Determining the Effect of Galectin Insertion on Membrane Organization

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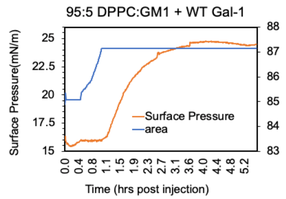
Background

The role of lectin protein and glycolipid interactions are a current and emerging interest in biochemistry due to their implications in biological functioning and pathology. Lectins are glycan-binding proteins that exist on the extracellular surface of membranes and recognize glycoconjugates on neighboring cells. Galectins more specifically exhibit conserved β-galactoside-binding sites unique to this family of lectins. Glycolipids are lipids that are covalently bound to sugar molecules at their head group and protrude from the cell membrane. Gangliosides are glycolipids covalently bound to complex oligosaccharides containing a sialic group. The use of specific saccharides at the cell surface by glycolipids to convey information is referred to as the 'sugar code' or glycocode. This code is read by lectin proteins to induce cellular changes. Lectins have extremely stereospecific carbohydrate binding domains that allow for lectins to bind with only a very specific glycolipid. This incredible binding specificity and different combinations of glycolipids per cluster allows for many complex signals to be read and interpreted by lectins, and translated into cellular change. The cellular changes regulated by the sugar code can have pathological implications when mutations and environmental conditions alter the lectin/glycolipid interactions causing cellular dysfunction. Lectins themselves are involved in inflammation, immune responses, cell migration, autophagy, and signaling. Their mutations are linked to heart disease, cystic fibrosis, cancer, as well as many other neurological diseases.

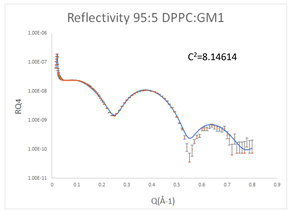
Results

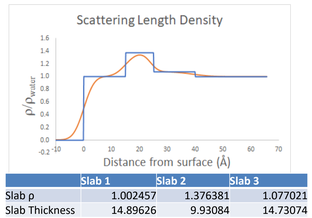
Langmuir Trough – WT Gal-1 Insertion Into Membrane



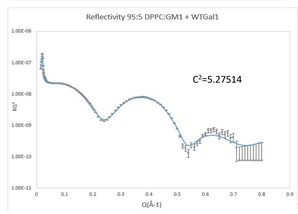
WT Gal-1 inserts into the membrane, evidenced by increase in surface pressure under constant area conditions.

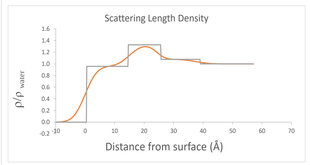
X-Ray Reflectivity – WT Gal-1 Inserts Into 95:5 DPPC:GM1





	Slab 1	Slab 2	Slab 3
Slab ρ	1.002457	1.376381	1.077021
Slab Thickness	14.89626	9.93084	14.73074

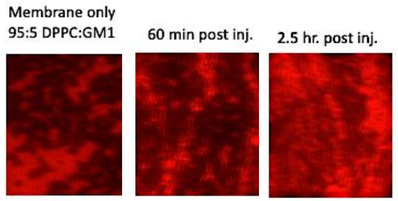




	Slab 1	Slab 2	Slab 3
Slab ρ	0.9784371	1.3871752	1.101412
Slab Thickness	14.92687	9.5221032	14.93081

WT Gal-1 inserts into head groups of monolayer containing 95:5 DPPC:GM1.

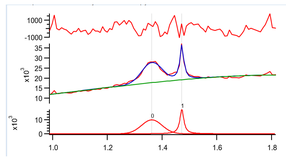
Fluorescence Microscopy of Langmuir Film – WT Gal-1 Shrinks Condensed Domains



WT Gal-1 causes reduction in LC domain size. Liquid expanded (LE) is fluorescent red, and liquid condensed (LC) is seen as black patches.

Grazing Incidence X-ray Diffraction – WT Gal-1 Impacts Lipid Packing

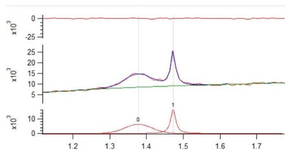
GIXD 95:5 DPPC:GM1



GIXD 95:5 DPPC:GM1 Calculated Values

Peak	Location (Å)	Area (Å²)	Full Width Half Max (FWHM)	L_c (Å)	Lattice Spacing
Peak 0	1.3627+/- 0.0008	933.09+/-20	0.087728+/- 0.002	64.459	4.6108
Peak 1	1.4724+/- 0.0003	474.16+/- 20	0.017401+/- 0.0008	324.97	4.6273
Total Area		1407.25+/- 40			

GIXD 95:5 DPPC:GM1 + WT Gal-1



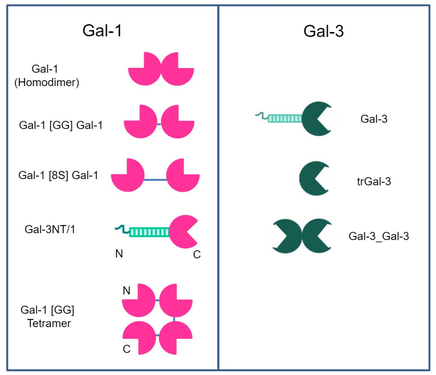
GIXD 95:5 DPPC:GM1 + WT Gal-1 Calculated Values

Peak	Location (Å)	Area (Å²)	Full Width Half Max (FWHM)	L_c (Å)	Lattice Spacing
Peak 0	1.3775+/-0.001	577.23+/-20	0.087917+/-0.003	64.471	4.561
Peak 1	1.47247+/- 0.0003	408.174+/-10	0.016119 +/- 0.0006	377.982	4.267
Total Area		985.408 +/-30			

WT Gal-1 reorganizes lipid packing structure of 95:5 DPPC:GM1 membrane

Discussion

- Gal-1 insertion into the 95:5 DPPC:GM1 membrane yields membrane reorganization.
- Data collected from multiple experiments verifies interactions with the lipid head groups and galectin protein.
- Further analysis of different mutant galectin proteins in different membrane conditions using collected XRD data will determine differences in membrane reorganization correlating to different galectin structure.



Methods /Techniques

Langmuir Trough

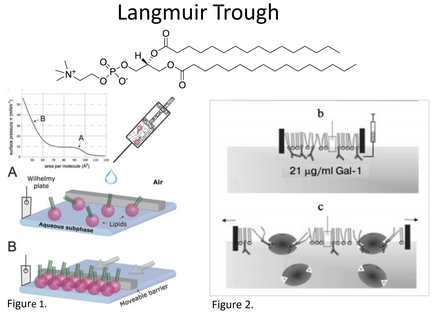


Figure 1. Figure 2.

Gangliosides deposited onto a liquid surface, will self-organize into 2D, monolayer film, known as a Langmuir monolayer. (Figure 1 and Figure 2). DPPC lipid above.

Liquid Surface X-Ray Scattering

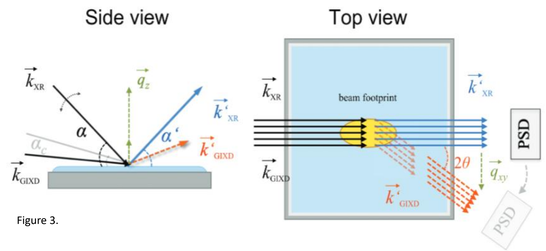


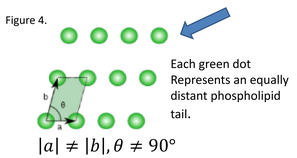
Figure 3.

X-ray Reflectivity (XR) : measurements yield information about the out-of-plane (vertical) monolayer structure.
Grazing Incidence X-ray diffraction (GIXD): measurements provide structural in-plane information of the monolayer

Lipids Pack Into Oblique Unit Cell

Oblique (monoclinic) Unit Cell

Figure 4.



Each green dot Represents an equally distant phospholipid tail.

$|a| \neq |b|, \theta \neq 90°$

Equations for calculations in Tables D and H

$$\text{Lattice Spacing} = \frac{2\pi}{qxy}$$

Spacing in between each lipid molecule

$$L_c = \frac{0.9 \times 2\pi}{FWHM}$$

Length of coherently scattering object

In (figure 4) we see an oblique unit cell with that shows the two different lengths of $\rightarrow$ a and $\rightarrow b$, which correlate to the two different diffraction lengths and *qxy* values used to determine the peak positions of lipids arranged in a semi crystalline manner.

References and Acknowledgements

References:

Majewski, J., André, S., Jones, E., Chi, E., and Gabius, H.-J. (2015) *Biochemistry (Moscow) 80*, 943–956.

Crystal Vander Zanden Advanced Biochemistry Lecture 18, University of Colorado at Colorado Springs, April 2020

Acknowledgements:

NSF's ChemMatCARS Sector 15 is supported by the Divisions of Chemistry (CHE) and Materials Research (DMR), National Science Foundation, under grant number NSF/CHE- 1834750. Use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory, was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357.

National Science Foundation: Any opinion, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

UCCS start up funds from the Department of Chemistry and Biochemistry

Chad Sallaberry and WIliam Stone for helpful discussions related to data analysis

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