Experimental Report

Proposal: **9-13-508** **Council:** 10/2012

Title: Glycerol and glucose behavior at membrane interfaces

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples:
glucose-C6H12O6
glycerol-C3H8O3
DMPC - C36H72NO8P
POPC -C42H82NO8P
POPS - C40H75NO10PNa
POPG - C40H76O10PNa
D2O

Instrument	Req. Days	All. Days	From	To
D11	0	2	09/07/2013	11/07/2013

Abstract:

The biological membrane is essentially an impermeable lipid barrier. This allows for environment differences between the inside and outside of the cell, such as metabolite concentrations and pH. Even though the membrane is impermeable, given enough time, virtually any molecule will diffuse through a protein-free lipid bilayer. Since the cell requires the fast transport of large or charged molecules through the membrane, the membrane contains proteins that specifically transport these across the membrane. Glycerol is a molecule that can diffuse freely through the membrane while its dimer, glucose, does not. Glucose is of particular importance as its excess in the bloodstream is toxic and is associated with the disease diabetes. Insulin is needed to signal the uptake of glucose into the cell. Recent studies however show that insulin resistance (one of diabetes type 2 characteristics) has been correlated by a change in the lipid make up and thus permeability of muscle cells. We propose to study the diffusion properties of glycerol and glucose through model membranes to obtain insight into this lipid composition based diffusion arrest behavior.

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Experimental report:

The lipid bilayer, the basic unit of biological membranes, is an essentially impermeable barrier. Because of this, the environment inside the cell can be significantly different from the environment surrounding it such as pH as well as ion compositions and concentrations. Also, within the cell, different microenvironments can exist because lipid membranes surround them; examples of these are the cell's organelles. However, given enough time, virtually any molecule will diffuse through a protein-free lipid bilayer. How long it takes to do so, varies enormously of course; those that are small and partition favorably into an oily phase will diffuse faster than those that are large or charged as shown in figure 1 (1). Since several cellular functions require the fast transport of large or charged molecules through the membrane, the membrane contains assisting proteins that specifically transport them across the membrane. For example, the Na and K pump is of particular significance to nervous cells, as it is responsible for responding to stimuli and transmitting impulses in short intervals of time.

As is schematically shown in figure 1, glycerol and glucose, the latter being the dimmer of the former, are good examples of molecules on either side of the line that divides permeable and non-permeable molecules in a passive diffusion mode, ie, without the assistance of transporter proteins. Glucose is of particular importance as its excess in the bloodstream is toxic and is associated with the disease diabetes. It is well known that the hormone insulin and its target G- protein coupled receptor (GPCR) are required for the uptake of glucose by cells. Recently it was discovered that one of the main symptoms of diabetes type II is the extreme built-up of lipids in muscles leading to insulin resistance (2). The current treatment for Diabetes type II consists of increasing the insulin concentration by either drug stimulation or by external application of insulin. Therefore understanding the behavior of these molecules at the interface of model membranes could hold clues to alternative passive triggers for the treatment of this disease.

Recent work by Pocivavsek et al. explored the role of glycerol on the mechanical stability of model lung surfactant monolayers made from mixtures of DPPC and POPG (3). They found that, in the absence of proteins, glycerol preferentially enriches the membrane –water interface. They also found that that the mechanical properties of the membrane are significantly affected by this enrichment resulting in the stiffening of the membrane. This stiffening is possibly explained by a vitrification effect at the surface of the membrane where carbohydrates are competing with and replacing water in the lipid headgroup region by hydrogen bonding with its polar moieties. This gave a possible explanation as to why, as far as lung surfactants are concerned, the body keeps a low concentration of these sugary molecules, otherwise, as this study seems to indicate, consistent high levels of them (predominantly glucose) have a toxic effect that could develop into breathing problems.

In experiment 9-13-508 we followed the behavior of both glycerol and glucose at the interface of water and a lipid membrane. We found that 50nm POPC vesicles

exposed to glycerol will diffuse through and swell the vesicles but keep their spherical structure intact (figure 2). Also, there is no clear sugary adlayer on the surface of the vesicles. An adlayer of sugary molecules has as previously reported in lipid monolayers (3). On the other hand, when exposed to glucose, the vesicles become ellipsoidal in shape. This is consistent with the idea that the membrane significantly rigidifies in the presence of sugary molecules which would tend to promote a flat membrane configuration.

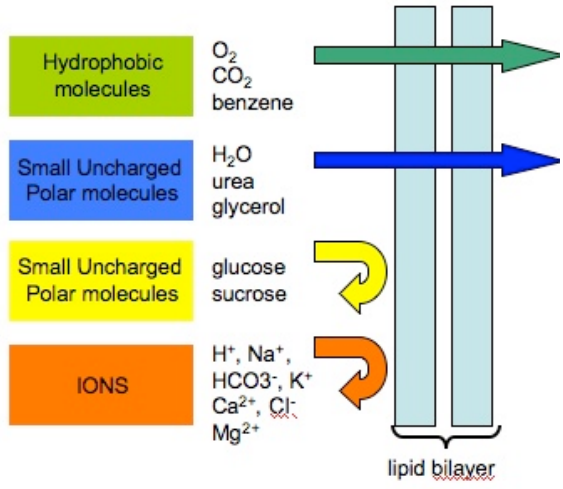

Figure 1. Relative permeability of a lipid bilayer to different classes of molecules (1).

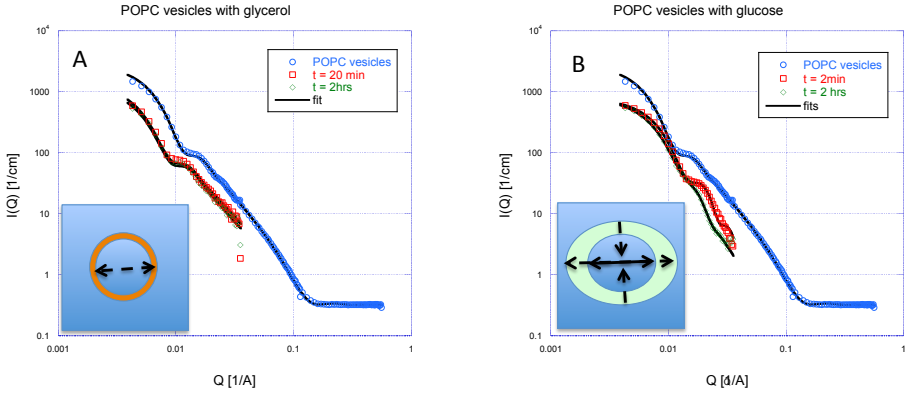

Figure 2. POPC vesicles in 100% D2O at 38°C in (A) a 50% by volume glycerol solution and (B) a 32% by volume saturated glucose (curves shifted for clarity).

(1) B Alberts, D. Bary, J. Lewis, M. Raff, K. Roberts, J. Watson. Molecular Biology of the cell. Third edition. Page 508 (2) Patrick Schrauwen and Matthijs K.C. Hesselink. Oxidative Capacity, Lipotoxicity, and Mitochondrial Damage in Type 2 Diabetes. Diabetes (2004) 53(6):1412-1417.

(3) Luka Pocivavsek, Kseniya Gavrilov, Kathleen D. Cao, Eva Y. Chi, Dongxu Li, Binhua Lin, Mati Meron, Jaroslaw Majewski, and Ka Yee C. Lee. Glycerol-Induced Membrane Stiffening: The Role of Viscous Fluid Adlayers. Biophysical Journal (2011) 101:118–127