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Building a biomimetic membrane for neutron reflectivity investigation: Complexity, asymmetry and contrast

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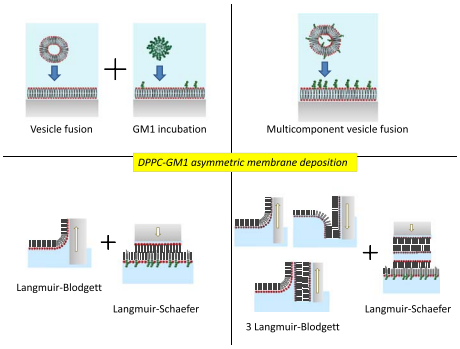
HIGHLIGHTS

- Biomimes of ganglioside-containing microdomains can be built by different methods.
- Langmuir–Blodgett deposition gives best GM1-containing membranes.
- Asymmetric supported membranes containing GM1 are also obtained by vesicle fusion.

GRAPHICAL ABSTRACT



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ABSTRACT

The preparation and investigation of model membranes is deserving growing interest both for the physics of complex systems, and for biology. The need of simplified models should preserve mimicking the qualifying characteristics of biological membranes, and keep non-invasive and detailed description. As a main feature, biological membranes are non-homogeneous in the disposition of components, both in the lateral and in the transverse direction. We prepared asymmetric supported membranes containing GM1 ganglioside in biomimetic proportion according to different protocols. Then, we studied their internal structure by neutron reflectometry, providing few-Angstrom sensitivity in the cross direction meanwhile avoiding radiation damage. This technique can also be profitably applied to study interactions at the membrane surface. The best protocol has proven to be the Langmuir-Blodgett/Langmuir-Schaefer depositions. Notably, also the simpler and most accessible protocol of vesicle fusion was found to be suitable for straightforward and good quality deposition of compositionally asymmetric membranes.

1. Introduction

The realization and structural characterization of model membrane systems is deserving growing interest both for the physics of auto-aggregating complex systems and for membrane biology. However, because of membranes complexity, the research in this field is far from trivial. Biological membranes are in fact composed by thousands of different components, whose disposition is inhomogeneous both in the lateral and in the transverse direction [1,2]. The need for dealing with simplified models should match the opportunity to mimic the

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qualifying characteristics of biological membranes, in different respects. As a typical example, the so-called Glycosphingolipid Enriched Microdomains (GEMs) [3] are membrane domains with prominent structural and functional roles. Besides the distinctive lipidic composition, a qualifying aspect for GEMs biomimetism is compositional asymmetry. In fact, glycolipids reside only in the outer leaflet of the cell [4,5]. Besides model construction, detailed and resolved structural observation is then required. In this respect, the use of neutron scattering offers the unique advantage of visibility modulation, *via* the isotopic H-D substitution, with no significant impact on the physicochemistry of the membrane, meanwhile avoiding radiation damage. These features have been largely exploited, by using neutron scattering and diffraction in the study of structural and dynamic properties of colloidal systems, including biocolloids [6–8]. Within this favourable frame, the neutron reflectometry technique has been implemented to the membrane biology edge and now it is increasingly employed to access the local structural properties of biomimetic single membranes [9–13] and in particular to achieve information about phospholipid membranes asymmetry [14,15].

In this context, we studied the structural characteristics of model supported membranes containing GM1 ganglioside, prepared according to different protocols. In fact, being GM1 a very important component of functional membrane domains, the deposition of model membranes bearing GM1 asymmetry and suitable for structural and morphological investigation, is of great interest. The features of bicomponent model systems with asymmetric GM1 distribution have been studied by means of molecular dynamics simulations [16]. On the other hand, building up experimental models with controlled asymmetric disposition of components is not trivial. The laborious layer by layer Langmuir Blodgett/Langmuir-Schaefer [17,18] deposition technique ensures ganglioside asymmetry, while the easier and most commonly used vesicle-fusion technique was never shown, although hypothesized [19], to allow for asymmetric membranes.

The study of the lateral pressure of mixed d_{75}DPPC-GM1 as a function of the area-per-molecule (π-A Langmuir isotherms) evidences that the mutual interactions between lipids of different species reflect in the surface arrangement of molecules, known as 'umbrella effect'. Supported membranes allow the investigation of membrane transverse structure by neutron reflection. We built supported single membranes (50 Å thickness) with macroscopic lateral extension (25 cm^2 area) by either layer-by-layer Langmuir-Blodgett/Langmuir-Schaefer deposition or vesicle fusion. The macroscopic extension of the single membrane allows for significant statistics, while keeping high detail in the description of the cross section. Neutron scattering takes also advantage from the isotopic H-to-D substitution to modulate the visibility of different components admixed in the membrane. We verified that either protocols are suitable for a good deposition of mixed systems, concerning in particular the macroscopic membrane integrity.

2. Materials and methods

d_{62}-DPPC, d_{75}-DPPC and d_{83}-DSPC were from Avanti Polar Lipids Co. GM1 ganglioside was extracted and purified according to [20]. D_2O (≥ 99% purity) was purchased by ILL.

Different membranes were been prepared by different protocols.

2.1. Langmuir films for isotherms and layer-by-layer membranes build-up

Phospholipids were dissolved in chloroform to a final concentration of 1 mg/ml, GM1 ganglioside was dissolved in chloroform:methanol (2:1 vol:vol) to the final 1 mg/ml concentration. Mixed systems were obtained by mixing appropriate amounts of the different organic solvent lipid solutions. 60 μl of the desired lipid solution were then deposited on the 450 cm^2 surface of a Langmuir trough filled with water kept at T = 22 °C. Monolayers were compressed up to collapse (~60 mN/m), while recording the corresponding (π-A) isotherms. For membrane deposition, layers were collected from the surface at 40 mN/m. All of the used monolayers are in the solid phase in these conditions.

2.2. Lipid vesicles and micelles

Unilamellar vesicles (roughly 100 nm diameter) were obtained by the following procedure: lipid powders (d_{75}-DPPC or d_{62}-DPPC:GM1 10:1 mol) were weighted in glass ball-shaped containers, dissolved in the appropriate organic solvent, then evaporated under continuous rotation so that lipid films were deposited over the balloons surface. Chloroform evaporation was completed under vacuum for 30 min. Then the films were submitted to a gentle stream of humidified nitrogen for 30 min, to disentangle multilayer compact stacks. Finally, a 150 mM NaCl water solution was added, to the final concentration of 0.5 mg/ml. The d_{62}-DPPC:GM1 10:1 mol system spontaneously forms unilamellar vesicles, whereas the d_{75}-DPPC multilamellar system was extruded through twinned polycarbonate filters (800 Å porosity) with a manual extrusor (LiposoFast, Avestin Inc.). Samples were then stored at 45 °C, above the chain gel-to-fluid transition, to ensure vesicle stability.

GM1 micelles were prepared by dissolving 1 mg of GM1 powder in 1 ml of pure water (MilliQ).

2.3. Membranes deposition

Solid supports were single crystals of silicon (5 × 5 × 1 cm^3) polished on one large face (111), cleaned before use with chloroform, acetone, ethanol and pure water in the sequence, and then treated with plasma cleaner. Supported membranes A and B were obtained by vesicle fusion, widely used for the deposition of membranes of selected phospholipids, applicable to neutron reflectivity measurements [11]. Vesicle solutions were incubated in the measuring cell during 40 min at 45 °C (d_{75}-DPPC) or 50 °C (d_{62}-DPPC:GM1 10:1). After 40 min the cell was thoroughly rinsed with deionized water to flush the excess vesicles and NaCl. Membrane A was then incubated with GM1, by injecting 25 μl of the GM1 micellar solution in the measuring cell. After 12 h at T = 55 °C, the cell was flushed with solvent. Supported membrane C and floating membrane D were layer-by-layer deposited on the silicon substrate by the Langmuir-Blodgett and Langmuir-Schaefer techniques. Asymmetric bilayers were built by completely removing and replacing the monolayer in the Langmuir trough in between different steps.

2.4. Pressure-area isotherms

Pressure-area (π-A) isotherms were recorded on a Nima Langmuir-Blodgett trough, using a Wilhelmy plate for pressure sensing. All (π-A) experiments were carried out at 22 °C (± 0.5), below the melting temperature of the used lipids. Water for the subphase was processed in a Milli-Q system (Millipore, Bedford, MA), to a resistivity of 18 MΩ·cm. Each lipid solution was spread over the water subphase and the organic solvent was allowed to evaporate completely, over 15 min. All isotherms were recorded using a barrier speed of 25 cm^2/min. Stability and reproducibility of Langmuir films were verified by performing various compression-expansion cycles on two different Nima Langmuir troughs.

2.5. Neutron reflectometry

In a neutron reflectivity experiment a neutron beam is sent at grazing angle to a stratified sample and the specular reflected beam is collected as a function of $q = (4\pi \sin\theta) / \lambda$, where θ is the incident angle and λ the neutron wavelength. The technique allows recovering the neutron scattering length density profile ρ(z) of a membrane along the transverse direction. Compositional asymmetry can be investigated by the use of selective deuteration. Measurements were performed on FIGARO [21] horizontal reflectometer at ILL (FR), in TOF mode, dq/q = 8%, reflection angles 0.8° and 3.2° or 0.8° and 2.8°.