

New effects in Langmuir and Langmuir-Blodgett monolayers from fluorescently labelled phospholipids and their possible use for water quality control

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Abstract. Secondary water contamination poses significant challenges to the sensitivity and selectivity of sensors used for its detection and monitoring. Currently only lab tests can detect these contaminants and by the time this happens the contaminated water has entered the city water supply system. Fluorescent chromophore NitroBenzoxaDiazole (NBD) is very suitable and had been successfully used in biosensor applications due to its high sensitivity to close proximity polarity of the medium. Over the years we have discovered 3 new effects in NBD-labelled phospholipids which can significantly improve the performance of biosensors. The phospholipid matrix provides flexible biocompatible environment for immobilization of selectively reacting enzymes, microorganisms, DNA, immunoagents, whole cells. Use of single layer (3.1 nm thickness) films at the air-water interface (Langmuir films) or deposited on solid support as Langmuir-Blodgett (LB) film gives fast response times for real time monitoring (no slow diffusion processes) and precise molecule ordering and orientation. The first new effect was fluorescence self-quenching in Langmuir and LB films. In the liquid phase films exhibit normal fluorescence. Upon transition to solid phase fluorescence intensity is almost completely self-quenched and fluorescence lifetimes in the nanosecond region decrease 2 times. This is easily measured. Usually large heavy metal atoms quench fluorescence. We observed the opposite new effect when LB film is deposited in the solid phase from a subphase containing heavy metals. The third new effect is the obtaining of nanosized cylinders with bilayer thickness, which remain stable at least for months, when LB monolayer is deposited in the phase coexistence region at thermodynamic equilibrium. This greatly increases reacting surface and sensitivity of possible sensors. Almost all possible optical experimental methods were used for this research. This includes polarized ATR FTIR and polarized UV-VIS spectroscopies, Fluorescence Lifetime Imaging Microscopy (FLIM), Scanning Near-field Optical Microscopy (SNOM).

1. Introduction

Reliable environmental monitoring strongly depends on the quality of chemical and biochemical sensors. There are still some unsolved problems especially when higher selectivity is required. Special

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problems pose secondary water contamination e.g. medicine disposal into drains [1, 2]. In this paper we propose a new class of materials – fluorescently labeled phospholipids, which can be used as chemical and biological sensors. We focus our attention on head labeled with nitrobenzoxadiazole (NBD) phosphatidyl ethanolamine. NBD molecule is considered one of the most suitable chromophores for sensor applications because of easily measurable fluorescence change on local changes of the polarity of the medium that surrounds the chromophore. In the nineties this effect was used for the fabrication of a number of biosensors by the Krull's group in Toronto [see e.g. 3]. More recently NBD labelled molecules were used as sensors for phosphatidylserine containing membranes [4], cysteine/homocysteine [5] and for selective chemical sensing of hazardous compounds and drugs of abuse [6]. In several hundred papers small percent (0.5 to 1 % typically) NBD labelled lipids were used in a mixture with other lipids to visualize domain structure in Langmuir films with fluorescence microscopy or in FRAP measurements. However we were the first to study these compounds as single component layers. Three new phenomena were discovered for this material that can be used in sensor applications. In our research we use the Langmuir and Blodgett (LB) method for investigation of organic monolayers at the air-water interface and for thin film deposition. The Nobel prize in mid-thirties winning LB method has renewed interest in it because this is the best method for supramolecular architecture offering discrete layer-by-layer film deposition, precise control of thickness and molecular orientation, considerably smaller number of defects compared to alternative methods. A small review of the method is given in our book chapter [7]. More recently Langmuir film from water insoluble molecules was used as a carrier to deposit e.g. adsorbed proteins from the water subphase or as matrix for deposition of inorganic nano particles [8].

2. New effects discovered by us

In the past 25 years we have focused on investigations of Langmuir and Langmuir-Blodgett (LB) films from fluorescently labelled phospholipids mainly Dipalmitoyl Phosphatidyl Ethanolamine head labelled with NitroBenzoxadiazole (DP-NBD-PE). Such long lasting focus allowed us to discover 3 new effects. First one is fluorescence self-quenching in Langmuir [9] and LB films [10]. Isotherms on Langmuir films from DP-NBD-PE on pure water subphase were measured on the 5th generation LB film system from Advanced Technologies Ltd. (Sofia, Bulgaria) at different temperatures (figure 1).

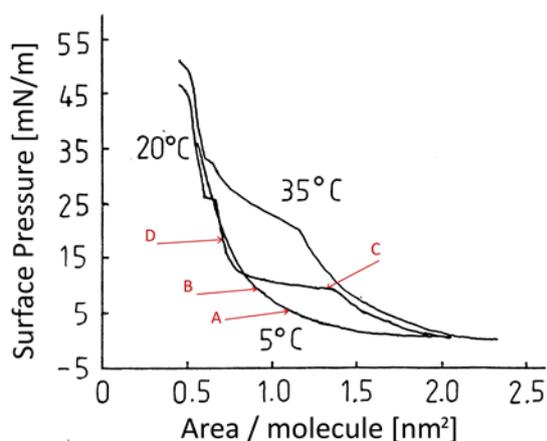


Figure 1. Isotherms of Langmuir films from DP-NBD-PE on pure water subphase at different temperatures (modified from [7]).

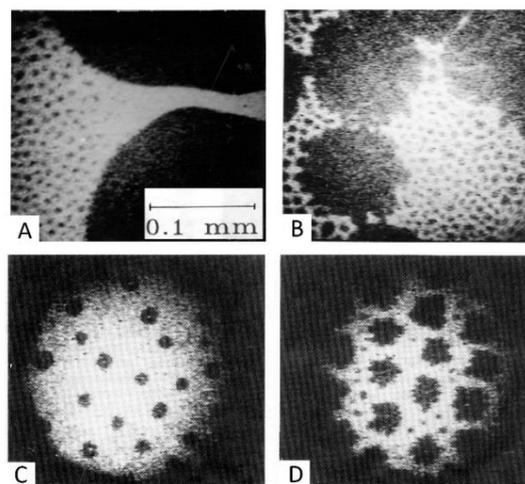


Figure 2. Fluorescence microscopy of a Langmuir film from DP-NBD-PE at different surface pressures indicated with red arrows on figure 1 and at different temperatures: 5° C – A) and B); 20° C – C) and D) (modified from [7, 9]).

During compression optical epi Fluorescence (FL) microscopy with high sensitivity camera is used to look at the monolayer. Some image examples are shown on figure 2. At 5° C there is no 1st order phase transition from liquid-expanded to liquid condensed phase as seen from both figures. In the solid phase the fluorescence is strongly self-quenched (dark domains) due to radiationless energy transfer between the NBD groups. In the liquid phase FL remains strong, only partly quenched, due to interaction with water. This phenomenon remains upon transfer of the monolayer to solid support as LB film (figure 3). During the transfer process the structure of the film is preserved as observed by FL microscopy.

Another new phenomenon is shown and described on figure 4. Large paramagnetic metal atoms are known as good FL quenchers. However we observe the opposite effect. If large metal ions in the water subphase interact with the FL self-quenched DP-NBD-PE molecules in the solid phase then they restore the FL. If the monolayer is in the solid phase the fluorescent is self-quenched. If large bivalent metal ions interact with DP-NBD-PE molecules they attach to the negatively charged head group and separate the molecules in the solid phase. If this separation is larger than the critical distance R_c FL is de-quenched and emission is restored. This is what we observe for Cadmium (Cd) ions. Cd is very health threatening contaminant. This effect can be used for express monitoring of heavy metal contamination in water.

The 3rd new effect – obtaining a highly developed surface from DP-NBD-PE monolayers goes beyond the scope of the current paper.

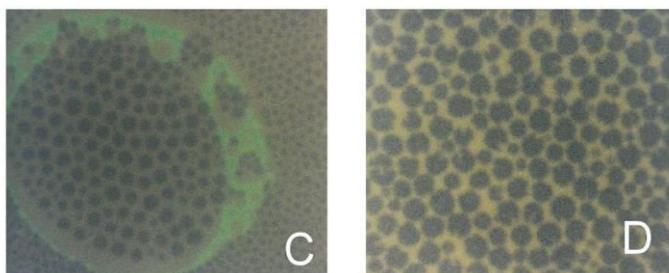


Figure 3. FL microscopy of an LB film from DP-NBD-PE deposited from pure water at room temperature. Some defects are seen in the left picture.

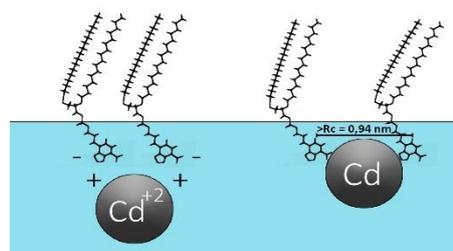


Figure 4. Second new effect. Large bivalent metal ions de-quench FL though typically they quench it.

Fluorescence spectroscopy of an LB film monolayers from DP-NBD-PE was recorded. When deposition is carried in the liquid phase below the main phase transition large well pronounced maximum at 530 nm is observed. If the deposition is carried in the solid phase above 26 mN/m then the fluorescence is almost completely quenched with just a slightly visible shoulder on the curve. This 95% quenching of the fluorescence is easily measurable. A model for the fluorescence self-quenching was developed [7]. It considers the probability for static quenching by the formation of emissionless traps consisting of pairs of statistical DP-NBD-PE molecules which are below the critical distance R_c . The model also considers the dynamic quenching due to Förster transfer of energy from DP-NBD-PE monomers to the traps. Assumptions in this model are: 1) statistical traps are formed according to two-dimensional equation of Perrin; 2) all DP-NBD-PE molecules that do not participate in the traps are uniformly distributed throughout the monolayer; 3) there is no diffusion during the lifetime of the excited state, 4) energy can move between and among fluorophores and traps, but once traps are reached energy immediately and without emission decreases; 5) passing of energy in more than one DP-NBD-PE molecule before reaching the trap is negligible. It is estimated that the distance at which the efficiency of Förster transfer of energy becomes 50% $R_0 = 2,55$ nm and that $R_c = 0,94$ nm. The insertion of the large metal ion (figure 4) between two DP-NBD-PE molecules increases the distance between the NBD chromophores above R_c . As a result de-quenching is observed and this

effect can directly be used for express monitoring of heavy bivalent or trivalent metal water contaminations as discussed below. Currently there are no express methods to do so.

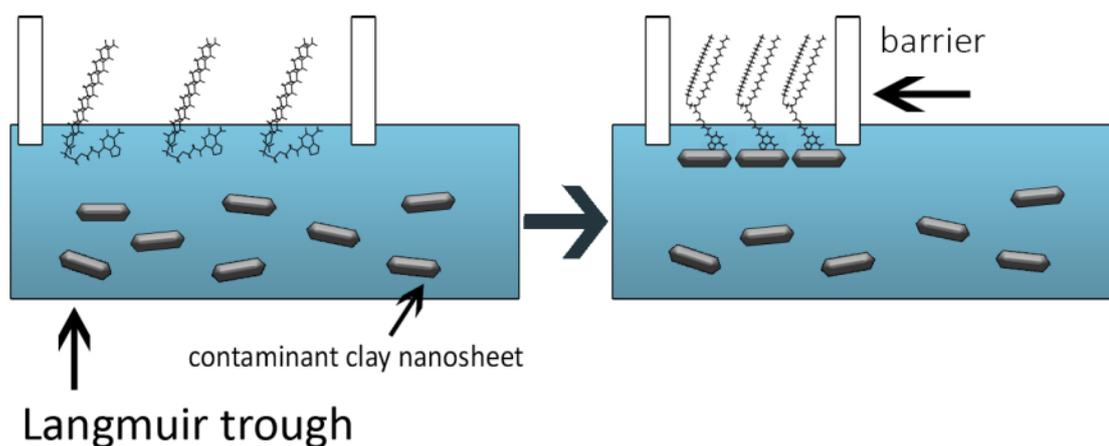


Figure 5. Langmuir trough method for monitoring of water purity.

3. Use of these new effects for measuring the quality of water in natural basins

The development of rapid, economical and sensitive techniques for characterization of the purity of natural and drinking water represents leading ecological problem. The surface properties of natural waters (sampled from rivers, lakes and gulfs) are already successfully used for evaluation of the ecology standard and purity of water basins. Recently it is proposed a Langmuir monolayer based technique which by measuring the surface pressure - area isotherm of the samples collected from a range of natural water basins, yields the so called “structural signatures” of water, which adequately predicted the quality and the purity of the basin [11]. Major advantage of the Langmuir monolayer technique is that it combines ease of use, high sensitivity and possibility for rapid field application with much lower price in comparison with the most commonly used chromatography techniques.

The “structural signatures” of samples of natural water result from the generalized scaling procedures applied to the surface pressure-area isotherms of the natural films. They appear to reflect in a quantitative and sensitive way the film composition, film solubility and the miscibility of its components, the kinetic mobility of surfactant molecules, and the compound’s surface concentration. It is suggested that certain classes of film-forming components or “end-members” may dominate the static and dynamic surface properties. Variation in the surface rheological parameters of source-specific surfactants is postulated to reflect organic matter dynamics in natural waters. The reported results demonstrate that natural films are complex mixtures of biopolymeric molecules covering a wide range of solubility, surface activities and molecular masses with a complex interfacial architecture.

An improvement of the method would be to use a Langmuir film from DP-NBD-PE molecules (figure 5). If some contaminants attach to the negatively charged NBD chromophore this will lead to changes in local polarity of the medium and subsequently change in the NBD fluorescence. Thus in addition to isotherm data also FL spectroscopy data will be available which can help with selectivity and sensitivity of the sensor. The described above effect in figure 4 can be used for monitoring heavy metal contamination.

Further extension of this method would be to mix selectively sensing molecules e.g. enzymes (figure 6) in the Langmuir film and use the FL self-quenching effect if some conformational changes in the enzyme when the analyte attaches leads to change in surface pressure, self-quenching and subsequent easily measurable optical signal. FL quenching can be measured not only by measuring

intensity of the signal but in addition FL maximum peak position and FL lifetime. Quenched FL has roughly 2 times shorter lifetimes of around 2 ns compared to unquenched signal (not shown).

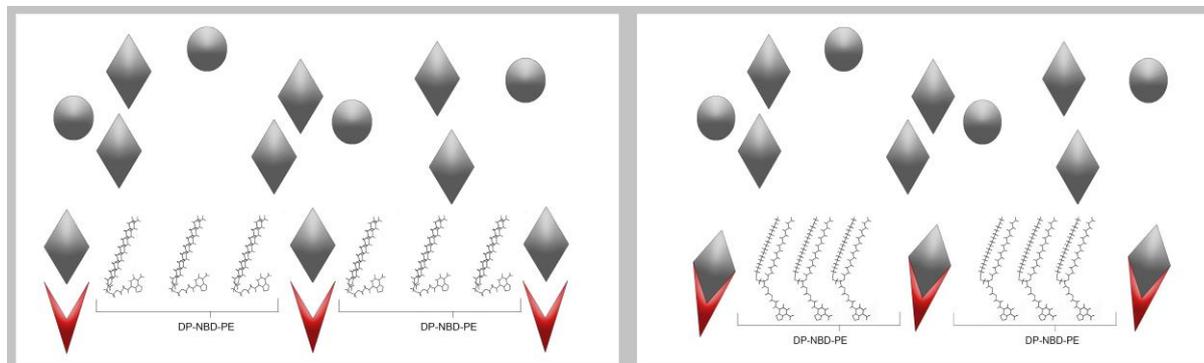


Figure 6. The use of FL self-quenching for biosensor applications. If a selectively sensing molecule is mixed with DP-NBD-PE the interaction with analyte can cause increase of lateral pressure and transition of part of the molecules from liquid to solid phase and FL self-quenching with easily measurable signal.

4. Conclusion

Over the years we have discovered 3 new effects in NBD-labelled phospholipids which can significantly improve the performance of biosensors. The phospholipid matrix provides flexible biocompatible environment for immobilization of selectively reacting enzymes, microorganisms, DNA, immunoagents, whole cells. Use of single layer (3.1 nm thickness) films at the air-water interface (Langmuir films) or deposited on solid support as Langmuir-Blodgett (LB) film gives fast response times for real time monitoring (no slow diffusion processes) and precise molecule ordering and orientation. The first new effect discovered was fluorescence self-quenching in Langmuir and LB films. In the liquid phase films exhibit normal fluorescence. Upon transition to solid phase fluorescence intensity is almost completely self-quenched and fluorescence lifetimes decrease 2 times. This is easily measured. Usually large heavy metal atoms quench fluorescence. We observed the opposite new effect when LB film is deposited in the solid phase from a subphase containing heavy metals. Possible use of these effects for monitoring water quality was discussed here.

Acknowledgements

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