Experimental report

12/02/2019

Proposal: 8-02-812 **Council:** 4/2017

Title: Membrane Interlinking by Glycolipids under Defined Repulsive Forces

Research area: Biology

This proposal is a new proposal

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Experimental team: Victoria LATZA

Local contacts: Bruno DEME

Samples: Phospholipids
glycolipids
salts (NaCl)

Instrument	Requested days	Allocated days	From	To
D16	7	7	13/03/2018	20/03/2018

Abstract:

Glycolipids bearing the neutral LewisX trisaccharide motif have been shown to be able to interlink lipid membranes via favourable saccharide-saccharide interactions, a phenomenon of documented biological relevance. Our recent experiments suggest that this behavior is not a unique property of the LewisX motif, but more prevalent among glycolipids than usually thought. Here we propose the systematic investigation by specular and off-specular neutron diffraction of lipid membranes containing glycolipids with various saccharide headgroups at defined mole fractions. In order to probe the strength of the membrane-interlinking, negatively charged lipids, which exert defined repulsive forces between the membranes, are introduced.

EXPERIMENTAL REPORT

EXPERIMENT N° **8-02-812** INSTRUMENT D16

DATES OF EXPERIMENT 13/03/2018 to 20/03/2018

TITLE Membrane Interlinking by Glycolipids under Defined Repulsive Forces

EXPERIMENTAL TEAM
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Important biological cell processes such as material transport and cell division involve spatiotemporal reorganization of membranes, including membrane adhesion, vesicle release, and the formation of lamellar structures[1]. Such reorganizations require continuous adjustments of the membrane interactions by the organism. The surface of biological membranes is mainly formed by the head groups of lipids and by membrane-bound polypeptides. It is known that glycolipids have a strong influence on membrane interactions. In contrast to phosphatidylcholine (PC) lipid vesicles, glycolipid membrane vesicles tend to aggregate[2]. Membrane-bound saccharides, like the neutral LewisX trisaccharide motif, induce membrane adhesion and were reported to play key roles in the aggregation of cells during embryonic development[3]. Knowledge about the influence of glycolipids on membrane interactions and the ensuing biological and medical implications has however remained limited to few examples. To elucidate how glycolipids affect membrane mechanical properties in terms of bending rigidity and inter-membrane confinement, we have established a methodology to extract such information from neutron diffraction from aligned membrane multilayers at controlled humidity and under bulk water and buffer conditions[4-6]. We also studied PC lipid membranes containing defined mole fractions of glycolipids bearing the LewisX trisaccharide (LewisX lipid)[4] . This behavior has been thought to be a result of the unique chemistry of the LewisX trisaccharide. However, experiments with other glycolipids which have gentiobiose, lactose ("lac"), or double-lactose ("lac2") head groups, showed a different picture.To investigate further we used the ternary lipid mixture approach: PC lipid membranes containing various fractions of glycolipid in the absence and presence of 0, 1 or 2 mol% of negatively charged phosphoglycerol (PG) lipid. Fig. 1B shows a set of small-angle x-ray scattering (SAXS, see Fig. 1B) results on mixtures with the glycolipid N-hexadecanoyl-lactosylceramide (*LaCer*-sat, see Fig. 1A) in buffer solution. It is seen that the negative charges have a strong effect on the lamellar spacing *d* (see Fig. 1C) especially for low glycolipid contents, while in the presence of glycolipids the *d*-spacing converges to a certain value. This shows that the membranes containing the highest fractions of glycolipids are less sensitive to charge, demonstrating that the sugar groups are able to bind the membranes together, confirming the feasibility of the approach.

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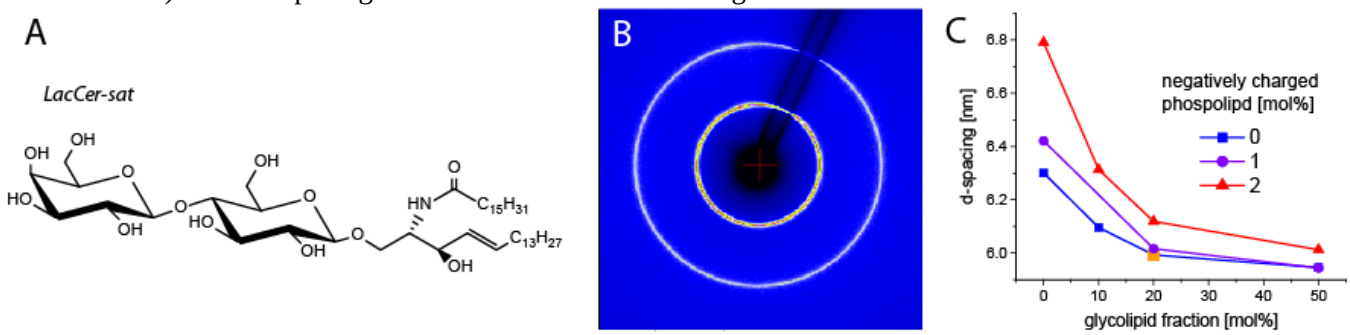
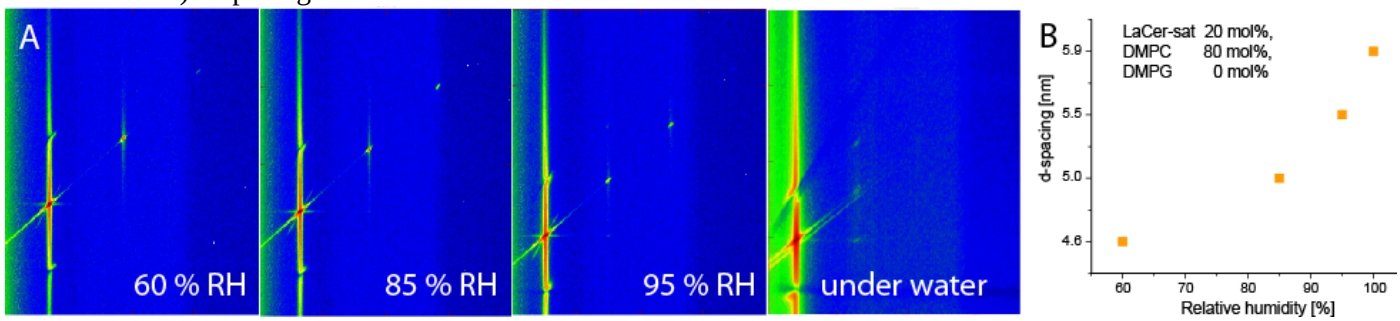
Fig. 1 A) Chemical structure of *LaCer*-sat. B) Exemplary SAXS 2D-scattering pattern, with first and second order reflections. C) Set of *d*-spacings for PC-membranes containing *LaCer*-sat.



During experiment 8-02-812 on D16 we investigated various ternary lipid mixtures in terms of *d*-spacing, phase-separation and mechanical properties of the membranes. As an example, with the sample *LaCer*-sat 20 mol%; PC lipid 80 mol% measurements where conducted at 3 different humidities and under bulk water conditions (see Fig. 3A. Under bulk water we can confirm the SAXS *d*-spacing of around 6 nm (see Fig. 3B, orange squares and Fig. 1C). From 60 to 95 % relative humidity (RH) the separation of the membranes is increasing (Fig. 3B).

Figure 3: A) Measurements conducted for *LaCer*-sat 20 mol%-sample at 60, 85 and 95 % relative humidity (RH) and under water. B) *d*-spacing as function of RH.



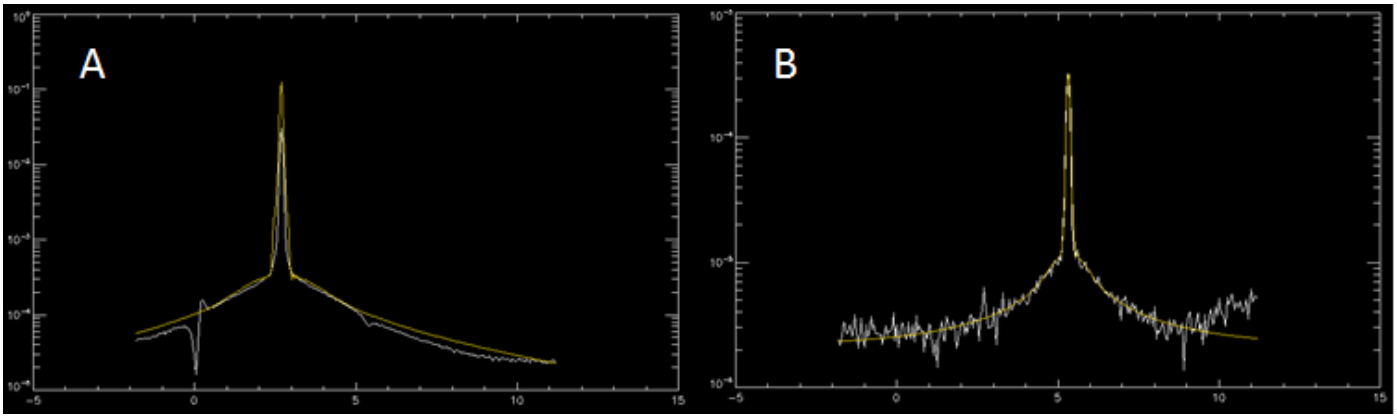
As shown in earlier work by Schneck et al.[4] the mechanical parameters bending modulus κ and compression modulus B can be extracted from the second Bragg sheet of the ND scattering signals. The membrane-displacement correlation functions can be expressed with the Caillé parameter η and the De Gennes parameter Λ for stacked membranes:

$$\eta = \frac{\pi k_B T}{2d^2\sqrt{\kappa B/d}} \quad \text{and} \quad \Lambda = \sqrt{\kappa/Bd}$$

For known lamellar periodicities d, the simulations are fully determined by the three free parameters η, Λ, and an adjustable size-cutoff. κ and η were calculated from the simulated results.

The intensity of the second Bragg sheet is low (Fig.4B), due to a nearby form factor minimum. The De Gennes parameter Λ is therefore fitted to the diffuse part of the first Bragg sheet (Fig.4A), while the specular intensity was ignored because of the breakdown of the kinematic approximation. The Caillé parameter is then obtained from the secon Bragg sheet, where the kinematic approximation is valid. For *LacCer-sat* 20 mol% (see Fig. 1A and B, Fig. 3) at 85 % RH we obtain κ at the order of 10 k_BT and B at the order of 10 MPa. Note that these results are still preliminary.

Figure 4: *LaCer*-sat 20 mol%-sample at 85 % RH A) 1. Bragg sheet (white) with simulation (yellow). Parameter Λ is optimized while ignoring the intensity of the specular peak, which in the first Bragg sheet violates the kinematic approximation. B) 2. Bragg sheet (white) with simulation (yellow). Caillé parameter η was optimized from the specular/diffuse intensity ratio, which in the second Bragg sheet is described by the kinematic approximation.



1. A. J. Bruce Alberts, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter, *Molecular Biology of the cell*, Garland Science, United States, 6th edn., 1983.
2. M. S. Webb and B. R. Green, *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1990, **1030**, 231-237.
3. B. F. Ivan Eggens, Tatsushi Toyokuni, Barbara Dean, Mark Stroud, Sen-itiroh Hakamori, *Journal of Biological Chemistry*, 1989, **264**.
4. E. Schneck, B. Demé, C. Gege and M. Tanaka, *Biophysical Journal*, 2011, **100**, 2151-2159.
5. E. Schneck, R. G. Oliveira, F. Rehfeldt, B. Demé, K. Brandenburg, U. Seydel and M. Tanaka, *Physical Review E*, 2009, **80**, 041929.
6. E. Schneck, F. Rehfeldt, R. G. Oliveira, C. Gege, B. Demé and M. Tanaka, *Physical Review E*, 2008, **78**, 061924.