report on the material properties of these novel mixed membranes and the mixing behavior of the two main membrane constitutents, a commercially available diblock copolymer (polybutadiene-*b*-poly(ethylene oxide, PBd-*b*-PEO) and a simple phospholipid (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine, POPC) and show how initially well-mixed membranes can be coerced into phase segregation and domain formation.

There is much to be gained by creating well-controlled lipid−polymer hybrid membrane systems, especially if the most desirable attributes of the pure systems are not only preserved in the hybrid but offset the inherent limitations of the simpler structures. In the case of liposomes, one key attribute is the relative ease with which natural lipid membranes can be functionalized with proteins and ligands,[1] making these systems useful for in vivo applications such as targeted drug delivery and controlled release. The biocompatible, nondenaturing interface of liposomal capsules also makes this a suitable material for the encapsulation and isolation of biomolecules for in vitro applications. However, the long-term stability of liposomes can be problematic for applications that require long shelf lives or prolonged monitoring times; membrane-specific interactions of other molecules in solution can also acutely reduce liposome stability. Surface functionalization of liposomes with polymers such as membrane-anchored polyethylene glycol (PEG) is a strategy that has been used in the drug delivery field to provide a steric "shield" that protects the membrane, increasing stability and reducing the premature release of encapsulated cargo.[2]

Polymersomes are self-assembled shells of amphiphilic block copolymers constituted of hydrophilic and hydrophobic covalently linked polymeric blocks.[3] Key attributes of these membrane systems are their long-term stability and tunability, as accomplished by varying the chemical makeup of their constituent copolymers. The selection of copolymer molecular weight, for example, controls the membrane thickness and hence the permeability and mechanical properties of the membrane.[4] Functional groups can be added to the copolymers to make stimuli-responsive

*Corresponding author. E-mail: kyle.vanderlick@yale.edu.

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polymersomes that respond to changes in environment such as temperature, pH, and light.[5] Although polymersomes benefit from increased stability, little is known about the potential toxicity of the long-term accumulation of these synthetic molecules in the body if used for in vivo applications; therefore, the dilution of block copolymers in composite membranes with biocompatible molecules such as lipids could reduce any possible health risks. These synthetic polymeric membranes also provide additional challenges for applications that require the incorporation of biological components into the membrane, such as targeting ligands or integral membrane proteins.

This study is motivated by the potential to incorporate the reciprocal advantages of both phospholipids and copolymers into the same vesicle membrane. Only a few studies on phospholipid−block copolymer mixed-vesicle membranes have so far been reported.[6,7] Here we investigate hybrid lipid−diblock copolymer giant unilamellar vesicles (HLP-GUVs), or "lipopolymersomes", and take advantage of the optical visualization of micrometer-scale vesicles to investigate the mixing and morphological properties of these composite membrane systems.

The mixing behavior of multicomponent membranes, in general, continues to capture much attention because of their relevance as model systems for functional, in vivo membrane heterogeneities referred to as "lipid rafts".[8] Although these dynamic, nanoscale lipid domains have not been directly observed in live cells, phase separation into coexisting lipid domains has been reported for many different lipid mixtures reconstituted into model membrane systems. These include the observation of solid-like domains within a fluid matrix[9] and membrane demixing into two coexisting fluid phases.[10] The significance of membrane heterogeneities has also led to much theoretical work on understanding the emergence, behaviors, and consequences of domains or inclusions within fluid, elastic membranes.[11,12] This previous body of work on mixed lipid membranes provides an existing framework from which we can build an understanding of the mixing behavior of lipid−copolymer hybrid membranes, and this novel system may also provide new insights into the phenomenology of multicomponent membranes.

Experimental Section

Materials. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[biotinyl(poly(ethylene glycol))-2000 (DSPE-PEG2000-Biotin) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Rhodamine Red-X conjugated NeutrAvidin (Rh-NA), (*N*-(6-tetramethylrhodaminethiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (rhodamine-DHPE), Oregon Green 488 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Oregon Green-DHPE), and *N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (BODIPY-DHPE) were purchased from Invitrogen Molecular Probes. Poly(butadiene-*b*-ethylene oxide) (PBd-*b*-PEO) diblock copolymer was purchased from Polymer Source Inc. as a custom-synthesized material. Its overall molecular weight (M_w) was reported to be 3800 with 46 Bd units and 30 EO units. The polydispersity (PDI) was reported to be 1.04. Other chemicals were purchased from Sigma, of the highest grade available, and were used as received. Water was produced by a Milli-G UF unit (Millipore, Bedford, MA) and had a resistivity of 18.2 MΩ cm.

Giant unilamellar vesicles (GUVs) were created using a modification of the electroformation method.[13] POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) was combined with PBd-*b*-PEO (poly(butadiene-*b*-ethylene oxide)) to make 0.5 mg/mL solutions in chloroform with the desired lipid fraction. A lipid/polymer mixed solution (50 μL) was spread on platinum electrodes that were held 8 mm apart in a home-built Teflon cell. Electroformation was carried out in sucrose solutions near 260 mOsm. An alternating electric field was applied across the wires at 3 V ac and 11 Hz with a sine wave function at room temperature for several hours. Vesicle observation and manipulation were conducted in phosphate-buffered (pH 7.4) glucose solutions of 265 mOsm.

Biotinylation of PBd-*b*-PEO. The biotinylation of the hydroxyl end group of PBd-*b*-PEO follows an established protocol.[14] PBd-*b*-PEO was dissolved in anhydrous dimethyl formamide to a concentration of 5 wt %, and excess *N*,*N*′-disuccinimidyl carbonate was dissolved in DMF at 50 °C and then added to the PBd-*b*-PEO solution. The equivalent molar amount of DMAP (dimethylamino pyridine, Aldrich) as a base, matching the amount of *N*,*N*′-disuccinimidyl carbonate, was also dissolved in DMF at 50 °C and added dropwise to the reaction vessel over the course of a few minutes. The reaction mixture was stirred at 60 °C for 8 h. After the completion of the coupling reaction, the DMF was removed by vacuum. The intermediate product was weighed and used in the biotinylation step without purification. Excess 5-(biotinoamido) penthylamine was dissolved in DMF at 50 °C and added to the reaction vessel. The reaction proceeded at 60 °C for 24 h. The final product was dried under vacuum and then dissolved in the solvent of choice for vesicle formation.

Confocal microscopy images were acquired on a TSC SP5 confocal microscope from Leica, equipped with LCS software and using a Leica 63×/1.3 N.A. Plan Apo DIC glycerin-immersion objective. Rh-NA was excited with a DPSS laser at 561 nm, and Oregon Green-DHPE and BODIPY-DHPE were excited with the 488 nm line of an argon laser. Samples were prepared in glass-bottomed culture dishes (P35G-1.5-14-C, MatTek) coated with 5 wt % (/vol) bovine serum albumin solution for 1 h at room temperature prior to use in order to prevent vesicles from adhering to the glass coverslip.[15−18]

Fluorescence recovery after photobleaching (FRAP) measurements were performed to monitor the fluidity of the membrane bilayer. For FRAP, the bilayer was labeled with 0.1% rhodamine-DHPE. The Axelord method was employed to estimate the diffusion coefficients from the half recovery time, $t_{1/2}$ values for complete fluorescence recovery, which in the case of a uniform circular

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beam profile is given by $D = 0.224\omega^2/t_{1/2}$, in which ω is the full width of the bleached spot.[19]

Vesicle Aspiration Test. Following previously published designs,[20−22] this test was conducted using an inverted optical microscope fitted with differential interference 40× contrast optics (Nikon TE200, Micron Optics) employing a Narshiage MHW-3 micromanipulator (Micron Optics) for pipet manipulation. In studies of membrane mechanics, eight vesicles were aspirated into a micropipet and the suction was first increased relatively quickly to draw out any wrinkles and tethers in the membrane. The suction was then decreased to nearly zero to initiate the experiment. The suction was then increased again until the vesicle broke. Vesicle images, recorded on video, were analyzed to obtain the membrane area at each suction level, and the Laplace equation was applied to translate the suction values to the isotropic membrane tension, τ. The plot of membrane tension as a function of areal strain gives the area expansion modulus, $K_a = d\tau/d(\Delta A/A_o)$, at sufficiently large strains.[23]

Results and Discussion

HLP-GUVs composed of POPC and PBd-*b*-PEO (Figure 1a, b), which individually form GUVs at room temperature, could be successfully fabricated within certain windows of compositional space. These HLP-GUVs were prepared by the electroformation technique as described previously.[13] To confirm the mixing behavior of both amphiphiles in forming hybrid-GUVs, GUVs were labeled with a low molar percentage of fluorescent phospholipid and observed via confocal laser microscopy. The formation of HLP-GUVs is dependent on the fractional composition of each amphiphile. At higher concentrations (above ~70 mol %) of diblock copolymer, most vesicles formed as well-mixed, single-phase, enclosed lipopolymersome membranes, where phospholipids could be homogeneously integrated into the diblock copolymer-rich matrix during vesicle formation. The phospholipid-based fluorescent probes did not show any preferential partitioning within the membranes on optically resolvable length scales, suggesting well-mixed single-phase bilayers, as shown in Figure 1c,d. Uniform membrane fluorescence was observed for HLP-GUVs prepared using different trace fluorescent lipids, Oregon Green-DHPE or BODIPY-DHPE. The bright, uniform fluorescence intensity observed in the membranes of all vesicles in these samples makes the possibility of unitary block-copolymer-based polymersomes coexisting with unitary phospholipid-based liposomes unlikely. However, at high phospholipid concentration, the majority of vesicles were POPC liposomes with only a small fraction of GUVs forming mixed lipopolymersomes of POPC and PBd-*b*-PEO. Although low-molecular-weight phospholipids are able to be integrated into the thick PBd-*b*-PEO-rich membrane, it appears to be energetically less favorable for higher-molecular-weight diblock copolymer molecules to be incorporated into the thinner, lipid-rich membrane environment, resulting in poor HLP-GUV formation. At intermediate lipid compositions (between ~35 and 65 mol %), no GUV formation of any kind was observed in our experiments. The regions of compositional space where lipopolymersomes can be formed are shown schematically in Figure 1e.

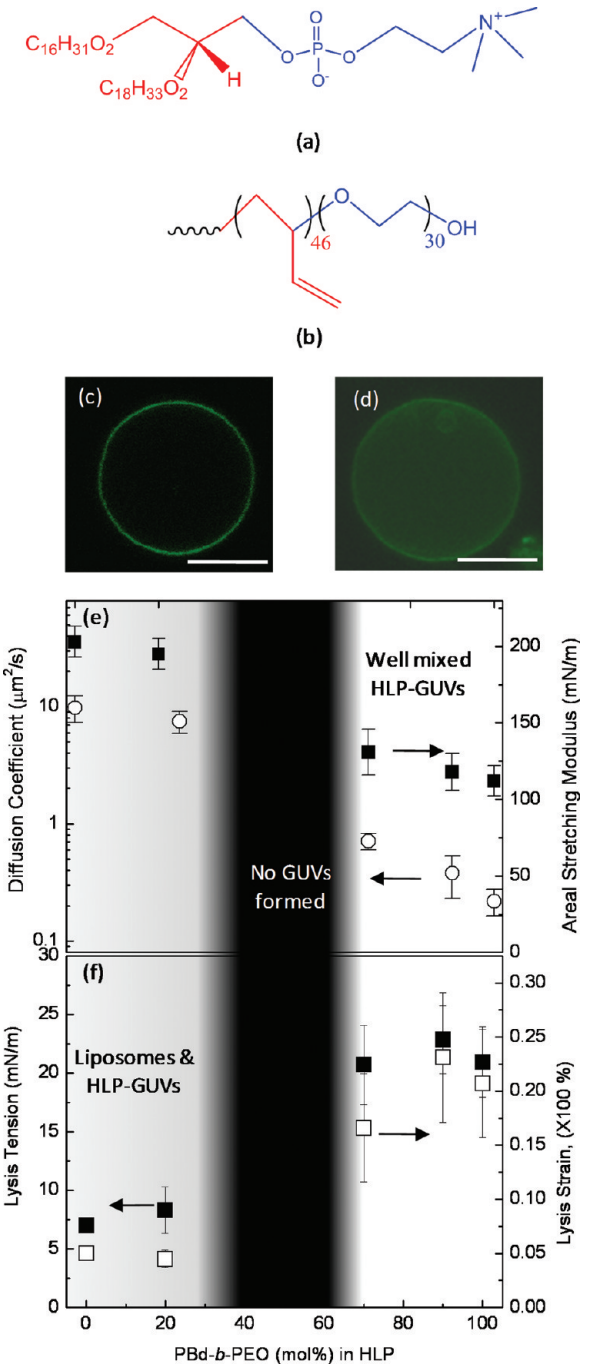

Figure 1. Structures of (a) POPC and (b) PBd-*b*-PEO. Confocal microscopic images of (c) a single image slice through the vesicle equator and (d) a reconstructed 3D image of a vesicle hemisphere from confocal image sections show a uniform fluorescence intensity of 0.5 mol % Oregon Green DHPE in hybrid POPC (30 mol %)−copolymer mixed vesicles. Scale bars represent 10 μm. (e) Areal stretching modulus (K_a, ■) and lipid lateral diffusion coefficient (D, ○) for varying compositions of HLP-GUVs. (f) Critical lysis tension (τ_c, ■) and lysis strain (α_c, □) of POPC/PBd-*b*-PEO GUVs. (e, f) Background shading represents the regions of compositional space where hybrid GUVs formed.

It should be noted that the observation of uniform, mixed lipopolymersome membranes by confocal microscopy does not rule out the possibility of intramembrane heterogeneities on length scales below that of optical resolution (~200−300 nm). However,

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nanoscale domains would seem to be energetically unlikely because of the anticipated high interfacial line tension of matching a polymer-rich membrane domain, whose hydrophobic thickness in the unitary form is approximately 8 nm, to a phospholipid-rich membrane domain, where the hydrophobic core thickness of a pure lipid bilayer is around 4 nm.[3] Therefore, in equilibrium, this interfacial energy cost would likely be minimized by coalescence into larger domains with higher area-to-circumference ratios. Hence, these images represent direct visual evidence of the efficient mixing of lipids and copolymers in hybrid giant vesicles.

The mechanical properties of hybrid lipopolymersomes were characterized by micropipet aspiration. The variation of membrane mechanical properties across compositional space provides additional evidence for the formation of homogeneously mixed HLP-GUVs. The membrane areal stretching modulus as a function of membrane composition is shown in Figure 1e. The stretching modulus of a POPC liposome ($K_a = 203 \pm 10$ mN/m) is approximately double that of the pure polymersomes ($K_a = 112 \pm 20$ mN/m). The stress (τ) versus strain (α) plots for the different membrane compositions (Figure 1f and Figure S1a in the Supporting Information (SI)) also reveal that the thick, entangled, interconnected layer of PBd-*b*-PEO block copolymer membranes is more robust (10-fold-higher membrane toughness) than POPC liposome membranes, with a higher critical lysis stress (τ_c) and strain (α_c) (18 mN/m and 19% for PBd-*b*-PEO-GUVs vs 7 mN/m and 5% for POPC-GUVs, respectively). Note that membrane toughness (U) is defined as the energy required to break the membrane (i.e., $U = \int_0^{\alpha_c} \tau \, d\alpha$). As the POPC concentration increased up to 30 mol %, the areal stretching modulus increased up to 17% (from 112 ± 10 to 131 ± 15 mN/m) but τ_c and α_c decreased by 17% (from 15.3 to 12.7 mN/m) and 40% (from 0.15 to 0.09), respectively. However, despite the decrease in lysis stress and lysis strain, the increase in the stretching modulus that results from the incorporation of lipid into the block copolymer membrane somewhat compensates with respect to the toughness of the hybrid membranes because this results in a greater applied tension being required to achieve a given strain.

Lateral lipid diffusion within the hybrid membranes was measured by FRAP (Figure 1f). Note that the measurements report the mobility of lipid-based fluorescent probes within these membranes. The lateral diffusion coefficient (0.22 ± 0.06 $\mu m^2/s$) in the block copolymer membranes of polymersomes is almost 2 orders of magnitude lower than that of pure POPC liposomes (9.8 ± 1.7 $\mu m^2/s$). The HLP-GUVs showed intermediate diffusion coefficients for the fluorescent lipid probe; representative FRAP recovery curves are shown in Figure S1b in the SI. The lipid lateral diffusion coefficient of HLP-GUVs increased in proportion to the amount of incorporated POPC in the lipopolymersome mixture, as shown in Figure 1e. However, at high POPC compositions, the diffusion coefficients showed a weaker dependence on the mixing ratio compared to the variations in diffusivity with the composition of block-copolymer-rich hybrid membranes. It is interesting that the fits to the FRAP recovery data (Figure S1b in the SI) are not as accurate for the hybrid membranes as is found for the unitary lipid or copolymer GUVs. This provides some indication that the intramembrane molecular dynamics of these mixed systems are not ideally described by a standard lateral diffusion model with a single diffusion coefficient.

The polymer-rich HLP-GUVs are relatively stable and intact for several weeks (Figure 2). However, at high lipid compositions, HLP-GUVs became less stable with visible aggregation and vesicle deformation occurring over time within these samples as shown in Figure 2d.

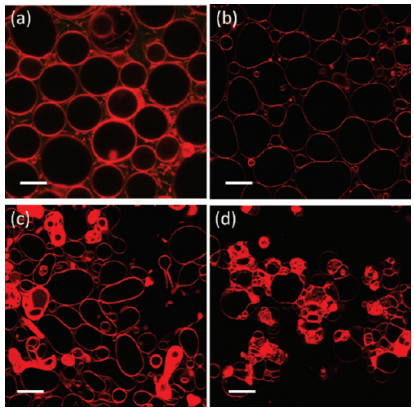

Figure 2. Confocal microscopy images of HLP-GUVs after 24 h. POPC/PBd-*b*-PEO: (a) 0/10, (b) 1/9, (c) 2/8, and (d) 9/1. Lipid-rich HLP-GUVs became unstable and were seen to aggregate strongly. However, block-copolymer-rich HLP-GUVs were stable with little deformation as the composition of phospholipid was decreased. The bar represents 10 μm.

After the successful formation of homogeneous HLP-GUVs, our next goal was to induce domain formation, thus creating structures with both biofunctionality (via the phospholipid regions) and the concurrent stability of diblock copolymers. Although much work has already been reported on domain formation in purely lipid membranes[9,10] and there are some reports of domain formation in entirely copolymeric vesicle membranes,[24] the lateral demixing of lipids from copolymers in hybrid vesicle membranes is yet to be reported. To induce membrane heterogeneities in HLP-GUVs, it is necessary to introduce an external driving force that can perturb the system from its initial equilibrium mixed state into a regime where compositionally distinct, coexisting domains are favored. Our chosen scheme to disturb the mixing behavior in the system was to biotinylate either the lipid or the copolymer species and then cross link these molecules[25,26] by the addition of the receptor protein NeutrAvidin (NA).[15]

Approximately 5 mol % biotinyl phospholipid (DSPE-PEG2000-biotin) was added to the lipid−copolymer mixture for vesicle preparation. The inclusion of the biotinyl lipids in the mixture did not alter the observed even distribution of fluorescently labeled lipids within the vesicle membranes, suggesting that the inclusion of the biotin-functionalized lipids alone does not cause the membrane to demix. These vesicles were incubated in an isoosmolar solution of rhodamine-labeled NeurtrAvidin (Rh-NA) such that vesicle morphology changes would not be induced by changes in the vesicle-encapsulated volume caused by creating an osmotic pressure gradient across the membrane. We found that Rh-NA binding to the biotinyl lipids induces lateral segregation within the vesicle membranes of the hybrid lipopolymersomes. Figure 3c−j demonstrates the evolution of membrane surface morphology with time. After 1 h, uniform fluorescence from Rh-NA was observed on the vesicle surface. This homogeneous membrane demixed over time, resulting in the morphology of many small spotlike clusters of Rh-NA bound to the biotinylated lipids. It should also be noted here that the PEG-2000 spacer (45 units) in

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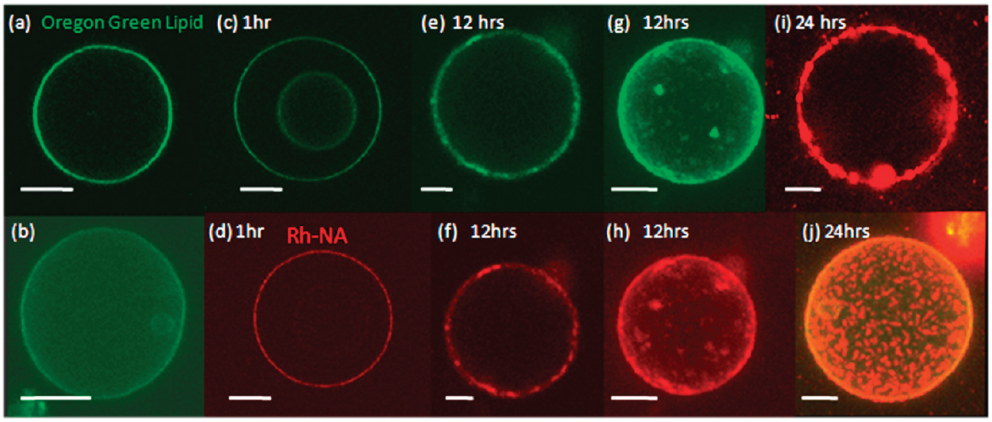

Figure 3. Confocal microscopy images of hybrid lipopolymersomes at different neutravidin incubation times. It should be noted that these images are of typical vesicles at each incubation time and that this does not show the same vesicle at each time point. (a) Single image slice through the vesicle equator and (b) reconstructed image of the vesicle hemisphere from confocal image sections. Uniform intensity from the Rh-NA is observed at the edge of the vesicle membrane after 1 h of incubation in neutravidin solution: (c) a homogeneous membrane composed of 5 mol % DSPE-PEG-biotin, 25 mol % POPC, PBd-*b*-PEO, and 0.5 mol % Oregon Green DHPE (green); here, one giant vesicle has formed inside another; (d) the same vesicles as in panel c showing Rh-NA (red) bound to the hybrid membrane. The inner vesicle cannot be observed because Rh-NA cannot cross the membrane of the outer vesicle. (e−h) After 12 h, small, red, spot domains of Rh-NA were observed in the membrane: (e) a single image section in a green (Oregon Green DHPE) channel, (f) an image section in a red (Rh-NA) channel, (g) 3D reconstruction of the vesicle hemisphere (green channel), and (h) 3D reconstruction of the vesicle hemisphere (red channel). After 24 h (i, j) many red spot domains are observed: (i) a single confocal section in the red channel and (h) a 3D reconstruction of the vesicle hemisphere (superposition of red and green channels). The scale bar represents 5 μm.

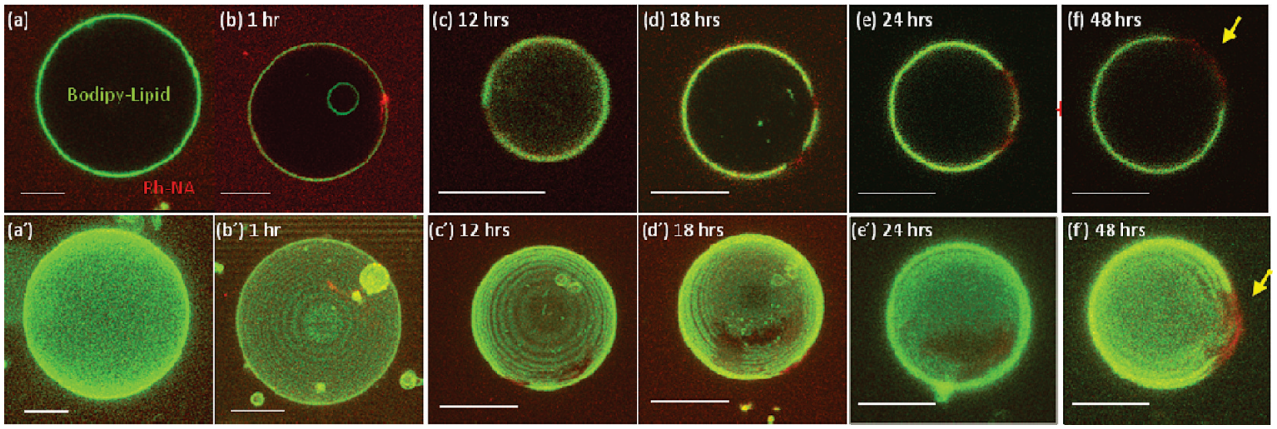

Figure 4. Confocal microscopy images of Rh-NA (red) bound to biotinyl PBd-*b*-PEO (30 mol %)/POPC hybrid vesicles labeled with BODIFY-DHPE (green) with different incubation times: (a−f) single image sections through the vesicle equator (superposition of red and green channels) and (a′−f′) 3D reconstruction of vesicle hemispheres. Arrows indicate large Rh-NA-rich domains with irregular curvatures that deviate from the native spherical curvature of the vesicle. The scale bar represents 10 μm.

the biotinylated lipid was essential to the extension of the biotin ligand from the membrane surface for Rh-NA binding; when biotinylated lipid without the spacer group was used, such as DOPE-biotin (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-biotinyl), no Rh-NA binding to the membrane was detected. This is likely due to the steric hindrance of the large hydrophilic PEG blocks (30 units) of the copolymers that create a polymer brush layer, burying the biotins and thereby preventing the receptor protein (Rh-NA) from accessing these ligands. This also provides strong evidence that the lipids and copolymers are initially well-mixed on the nanoscopic scale of the protein.

Significantly different resultant domain morphology was generated with the biotinylation of the block copolymer instead of the lipid. Figure 4 reveals the morphology change of HLP-GUVs where PBd-*b*-PEO is biotinylated and Rh-NA had been added to cross link the biotinylated copolymers. It can be seen that the NA-linked copolymers ripen into one large domain within the GUV matrix. These domains are also observed to deform the vesicle in these regions from its native spherical morphology (Figure 4e,f,l). This is plausibly due to the enhanced stiffness of the protein cross-linked, copolymer-rich membrane domain.

It should be noted that unitary polymersomes (or liposomes) containing biotinylated copolymers (lipids) do not exhibit domain formation when treated with Rh-NA under our experimental conditions: the vesicles exhibit uniform Rh-NA fluorescence over the entire membrane. Therefore, the domain formation observed in the hybrid system is not solely driven by preferential, lateral protein−protein interactions on the membrane surface but is also driven by lipid−copolymer interactions within the hybrid membrane itself. Therefore, the NA acts as an isothermal trigger that tips the free-energy landscape of the hybrid vesicles into a state that favors demixing to create heterogeneous surface textures of compositionally distinct domains. This response of the hybrid lipopolymersomes to external stimuli might therefore be utilized

in applications that require capsules with environmentally sensitive features (e.g., in vivo therapeutic delivery or environmental detection assays).

It is of interest to consider the possible molecular-level structures of these heterogeneously mixed membranes because there is a noteworthy mismatch between the thicknesses of unitary lipid (4 to 5 nm) and block copolymer (~8 nm) bilayers. Indeed, energetic constraints in accommodating these mismatches may, at least in part, be responsible for our findings that either no GUVs or GUVs with significantly heterogeneous compositions formed in some regions of compositional space (Figure 1e,f). The mismatch in thickness between lipid-rich and copolymer-rich domains in hybrid membranes could lead to a large energy cost at the domain boundaries as a result of water molecules potentially being able to access hydrophobic residues of the thicker copolymer-rich membrane. This energetically unfavorable configuration is plausibly avoided by having a mixed interfacial region at the boundaries of the domains that allows a more gradual change in membrane thickness. Such a boundary region would maintain the isolation of hydrophobic residues from directly contacting the aqueous phase. Speculatively, this interfacial zone between domains may also involve some interdigitation of block copolymer species in attaining a thinner membrane structure.

The difference in domain morphology between NA cross linking of lipids and that of copolymers could result from differences in the dynamics within lipid-rich and polymer-rich membranes. Copolymer diffusion in polymersome membranes is at least an order of magnitude slower than the diffusion of lipids in fluid liposome membranes (Figure 1e). Therefore, polymer-rich domains are likely to diffuse more rapidly through a lipid-rich membrane matrix than are lipid-rich domains within a polymer-rich matrix because domain mobility is dependent upon the viscosity of the continuous phase. Therefore, it would be quicker and easier for the polymer-rich domains to interact and coalesce and thus ripen into a single large domain as shown in Figure 4. Conversely, the slower dynamics of the viscous, entangled matrix of polymer-rich membranes could retard the dynamics of lipid-rich membrane inclusions as these domains grow. This could result in the dynamic arrest of size-limited domains within the polymer-rich membrane, jamming the system in a state that prevents further domain coalescence, as seen in Figure 3. Indeed, the small lipid-rich domains in Figure 3 were observed to be static on observational timescales, consistent with our proposed explanation for the differences in resultant morphologies.

Differences in interactions between domains in these two systems may also play a role in the morphological differences reported. Theoretical models have shown that interactions between inclusions (e.g., domains) in membranes can be attractive or repulsive depending upon factors such as the elastic properties of the membrane and its inclusions.[11] Lipid-rich domains likely represent comparatively flexible, thin inclusions within a thicker, more rigid copolymer-rich matrix whereas the converse system likely yields thicker, more rigid copolymer-rich inclusions residing in a thinner, more flexible lipid-rich matrix. These contrasting differences in elastic deformations within the membranes of these two systems may lead to attractive interactions between inclusions that encourage ripening into a single, large domain (e.g., copolymer-rich domains) or repulsive interactions between inclusions that frustrate domain ripening by coalescence, leading to many small (meta)stable domains (e.g., lipid-rich domains).

Conclusions

We have presented a novel membrane platform by fabricating hybrid lipid−diblock copolymer giant unilamellar vesicles. We have shown that within the permitted windows for hybrid lipopolymersome GUV formation these lipopolymersomes form homogeneous, well-mixed membranes and have characterized the lateral diffusivity and mechanical properties of these hybrid membranes. We have further demonstrated that it is possible to control the formation of compositional heterogeneities within these membranes by binding neutravidin to either biotinylated lipids or copolymers. The resultant morphologies are dependent on which species carries the biotin functionality. These novel hybrid capsules could find application in technologies where it would be advantageous to combine the structural stability and mechanical strength of polymer membranes with the biocompatibility and biofunctionality of lipid membranes (e.g., drug delivery, controlled release, and environmental detection assays). These systems may also provide a useful platform in the emerging field of nanotoxicology in elucidating details of the interactions of natural lipids with synthetic block copolymers that might point to potential systemic pathogenesis from the long-term buildup of synthetic block copolymers in the body from use in biomedical therapies.

Supporting Information Available: Micropipet aspiration and FRAP data. This material is available free of charge via the Internet at http://pubs.acs.org.