Experimental report

08/04/2024

Proposal: 8-02-977 **Council:** 10/2022

Title: What's underneath? gangliosides rearrangement in cancer diseases

Research area: Biology

This proposal is a new proposal

Main proposer: Caterina RICCI

Experimental team: Giovanna FRAGNETO
Elena DEL FAVERO
Caterina RICCI

Local contacts: Ben HUMPHREYS
Nicolo PARACINI

Samples: DPPC, cholesterol
GM3, GM35Ngc

Instrument	Requested days	Allocated days	From	To
D17	4	2	17/05/2023	19/05/2023

Abstract:

The two major sialic acid variants that can be found in mammals gangliosides are N-acetylneuraminic acid (Neu5Ac) and N-glycolyneuraminic acid (Neu5Gc). Normally Neu5Gc-sialoconjugates are absent from human tissues. However, expression of N-glycolylated gangliosides has been observed in a wide range of human cancers. GM3(Neu5Gc) has been considered as a tumor-specific antigen and as a target for cancer immunotherapy. Moreover, membrane glycosphingolipids regulate lateral membrane movements via cholesterol and sphingolipid-rich membrane rafts. Changes in rafts have been shown to promote proliferative signaling through lateral membrane microdomain re-organisation.
Thus, the analysis of the arrangement of GM3 in lipid rafts and the observation of the differences in the behaviour of GM3Neu5Gc can be transferred in information for the development and optimization of novel therapeutic strategies based on targeting lipid rafts of cancer cell membranes.

We performed Neutron Reflectometry NR experiments (8-02-977 on D17) on bilayers of dDPPC (deuterated chains): cholesterol: GM3 (monosialodihexosylganglioside) ganglioside with two different sialic acid (NEu5Ac and Neu5Gc, from here GM3 and GM3GLY) at different relative concentrations (10:2.5:1 mol and 10:1.5:1 mol) in four contrast solvents (in Fig1 samples with dDPPC:chol:GM3 10:1.5:1 at different contrasts are reported). Membranes were prepared by Langmuir-Schaefer technique at PSCM laboratories, forming bilayers in which cholesterol and GM3 are present only in one of the two layers, respectively in the inner and outer layer. The measurements were performed before and after the annealing, to observe the redistribution of the cholesterol and of the gangliosides.
In Figure 1, right, the profiles of the samples (chol 1.5) with the different gangliosides are reported. We observed modifications of the intensity profile upon the incorporation of the different gangliosides. Also differences pre and post annealing are visible: in Figure 2 the intensity profiles of the samples with different gangliosides pre and post annealing in H2O are reported. This suggests a redistribution of the cholesterol linked to the presence of the ganglioside, as already observed in presence of GM1. In figure 3 the curves of each sample before and after the annealing are reported.

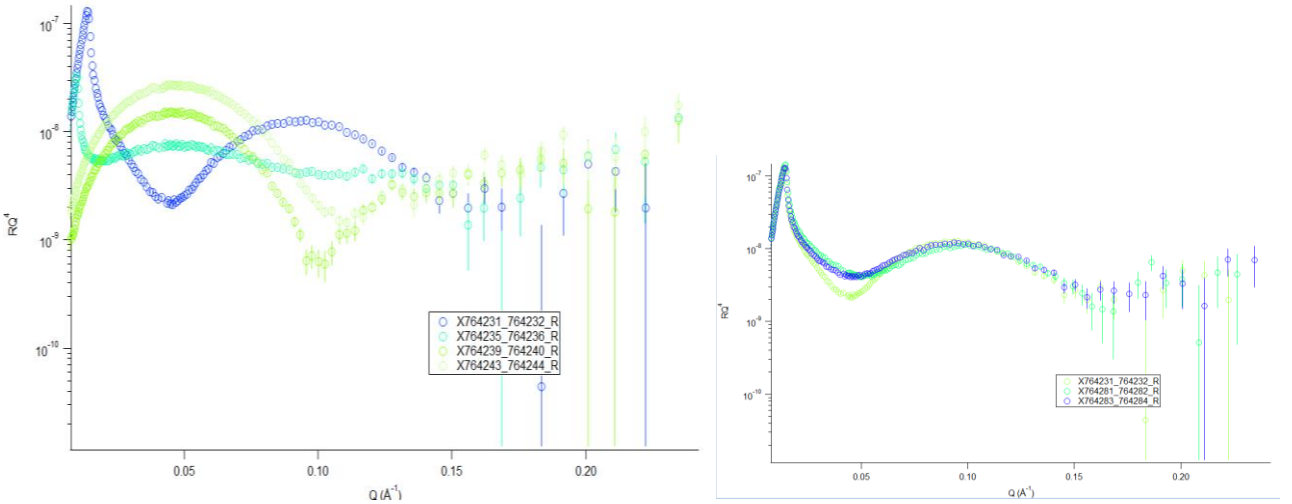

Figure 1: Left: NR spectra (RQ4 vs Q) of dDPPC:chol:GM3 10:1.5:1 in H2O, D2O, 4MW and SMW. Right: Samples with GM3 (light green), GM3GLY (blue) and only DPPC in D2O

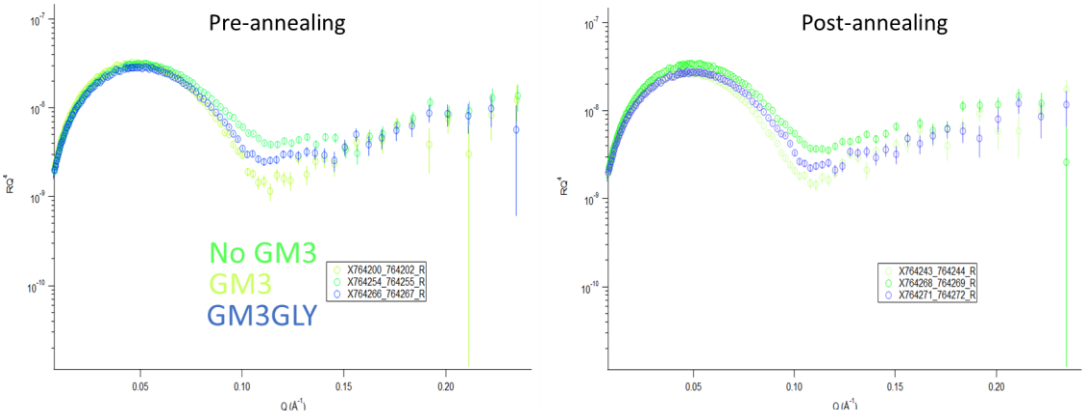

Figure 2: dDPPC:chol:GM3 10:1.5:1 mol in H2O before and after the annealing are reported

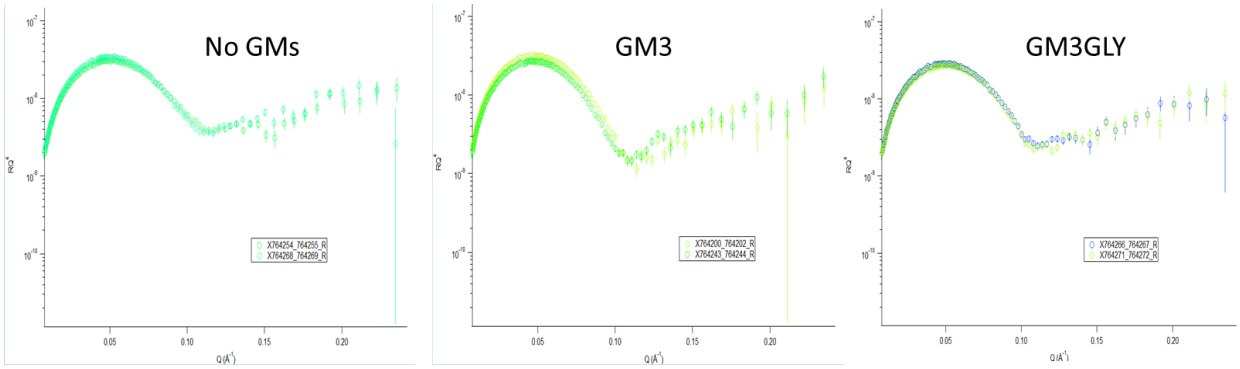

Figure 3 NR spectra (RQ4 vs Q) of samples without GM3, with GM3 and GM3 gly before and after the annealing

Further analysis is in progress to quantify the variations connected to the introduction of the gangliosides.