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Interface-Mediation of Lipid Bilayer Organization and Dynamics

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Abstract

We report on the morphology and dynamics of planar supported lipid bilayer structures as a function of pH and ionic strength of the aqueous overlayer. Supported lipid bilayers composed of three components (phosphocholine, sphingomyelin and cholesterol) are known to exhibit phase segregation, with the characteristic domain sizes dependent on the amount and identity of each constituent, and the composition of the aqueous overlayer in contact with the bilayer. We report on fluorescence anisotropy decay imaging measurements of a rhodamine chromophore tethered to the headgroup of a phosphoethanolamine, where anisotropy decay images were acquired as a function of solution overlayer pH and ionic strength. The data reveal a two-component anisotropy decay under all conditions, with the faster time constant being largely independent of pH and ionic strength and the slower component depending on pH and ionic strength in different manners. For liposomes of the same composition, a single exponential anisotropy decay was seen. We interpret this difference in terms of bilayer curvature and support surface-bilayer interactions, and the pH and ionic strength dependencies in terms of ionic screening and protonation in the bilayer headgroup region.

TOC image

Keywords

Supported lipid bilayer; fluorescence anisotropy decay imaging; pH; ionic strength

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Introduction

Chemical sensing requires two distinct functions; molecular selectivity and signal transduction. Achieving chemical selectivity is a substantial challenge that has been mastered to a significant extent by living biological systems, and the transduction of a chemical signal into one that is quantifiable (*e.g.* photons or electrons) is comparatively well established.^{1, 2} The components that perform these two functions are connected directly, with the transducer typically functioning as the support or mount for the chemically selective component.

The use of biomolecules as selective entities is appealing based on the known chemical selectivity of transmembrane proteins and enzymes. The ability of transmembrane proteins to function is mediated by the composition and morphology of the plasma membrane surrounding them, for reasons of protein folding and mobility, and the replication of such a system in the laboratory is not a simple task. The mammalian plasma membrane is known to contain in excess of 500 unique molecular constituents,³ and there is a limited amount of detailed information available on the fluidity or spatial heterogeneity of the plasma membrane. It is believed, however, that the plasma membrane constituents form “lipid raft” structures, which are typically considered to be local phase segregated regions within the bilayer. One approach to developing biosensors that use transmembrane proteins is to design synthetic bilayers containing a limited number of components,^{4, 5} but a significant limitation to this approach lies in the ill-understood relationship between bilayer composition and protein folding. We have undertaken a series of studies of model bilayer structures with an eye toward understanding the relationship between composition and environmental factors, and the observed phase-segregated organization of this class of model systems.^{6–18} In this work we focus on model lipid bilayer structures covered by an aqueous overlayer and supported on a planar mica surface.

In this structural format, supported lipid bilayers exhibit phase segregation, akin to lipid raft structures,^{4, 5, 14–16, 19, 20} with cholesterol-rich regions separating from phosphocholine-rich regions. The characteristic sizes and shapes of these cholesterol-rich domains are determined by the minimization of the sum of interfacial energies within the system. For a simple two-component system one would expect a single phase boundary, however this is not the case for supported bilayers. The lowest energy structure is determined from the energies of interaction between the support and the aqueous overlayer, with each bilayer phase and the phase boundaries in the bilayer plane.^{19, 20} We can thus exert some control over the domain size of the cholesterol-rich regions of our supported bilayers through changes in the composition of the aqueous overlayer (*e.g.* ionic strength and pH).

Controlling model bilayer phase segregation is an important first step in establishing control over the folding of transmembrane proteins, but it is not clear from data showing mesoscopic phase segregation how the fluidity and local dynamics of the bilayer constituents are influenced by changes in the bilayer morphology. Consequently, we incorporate a small amount (*ca.* 1 mol%) of a tethered fluorescent probe into these bilayer structures and monitor rotational diffusion dynamics to gauge how the local environment of the chromophore is affected by variations in the aqueous overlayer pH and ionic strength. A

rhodamine derivative that is bound covalently to the headgroup of phosphoethanolamine is used as the chromophore. The tethered chromophore exhibits dynamics that can be modeled in the context of a hindered rotor, a well understood model.^{21, 22} Our data point to the dynamics of the rhodamine chromophore sensing two distinct environments within the supported bilayer, which depend differently on the pH and ionic strength of the aqueous overlayer. The sub-population of the tethered chromophore that exhibits pH and ionic strength dependencies appears to reside in an environment characterized by stronger interactions with the chromophore compared to the sub-population in a pH and ionic strength-independent environment. We consider the chemical basis for this behavior.

Experimental Section

Chemicals

1,2-Dioleoyl-*sn*-phosphatidylcholine (DOPC), cholesterol (ovine wool), sphingomyelin (chicken egg) and sulforhodamine tagged 1,2-dioleoyl-*sn*-phosphatidyl-ethanolamine (SR-DOPE), dissolved in chloroform were purchased from Avanti Polar Lipids and used as received. Trizma[®] Tris buffer (Sigma Aldrich), NaOH (Spectrum, Pellets, Reagent, ACS), CaCl₂ (Purified Grade, Anhydrous), HCl (ACS/Electronic Grade), NaCl (ACS Grade, Crystals), were purchased from CCI and used without further purification. High-grade muscovite mica used as the support was purchased from Ted Pella, Inc.

Fluorescence Anisotropy Decay Imaging (FADI)

An instrument based on an inverted microscope (Nikon Eclipse Ti-U) is used to obtain fluorescence anisotropy decay images of the planar bilayers.^{11, 12} Detection of time-resolved data is achieved with a polarized dual channel confocal scanning instrument (Becker & Hickl DCS-120) attached to a microscope port and controlled by a galvo-drive unit (Becker & Hickl GDA-120). The confocal scanner is equipped with a polarizing beam splitter and two avalanche photodiode detectors (ID-Quantique ID100) for the acquisition of fluorescence lifetime and anisotropy decay images. Polarized fluorescence transients used in the generation of fluorescence lifetime and anisotropy images are acquired using time-correlated single photon counting detection electronics (Becker & Hickl SPC-152, PHD-400N reference diode). The time-correlated single photon counting (TCSPC) and confocal scanning instruments are controlled by commercial software (Becker & Hickl) run on a windows-based PC.

The light source for this instrument is a synchronously pumped, cavity-dumped dye laser (Coherent 702-2) excited using a passively-mode locked Nd:YVO₄ laser (Spectra Physics Vanguard). The output of the excitation laser is a train of 13 ps pulses at a repetition rate of 80 MHz. The second harmonic and third harmonic outputs of this laser (532 nm, 355 nm) are used to pump the dye lasers. In this work the dye laser was excited with the second harmonic output (2.5 W average power) and Pyrromethene 567 (Exciton) was used as the laser dye. The dye laser repetition rate, determined by the cavity dumping electronics, is 4 MHz (250 ns between pulses), with 5 ps pulses at 563 nm.

Time-Resolved Fluorescence Measurements

The system used to acquire anisotropy decay data on vesicles suspended in solution has been described elsewhere and we provide only a brief overview here.²³ The light source used for this instrument is identical to that used for the TCSPC imaging measurements (*vide supra*). Emission collection was accomplished using a 40× reflecting microscope objective (Ealing). Emission polarization was selected using a polarizing cube beamsplitter (Newport) with separate detection channels for vertical and horizontal polarizations. Spectral selection was performed using subtractive double monochromators (Spectral Products CM112) equipped with microchannel plate PMT detectors (Hamamatsu R3809U-50). A photodiode (Becker & Hickl PHD-400) was the reference channel detector. Commercial TCSPC electronics (Becker & Hickl SPC-132) were used to process the detector signals, and the data was acquired using software written using LabVIEW®. Data processing is the same as described previously.²³

Bilayer Preparation

The vesicles used in these experiments were formed from a mixture of the constituent species; 49 mol% DOPC, 40 mol% sphingomyelin, 10 mol% cholesterol, and 1 mol% SR-DOPE. After the chloroform was removed by evaporation, Milli-Q H₂O was added to make the overall lipid concentration 1 mg/mL. The vial was placed in a 50 °C water bath for 30 minutes, vortexed for 2 minutes, and sonicated for 30 minutes at 50 °C.²⁴ The resulting vesicles were deposited onto mica, and Trizma® buffer solution (10 mM, pH 7.5, 100 mM NaCl) and CaCl₂ (2 mM aqueous solution) were added. After ten minutes of exposure time the bilayers formed by vesicle fusion were rinsed with the intended aqueous overlayer: pH between 4 and 10 at ionic strength $I = 0.05$, or ionic strength in the range of 0.05 to 0.4 at pH 7. Suspended vesicles used for the TCSPC measurements are the same as those used for deposition of the planar supported bilayers. The vesicles are suspended in solutions with the same ranges of pH and ionic strength as the planar bilayers.

FADI Data Collection

For each pH and ionic strength studied, a total of 3 planar bilayers were prepared. Two measurements were performed on each sample, for a total of 6 measurements. A 40× objective was used data collection. For this optical configuration the depth of focus is on the order of 1 μm. The depth resolution of the measurement is limited by the thickness of the bilayer, which is *ca.* 6 nm for the bilayers studied here. In-plane resolution of the acquired image is 256 × 256 pixels, with the physical dimension subtended by each pixel being determined by the objective used. For the 40× objective, each pixel corresponded to a 2.5 μm diameter area on the sample, *ca.* ten times the diffraction limit. For each pixel, two TCSPC channels of data are acquired, one for vertical polarization and one for horizontal polarization. Each channel has 256 time resolution elements. For a time window of 10 ns, data points were separated by 39 ps. A frame scan was used to produce an image of a defined area. These measurements used 60 second frame acquisition times and 30 frames were collected.

Data Analysis

Fluorescence lifetime and anisotropy decay images were obtained and processed using commercial software (Optispec, Becker & Hickl). The anisotropy decay data and images were prepared using SpcImage software (Becker & Hickl). From each of the six measurements, three 25×25 pixel squares of data were extracted, for a total of 18 sets of data for each pH and ionic strength. Transients of a given polarization in the extracted region were summed and used to create the induced orientational anisotropy function, $R(t)$. Regression of the anisotropy decay data was performed using Microcal Origin[®] v 9.0.

Results and Discussion

The morphological changes in supported bilayers that occur with changes in overlayer pH and ionic strength are expected based on the minimization of the overall free energy of the system.^{4, 5} While the thermodynamic treatment of bilayers provides the fundamental energetic basis for phase segregation, such information does not generally provide molecular-scale insight into the structural properties system, including intermolecular interactions that ultimately determine the details of system organization. Relating changes in the observed dynamics on molecular length scales to macroscopic system morphology remains to be achieved, and the data presented here will hopefully provide some useful information for making that connection.

We are concerned in the long-term with how changes in the aqueous overlayer of the lipid bilayer give rise to observed changes in the morphology and dynamics of the phase-segregated, cholesterol-rich regions of the supported bilayer structures. These phase-segregated regions are seen only when sphingomyelin is present in the bilayer.^{5, 11, 12} It is possible, however, that phase-segregated cholesterol-rich regions are present in the absence of sphingomyelin but their characteristic domain size is smaller than can be resolved by optical microscopy (*ca.* 250 nm). This is an issue we intend to address in future work by comparing rotational and translational diffusion data in these systems.

In this work we focus on understanding the rotational diffusion dynamics of a headgroup-tethered chromophore present in a supported lipid bilayer (Fig. 1) under conditions where the aqueous overlayer pH and ionic strength are varied in a systematic manner. It is seen that the morphology of these systems depends on these overlayer properties (Fig. 2). For these measurements, the motion of the tethered rhodamine is treated in the context of the hindered rotor model by virtue of its structure and manner of incorporation into the bilayer.^{21, 22, 25–29} In this model, the chromophore is considered to move with a restricted conic volume, and while executing this precessional motion it “wobbles” about its tethering bond. We collect polarized emission transients $I_{||}(t)$ and $I_{\perp}(t)$ and combine them to create the induced orientational anisotropy decay function, $R(t)$ (Eq. 1).

$$R(t) = \frac{I_{||}(t) - I_{\perp}(t)}{I_{||}(t) + 2I_{\perp}(t)} \quad (1)$$

And $R(t)$ is related to the motion and extent of confinement of the chromophore, (Eq. 2).

$$\begin{aligned}
 R(t) &= R(\infty) + (R(0) - R(\infty)) \exp(-t/\tau_{HR}) \\
 \cos\theta_0 &= 0.5 \left(8 \left(\frac{R(\infty)}{R(0)} \right)^{1/2} + 1 \right)^{1/2} - 0.5 \\
 \tau_{HR} &= \frac{7\theta_0^2}{24D_w}
 \end{aligned}
 \tag{2}$$

Where $R(0)$ is the zero-time anisotropy, related to the angle between the excited and emitting transition dipole moments, $R(\infty)$ is the infinite time anisotropy, which is determined by the extent to which the chromophore motion is restricted by its local environment, θ_0 is the cone semi-angle in which the chromophore is able to reorient and D_w is the “wobbling” diffusion constant, which describes the motion of the chromophore about its tethering bond.^{21, 22} In this model, the anisotropy decays as a single exponential. For free rotors, the anisotropy decay can exhibit up to five exponential decay components, depending on the axes of excitation and emission and on the shape of the volume swept out by the rotating species.³⁰ The observation of a multi-component decay in a homogeneous medium is not consistent with the hindered rotor model, owing to the tethering of the chromophore precluding facile rotational motion about more than the tethering axis. For the planar supported bilayers considered here we observe a two-component anisotropy decay and we assert that this two-component decay reflects two distinct chromophore populations that do not exchange population on the time scale of the measurement. For a heterogeneous system this is feasible and we note that the heterogeneity is not resolvable optically using our anisotropy imaging instrumentation.

While the pH and ionic strength dependencies of the tethered chromophore anisotropy decay dynamics are different, and their functional forms are expected (*vide infra*). It is important to consider that for both data sets the fast anisotropy decay component (*ca.* 300 ps) is consistent with the chromophore experiencing an environment that is slightly more resistive to rotational motion than water.³¹ As would be expected if this is the case, changes in the overlayer pH and/or ionic strength should give rise to only small changes in the fast decay time component. The slow decay component, however, requires further consideration. The first issue is that the slow decay time constant is consistent with an environment that is much more viscous than water. Such data suggest that the chromophore population exhibiting a long anisotropy decay time does so because of interactions with the polar glycerol region of the lipid bilayer structure. While the nonpolar acyl chain region may well exhibit high local viscosity due to chain-chain interactions, the polarity of the rhodamine chromophore precludes significant direct interaction with this region of the bilayer.

To interpret the anisotropy decay imaging (FADI) data, determining the confining cone angle is essential, requiring the quantitative measurement of $R(0)$ and $R(\infty)$ (Eq. 2). The imaging instrument used to acquire the FADI data is optimized for spatially resolved data acquisition, but not for the quantitation of polarized emission transient absolute intensities. To evaluate the zero- and infinite-time anisotropies for our bilayer structures, we have constructed the same bilayer systems as an aqueous suspension of 50 – 100 nm diameter vesicles.²⁴ In this structural motif, we acquire anisotropy decay data using a TCSPC instrument capable of quantitating the relative intensities of polarized emission transients.²³

We extract $R(0)$ and $R(\infty)$ from these experimental data and apply those results to the analysis of $R(t)$ for the planar supported bilayers.

From these $R(0)$ and $R(\infty)$ data for vesicles we extract a single anisotropy decay time constant, in contrast to the observed two component decay for planar bilayers, and calculate a cone angle (Eq. 2) of $\theta_0 \sim 90^\circ$ for all ionic strength and pH measurements. The implications of these results are significant and can be understood as follows. For the planar bilayers, the fast anisotropy decay data we expect the bilayer structure to exhibit a random orientational distribution of local environments, giving rise to $R(\infty) \sim 0.08$, and for the slow anisotropy decay data, where the chromophore interacts strongly with the bilayer headgroup, we could reasonably expect $R(0) \sim 0$, corresponding to $\theta_0 \sim 90^\circ$. For such a two-population system, we would expect to recover a value of $R(\infty)$ that is the weighted average of that for the two distributions, and given the S/N ratio of our $R(t)$ data, it is not likely that we could distinguish such an average value from its component values. The $R(\infty)$ data acquired for vesicles are thus consistent with our observation of a two-component population distribution where population exchange between the two components is facile (*vide infra*). Under the assumption that θ_0 for the fast decay component is *ca.* 55° and for the slow decay component θ_0 is 90° , we can evaluate the fractional contribution of each component to the experimental data for the vesicles. For the planar bilayers we extract values for the wobbling diffusion constant for each population. This information provides insight into the local environment(s) as functions of pH (Table 1, Fig. 3a) and ionic strength (Table 2, Fig. 4a). Using these values for D_w , we take the vesicle decay time constant (Tables 3 and 4) as the weighted average of the time constants for each component, and thus determine the fractional contribution of each (Fig. 5). We consider these results in detail below.

It is important to note that we extracted $R(\infty)$ data from vesicles of the same composition as those used for the creation of the supported bilayer, but with a characteristic diameter of *ca.* 50 nm. For systems characterized by significant curvature, it is reasonable to expect morphology that is similar to that seen for a planar bilayer, but characterized by more facile exchange dynamics because of the structural freedom available to constituents of the bilayer leaflets.³² This expectation is realized for the system examined here. For the planar bilayer structure we are able to resolve discrete populations of chromophore that do not exchange on the timescale of the chromophore motion, while for vesicles we observe a single exponential decay time constant that is the weighted average of the two time constants seen for the planar bilayer. It is useful to compare the fraction of each chromophore population for the vesicles to the corresponding data for the supported planar bilayer. As noted above, we use the fast and slow time constants from the supported planar bilayer to extract the fractional contribution of each to the weighted average for the vesicles. The relative fraction of the fast and slow chromophore populations depends differently on pH (Fig. 3b, Table 1) and ionic strength (Fig. 4b, Table 2) for the planar supported bilayers and vesicles (Fig. 5, Tables 3 and 4). The different dependencies provide some insight into the factors at play in mediating bilayer organization. For the planar supported bilayers, the diffusion constants depend on pH and ionic strength, but the fractional contributions of the two chromophore populations do not change with either pH or ionic strength (Figs. 3 and 4), within the uncertainty of the measurements. This result stands in contrast to the data for vesicles (Fig. 5), where the fractional contributions of the two chromophore populations change with both ionic strength

and pH. One structural difference between planar supported bilayers and vesicles is that the vesicle bilayer exhibits significant curvature, as noted above, which plays some role in mediating the facile exchange between the two chromophore populations.³³ Another structural difference has to do with pH and ionic strength gradients that can exist across the bilayer for the two structural motifs. For the planar supported bilayer, the (silica) surface on which the bilayer is supported is negatively charged and for the vesicle, the solution contained within it is nominally the same as that in which the vesicles reside. Thus there is not an environmental gradient across the bilayer comprising the vesicles, whereas there is an environmental gradient for planar bilayer layers. The (weak) adhesion to the support and the existence of a charge gradient across the bilayer could both serve to minimize lateral mobility within the bilayer. Indeed, this situation is not without parallel in mammalian plasma membranes, where membrane stiffness is influenced by the cytoskeleton.^{34–37} For the vesicles, where there is neither a charge or mobility gradient across the bilayer, there are no analogous impediments to molecular motion in the plane of the bilayer. As such, translational diffusion within the vesicle bilayer is more facile, leading to the observed single exponential anisotropy decay. As the bilayer headgroups are screened by increasing ionic charge, or their extent of protonation varies, it would be reasonable to expect changes in the fractional contribution of the fast and slow processes, as is seen experimentally. We consider next the basis for the pH and ionic-strength dependence of the anisotropy decay data.

The pH-dependent data were acquired at a fixed overlayer ionic strength of $I = 0.05$ (Table 1). The data show that the slow anisotropy decay time constant reaches a maximum of 2.8 ns in the pH 7–8 range and then decreases to 1.6 ns at pH 4 and to 2.1 ns at pH 10. We believe that the pH dependence is associated with changes in the bilayer headgroup local environment rather than any change in the chromophore; no pH-dependent spectral changes are seen for the rhodamine chromophore over this pH range (Fig. 6). The three species contained in the bilayer are phosphocholine, sphingomyelin, and cholesterol, each of which has functionalities that undergo protonation/deprotonation equilibria. We can understand possible reasons for these effects based on the chemical structures of the bilayer constituents (Fig. 1). At low pH the phosphocholine headgroup, which is typically depicted as zwitterionic, is expected to contain a protonated phosphate functionality and thus be cationic. Cholesterol has a $pK_a > 15$,³⁸ and for our experimental conditions is not expected to exhibit any change of protonation. Sphingomyelin contains alcohol and amide protons, with pK_a s in the region of 18 and 26, respectively.¹⁸ As a consequence, these labile protons are not expected to play a significant role in the pH dependence we observe. However, the sphingomyelin phosphate functionality is expected to participate in acid-base equilibria analogous to that for phosphocholine over the pH range of our experiments. For all pH values examined here, the phosphocholine and sphingomyelin phosphate moieties thus mediate the pH-dependent organization of the system. Near pH 7 the zwitterionic nature of the lipid headgroups can exhibit significant intermolecular charge-based interactions, leading to changes in the ability of the chromophore to execute rotational motion. For low pH, the phosphate head groups are protonated, giving rise to charge repulsion between the phosphate headgroups, and consequently greater freedom of motion for the chromophore. For high pH conditions, sufficient OH^- will be present in solution to associate with choline

headgroup moieties, leaving anionic repulsion between the phosphate headgroup functionalities to produce a result at least somewhat analogous to that seen for low pH conditions. We expect the change in extent of protonation in the bilayer constituents will alter the (cholesterol) domain sizes in the supported bilayers, and this situation is realized experimentally (Fig. 2). There are other factors that can and do affect the domain structure of these films, and we consider some of them below.

The dependence of the chromophore anisotropy decay time on overlayer ionic strength is different than the pH-dependence. The dependence on ionic strength is monotonic, with the slow anisotropy decay time constant ranging from 2.75 ns at $I = 0.05$ to 1.4 ns at $I = 0.4$. We attribute this trend to the increasing extent of charge compensation and screening in the bilayer with increasing ionic strength, leading to a reduction in the overall energy of intermolecular interactions between bilayer constituents and thus an environment that provides progressively less constraint on the motional freedom of the tethered chromophore. We note that, as with the pH-dependence, changes in ionic strength are expected to alter the organization of the bilayer because of the effect on intermolecular interactions within the bilayer headgroup region.

The above discussion focuses on protonation/deprotonation and charge compensation/screening of the phospholipid species in the bilayer. The rhodamine chromophore is cationic and it is tethered to a phospholipid by means of an amino functionality, which can participate in protonation/deprotonation equilibria. Thus the probe is potentially involved in at least some of the changes associated with pH and ionic strength. We have found no evidence for dissociation of the probe from its tethering phosphoethanolamine moiety, however, and as noted above, the chromophore exhibits no spectral changes over the pH region of 4–10. Whatever roles the chromophore charge or tethering bond play in the observed dynamics do not appear to play a significant role in the changes we observe.

As noted above, we observe both microscopic and macroscopic changes in the morphology of our phase-segregated supported bilayers as a function of pH and ionic strength. For the planar supported bilayers, there are nine distinct interactions that play a role in mediating bilayer morphology; cholesterol-mica, phosphocholine-mica, sphingomyelin-mica, cholesterol-phosphocholine, cholesterol-sphingomyelin, phosphocholine-sphingomyelin, cholesterol-overlayer, sphingomyelin-overlayer, and phosphocholine-overlayer. While it is not feasible to distinguish the contributions of each interaction given the data we report here, the dominant changes in interactions we probe for the planar supported bilayer are those between the aqueous overlayer and the bilayer constituents. Differences in our results for the unilamellar vesicles and planar supported bilayers reflect the absence or presence of interactions between the bilayer constituents and the mica support. The issue of how the change in overlayer properties (pH and ionic strength) influences the mica support remains unresolved because the permeability of the bilayer has not been evaluated. If the time-resolved spectroscopic results for bilayers supported on ITO are similar to those reported here, it may be possible to gauge the permeability of the bilayer through electrochemical studies. Such studies could not only address the permeability of the bilayers but also could assist in understanding the effect of potential gradient across the bilayer through a series of measurements at controlled support potentials.

Conclusions

Synthetic supported bilayer structures are important to a number of applications ranging from the creation of biomimetic interfaces to the hosting of transmembrane proteins for chemical sensing applications. Understanding the relationship between supported bilayer morphology and its environment is a key issue in determining the utility of any specific system. The morphology of such supported bilayers represents the global minimization of the interactions between the bilayer constituents, the support and the overlayer in contact with the bilayer. While this is true for both planar supported bilayers and for vesicles, there is a distinct difference in the mobility and dynamics of species incorporated into these two bilayer structures because of bilayer curvature and the inner leaflet being in contact with a liquid versus a solid. By varying overlayer pH and ionic strength, we can exert systematic change on three of the nine interfaces in our ternary supported planar bilayers. This systematic control gives rise to rigidity of the bilayer with increasing pH and decreasing ionic strength. Using a headgroup-tethered chromophore to probe the interface with the aqueous overlayer, we have found that its anisotropy decay dynamics are sensitive to the overlayer properties. We understand these effects in the context of protonation/deprotonation equilibria or charge screening effects, providing us with a facile means of controlling bilayer morphology. Future experiments will focus on controlling the potential gradient across the bilayer through control over support surface charge and the electric double layer. These variables should provide additional control over bilayer properties.

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Figure 1.
Structures of the bilayer constituents used in this work. From left to right: Cholesterol, DOPC, sphingomyelin, SR-DOPE.

Figure 2. Fluorescence lifetime images for planar supported bilayers over the pH range of 4 to 10, as indicated. The scale bar in each image is 10 μm .

Figure 3.

(a). Wobbling diffusion constant, D_w , as a function of pH, for the two resolved chromophore populations in planar supported bilayers. (b) Fractional contribution of each anisotropy decay component.

Figure 4.

(a). Wobbling diffusion constant, D_w , as a function of ionic strength, for the two resolved chromophore populations in planar supported bilayers. (b) Fractional contribution of each anisotropy decay component.

Figure 5. Relative fractional contribution of each anisotropy decay component as a function of pH (a) and ionic strength (b) for vesicles.

Figure 6.
Normalized absorbance and emission spectra of SR-DOPE as a function of solution pH.
These data were acquired for solutions at the pH values indicated.

Table 1

Anisotropy decay time constant for SR-DOPE as a function of pH at constant ionic strength ($I = 0.05$) in planar bilayer structures. As discussed in the text, for the fast time constant, τ_1 , $\theta_0 = 55^\circ$ and for the slow time constant, τ_2 , $\theta_0 = 90^\circ$. Anisotropy decay data were best fit by a two-component exponential decay, $f(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$.

pH	$A_1 \pm 1\sigma$	$\tau_1 \pm 1\sigma$ (ps)	$D_w \pm 1\sigma$ (MHz)	$A_2 \pm 1\sigma$	$\tau_2 \pm 1\sigma$ (ps)	$D_w \pm 1\sigma$ (MHz)
4	0.035 ± 0.007 (43±9%)	300 ± 26	896 ± 78	0.051 ± 0.005 (57±9%)	1603 ± 160	449 ± 46
5	0.036 ± 0.006 (40±9%)	339 ± 20	793 ± 43	0.053 ± 0.011 (60±9%)	2022 ± 123	356 ± 21
6	0.021 ± 0.005 (36±11%)	262 ± 37	1026 ± 145	0.038 ± 0.010 (64±11%)	2344 ± 202	308 ± 27
7	0.053 ± 0.009 (45±9%)	378 ± 45	711 ± 85	0.065 ± 0.005 (55±9%)	2760 ± 157	260 ± 16
8	0.064 ± 0.017 (49±15%)	389 ± 55	691 ± 98	0.066 ± 0.011 (51±15%)	2836 ± 224	254 ± 19
9	0.065 ± 0.007 (45±6%)	302 ± 38	890 ± 112	0.081 ± 0.011 (55±6%)	2421 ± 170	297 ± 21
10	0.036 ± 0.009 (44±14%)	360 ± 37	747 ± 77	0.046 ± 0.012 (56±14%)	2107 ± 137	343 ± 21

Anisotropy decay time constant for SR-DOPE as a function of ionic strength at constant pH (pH = 7) in planar bilayer structures. As discussed in the text, for the fast time constant, τ_1 , $\theta_0 = 55^\circ$ and for the slow time constant, τ_2 , $\theta_0 = 90^\circ$. Anisotropy decay data were best fit by a two-component exponential decay, $f(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$.

Table 2

Ionic Strength	$A_1 \pm 1\sigma$	$\tau_1 \pm 1\sigma$ (ps)	$D_w \pm 1\sigma$ (MHz)	$A_2 \pm 1\sigma$	$\tau_2 \pm 1\sigma$ (ps)	$D_w \pm 1\sigma$ (MHz)
0.05	0.063 ± 0.005 (77±9%)	323 ± 29	832 ± 75	0.019 ± 0.004 (23±9%)	2781 ± 164	260 ± 16
0.10	0.046 ± 0.011 (77±24%)	336 ± 53	800 ± 126	0.014 ± 0.004 (23±24%)	2528 ± 177	284 ± 19
0.20	0.040 ± 0.010 (73±23%)	254 ± 54	1058 ± 225	0.015 ± 0.003 (27±23%)	2191 ± 222	329 ± 32
0.30	0.041 ± 0.003 (68±8%)	242 ± 32	1111 ± 147	0.019 ± 0.004 (32±8%)	1709 ± 124	420 ± 29
0.40	0.045 ± 0.006 (66±13%)	209 ± 27	1286 ± 166	0.023 ± 0.007 (34±13%)	1407 ± 195	511 ± 70

Table 3

Anisotropy decay time constant for SR-DOPE as a function of pH at constant ionic strength ($I = 0.05$) in 50 nm diameter vesicles. Anisotropy decay data were best fit by a one-component exponential decay, $f(t) = A_1 \exp(-t/\tau_{HR})$.

pH	$\tau_{HR} \pm 1\sigma$ (ps)	X_{fast} (%)	X_{slow} (%)
4	1126 ± 114	37 ± 12	63 ± 12
5	1221 ± 47	48 ± 5	52 ± 5
6	1063 ± 18	62 ± 4	38 ± 4
7	1178 ± 64	66 ± 4	34 ± 4
8	1306 ± 68	63 ± 5	37 ± 5
9	1242 ± 49	56 ± 5	44 ± 5
10	1231 ± 66	50 ± 6	50 ± 6

Table 4

Anisotropy decay time constant for SR-DOPE as a function of ionic strength at constant pH (pH = 7) in vesicles. Anisotropy decay data were best fit by a one-component exponential decay, $f(t) = A_1 \exp(-t/\tau_{HR})$. Using (Eq. 2) the cone angle (θ_0) and diffusion constant (D_w) were calculated.

I	$\tau_{HR} \pm 1\sigma$ (ps)	X_{fast} (%)	X_{slow} (%)
0.05	1239 $\pm$ 37	63 $\pm$ 3	37 $\pm$ 3
0.1	1037 $\pm$ 47	68 $\pm$ 4	32 $\pm$ 4
0.2	1047 $\pm$ 89	59 $\pm$ 7	41 $\pm$ 7
0.3	1004 $\pm$ 14	48 $\pm$ 5	52 $\pm$ 5
0.4	1060 $\pm$ 83	29 $\pm$ 14	71 $\pm$ 14