

Monolayers from fluorescently labelled phospholipids [♣]

G. R. IVANOV*, G. AS. GEORGIEV^a, Z. I. LALCHEV^a

Advanced Technologies Ltd., compl. Yavorov, bl. 38, 1111 Sofia, Bulgaria

^aDepartment of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski,, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria

Organic monolayers from fluorescently labeled phospholipids, mainly DPPE-NBD, were deposited on solid supports under special conditions that form stable nanometer wide bilayer cylinders that protrude from the monolayer. This molecule was frequently used in sensor applications, due to its sensitivity to environmental changes. The proposed configuration should provide both fast response times (ultra thin films) and increased sensitivity (greatly increased surface area). Atomic Force Microscopy (AFM) can clearly distinguish between the different phases. The height difference between the solid-expanded and the liquid-expanded phases was measured to be 1.4 nm, while the bilayer thickness was 5.6 nm. The solid domains showed a 20 % decrease in the fluorescence lifetime in comparison to the monolayer, as measured by Fluorescence Lifetime Imaging Microscopy. This difference in lifetimes is explained in the model of fluorescence self quenching in the solid phase, due to the molecules being closer to each other.

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1. Introduction

Investigation of monolayers from fluorescently labeled phospholipid Dipalmitoyl Nitroben-zoxadiazole Phosphatidylethanolamine (DPPE-NBD) serves 3 main purposes: 1) these molecules closely mimic the behavior of the biological membranes in living organisms [1]; 2) similar structures from this compound have been successfully used as biological sensors, because the NBD group is an excellent sensor for changes in its environment [2]; 3) this molecule is suitable for a better understanding of ultra thin organic films, because it forms stable monolayers at the air-water interface, easily builds multilayers (unlike the phospholipids) and the chromophore allows many additional experimental techniques to be used. Our previous work on this compound has allowed us to discover several new phenomena. For the first time, fluorescence self quenching in Langmuir films has been observed [3]. In the solid phase, due to the close proximity of the molecules, their fluorescence was strongly quenched and they were observed as dark domains in the liquid phase. Multilayer cylinders with several hundred nanometer diameters were observed in Langmuir-Blodgett (LB) monolayers [4]. Their height corresponded to the bilayer thickness, if the deposition was carried out below the equilibrium

spreading pressure of 19.6 mN/m, and was much higher if the deposition was at higher pressures. The bilayer structures remained stable for at least several months, indicating that this was not a kinetic phenomenon. Contrary to expectations, paramagnetic cobalt ions were found to act as de-quenchers, when added to the water subphase during deposition. This was interpreted as an increase in the distance between the molecules in the solid phase, and a consequent decrease in self quenching. In this work, we add the technique of fluorescence lifetime measurements, both integrally and microscopically, to get a better understanding of the above phenomena.

2. Results and discussion

The most probable conformations for the DPPE-NBD molecule, obtained from molecular conformational modeling at a simulated air-water interface, are shown in Fig.1.

The equilibrium spreading pressure (ESP) of the DPPE-NBD was measured to be 19.6 mN/m. Thus, all depositions described here are below the ESP.

We have deposited monolayers from DPPE-NBD from pure water, simultaneously on silicon wafers for the AFM

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study and on glass substrates for the fluorescence study. The depositions were at different pressures from the main transition at 6.8 mN/m to the solid phase at 35 mN/m. Friction force microscopy of an LB film deposited in the phase coexistence region is shown on Fig. 2. One can see that the solid domains are not homogeneous, but are composed from several segments.

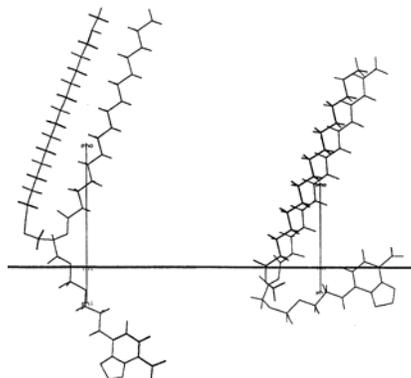


Fig. 1. Most probable conformations of a DPPE-NBD molecule: on the left – in the solid phase; on the right – in the liquid phase. The solid line represents the air-water interface. Also shown are the hydrophilic (ϕ) and the hydrophobic (pho) centres of the molecules, as well as the hydrophilic dipole.

Fluorescence Lifetime Imaging Microscopy (FLIM) reveals that, as expected, the solid domains have shorter fluorescence lifetimes of around 1.7 ns. In the liquid phase, the lifetimes were around 2.3 ns. From these images, one would expect that with increasing deposition pressure when the area occupied by the solid phase (with a significantly shorter lifetime) increases compared to the liquid phase, then the average lifetime would decrease. However, we observed the opposite from the integral fluorescence lifetime measurements. Thus, the average lifetime for the sample deposited at 7 mN/m was 1.29 ns; for the sample deposited at 11 mN/m it was 1.95 ns; for the sample deposited at 35 mN/m it was 2.45 ns. The results were best fitted with 3 exponentials, and the decrease in the lifetime affected all 3 components and was not due to changes in fractal lifetimes.

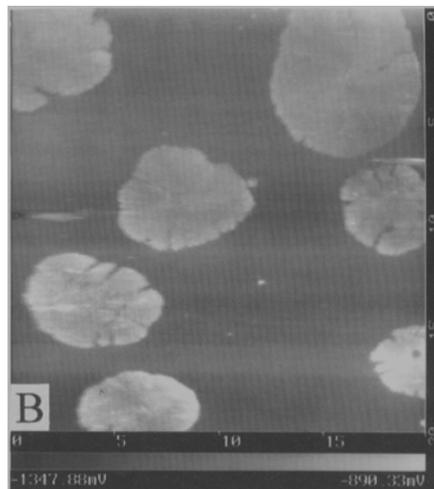


Fig. 2. Friction force microscopy of a LB monolayer from DPPE-NBD, deposited in the phase coexistence region in the main transition at 14 mN/m and 0.5 mM NaCl in the water subphase. 20 μm scan size.

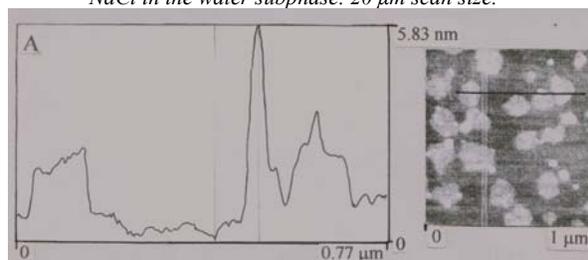


Fig. 3. Atomic force microscopy and a line cross section of a LB monolayer from DPPE-NBD, deposited in the phase coexistence region in the main transition at 8 mN/m. One can see the height difference between the solid and liquid phases of 1.4 nm. Also, bilayer thick cylinders with heights of around 5.8 nm and widths of around 100 nm is seen.

We believe this abnormal behavior is due to the fluorescence of the high cylinders. Molecules in them are not well ordered, and exhibit liquid phase fluorescence. The number of DPPE-NBD molecules they contain significantly increases with increasing deposition pressure. Thus, this liquid phase fluorescence starts to dominate, although the area of the solid phase increases at higher deposition pressures.

3. Conclusions

Here we have proposed a way to deposit monolayer thick films from a fluorescently labelled phospholipid, with a clearly pronounced 3D structure in the form of bilayer thick cylinders. We have investigated the morphology of this structure with nanometre resolution. Through fluorescence kinetics measurements (both integral and on point by point measurements), we have shown that the molecules in these cylinders are in a liquid

phase conformation, with limited self-quenching and larger fluorescence kinetic lifetimes. This structure has significant potential for chemical sensor applications, having the possibility of simultaneously short reaction times (very thin monolayer, no diffusion for the reagents) and enhanced sensitivity (well developed structure with more reaction sites). These structures are expected to be stable with time, because depositions were carried out below ESP, and 2 month studies show no visible changes.

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*Corresponding author: info@at-equipment.com