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Xinxin Woodward, Eric E. Stimpson, Christopher V. Kelly

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Single-lipid tracking on nanoscale membrane buds: The effects of curvature on lipid diffusion and sorting

Xinxin Woodward, Eric E. Stimpson, and Christopher V. Kelly[&]

Department of Physics and Astronomy, Wayne State University, Detroit, MI
[&] Corresponding author. Email: cvkelly@wayne.edu

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Membrane curvature, single-particle tracking, molecular sorting, diffusion, single-molecule localization microscopy, Monte Carlo

ABSTRACT

Nanoscale membrane curvature in cells is critical for endocytosis/exocytosis and membrane trafficking. However, the biophysical ramifications of nanoscale membrane curvature on the behavior of lipids remain poorly understood. Here, we created an experimental model system of membrane curvature at a physiologically-relevant scale and obtained nanoscopic information on single-lipid distributions and dynamics. Supported lipid bilayers were created over 50 and 70 nm radius nanoparticles to create membrane buds. Single-molecule localization microscopy was performed with diverse mixtures of fluorescent and non-fluorescent lipids. Variations in lipid acyl tales length, saturation, head-group, and fluorescent labeling strategy were tested while maintaining a single fluid lipid phase throughout the membrane. Monte Carlo simulations were used to fit our experimental results and quantify the effects of curvature on the lipid diffusion and sorting. Whereas varying the composition of the non-fluorescent lipids yielded minimal changes to the curvature effects, the labeling strategy of the fluorescent lipids yielded highly varying effects of curvature. Most conditions yield single-population Brownian diffusion throughout the membrane; however, curvature-induced lipid sorting, slowing, and aggregation were observed in some conditions. Head-group labeled lipids such as DPPE-Texas Red and POPE-Rhodamine diffused >2.4x slower on the curved vs. the planar membranes; tail-labeled lipids such as NBD-PPC, TopFluor-PPC, TopFluor-PIP2, $DiIC_{12}$, and $DiIC_{18}$ displayed no significant changes in diffusion due to the membrane curvature.

1) INTRODUCTION

Nanoscale curvature of the plasma membrane is tightly regulated during endocytosis and exocytosis via the dynamics and sorting of membrane components [1–4]. For example, signaling lipids such as PIP_2 are concentrated at sites of endocytosis and exocytosis, although the mechanisms of their sorting are unknown [5,6]. Recent studies of lipid distributions show a possible inherent sorting of lipids at curvature sites that correlate to the lipid molecular shape [7–9] and a significant slowing of single-lipid diffusion correlated to membrane curvature [10–12]; however, some studies show no effects of curvature on molecular distribution and dynamics [13–16]. These potentially contradictory results and the underlying biophysical interplay between lipids and curvature are poorly understood despite numerous studies demonstrating the importance of lipid-composition-dependent bending rigidity, phase behavior, and molecular sorting [17].

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