Emergence of compositionally tunable nanoscale dynamical domains in model binary lipid biomembranes: Supplementary information

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Preparation of Langmuir monolayer and supported lipid bilayer

i). Langmuir-Blodgett (LB) Technique:

Lipids and dyes are dissolved in chloroform and spread on the surface of DI water filled Langmuir trough and left for 15 mins for the chloroform to evaporate. Subsequently two isotherm cycles are performed by compressing and relaxing the teflon barriers. Target pressure for all our experiments was chosen to be 32 mN/m for a sub-phase temperature of $T = 15 \, ^{\circ}C$ and the compression cycle recorded for different sample ratios is shown in Figure 1.

ii). Free Langmuir monolayers (FLM)

We have a unique experimental platform by using a combination of LB trough and fluorescence laser scanning confocal microscope (LEICA TCS SP5, Germany) microscope as shown
in Figure 2. Compressed monolayers can be directly imaged (Figure 3) on top of water surface using this custom made setup. We can directly capture the formation of domain morphology, study the dynamics of monolayer at the interface etc.,\textsuperscript{1,2}

For this set of experiments, we used BODIPY C12 HPC. This dye is observed to prefer the L-rich phase preferably.

![Figure 1: LB compression cycle for different compositions of DLPC and DPPC](image)

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![Figure 2: (a)Photo of experimental setup: a combination of LB and confocal microscope b) zoomed version of the optical window region](image)

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**iii). Supported Lipid Bilayer**

The L1P0 and L0P1 sample (SI Figure 4) shows homogeneous fluorescence intensity with no signature of phase separation. Phase co-existence in this two component system is observed
Figure 3: Confocal images of a)L1P0 b)L3P1 c)L1P1 d)L1P3 mono layers floating on air-water interface. Scale bar in the figure corresponds to 25 µm

only for a certain range of composition. Two extreme cases of pure DLPC and DPPC bilayers shows homogeneous intensity distribution all across the sample as shown in Figure 6.

Figure 4: Confocal images of a)DLPC and b)DPPC bilayers showing homogeneous intensity. Scale bar in the figure corresponds to 10 µm

Figure 5 shows the confocal images for compositions that show co-existing phases. Strikingly different stripe like P-rich phase are observed in case of L5P1 and L3P1 bilayers. We could not reason out the origin of such features observed only in low DPPC fraction cases as the corresponding free Langmuir monolayer(FLM) and supported Langmuir monolayer(SLM) shows only circular type of domains shown in Figure 6. Similar observations have been reported earlier.\textsuperscript{3,4}

However, with increasing DPPC concentration (L1P1-L1P3), bilayers shows circular domains shown in Figure 6. This evolution of structure on increasing DPPC fraction is reported to be due to pearling of stripe like phase into hexagonally packed (Figure 7) circular domains with a certain inter-domain interaction force.\textsuperscript{5,6} Since the size and density of the circular domains increases with increasing $X_{DPPC}$ it seems reasonable to identify them as the DPPC
Figure 5: Confocal microscopy images of (a) L3P1 (b)L1P1 (c)L1P2 and (d)L1P3 bilayers. Blue arrows in (a) indicates the S phase for L3P1. For (b-d), S phase are seen as circular domains in a background of F phase. Scale bar in each image corresponds to 10 $\mu m$.

Figure 6: Confocal image of L5P1 sample: Comparison of FLM, SLM, SLB.
iv). Control Experiment : Vesicle fusion Technique:

This was done as a control experiment to rule out any possible role of the preparation technique in the formation of domains.

(a). Preparation of small unilamellar vesicles (SUVs)

1 mM lipids were dissolved in chloroform. The solution was desiccated in vacuum to dry the lipids from the chloroform for 2 hours. Dried lipids were added with 1 ml of water and PBS buffer (pH 5.5). The solution was incubated at 40°C for 3 to 4 hours. It was then freeze-thawed before extrusion or sonication. To prepare SUV, the solutions were extruded 21 times in an extruder with 100 nm polycarbonate filter. The prepared SUVs were then stored at 4°C for further use.
(b). Supported Lipid Bilayer (SLB) through vesicle route

Coverslips were cleaned by sonicating in ethanol for 20 mins followed by heat treatment in hot air oven for 2 hours and plasma cleaning for 10 mins. Stored SUVs were taken and added on a clean coverslip. Further, the coverslip with SUV were incubated for 1 hour at a temperature higher than the melting temperature of the lipid used. The coverslips were then washed with buffer or water and used for further preparation. Figure 8 shows the confocal image of the L1P1 bilayer prepared using this technique. We observe clearly phase separated regions and the difference being only in the size of the domains.

![Confocal image of L1P1 bilayer prepared using vesicle fusion. Scale bar in the figure corresponds to 25 µm](image)

Figure 8: Confocal image of L1P1 bilayer prepared using vesicle fusion. Scale bar in the figure corresponds to 25 µm

v). Nanodomains observed using AFM

Since confocal microscopy cannot reveal possible presence of nanoscale domains, we have performed AFM to image the very same bilayer systems as has been successfully used earlier. Most of the samples appeared homogeneous except for some cases. Figure 9 shows structural heterogeneity observed in the samples. Small area scans done using AFM could detect
presence of domains that are unresolved under confocal imaging. In the L-rich phase of L3P1 and L1P1, we observed nanodomains as shown in Figure 9. We observe gel like domains of \( \sim 200 \, \text{nm} \) and \( \sim 100 \, \text{nm} \) for L3P1 and L1P3 respectively dispersed in the \( L_d \) phase.

![AFM images showing nanoscale features that are not resolved under confocal imaging](image)

**Figure 9:** AFM images showing nanoscale features that are not resolved under confocal imaging

**Lipid Dynamics : Specific cases**

**i) Reference : DLPC and DPPC**

Diffusion measurements performed on DLPC gives a diffusion co-efficient of \( 5.0 \pm 0.8 \) \( \mu \text{m}^2/\text{s} \) shown in Figure 10. DPPC \( (T_m = 41 \, ^\circ C) \), a gel like membrane at room temperature shows no signature of diffusion as bleaching is observed during the Fluorescence correlation Spectroscopy (FCS) measurement. We have also performed fluorescence recovery after photo bleaching (FRAP) experiments on DPPC which shows no recovery of intensity shown in Figure 11 a-c, for comparison, recovery of intensity observed in DLPC samples are shown in Figure 11 d-f.
Figure 10: Distribution of diffusion co-efficient values measured from DLPC sample using confocal FCS.

Figure 11: Time lapse images of FRAP experiment on (a-c) DLPC bilayer showing recovery of intensity within 10 sec and (d-f) DPPC showing no recovery of intensity for about 20 sec. Scale bar in each figure corresponds to 25 µm.
ii) Control experiment : Tail tagged Dye

We used a tail tagged dye, BODIPY C12 HPC, to study the nature of domains. SLBs tagged with ATTO 488 DMPE (head tagged) is observed to prefer the P-rich phase and BODIPY C12 HPC (tail tagged) is observed to prefer L-rich phase. Figure 12 shows comparison of confocal images of L1P1 tagged with this probes. We also observe that, contrast between the two phases is better in case of ATTO samples compared to Bodipy tagged samples.

Comparison of dynamics data between the two dyes show relatively fluid like behavior in the tail tagged case in comparison to the head tagged ATTO samples, as the ordering of the membrane increases towards the head region. Measurements on this samples are summarized in Table 1. Even though, dye contrast is better for ATTO sample compared to BODIPY. Dynamics show difference between the two phases is smaller for ATTO case compared to BODIPY.

![Confocal images of L1P1 tagged with a) BODIPY C12 HPC and b)ATTO 488 DMPE showing phase preferences of the probe. Scale bar in the figure corresponds to 25 µm and 10 µm](image)

References


(2) Kandar, AK and Bhattacharya, R and Basu, JK. *Physical Review E* 2010, 81, 041504.
Table 1: FCS results : BODIPY probe

<table>
<thead>
<tr>
<th>Sample</th>
<th>( \tau_0 (ms) )</th>
<th>D ( ( \mu m^2/s ) )</th>
</tr>
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<tbody>
<tr>
<td>L1P0</td>
<td>3.2 ± 0.56</td>
<td>6 ± 1.0</td>
</tr>
<tr>
<td>L3P1 F</td>
<td>2.3 ± 0.6</td>
<td>9 ± 1.8</td>
</tr>
<tr>
<td>L3P1 S</td>
<td>6.0 ± 1.5</td>
<td>4 ± 1.3</td>
</tr>
<tr>
<td>L1P1 F</td>
<td>2.0 ± 0.4</td>
<td>11 ± 1.5</td>
</tr>
<tr>
<td>L1P1 S</td>
<td>5.0 ± 1.2</td>
<td>4 ± 1.1</td>
</tr>
<tr>
<td>L1P3 F</td>
<td>5.8 ± 1.4</td>
<td>4 ± 0.9</td>
</tr>
</tbody>
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(All the deviations from the transit time and Diffusion data are between 12-25 %)


