Methanol accelerates DMPC flip-flop and transfer: A SANS study on lipid dynamics

M. H. L. Nguyen,[1] M. DiPasquale,[1] B. W. Rickeard,[1] C. B. Stanley,[2] E. G. Kelley,[3] and D. Marquardt[1]

Lipid bilayers form the structural backbone of cellular membranes and possess marked lateral and transversal organization of lipids. This strict lipid organization has implications in vital cellular processes, including protein function and localization, vesicle fusion and budding, and apoptosis. Lipids undergo diffusive motions that can disrupt their carefully assembled organization. This study focused on lipid exchange (between bilayers) and transverse diffusion (lipid flip-flop) as the former pertains to how lipids arrive, remain, and leave cellular membranes, whereas the latter disrupts the maintained membrane asymmetry (i.e., the compositional difference between leaflets) found in cells. In essence, both dynamical actions are intrinsically linked to bilayers and their compositional stability. Here, we examined how the common organic solvent methanol impact these dynamics.

In this work, we applied very small angle neutron scattering (VSANS) to monitor lipid mixing of two distinct 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) populations, one chain perdeuterated DMPC (d-DMPC) and the other fully protiated DMPC (h-DMPC), in the presence of an increasing deuterated methanol concentration. The measurements were achieved by setting the ratio of H_2O and D_2O (here 45 % D_2O) such that the water solvent neutron scattering length density was matched to uniformly mixed d-DMPC/h-DMPC vesicles. Unmixed vesicles will thus display contrast versus the water solvent, resulting in heightened scattering intensity, whereas fully mixed samples will display scattering intensities akin to the solvent background (i.e., a flat and featureless curve). Thus, as h-DMPC and d-DMPC large unilamellar vesicles begin to mix via lipid monomers transferring within and between bilayers, the measured intensity will decay and eventually reach an intensity baseline, corresponding to a single population of completely mixed vesicles (shown in Fig. 2a). Fig 1 illustrates such a strategy [1]. Further, a normalized intensity decay was calculated from the collective scattering curves of each sample and analyzed with a model for exchange/flip-flop (Fig. 2b). With this experimental setup, we quantified both DMPC flip-flop (k_f) and exchange (k_e) rates under the influence of methanol.

Increasing methanol concentrations had a profound effect on the kinetics of DMPC monomers in free-floating vesicles. Despite differences in vesicle size and investigative techniques, our unperturbed DMPC flip-flop and transfer rates agree and contrast previous findings. For example, Gerelli *et al*. used neutron reflectometry to measure DMPC flip-flop and exchange between vesicle dispersions and adsorbed planar bilayers [2]. Their exchange half-times for fluid-phase DMPC coincide with values found here (timescale of hours), whereas flip-flop was magnitudes faster (≤ 2.5 min). As recently shown however, the incomplete surface coverage of planar bilayers results in microscopic defects, which can facilitate lipid flip-flop and thus result in flip-flop rates on the order of seconds to minutes [3]. This study, with fully sealed vesicles, bypasses such issues.

More significantly, our results reveal that methanol accelerates both fluid-phase DMPC flip-flop and transfer rates. The flip-flop rate increases exponentially, whereas the exchange rate increases linearly under the studied concentrations (Fig. 2c). Methanol has by far the weakest hydrophobic character in the short-chain alcohol group yet seems to perturb the membrane through a similar fashion as longer chained alcohols and alkyl diols. Although it has been shown that other short-chain alcohols affect inward flipping rates, to our knowledge, we provide new insights with regards to both flip-flop and transfer rates in the presence of methanol. In general, at low methanol levels, DMPC undergoes slower flip-flop than transfer, but at methanol volume fractions greater than 2 %, the situation is reversed. Interestingly, these observations suggest that methanol affects the two dynamical processes in distinct ways.

Dynamic light scattering (DLS) measurements of size and polydispersity were taken before and after incubation at multiple methanol concentrations, neither of which revealed significant changes, maintaining a vesicle diameter of 140 nm and a polydispersity index of 0.15 ± 0.02. Significantly, these results suggest fusion events did not occur as an increase in mean particle size and a polydispersity index would have been observed. Furthermore, SANS and small angle x-ray scattering (SAXS), were applied. SANS and SAXS are complementary techniques used to probe sample structure and are known to be extremely sensitive to membrane lamellarity and lipid bilayer structure. Both SANS and SAXS yielded curves that displayed diffuse scattering (i.e., no detectable sharp Bragg peak) demonstrating that multilamellar bilayers did not evolve under the presence of d-methanol. Collectively, DLS, SAXS and SANS indicate that methanol did not

1 University of Windsor, Windsor, Ontario ON N9B 3P4, Canada
2 Oak Ridge National Laboratory, Oak Ridge, TN 37831
3 NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD 20899

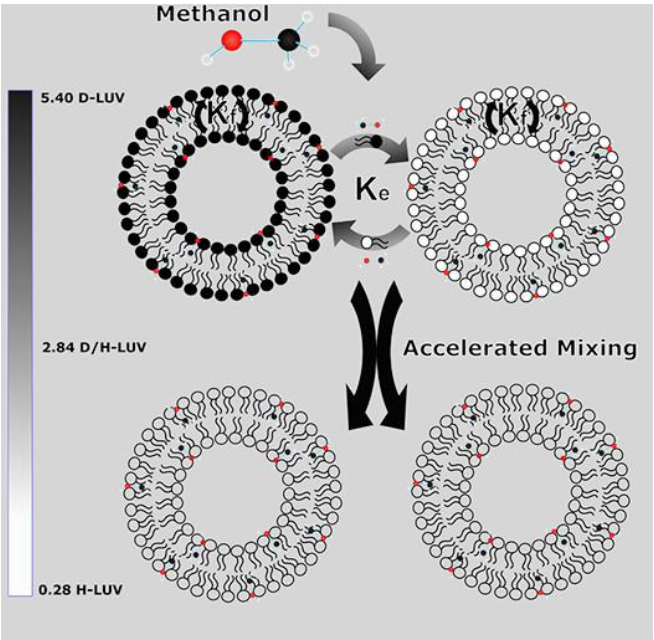

FIGURE 1: Contrast matching strategy employed. Vesicles composed solely of d-DMPC d_{54}-DMPC) and h-DMPC are placed together in a H_2O/D_2O (55/45) mixture with varying amounts of methanol, contrast matched to fully mixed vesicles of d-DMPC and h-DMPC. Over time, intensity loss can be monitored as vesicles mix (via exchange and flip-flop) and near the contrast match point.

alter the vesicles' morphological structure at these concentrations. To determine if a defect-mediated mechanism is responsible, we examined pertinent bilayer properties. Previous in silico studies revealed that area per lipid (A_L) generally increases with increasing alcohol concentrations and this led to greater occurrences of bilayer defects [4]. Such defects can permit lipid headgroups to traverse the bilayer core and flip-flop. A joint refinement of SANS and SAXS data revealed that this was not the case; the scattering profiles of pure lipid and methanol-treated samples are near indiscernible. In terms of relevant bilayer structural parameters, the A_L and bilayer thickness were essentially unchanged. Thus, the most likely explanation must involve methanol inducing short-range and perhaps short-lived defects, which are thus difficult to discern via methods (as those used here) that measure an ensemble structural average.

Time-resolved SANS in combination with structural SANS, SAXS, and DLS measured the dynamical and structural effects of methanol on DMPC liposomes. Though structural deviations were not detected in the concentration regime studied, DMPC flip-flop and exchange were markedly enhanced. Our findings highlight an additional complication when externally adding biomolecules, whether it be protein, peptides or drugs. For example, in antimicrobial peptide (AMP) studies, though AMP attack is better simulated by external addition, these studies have the potential to incorrectly assign the cause of the enhanced lipid kinetics to the AMP. Ultimately, this work highlights the importance of understanding the interplay between the system of interest and the carrier solvent on lipid mobility whether it be model lipid systems or *in vitro* screening in cell biology.

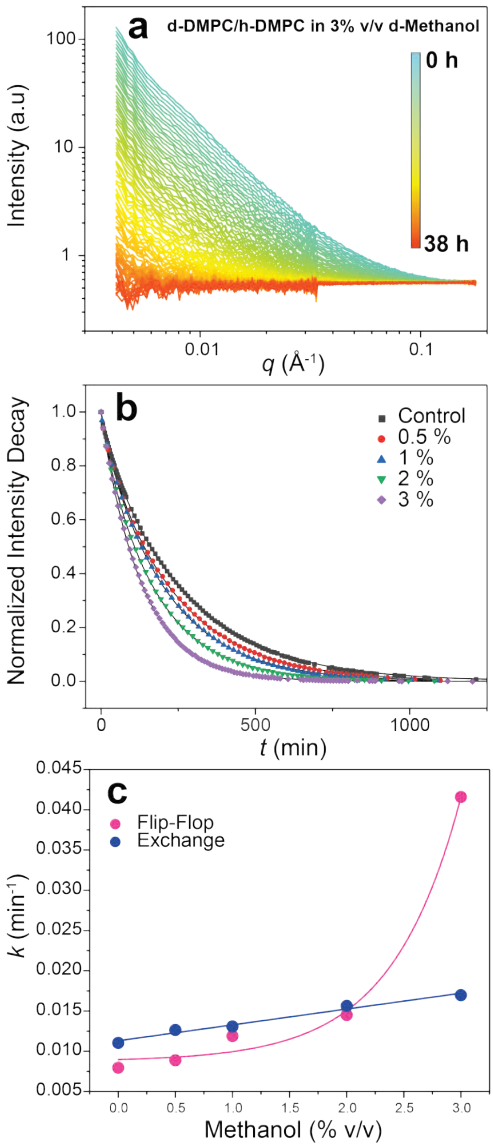

FIGURE 2: (a) SANS curves of d-DMPC and h-DMPC vesicles with 3% (v/v) d-methanol solvent. Periodic measurements were conducted at 37 °C over 38 h. (b) Normalized contrast decay curves of increasing d-methanol presence; continuous lines indicate fitted curves used to derive flip-flop and lipid exchange rate constants. Each data point represents the normalized integrated intensity of a single SANS curve like those found in (a). (c) Plot of measured flip-flop and lipid exchange rate constants as a function of d-methanol volume percent. Solid lines represent curves of best fit.

References

[1] M. Nguyen, M. DiPasquale, B. Rickeard, C. Stanley, E. Kelley, D. Marquardt, Biophysical Journal, **116**(5), 755 (2019).

[2] Y. Gerelli, L. Porcar, L. Lombardi, G. Fragneto, Langmuir, **29**(41), 12762 (2013).

[3] D. Marquardt, F. A. Heberle, T. Miti, B. Eicher, E. London, J. Katsaras, G. Pabst, Langmuir, **33**(15), 3731 (2017).

[4] H. V. Ly, M. L. Longo, Biophysical Journal, **87**(2), 1013 (2004).