Separation and Identification of Raft Associated Membrane Species Using a Patterned Supported Lipid Bilayer Extractor

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Abstract:  
New methods toward manipulation and study of membrane species within a lipid bilayer platform are desired because lipid bilayers are able to maintain native conformation and function of intrinsic membrane species. This report demonstrates design and use of a bilayer patterned device for separation, sorting, and categorization of membrane biomolecules based on their affinity for different co-existing lipid phases within a patterned, heterogeneous supported lipid bilayer.

Summary:  
Membrane proteins and glycolipids are the primary targets for therapeutic development, but processing and handling any membrane-bound species while maintaining intact structural information, proper orientation, and necessary lipid associations remains a large bottleneck to characterizing and understanding their structure-function behavior [1]. Many purification strategies rely on the use of harsh chemicals and conditions that cause denaturation (in the case of proteins) and disrupt orientational order necessary for binding to soluble species and maintaining critical lipid membrane interactions or environments (e.g., lipid microdomains) that are important for function [2].

We recognized that the physico-chemical properties and spatial chemical heterogeneity of the supported bilayer can be tailored to facilitate carrying out unit operations on membrane-bound species. Our new approach separates membrane-bound species based on partitioning preference of a species between two lipid phases within a supported lipid bilayer. This separation process in the two-phase lipid bilayer is somewhat analogous to a classical single stage liquid-liquid extraction except here the immiscible “liquids” are two immiscible but mobile lipid bilayers that meet along an interface inside a microfluidic device. One kind of lipid domain, termed a lipid raft, is defined as a cholesterol and sphingolipid rich lipid phase. The other domain in our system is a more fluid phospholipid-rich domain. This design can be used to identify and isolate lipid raft residents from species that prefer more disordered lipid environments by tracking their partitioning behavior in the heterogeneous bilayer [3].

In this report, we use microfluidics to construct heterogeneous SLBs with controllable phase patterns. As illustrated in Figure 1, via laminar flow patterning, we create parallel stripes of lipid raft phase (black) and POPC-rich phase (grey) in the microchannel and load a mixture of membrane-bound biomolecules to sort (white and dark grey disks) in the side channel. The interface between the phases is contiguous, allowing molecules to diffuse across and partition into a preferred phase as they are transported down the main channel towards collection ports.

Figure 1: Illustration of the separation device. Laminar flow configuration in a microfluidic device is used to create parallel stripes of lipid raft phase (black) and POPC-rich phase (grey) in the microchannel and to load a mixture of membrane-bound biomolecules to sort (white and dark grey disks) in the side channel. The interface between the phases is contiguous, allowing molecules to diffuse across and partition into a preferred phase as they are transported down the main channel towards collection ports.
By modifying the chemical composition of the lipid phases, channel dimensions, or the convection rate of the mixture down the channel, extraction efficiency can be tuned. We demonstrate separation and sorting of two fluorescently labeled lipid species using this device: the glycolipid, GM1, an important cell signaling molecule and lipid raft marker labeled in the headgroup, and BODIPY-DHPE, a fluorescently-labeled phospholipid.

For this prototype single stage, the extraction efficiency is defined as the amount of species extracted into the raft region normalized by the total amount of that species that entered the separation region by the end of the experiment. This quantity is a function of time because the initial input (the mixture) is a discrete plug of material. Alexa 594-GM1 entering the separation region with the POPC-rich phase is more significantly extracted to the raft phase, as expected because it is a known raft marker, while the BODIPY-DHPE remains primarily in the POPC-rich phase. For this device design and experimental conditions, almost 30% of the Alexa 594-GM1 is extracted while close to 15% of the BODIPY-DHPE is extracted into the raft phase (Figure 3). Once prepared, this device achieves an affinity-based separation and in particular, can separate and discriminate lipid raft residents from non-raft species outputting two spatially separated phases without the need for operator intervention or complicated device design.

References: