Experimental report

21/09/2022

Proposal: **8-02-966**

Council: 4/2021

Title: Floating lipid bilayers stabilizedby adhesion-inducing glycolipids

Research area: Biology

This proposal is a new proposal

Main proposer: **Emanuel SCHNECK**

Experimental team: Emanuel SCHNECK
Lukas BANGE
Tetiana MUKHINA
Valeria Maria RONDELLI

Local contacts: Samantha MICCIULLA
Giovanna FRAGNETO

Samples: Phospholipids
glycolipids

Instrument	Requested days	Allocated days	From	To
D17	3	0		
FIGARO	3	3	01/10/2021	04/10/2021

Abstract:

Glycolipids influence the behavior of lipid bilayers in various ways with consequences for the functions of biological membranes. Based on our earlier work we have identified glycolipid/phospholipid binary mixtures comprising synthetic glycolipids that either strengthen bilayer adhesion or lead to enhanced chain ordering. Here we intend to study floating lipid bilayers (FLBs) incorporating different types of glycolipids at defined lateral densities and to structurally characterize them by NR. We are going to investigate (i) how the glycolipids affect the water layer thickness and FLB stability, (ii) whether glycolipids are recruited to the inner leaflet to increase the number of favourable sugar contacts and (iii) whether or not certain glycolipids reduce the bilayer fluidity and thereby slow down the flip-flop of phospholipids within the bilayer. Moreover, we will explore to what extent glycolipid-induced adhesion on the long trerm is suited to selectively promote the fusion or bilayer formation of native biomembrane vesicles.

EXPERIMENT N° **8-02-966** INSTRUMENTS **FIGARO**

DATES OF EXPERIMENTS **31/09/2021** to **04/10/2021**

TITLE Floating Bilayers

EXPERIMENTAL TEAM

Lukas Bange, Emanuel Schneck, Tetiana Mukhina, Valeria Rondelli, Giovanna Fragneto

LOCAL CONTACT Giovanna Fragneto

Date of report: September 21, 2022

Glycolipids can influence the behavior of lipid-based membranes in various ways, one aspect being the interactions between two adjacent membranes. High glycolipid concentrations substantially reduce hydration repulsion [1], but even low concentrations can already significantly strengthen membrane adhesion via weak sugar-sugar interactions [2].

Experiment 8-02-966 was aimed to investigate by neutron reflectometry (NR) the effect of such sugar-sugar binding on the structure and stability of solid-supported "floating lipid bilayers" (FLB) in which one lipid bilayer is used as a soft support for a second bilayer that floats on top of it. For the preparation we have used a combination of the Langmuir-Blodgett (LB) and Langmuir-Schaefer (LS) techniques to sequentially deposit four lipid monolayers onto solid substrates. While the first monolayer was always of pure phospholipid, layers 2-4 contained 20 mol% glycolipids, namely LacCer-sat, Trihexo-sat, or GM1 [2, 3]. Layers 1-2 were based on DSPC (forming densely-packed and tail-ordered layers up to 55 °C), while layers 3-4 were based on DPPC (tail melting temperature 41 °C). Ellipsometry experiments in our home laboratory allowed us to identify conditions under which the transfer preparation was most successful. Reproducibly excellent bilayer coverage was only achieved when completely dipping of the solid substrates under water. When incomplete coverage nevertheless occurred, then distinct mm-sized uncovered regions were found, which is in contrast to the usual picture of having bilayers with water-filled defects on the nanometer scale. This notion is of great relevance for the NR analysis (see further below).

One question to be answered by NR was whether preferential sugar-sugar interactions would accumulate glycolipids, via flipflop processes, at the inner leaflet of the outer bilayer. Fig. 1A shows NR curves in 3 water contrasts for a sample composed of tail-deuterated phospholipids but fully hydrogenous Trihexo-sat. The temperature was 50 °C, such that the outer bilayer was in the fluid state. The solid lines superimposed to the data points are simulated reflectivity curves corresponding to the best-matching parameters in a common model. The model is based on a rough-slab description of the volume fractions of phospholipid tails and headgroups, glycolipid tails and headgroups, and water, in addition to silicon and silicon oxide (see Fig. 1B). The number of free parameters is minimized by including physical constraints on symmetry and on coupled solvent-excluded volumes of headgroups and tails. Moreover, the H/D-exchange of the OH groups of the sugars is taken into account.

One fit parameter was the distribution of Trihexo-sat between the two leaflets of the outer bilayer. The best-matching model (corresponding to the solid lines in Fig. 1A) requires that Trihexo-sat is evenly distributed over the two leaflets, as seen in the volume fraction profiles in Fig. 1B. Fig. 1C shows the corresponding scattering density (SLD) profiles for the 3 water contrasts. The dashed lines in Fig. 1A and C indicate modeled reflectivity curves and SLD

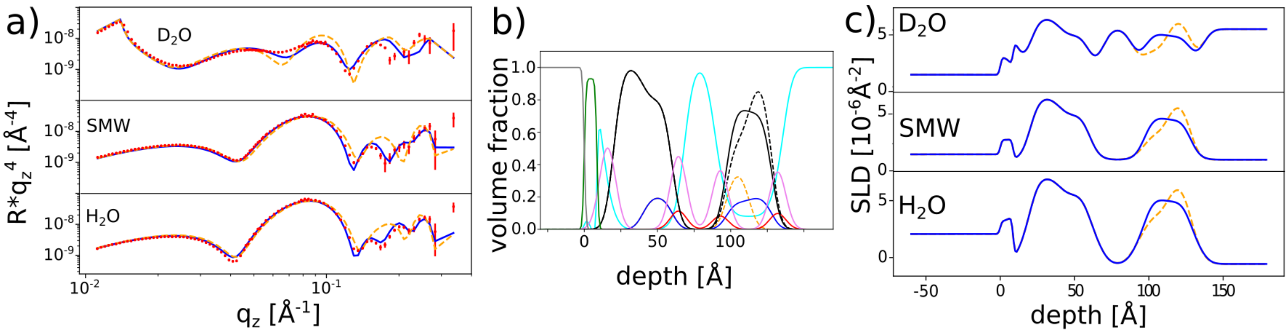

Figure 1: (a) Experimental reflectivity data (symbols) and model (lines) for a sample composed of tail-deuterated phospholipids but fully hydrogenous Trihexo-sat in three water contrasts. Solid blue line: best-matching model with glycolipids equally distributed over both leaflets. Dashed orange: when assuming the glycolipid concentrated in inner leaflet. (b) Volume fraction profiles of Si (grey), SiO_2 (olive), water (cyan), PC tails (black), PC headgroups, glycolipid tails (blue/orange), glycolipid headgroups (red). Solid lines: best-matching distribution. Dashed lines: When assuming the glycolipid concentrated in inner leaflet. (c) Corresponding SLD profiles. Solid blue line: best-matching model. Dashed orange: when assuming the glycolipid concentrated in inner leaflet.

profiles, respectively, under the assumption that all Trihexo-sat would be recruited to the inner leaflet. Obviously the experimental data are incompatible with this scenario. Overall, this NR study has demonstrated that:

1. FLBs with high coverage were formed for all examined systems except for the FLBs containing GM1, likely because of electrostatic repulsion.

2. The FLBs were remarkably ordered and stable, likely due to the attractive sugar-sugar interactions, and the glycolipid species was found to determine the inter-bilayer water layer thickness.

3. As suggested by ellipsometry, treating incompletely covered samples as weighted sum of reflectivity curves from covered and uncovered regions reproduces the reflectivity curves much better than dealing with averaged SLD profiles.

4. Selective deuteration led us to conclude that favorable sugar-sugar interactions with the supporting bilayer do not lead to an uneven distribution of glycolipids in the FLBs.

References

[1] Matej Kanduč, Alexander Schlaich, Alex H. de Vries, Juliette Jouhet, Bruno Demé, Roland R. Netz, and Emanuel Schneck. Tight cohesion between glycolipid membranes results from balanced water–headgroup interactions. Nat. Comm., 8(14899), 2017.

[2] Victoria M. Latza, Bruno Demé, and Emanuel Schneck. Membrane adhesion via glycolipids occurs for abundant saccharide chemistries. Biophysical Journal, 118(7):1602–1611, 2020.

[3] V Rondelli, G Fragneto, S Motta, E Del Favero, P Brocca, S Sonnino, and L Cantù. Ganglioside gm1 forces the redistribution of cholesterol in a biomimetic membrane. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1818(11):2860–2867, 2012.