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Applications of Brewster angle microscopy from biological materials to biological systems

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ABSTRACT

Brewster angle microscopy (BAM) is a powerful technique that allows for real-time visualization of Langmuir monolayers. The lateral organization of these films can be investigated, including phase separation and the formation of domains, which may be of different sizes and shapes depending on the properties of the monolayer. Different molecules or small changes within a molecule such as the molecule's length or presence of a double bond can alter the monolayer's lateral organization that is usually undetected using surface pressure-area isotherms. The effect of such changes can be clearly observed using BAM in real-time, under full hydration, which is an experimental advantage in many cases. While previous BAM reviews focused more on selected compounds or compared the impact of structural variations on the lateral domain formation, this review provided a broader overview of BAM application using biological materials and systems including the visualization of amphiphilic molecules, proteins, drugs, extracts, DNA, and nanoparticles at the air-water interface.

Contents

Abbreviations: AFM, atomic force microscopy; ANS, 1-anilino-8-naphthalene sulfonate; BLES, bovine lung extract surfactant; BAM, Brewster angle microscopy; BSA, bovine serum albumin; CLSE, calf lung surfactant extract; CMC, critical micellar concentration; DM-, dimyristoyl; DMG, dimyristoyl glycerol; DODAB, dioctadecyldimethylammonium bromide; DO-, dioleoyl; DP-, dipalmitoyl; DPGG, dipalmitoyl galloylglycerol; DS-, distearoyl; EmrE, *Escherichia coli* multidrug resistance protein E; PLP, Folch-Lees proteolipid protein; GM, gangliosides; GIXD, grazing Incidence X-ray diffraction; LC, liquid condensed; LE, liquid expanded; MBP, myelin basic protein; NAE, *N*-acylethanolamines; NP, nanoparticle; OTS, octadecyltrichlorosilane; PO-, palmitoyloleoyl; -PA, phosphatidic acid; -PC, phosphatidylcholine; -PE, phosphatidylethanolamine; -PG, phosphatidylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate; -PS, phosphatidylserine; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PAF, platelet activating factor; PEG, polyethylene glycol; SM, sphingomyelin; SP-A, surfactant protein A; SP-B, surfactant protein B; SP-C, surfactant protein C; SP-D, surfactant protein D; TMCL, tetramyristoyl cardiolipin; TRCDA, tricosadyinoic acid; VILIPs, VIsinin-Like proteins.

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1. Introduction

Monomolecular films formed at interfaces are the site of many biologically relevant interactions. Thus, monomolecular films, commonly known as monolayers, have been extensively used as models for biomembranes [1–3] and to analyze processes that occur at the surface of membranes that often include lipids and or proteins [4]. The use of monolayers as biomimetic models allows for a stringent control of experimental factors, such as film composition [5]. In addition, direct visualization enables a more detailed analysis of the film architecture and changes upon their interactions with other molecules. To this end, optical microscopy methods are among the most popular imaging techniques, whereby Brewster angle microscopy (BAM) can be performed label free [6,7]. BAM was developed concurrently and independently by two groups [7,8] and its experimental set-up and principle are shown in Fig. 1. A laser beam is polarized in the parallel plane and directed at the Brewster angle (~53° for water) onto the air water interface (Fig. 1A). Under these conditions the light is not reflected (Fig. 1B). However the addition of a film at the air-water interface changes the refractive index resulting in light reflection off the films into a camera that provides real-time images of the interface (Fig. 1A). The experimental set-up includes a Langmuir trough that provides additional information on film packing by recording changes in surface pressure upon reduction of the molecular area changes that are displayed as pressure-area isotherms. Additionally, portions of the monolayer that protrude from the surface appear brighter and this qualitative information is also useful to analyze the phase behavior of the monofilm. Recently, it was shown that BAM is a technique that can provide quantitative structural analysis in addition to the qualitative optical information [9]. This is based on its ability to image anisotropy due to differences in the reflective properties of the lipid polar head group. This topic has been reviewed in detail by Roldán-Carmona and colleagues [9]. Fig. 2 shows a typical isotherm and BAM images observed during the compression of a dipalmitoylphosphatidylcholine (DPPC) monolayer. Firstly, at large molecular areas the films exist in the so called “gas phase” whereby all molecules are well distributed with very limited interactions between the lipid molecules. Thus no lateral surface pressure is observed. However, as the area is decreased, the lipid acyl chains and headgroups begin to orient themselves and begin to interact adopting the liquid-expanded phase (LE). As the monolayer is compressed some lipids, like DPPC, displays a plateau region, denoting the coexistence of LE and a more rigid liquid condensed (LC) phases leading to the formation of domains. Subsequent compression induces tighter lipid packing and more intermolecular interactions. The resulting steeper slope of the isotherm reflects a greater change in surface pressure over a comparable small decrease in area. Once a maximum pressure is reached, film compression results in the collapse of the films into the aqueous subphase or the formation of multilayers. This collapse pressure is a measure for film stability and can be used to quantify the impact of destabilizing ligands [10,11]. Amphiphilic monolayers at the air water interface have a great potential as models of biological membranes which was extensively reviewed elsewhere [12].

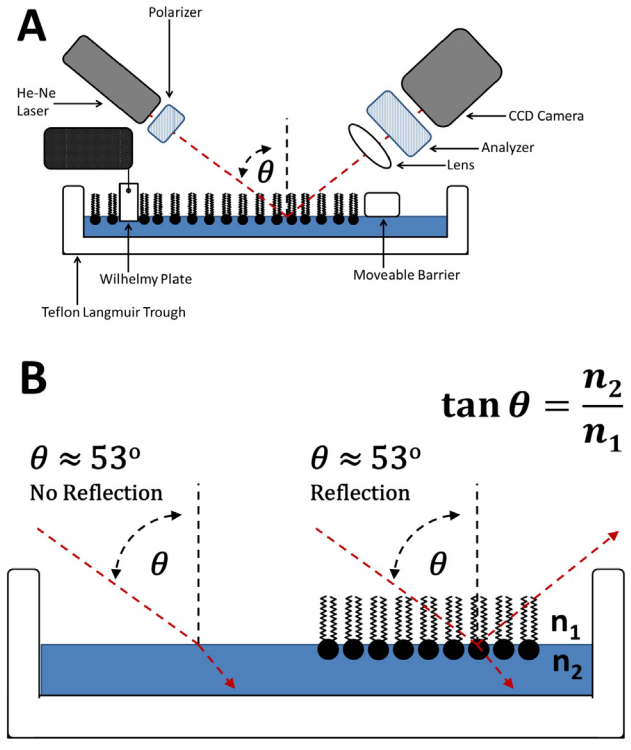

Fig. 1. Schematic of the experimental set up of Brewster angle microscopy (A). Schematic diagram of the principle of Brewster angle microscopy (B).

There are other powerful imaging techniques that are extensively used in the field of monolayer analysis including fluorescence microscopy, which also allows for the visualization of monolayers at the interface utilizing the inclusion of fluorescent molecules [13–17]. BAM has been extensively reviewed [4,18–29] and it has shown promise when viewing changes within the LC domain morphology, specifically in regards to chirality and texture as reviewed in detail elsewhere [27,30]. This review will provide a broader overview of the various biologically relevant applications of BAM for the direct visualization of amphipathic molecules, proteins, drugs, extracts, DNA and nanoparticles (NPs).

2. Biological applications of BAM

2.1. BAM studies of lipids/amphiphilic molecules at the air–water interface

While BAM has been used to visualize a vast array of amphipathic compounds, lipids and surfactants are among the most widely investigated biomolecules. The majority of these experiments aim to understand the driving forces behind specific interactions, as well as the effect of those interactions on lateral film organization. In addition, investigations into small structural changes within a molecule and the concomitant effect on the lateral film organization have been conducted as will be reviewed below.

Albalat and colleagues compared the monolayer behavior of synthetic 1,2-diacyl glycerol amino acid-based surfactants with natural phospholipids [31]. These novel surfactants are biocompatible and antimicrobial and thus are very interesting as preservatives for medical and pharmaceutical applications [32]. Such amino based surfactants were found to be effective against viruses, bacteria, and even tumors [33]. The similarities between the amino acid based surfactants and naturally occurring phospholipids became apparent when both the headgroup and alkyl chain length of these surfactants were systematically screened. A chain length of 12 to 18 carbons displayed temperature dependent phase behaviors comparable to phospholipids. For example, the monocationic derivative of 1,2-diacyl glycerol amino acid surfactant, 1414RAc (Fig. 3-a) exhibited phase co-existence in the surface pressure-area isotherms that was visualized by BAM (Fig. 3-b). Moreover, the observed domains were similar to saturated glycerophospholipids [31].

BAM has been further utilized to visualize the effect on the overall lateral film organization when the lipid headgroup structure was altered while maintaining the acyl chain composition. Minones and colleagues [34] compared DPPC, dipalmitoylphosphatidylglycerol (DPPG), and dipalmitoylphosphatidylserine (DPPS) monolayers at the air water interface. While the three lipids contained two fully saturated 16 carbon acyl tails, they carry very distinct headgroups: a zwitterionic phosphocholine, a negatively charged phosphoglycerol, and the amino group containing phosphoserine, which is also negatively charged. The data clearly showed that the headgroup played a significant role in the lateral film organization at constant pH and temperature [34]. DPPC, at pH of 6 and 20 °C, displayed LC domains (Fig. 4-a) that appeared in