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Single Molecule Probes of Lipid Membrane Structure

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Abstract

Biological membranes are highly heterogeneous structures that are thought to use this heterogeneity to organize and modify the function of membrane constituents. Probing membrane organization, structure, and changes therein are crucial for linking structural metrics with function in biological membranes. Here we report the use of single molecule fluorescence studies to measure membrane structure at the molecular level. Several groups have shown that polarized total internal reflection fluorescence microscopy (PTIRF-M) using p-polarized excitation can reveal single molecule orientations when spherical aberrations are introduced into the optics train. We use this approach to measure the orientation of fluorescent lipid analogs doped into Langmuir-Blodgett films of DPPC and arachidic acid. We compare two commonly used fluorescent lipid analogs, BODIPY-PC and DiIC₁₈ which have their fluorophores located in the tailgroup and headgroup, respectively. We find the tilt orientation of BODIPY-PC is very sensitive to the surface pressure at which DPPC films are transferred onto the substrate. At low surface pressures, the tailgroups are largely lying in the plane of the film and evolve to an orientation normal to the surface as pressure is increased. For DiIC₁₈ however, no evolution in orientation with surface pressure is observed which is consistent with the headgroup located fluorophore being less sensitive to changes in membrane packing. Single molecule orientation measurements of DiIC₁₈ in multilayer films of arachidic acid are also measured and compared with previous bulk measurements. Finally, single molecule measurements are utilized to reveal the ordering induced in DPPC monolayers following the addition of cholesterol.

Keywords

Single molecule; Langmuir Blodgett; DPPC; Orientation; Cholesterol

Introduction

Biological membranes form the defining structural features of cells that both compartmentalize organelles within the cell and provide a semi-permeable barrier separating cells from their surroundings. Increasingly, the compositional and structural complexity of membranes is slowly being revealed. For example, it now appears that thousands of different lipids are incorporated into lipid membranes, totaling approximately 5% of a cell's genes.¹ It is thought that this large lipid variety is used to fine tune membrane properties and function, but direct links are often lacking. Biological membranes also support a large degree of structural heterogeneity that may be used to control or influence specific functional roles. For instance, small cholesterol rich domains, termed lipid rafts, are thought to organize and segregate membrane constituents to modify their function.^{2–8} Biochemical studies first

suggested their existence, but direct physical measurements of lipid rafts have proven elusive, presumably because of their small size.

Because of the importance that membranes play in biological function, a host of techniques have been applied to probe the microscopic structure present in both natural and model membranes. Fluorescence microscopy and atomic force microscopy (AFM) have proven especially useful for understanding the phase partitioning in model membranes.^{9–15} Bulk lipid order and orientational information has been obtained by methods such as quasielastic neutron scattering (QENS)¹⁶, NMR relaxation studies^{17–20}, small angle x-ray diffraction²¹, and wide angle x-ray scattering.²² Structural information can also be obtained from optical techniques such as infrared (IR) spectroscopy^{23–25}, polarized total internal reflection fluorescence (PTIRF) microscopy^{26, 27}, variable acquisition angle polarized TIRF (VAATIRF)²⁸, and polarized epifluorescence (PEF).^{27, 28} These techniques, however, often average over large populations of molecules and therefore provide an ensemble view of membrane organization.

The recent advances in single molecule fluorescence detection provide new opportunities for probing membrane structure at the molecular level. Single molecule fluorescence detection is now well established using both near-field and far-field approaches.^{29–34} Moreover, these measurements have been extended to probe the three-dimensional orientation of fluorescent molecules doped into samples. For example, several groups have shown that polarized total internal reflection fluorescence microscopy (PTIRF-M) measurements with p-polarized excitation can probe single molecule orientation in a sample.^{26, 35–42} By defocusing the optics, distinct emission patterns are observed in the single molecule fluorescence image which reflect the orientation of the molecule in the sample. As has been shown, these emission patterns can be modeled to extract the orientation of the emission dipole moment. Here we show that this provides a powerful new approach for probing membrane structure.

Langmuir-Blodgett monolayers of dipalmitoylphosphatidylcholine (DPPC) and arachidic acid (AA) are doped with small amounts of commonly used fluorescent lipid analogs. Single molecule fluorescence images taken with slightly defocused PTIRF-M, reveal changes in the single molecule orientation as the surface pressure of the monolayer is changed. The reorientations of the reporter dye reflect the evolution in order within the film as the surface pressure is increased and demonstrates that this technique can be used to probe membrane structure at the molecular level. Having shown that single molecule emission patterns can track membrane order, similar measurements are made on DPPC monolayers incorporating cholesterol. The addition of cholesterol is known to lead to dramatic ordering in membranes^{43–45} which is reflected in the single molecule emission patterns. These measurements yield both the orientation and location of the dye molecules in the film which should be particularly informative in cellular applications. Unlike ensemble averaging techniques, the entire orientation distribution is measured in single molecule measurements which will be very useful for heterogeneous membrane systems. Finally, since only trace quantities of the reporter dye are incorporated into the membranes, this approach is less perturbative than conventional fluorescence approaches.

Materials and Methods

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Avanti Polar Lipids, Alabaster, AL), arachidic acid, and cholesterol (Sigma Aldrich, St Louis, MO) were obtained at >99% purity and used without further purification. The fluorescent lipid analogs 2-(5-butyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene-3-nonanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (β -C₄-BODIPY 500/510 C₉ HPC) (BODIPY-PC) (Invitrogen Corporation, Carlsbad, CA, B3794) and 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine

perchlorate (DiIC₁₈) (Invitrogen Corporation, Carlsbad, CA, D-282) were doped into lipid films at a concentration of approximately 1×10^{-8} mol%.

DPPC and arachidic acid were dissolved in chloroform (1 mg/ml), doped with an appropriate reporter dye, and dispersed on a subphase of ultra-pure water (18 M Ω) in a Langmuir-Blodgett trough (Type 611, Nima Technology, Coventry, England). Once dispersed on the subphase, the chloroform was allowed to evaporate for 15 minutes. DPPC monolayers were compressed at a speed of 100 cm²/min to approximately 42 mN/m and then expanded at a speed of 80 cm²/min. The compression cycles were repeated twice to anneal the monolayer. The monolayer film was then compressed to the target pressure and held there for approximately 15–20 minutes. The film was then transferred in a heads down geometry onto a Piranha cleaned glass substrate at a dipping speed of 25 mm/min. For DPPC films containing cholesterol, the appropriate amount of cholesterol (from a 1 mg/ml in chloroform stock solution) was added to the DPPC solution to make the appropriate concentration in mol%.

For arachidic acid films, glass slides were first treated in a 3% octatriethoxysilane (OTS) / toluene (v/v) solution for 6 hours and rinsed with water (18 M Ω) to produce a hydrophobic surface. The first monolayer of pure AA was transferred onto the OTS treated substrate with tailgroups down. A second layer of AA doped with $\sim 10^{-8}$ mol% DiIC₁₈ was then deposited on top to produce a Y-type film. All films were prepared at a temperature of $\sim 21^\circ\text{C}$.

The films were imaged using a total internal reflection fluorescence microscope (TIRF-M) (Olympus IX71, Center Valley, PA) equipped with a 100x objective (1.45 NA achromat). The 514 nm line from an argon ion laser (Coherent Innova 70 Spectrum, Santa Clara, CA) was directed through half-wave and quarter-wave plates (Newport, Irvine, CA) to generate p-polarized excitation. Excitation was directed through the objective and fluorescence collected in an epifluorescence geometry with the optics defocused ~ 500 nm. The fluorescence was filtered with a combination of a dichroic mirror and long pass filters (Chroma, Rockingham, VT) and imaged onto a CCD camera (Cascade 650, Roper Scientific, Tucson, AR). Image collection was controlled with Slidebook software (Version 4.2, Intelligent Imaging Innovations, Denver, CO) and analyzed using MatLab software (Natick, MA).

Results and Discussion

To explore the structural information available from single molecule orientation measurements, PTIRF-M studies on the fluorescent lipid analogs BODIPY-PC and DiIC₁₈ doped into Langmuir-Blodgett (LB) films were studied at a range of surface pressures. For these studies, lipid monolayers were formed from DPPC, arachidic acid (AA), and DPPC/cholesterol mixed monolayers. Figure 1 shows the structures of DPPC, AA, cholesterol, and the two fluorescent lipid analogs that were doped into the films. The two fluorescent lipid analogs shown in Fig. 1 were chosen for the placement of the fluorophore. For DPPC monolayers transferred onto glass with headgroups down, the BODIPY-PC probe with the fluorophore in the tailgroup is expected to be more sensitive to changes in membrane packing while the DiIC₁₈ with the fluorophore in the headgroup, should be less sensitive and will act as a control in these studies. Each of these fluorophores has an emission dipole aligned along the long axis of the chromophore as shown schematically in Fig. 1. For the BODIPY chromophore, anisotropy measurements suggest there is an angle of $\sim 13^\circ$ between the absorption and emission dipoles.⁴⁶

Figure 2 shows a pressure-area isotherm for DPPC with arrows denoting the surface pressures at which monolayers were transferred onto a glass substrate. The pressure-area

isotherm for DPPC shows the phase coexistence plateau region where liquid expanded (LE) and liquid condensed (LC) phases coexist. Previous studies have shown the propensity of fluorescent lipid analogs to partition into the more expanded phase in these films. Also shown in Fig. 2 are atomic force microscopy (AFM) images of the DPPC monolayers transferred at pressures of 25 mN/m and 40 mN/m. Height differences indicative of coexisting phases remain visible in the AFM image of the film transferred at 25 mN/m, where a single phase is expected for an ideal single component film. The departure from ideal behavior is common and arises from small amounts of impurities.⁴⁷ At 40 mN/m, the AFM image reveals a nearly homogeneous film with only small, < 15 nm width, structures remaining.

As an example of how out-of-focus PTIRF-M measurements can probe lipid membrane organization, Fig. 3 shows a typical single molecule fluorescence image taken on a DPPC monolayer doped with $\sim 10^{-8}$ mol% of the reporter molecule BOPIPY-PC. This monolayer was transferred onto a glass substrate at a surface pressure of 25 mN/m. Each bright spot in the image reflects the emission from a single BODIPY-PC molecule in the monolayer. With the optics defocused ~ 500 nm, the single molecule fluorescence shows distinct shapes that reflect the orientation of the BODIPY-PC fluorophores in the monolayer. As shown previously by others, donut-like shapes in the fluorescence image reflect emission dipoles oriented normal to the substrate.^{35–42} At this defocus length, these features become asymmetric as the dipole orients away from the normal and appear as elliptical bright spots surrounded by wings when the emission dipole lies in the plane of the membrane. As seen in Fig. 3a range of single molecule emission patterns are observed that qualitatively reflect the order present in the lipid membrane at this surface pressure.

Representative single molecule emission patterns, extracted from the full image, are also shown in Fig. 3. The top single molecule emission feature shows the donut-like shape indicative of molecules oriented normal to the surface. The middle image shows an asymmetric emission suggesting a molecule tilted away from the normal while the bottom image reveals the emission pattern measured for molecules lying in the plane of the film. As shown previously^{35–42}, the degraded imaging system that leads to the emission shapes can be modeled to extract the emission dipole orientation. For LB films this is simplified because the thinness of the film leads to all dye molecules being located at the same z position.

Figure 3 shows modeled emission patterns for the three extracted single molecule features, along with the angles used in the modeling. As shown in Fig. 4, here we use the convention that ϕ represents the polar angle and θ denotes the azimuthal angle. Figure 3 also shows a schematic representation of the emission dipole orientation for each extracted emission pattern.

To explore the use of single molecule emission measurements to reveal membrane structure, DPPC monolayers were doped with $\sim 10^{-8}$ mol% BODIPY-PC and transferred onto substrates at surface pressures of $\pi = 3$ mN/m, 25 mN/m, and 40 mN/m. Figure 5 shows representative emission images taken on monolayers transferred at these pressures. Qualitatively, comparison of the emission images shows an evolution in the emission patterns as the surface pressure increases and the films become more ordered. At low surface pressure (3 mN/m) where there is maximal orientational freedom around the probe molecule, the single molecule emission patterns consist primarily of patterns associated with emission dipoles lying in the plane of the film. Relatively few donut-like single molecule emission patterns are observed at this pressure where the average area per molecule is approximately $75 - 80 \text{ \AA}^2/\text{molecule}$.

As shown in Fig. 5, as the surface pressure of the film is raised, an increase in the appearance of donut-like emission patterns is observed. For example, the emission image shown in Fig. 5 for the DPPC monolayer transferred at 40 mN/m shows single molecule patterns that are dominated by donut-like features. These features arise from emission dipoles oriented normal to the membrane plane and are consistent with the tailgroups of the fluorescent probe positioned vertically in the monolayer. At this surface pressure, the area per molecule of DPPC is $\sim 40 \text{ \AA}^2/\text{molecule}$ which is approximately half that for monolayers transferred at 3 mN/m, where few donut-like features are observed.

Using the same procedure outlined in Fig. 3, the three-dimensional orientation of each emission feature in the images can be extracted. These orientations are mapped schematically below each of the measured emission images shown in Fig. 5. A clear trend towards vertically oriented emission dipoles is seen as the film surface pressure is increased. A more quantitative view is found in population histograms of the polar angle (ϕ) extracted from the modeled emission patterns. For each pressure studied, at least three different films were analyzed at various locations to create the histograms shown in Fig. 5. In all, at least 700 individual molecules were analyzed at each surface pressure with the exact number for each histogram shown in Fig. 5. At a surface pressure of 3 mN/m, this measure of the tilt from the membrane normal shows that most of dye probes have their emission dipoles lying in the plane of the film with an average tilt angle of $67.4^\circ \pm 5^\circ$. As the pressure is increased to 25 mN/m, a bimodal histogram is found with roughly $\sim 39\%$ of the molecules oriented in the plane ($\sim 81^\circ$) and $\sim 42\%$ oriented normal to the plane ($\sim 10^\circ$), with the remaining 19% approximately evenly spread through the remaining orientations.

The AFM measurements in Fig. 2 show that expanded phases in the DPPC monolayer persist even at 25 mN/m. The bimodal distribution of BODIPY-PC orientations seen in Fig. 5, therefore, reflects dye molecules located in the differing phases. Emission dipoles oriented normal to the surface arise mainly from dye molecules in condensed regions of the film while those located parallel to the film plane reflect expanded regions. Even though the AFM measurements show a nearly 95% to 5% condensed to expanded phase distribution in the films by area, the dye orientations in the histogram are nearly evenly divided between upright and parallel due to the selective partitioning of BODIPY-PC into the expanded phase. If the bimodal distribution is combined, the average tilt angle at 25 mN/m is $48.7^\circ \pm 5^\circ$.

At a surface pressure of 40 mN/m, the increased packing in the film produces a histogram with the majority of probes oriented normal to the film. At this elevated surface pressure, approximately 68% are oriented normal to the surface and only $\sim 12\%$ lie in the plane of the film producing an overall average tilt angle of $22.4^\circ \pm 5^\circ$. These results show a clear trend towards vertically oriented tailgroups as the surface pressure increases.

Similar measurements to that shown in Fig. 5 were conducted on DPPC monolayers incorporating the fluorescent lipid analog DiIC₁₈. As shown in Fig. 1, this probe has the fluorophore located in the headgroup with the transition dipole aligned along the long axis of the conjugated ring system. This positions it along the plane of the membrane when incorporated into a lipid monolayer as shown schematically in Fig. 1. With the fluorophore located in the headgroup, DiIC₁₈ should be much less sensitive to membrane packing in LB monolayers transferred in headgroup down geometry. These studies, therefore, act as a control to confirm that membrane packing is the predominant metric leading to changes in the observed emission patterns.

Figure 6 shows representative single molecule emission images of $\sim 10^{-8}$ mol% DiIC₁₈ incorporated into DPPC monolayers transferred at surface pressures of 3 mN/m, 25 mN/m,

and 40 mN/m. As before, Fig. 6 also shows schematic representations of the extracted emission dipole orientations and population tilt histograms for each surface pressure studied. As before, at each pressure several areas were imaged from at least three different films to construct the histograms. In contrast to the studies carried out with BODIPY-PC, the tilt histograms measured for DiIC₁₈ are not sensitive to the surface pressure or packing of the DPPC monolayer. The histograms reveal that most of the probes are oriented with their emission dipoles lying in or near the plane of the film. At a surface pressure of 3 mN/m, for example, approximately 88% of the dye molecules are oriented in the plane of the film (81°). As surface pressure is increased to 25 mN/m and 40 mN/m, approximately 96% and 94% are oriented in the plane of the films, respectively. From the population histograms, average tilt angles of $80.7^\circ \pm 5^\circ$, $83.5^\circ \pm 5^\circ$, and $81.9^\circ \pm 5^\circ$ are calculated for surface pressures of 3 mN/m, 25 mN/m, and 40 mN/m, respectively, showing no appreciable changes in tilt angle with surface pressure. Since these films are deposited with the headgroups on the glass substrate and DiIC₁₈ has its emission dipole in the headgroup aligned along the plane of the film, the insensitivity to membrane packing is expected. The BODIPY-PC, on the other hand, with the emission coming from the tailgroup region is much more sensitive to membrane packing as shown in Fig. 5.

Other groups have used polarized fluorescence techniques to measure the ensemble orientation of fluorescent probes doped into thin films. In particular, several studies have reported on the orientation of DiIC₁₈ doped into thin films of arachidic acid. In one study, LB films consisting of three layers of pure cadmium arachidate followed by two layers of arachidic acid doped with DiIC₁₈ were studied. These measurements found a mean tilt angle of $75^\circ \pm 4.1^\circ$ away from the membrane normal.⁴⁸ A similar tilt angle of $77^\circ \pm 5^\circ$ was measured using polarized epifluorescence and polarized total internal reflectance fluorescence measurements.²⁷ This study measured the DiIC₁₈ tilt angle in LB films consisting of a doped bilayer of arachidic acid deposited on top of a pure bilayer of cadmium arachidate.

Figure 7 shows the results from single molecule measurements on DiIC₁₈ doped into an arachidic acid film. For these measurements, a pure arachidic acid monolayer was transferred in a tailgroup down geometry onto an OTS treated glass substrate. A second monolayer doped with $\sim 10^{-8}$ mol% DiIC₁₈ was then transferred onto the first. Both layers of the film were transferred at a surface pressure of 35 mN/m to match that used in previous studies. The results shown in Fig. 7 reveal that most of the DiIC₁₈ molecules are oriented with their emission dipoles lying in or near the plane of the film. Averaging together all the orientations observed results in an average orientation of $71^\circ \pm 5^\circ$ which is close to that measured in the previous studies on similar films.

Having shown that single molecule orientation measurements can probe membrane structure, the influence of cholesterol on monolayers of DPPC is investigated. Cholesterol is ubiquitous in biological membranes and influences a vast number of biological functions.^{4, 44, 45, 49–51} Cholesterol inserts into membranes with its rigid four ring structure aligned along the tailgroups of the lipids in the bilayer and its single hydroxyl group oriented towards the headgroups. The incorporation of cholesterol leads to dramatic changes in the biophysical properties of membranes which has been the subject of many excellent reviews.^{44, 45, 49, 50}

In general, the incorporation of cholesterol leads to ordering in lipid membranes.⁴⁵ Cholesterol increases the average motional order of the hydrocarbon chains in lipid bilayers, thus leading to a decreased average area per lipid and a new physical state known as the liquid-ordered (l°) state.^{4, 44, 45, 50, 51} For phospholipids with chain lengths up to

approximately 16 carbon atoms, this cholesterol induced ordering leads to an overall increase in membrane thickness and decrease in membrane permeability.

Figure 8 shows single molecule emission measurements of DPPC monolayers doped with increasing amounts of cholesterol at a surface pressure of 25 mN/m. As shown previously in Fig. 5 and reproduced here in Fig. 8, pure monolayers of DPPC doped with BODIPY-PC exhibit a predominantly bimodal distribution of dye molecule orientations at this surface pressure. With no added cholesterol to the DPPC monolayer, approximately 42% of the dye molecules are oriented normal to the surface ($\sim 10^\circ$). As seen in Fig. 8, the addition of small amounts of cholesterol into the DPPC monolayer leads to a dramatic shift in the single molecule orientations. With the addition of just 5 mol% cholesterol, the population histogram shows that $\sim 59\%$ of the dye molecules orient normal to the surface. This increases to $\sim 65\%$ at 10 mol% and finally $\sim 70\%$ at 33 mol%. These measurements agree with previous studies illustrating the ordering effect that cholesterol has on lipid membranes and illustrates the utility of single molecule measurements in more complicated and hence biologically relevant membrane models.

The results presented here suggest that single molecule emission patterns can be used to reveal structural changes in membranes. This technique compliments other approaches for studying membrane structure and provides several advantages. There is minimal perturbation to the membrane from the fluorescent lipid probe because of the small amount needed. Typical fluorescence studies of membranes use dyes doped at concentrations ranging from ~ 0.25 to ~ 2 mol%. While this is typically considered non-perturbative and does not affect the monolayer transition pressures, miscibility studies on mixed membranes have found an effect from the added fluorescent lipid analogs at concentrations as low as 0.05 mol%.⁵² To avoid crowding in the single molecule emission images, dye concentrations of approximately 10^{-8} mol% were utilized here which are several orders of magnitude lower than that in typical studies.

Probing membrane order at the single molecule level also has the advantage that the entire distribution of orientations is measured. Even simple membranes can be highly heterogeneous and this heterogeneity can be lost in bulk measures of average properties. For example, the results shown in Fig. 5 for DPPC monolayers transferred at 25 mN/m reveal a bimodal tilt distribution. This would be lost in average measures of tilt and reflects the structure remaining in this monolayer even at pressures where single phase behavior is expected for an ideal single component film. Moreover, because this is a microscopy technique, both dye orientation and location are measured which may prove especially informative in natural biological membranes where correlations may provide new insights.

Conclusions

Molecular orientations measured at the single molecule level are used to probe the microscopic order in model membranes. Using TIRF-M with p-polarized excitation, the single molecule emission from the fluorescent lipid analogs BODIPY-PC and DiIC₁₈ doped into LB films of DPPC and AA are measured. As shown by others, defocusing of the optics leads to distinct emission patterns in the single molecule images which reflect the orientation of the dye.^{35–42} DPPC monolayers doped with $\sim 10^{-8}$ mol% BODIPY-PC were deposited at a range of surface pressures and changes in the single molecule orientations tracked the increasing order in the monolayer. At low surface pressures, the emission dipole in the tailgroup of the BODIPY-PC was mostly lying in the plane of the film which evolved to a more upright orientation at high surface pressures where the area per molecule is reduced and the order of the monolayer increased. Similar experiments using DiIC₁₈ found no significant change in emission dipole orientation with surface pressure, as expected since its

fluorophore is located in the headgroup. Measurements of DiIC₁₈ in films of AA transferred at 35 mN/m found an average tilt away from normal of $71^\circ \pm 5^\circ$, which compares favorably with previous bulk measurements.^{27, 48} Incorporation of cholesterol into monolayers of DPPC is shown to increase the number of BODIPY-PC dye molecules oriented normal to the membrane, consistent with the known propensity of cholesterol to order membrane structure. These results show that orientational measurements taken at the single molecule level provide a useful approach for probing membrane structure.

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Figure 1.

Pressure-area isotherm for DPPC with arrows denoting pressures used to transfer monolayers onto the glass substrate. Below are AFM images of typical films transferred at the higher pressures.

Figure 2.

Typical single molecule fluorescence image of a DPPC monolayer doped with BODIPY-PC. This monolayer was transferred onto a glass substrate at a surface pressure of 25 mN/m. Representative single molecule emission patterns have been extracted to illustrate the range of patterns observed. Modeling of the emission patterns leads to the orientation of the emission dipole.

Figure 3. Schematic of an emission dipole oriented in space showing the convention used here for the polar (ϕ) and azimuthal (θ) angles.

Figure 4.

Single molecule fluorescence images of DPPC monolayers doped with BODIPY-PC and transferred at the surface pressures shown at the top. As the pressure increases, more donut-like features are observed reflecting the increased packing and order in the monolayers. The orientation for each molecule is shown schematically in the middle row of images with the polar angle (ϕ) population histograms plotted below.

Figure 5.

Single molecule fluorescence images of DPPC monolayers doped with DiIC₁₈ and transferred at the surface pressures shown at the top. As in Fig. 5, the top shows representative single molecule fluorescence images, the middle displays schematic representation of the emission dipole orientations, and polar angle (Φ) population histograms are shown below. With the fluorophore aligned along the long axis of the headgroup in DiIC₁₈, and inserting into the head group region of the monolayer, this probe is less sensitive than BODIPY-PC at sensing changes in membrane packing.

$$\Pi = 35 \text{ mN/m}$$

Figure 6.

Single molecule measurements of DiIC₁₈ in Y-type bilayers of arachidic acid at a surface pressure of 35 mN/m. From these measurements, the average tilt angle (ϕ) calculated from the histogram is approximately $71^\circ \pm 5^\circ$. This is in good agreement with bulk measurements in similar systems.

Figure 7.

Single molecule emission images and tilt histograms from measurements on DPPC monolayers as a function of mol% cholesterol. All monolayers were doped with $\sim 10^{-8}$ mol % of the reporter dye BODIPY-PC and transferred at the same surface pressure of 25 mN/m. As the percentage of cholesterol is increased, the films become more ordered as seen in the increased observation of donut-like single molecule emission patterns in the images. Analysis of the images reflects this ordering as an increase in the number of dye molecules with emission dipoles oriented normal to the film (tilt angle $\sim 10^\circ$).