

Characterization of Thermally Induced Bilayer Distortions

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Abstract:

Supported lipid bilayer (SLB) is a promising biologically inspired surface coating and model system for reconstructing membrane processes. We apply thermal stress to SLB and report on the resulting structural distortions. Thermal stress induces long, thin, flexible protrusions (worms) in the SLB that are unstable and eventually collapse into giant vesicles. We examine the length distribution, and collapse of SLB worms as a means toward better understanding the material properties of SLB.

Introduction:

Lipids are molecules composed of a hydrophilic head and hydrophobic tail. In the presence of water these molecules self assemble into a bilayer structure. Supported lipid bilayer (SLB) is a lipid bilayer that has been deposited on a substrate such as glass [1,2]. SLB holds great promise as a biocompatible surface coating and a model for studying membrane processes such as membrane embedded proteins [3]. Understanding SLB mechanics could potentially lead to a more efficient membrane permeable drug delivery system. We studied the response of the SLB to thermal stress and the resulting structural distortions.

Experimental Procedure:

We prepared a vesicle solution of 97% 1, 2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 3% of the fluorescent phospholipid N-(7-nitrobenzo-2, 3-diazol-4-yl)-dimyristoyl-phosphatidyle ethanolamine (NBD – DMPE). Our vesicle preparation followed a standard protocol [4]. Briefly, a lipid film was dried on the surface of a clean glass vial and placed under vacuum for 24 hrs. Then, the film was hydrated with 150 mM PBS (buffer), freeze/thawed, and extruded through a series of membranes with decreasing pore sizes (200 nm, 100 nm, 20 nm).

A clean piece of borosilicate glass was exposed to buffer for ~ 30 minutes in a temperature-controlled flowcell. After the temperature stabilized to 30°C, the buffer was replaced by vesicle solution. The glass was exposed to 0.1 mg/mL vesicle solution for 15 min before rinsing with buffer to remove vesicles from the bulk solution. The glass surface was then imaged in epifluorescence using an inverted microscope and a CCD camera. A spot was bleached by reducing an aperture and increasing the current of the LED light source. An image was taken directly after the bleach and 30 minutes later to check for fluorescence recovery. The surface was imaged at 30 seconds intervals as the temperature was increased to 37°C.

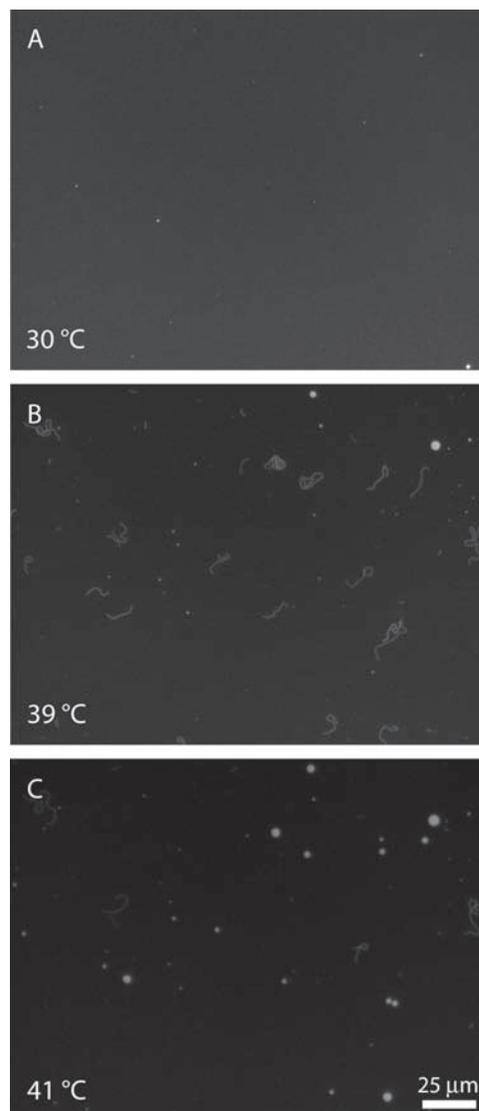


Figure 1: A. Bilayer at 30°C.
B. Bilayer at 39°C worms grow.
C. Worms at 41°C bilayer collapse.

Results and Conclusion:

We checked the bleached spot for recovery. Recovery indicated that the surface was covered in SLB. At 37°C, we observed the appearance of bright round spots—as the temperature increased these spots elongated into varied-sized, floppy worm-like structures or simply “worms” (Figure 1). While the temperature continued its increase, some of the worms continued to grow; others remained constant, while others collapsed into bright round spots.

We hypothesized that the worms were a result of thermal expansion. Since the SLB was confined to a fixed area (i.e. borosilicate glass) and it expanded faster than the area it was confined to, it tended to protrude from that surface. However, these protrusions were unfavorable and thus collapsed to a more stable geometry, i.e. giant vesicles.

We analyzed the growth and collapse (Figure 2) of the worms and obtained a rate of growth and collapse. We analyzed the length distribution of the worms (Figure 3).

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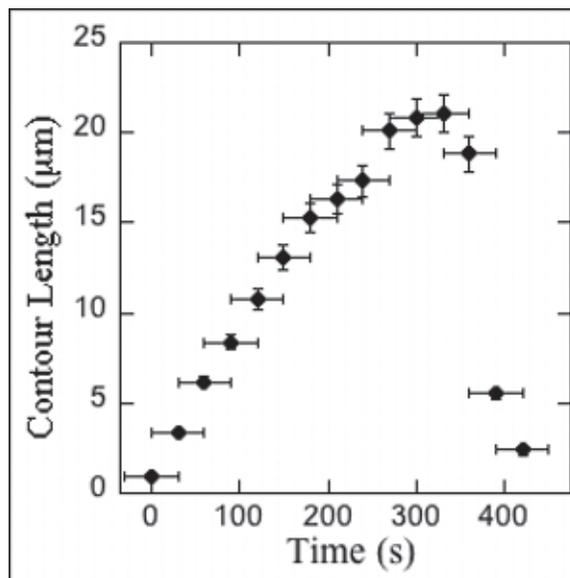


Figure 2: Growth and collapse of a worm.

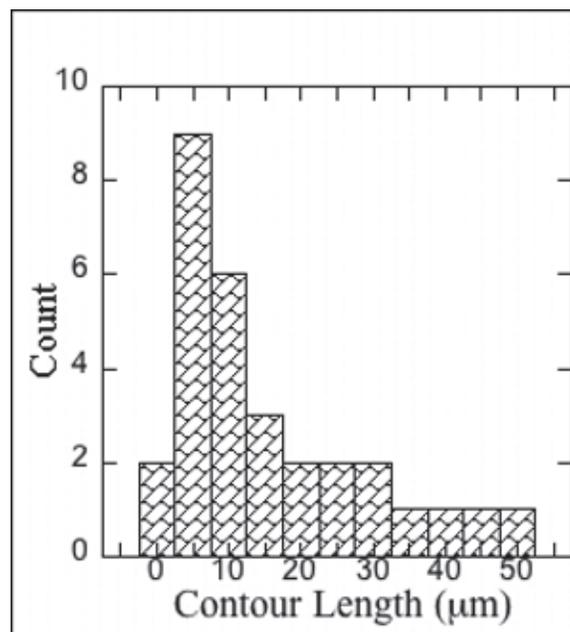


Figure 3: Length distribution of worms.