INFLUENCE OF CALCIUM ON LIPID DOMAIN FORMATION IN AGAROSE SUPPORTED LIPID BILAYERS

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Abstract

Cell membranes are complex entities that their local structure modulates important biological functions. Because of their complexity, we used simplified agarose supported lipid bilayer model system in conjunction with bimFCS (Huang 2011) to study the effect of calcium ions on PIP_2. We found lipid diffusion in the agarose supported bilayer to be faster than in a glass supported bilayer. Increasing calcium concentration of the system induced formation of PIP_2 clusters that were immobile and were reversibly formed and dissolved at the change of calcium concentrations.

Motivation

Phosphatidylinositol-(4,5)-bisphosphate (PIP_2) has been known to form clusters in cell membrane and has an important factor in many cellular events. However, the mechanisms behind the formation and maintenance of PIP_2 clusters still remain allusive (Wang 2012). Here, we test the effect of physiologically relevant concentration of calcium on formation of PIP_2 clusters in agarose supported lipid bilayer system that models cell membrane using bimFCS.

Approach:
Agarose Supported Lipid Bilayer

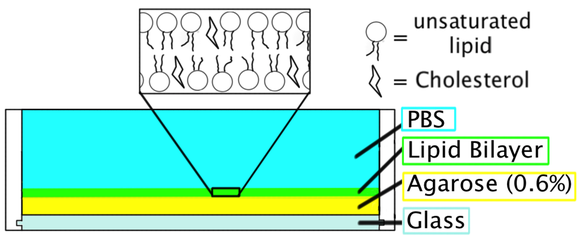

Figure 1. Schematic of agarose supported lipid bilayer: Agarose and lipid mixture are spin coated on top of clean glass. The sample is hydrated with 3X PBS to form lipid bilayer.

Agarose supported lipid bilayer system was used in order to reduce the interference from the solid support to the lipid bilayer. It has been observed that the solid support (typically glass support for the lipid bilayer) slows the diffusion of marked lipids within the bilayer.

Methods:
bimFCS (Binned-imaging Fluorescence Correlation Spectroscopy)

1. Select a region to record the TIRF data with EMCCD camera (55,000 frames at 900fps).
2. Load images as 3D intensity matrix into custom analysis software (IgorPro).
3. Calculate fluorescence autocorrelation functions for each pixels and super-pixel (binned 1x1 to 12x12) and average these over the frame to yield $G(\tau)$.

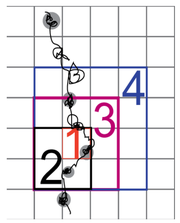

Figure 2. By altering binning of the camera pixel during analysis, different area for FCS analysis can be pro-duced. Information about possible domain smaller than the pixel size (grey dots) can be extrapolated from the series of FCS data generated.

4. Fit each of the 12 curves with square pinhole diffusion function to obtain D_n for each bin n, and calculate $t_{D,n}$ from $\omega^2_n = 4D_n * t_{D,n}$

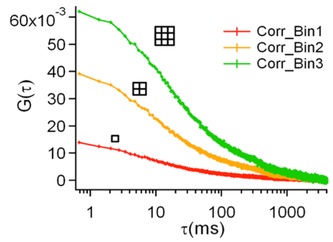

Figure 3. Auto-correlation (FCS) curves of measured intensity fluctuations in super-pixels.

5. Graph $t_{D,n}$ versus ω^2_n, and obtain t_0 and D_{eff}

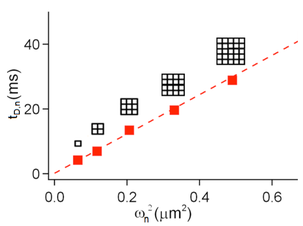

Figure 4. $t_{D,n}$ versus ω^2_n t_0 plot. The slope of the fit is used to calculated an effective diffusion coefficient, D_{eff}. In addition, x- and y- intercepts signify the type of interaction between tracer and matrix.

Reference

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Ellenbroek, W. G., Janmey, P. A. and Liu, A. J. (2011) Divalent Cation-Dependent Formation of Electrostatic PIP_2 Clusters in Lipid monolayers. *Biophys* 101, 9, 2178–2184

Huang, H., and Pralle, A. (2011) Continuous monitoring of membrane protein micro-domain association during cell signaling. arXiv:1101.5087

Veatch S. L., Keller S. L. (2003) A closer look at the canonical "Raft Mixture" in model membrane studies. *Biophys* 84, 725–726

Results:

1) Rhodamine-PE diffusion in solid supported lipid bilayer

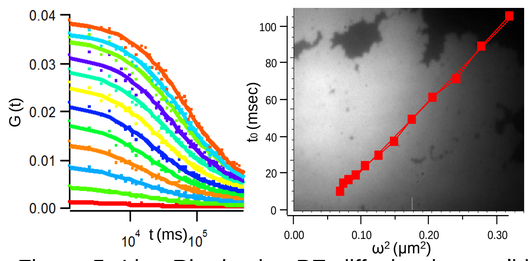

Figure 5. Liss Rhodamine-PE diffusion in a solid supported DOPC bilayer measured by bimFCS . ($D = 1.2 \pm 0.2\ \mu m^2/sec$)

2) Rhodamine-PE diffusion in agarose supported lipid bilayer

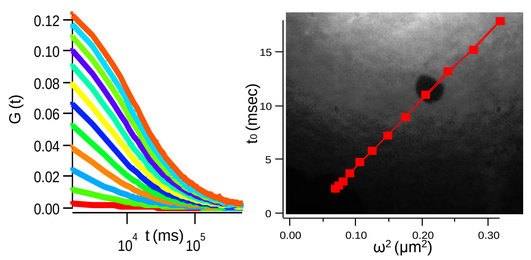

Figure 6. BimFCS on Liss Rhodamine-PE marker within the agarose supported DOPC bilayer. Diffusion is significantly faster than in the solid supported system. ($D = 6.2 \pm 1.2\ \mu m^2/sec$)

3) PIP_2-TopFluor diffusion in agarose supported lipid bilayer

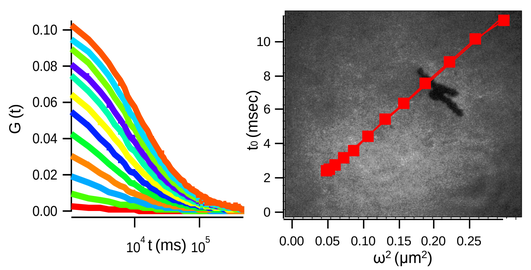

Figure 7. BimFCS on PIP_2-TopFluor marker within the agarose supported DOPC/PIP_2 bilayer. PIP_2-TopFluor in DOPC/PIP_2 diffuses nearly fast as Rhodamine-PE in a pure DOPC bilayer ($D = 6.3 \pm 0.9\ \mu m^2/sec$).

Acknowledgement

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4) High calcium concentration induces PIP_2-cluster formation

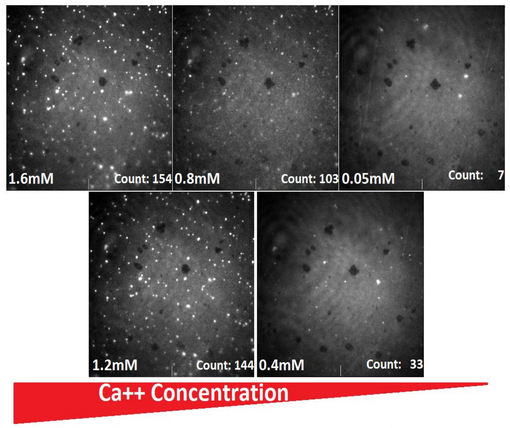

Figure 8. Changing concentration of calcium by adding EGTA to PSS on agarose supported DOPC/PIP_2 bilayer.

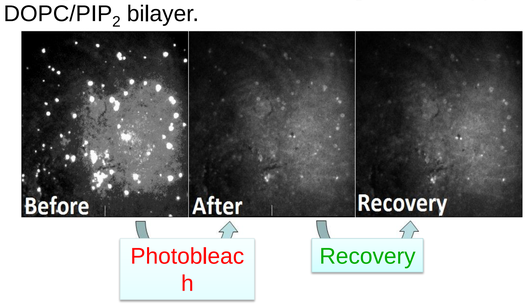

Figure 9. At 1.5mM calcium concentration, PIP_2-TopFluor clusters were photobleached with 0.2mW laser power for 10 minutes. The clusters remained at the same location for 45 minutes, and did not recover in that time.

Summary:

Support / Condition	t_0 (msec)	Diffusion Coefficient ($\mu m^2/sec$)
Solid (Glass) / [DOPC/ Liss Rhod PE] ` (N = 5)	-2 ± 3	1.2 ± 0.2
Agarose (0.6%) / [DOPC/ Liss Rhod PE] (N = 35)	0 ± 1	6.2 ± 1.2
Agarose (0.6%) / [DOPC/ PIP_2/ PIP_2-TopFluor] (N = 16)	1 ± 1	6.3 ± 0.9

Table 1. From bimFCS diffusion measurement, less interference from the support to diffusion in lipid bilayer is shown by increase in diffusion speed.

Calcium clusters were observed to be:

1. Form even at low calcium concentration, but form significantly larger size between 0.8mM – 1.2 mM.
2. Immobile once formed, even through changing calcium concentration.
3. Can be disassembled by decreasing calcium concentration.
4. BimFCS diffusion measurement showed no difference between no calcium and high calcium (2.0mM).