Experimental report

12/09/2018

Proposal: 8-04-812 **Council:** 4/2017

Title: Lipid dynamics in yeast cell membranes

Research area: Biology

This proposal is a new proposal

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Samples: yeast lipid extracts

Instrument	Requested days	Allocated days	From	To
IN16B	2	2	13/06/2018	15/06/2018

Abstract:

Cell membranes are complex lipid-protein assemblies that regulate a wide variety of cell functions from metabolism to cell-cell interactions, including the immune response. A wealth of knowledge has been produced about the biophysics and chemistry of simple lipid systems, but there have been few structural studies and even fewer dynamical studies of the complex lipid mixtures found in typical cell membranes. We recently developed methods for the production and purification of deuterated Pichia pastoris yeast lipids at large scale in collaboration with the ILL D-lab and the PSCM, and have recently also grown a number of other yeast strains at the Lund Protein Production (LP3) facility in both normal and deuterated form. We would, therefore, like to attempt the first inelastic and quasi-elastic experiments to compare the dynamical behavior of yeast lipid mixtures to the dynamics observed in simple membranes. In the first instance, we would like to measure the incoherent dynamics and characterize molecular diffusion of the total lipid extracts using non-deuterated lipids.

Experiment report: 8-04-812 Lipid dynamics in yeast cell membranes

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Cell membranes are complex lipid-protein assemblies that regulate a wide variety of cell functions from metabolism to cell-cell interactions, including the immune response. A wealth of knowledge has been produced about the biophysics and chemistry of simple lipid systems, but there have been few structural studies and even fewer dynamical studies of the complex lipid mixtures found in typical cell membranes. We recently developed methods for the production and purification of deuterated Pichia pastoris yeast lipids at large scale in collaboration with the ILL D-lab and the PSCM, and have recently also grown a number of other yeast strains at the Lund Protein Production (LP3) facility in both normal and deuterated form.

We carried out the first QENS experiment on IN16B in June 2018 to investigate the dynamics of lipid extracts from *Pichia pastoris yeast*[1] that contain range of phospholipids (PC, PE, PS, PI, cardiolipin), nonpolar lipids (triglycerides, diglycerides) as well as ergosterol. The study was motivated by our earlier neutron reflection and diffraction results that indicate significant differences in both membrane structure[2, 3] and the interaction with the antifungal therapeutic Amphotericin B[4]. Another difference to typical model lipid systems is that these cell lipids bear a mixture of hydrocarbon chains that vary in length between 14-18 carbons and have a significant portion (up to 40%) of polyunsaturated chains (mainly linoleic 18:2, and linolenic 18:3). Our aim was to investigate the incoherent dynamics of yeast lipid extracts to characterize molecular diffusion at the ns timescales probed by IN16B. In this first experiment our main goal was to determine how the dynamics compare to a well-characterised model lipid, such as DMPC, that has previously been investigated thoroughly by others[5, 6]. The samples consisted of sonicated vesicles of the lipids dispersed in D_2O at a concentration of 50-100mg/ml (5-10wt%). In these samples the dynamics are faster than in hydrated membranes stacks but were thought to be a good starting point for determining the relative mobility of the lipids compared to DMPC while using a relatively small quantity of lipids.

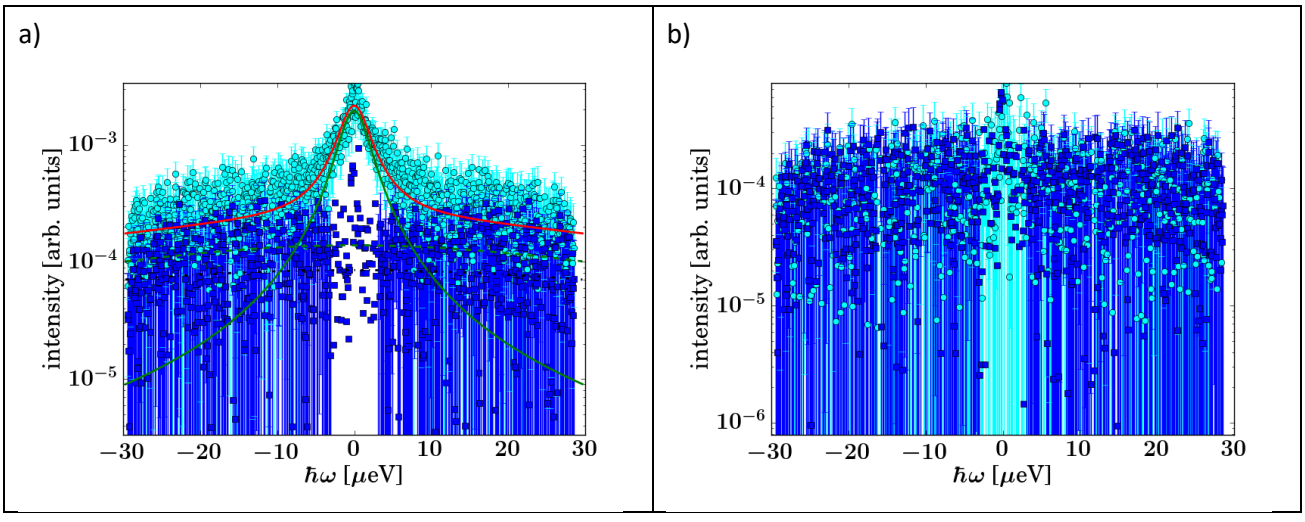

Figure 1. a) 50mg/ml DMPC vesicles at 303K, for q=0.69/A, T=303K, showing the sample (cyan circles) and pure solvent (blue squares) signal subsequent to empty ell subtraction with a tentative fit (lines) as explained below and b) yeast lipid extract vs pure solvent under the same conditions.

DMPC shows a clear quasi-elastic signal above the solvent background (Figure 1a), that can be fitted with a one Lorentzian with a Brownian q-dependence (green solid line), one with jump diffusion q-dependence (green dashed line), and one Lorentzian for the D_2O solvent (black dashed line). This preliminary model gives a Brownian diffusion coefficient of D = (6.26±0.24) $Å^2$/ns. On the contrary, the yeast lipid extract shows now detectable QENS signal in the IN16B window, and the sample and solvent signals in Figure 1b) are nearly identical. Furthermore, 30mol% of DMPC mixed into a perdeuterated yeast lipid sample (data not shown) did not have any QENS signal that could be attributed to the incoherent DMPC dynamics, suggesting that the yeast

lipid matrix also modified the dynamics of this model lipid. The fully deuterated yeast lipid did not exhibit any measurable coherent signal in the IN16B window either. Clearly, there is a significant difference in the molecular dynamics of yeast lipids and DMPC that warrant further investigation.

1. de Ghellinck, A., et al., *Production and Analysis of Perdeuterated Lipids from Pichia pastoris Cells.* Plos One, 2014. **9**(4): p. 9.
2. Gerelli, Y., et al., *Multi-lamellar organization of fully deuterated lipid extracts of yeast membranes.* Acta Crystallogr D Biol Crystallogr, 2014. **70**(Pt 12): p. 3167-76.
3. Luchini, A., et al., *The impact of deuteration on natural and synthetic lipids: A neutron diffraction study.* Colloids and Surfaces B: Biointerfaces, 2018. **168**: p. 126-133.
4. de Ghellinck, A., et al., *Lipid polyunsaturation determines the extent of membrane structural changes induced by Amphotericin B in Pichia pastoris yeast.* Biochimica et Biophysica Acta (BBA) - Biomembranes, 2015. **1848**(10, Part A): p. 2317-2325.
5. Busch, S., et al., *The picosecond dynamics of the phospholipid dimyristoylphosphatidylcholine in mono- and bilayers.* Soft Matter, 2012. **8**(13): p. 3576-3585.
6. Busch, S., et al., *Molecular Mechanism of Long-Range Diffusion in Phospholipid Membranes Studied by Quasielastic Neutron Scattering.* Journal of the American Chemical Society, 2010. **132**(10): p. 3232-3233.