Synthetic nano-structured supported membrane platform to investigate lipid diffusion and clustering

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Aim

We aim to develop a supported lipid membrane platform which in addition to controlling the content of important raft-forming lipids in the supported membrane will incorporate nanoscale pinning points for which can either be pinned immobile or released by external actuation. The biological aim is to use these aspects of the platform to investigate in detail the relationship between lipid diffusion, diffusional anisotropy, raft forming lipid composition and the external organization imposed by the cytoskeleton. The generic membrane platform with embedded, controlled attachment points for pinning will be realized by researching the integration of polymer modified magnetic nanoparticles and amphiphilic block copolymers into supported membranes.

Background and hypothesis

Recently, we (Kraut and collaborators) have demonstrated by spatio-temporal cross correlation using TIRF-FCS;[1, 2] that heterogeneous organizational features in the plasma membrane of cells depend on local lipid composition and on the cytoskeleton, as previously suggested,[3, 4] but that these two determinants exert distinct and separable effects upon short length-scale diffusion vs. longer length scale organization.[5] Lipids such as cholesterol and sphingolipids strongly affect the fluidity of the cell membrane and are thought to do so by the organization of the membrane into nanoscale domains of non-mobile lipids, so-called “lipid rafts”.[6] In addition to lipid-based self-organization, our studies suggest that the cytoskeleton imposes further restrictions to local lipid mobility by introduction of immobile obstacles at its attachment points to the membrane.

Rachel and collaborators have recently designed and analyzed fluorescently tagged short peptide probes to trace the organization and diffusion behavior of sphingolipid- and ganglioside-carrying domains in the cell membrane. The best-characterized such probe to date is based on the Sphingolipid Binding Domain (SBD) of the Alzheimer’s associated Aβ peptide (identified by Fantini and coworkers).[7] The SBD probe is internalized via a raft- and sphingolipid-dependent mechanism[8-10] and interacts relatively specifically with a constellation of cholesterol, sphingomyelin, and gangliosides at the outer cell membrane.[8, 9] In order to analyze SBD’s biophysical behavior on both artificial and real cell membranes, a variety of methods are employed including Fluorescence Correlation Spectroscopy (FCS; collaboration with Dr. Thorsten Wohland, NUS, Singapore), Surface Plasmon Resonance (SPR; collaboration with Dr. Susana Geifman-Shochat, NTU, Singapore), and Atomic Force Microscopy (AFM). With this combination of techniques, it was determined that the SBD peptide, through interacting with a subset of sphingolipids and cholesterol, marks a small, low-mobility domain at the cell surface that is required for efficient internalization into the cell. Furthermore, this and other available probes (Cholera toxin (CtxB) and a raftophilic lipid probe from JADO-tech, GmbH, Dresden) could be used to demonstrate a characteristic bimodal distribution of mobilities for both ordered lipids and sphingolipid-associating peptide probes,

which differed markedly from the disordered lipids and non-domain-associated transmembrane markers. The proportion of probes in the fast and slow populations changed markedly when raft lipids or cytoskeleton were disrupted, or when components of the uptake machinery (such as flotillin) were removed. Namely, the slow population disappears under these organization-disrupting conditions. This suggests that the slow-diffusing population is dependent on the presence of functional, intact endocytic domains and cytoskeleton, plus sphingolipids and cholesterol .[10] The relative contributions of these components, and the requirements of their organization/distribution for determining diffusion behavior is, however, unknown.

We are now using a TIRF-based FCS method [11] to analyze large-scale heterogeneities in diffusion behaviors at the cell membrane. With TIRF, an entire membrane region can be measured simultaneously, where in effect each pixel of a 20x20 region, for example, yields a single FCS measurement. Overlaid on this technique, a novel method of analysis that correlates the degree of similarity between diffusion values of neighboring pixels, which are different regions in the membrane, serves essentially as a quantifier of membrane diffusion anisotropy. This quantifier is termed the ΔCCF, which indicates the difference between the forward and backward cross-correlation functions between neighboring pixels, calculated over time. A distribution of ΔCCF values far from zero indicates high levels of anisotropic diffusion in the membrane. [2] Applying such analysis to SBD and other peptide and lipid probes of liquid ordered and liquid disordered domains has shown that cytoskeleton-disrupting treatments as well as sphingolipid-, glycolipid-, or sphingomyelin-inhibition or degradation drastically alters the large-scale anisotropic character of the membrane.[1] Add-back of sphingolipids to the membrane leads to reestablishment of anisotropy for both peptide and lipid probes, and recovery of original slow diffusion rates, suggesting that sphingolipids have an organizational role in membrane heterogeneity.

Homogenization of the ΔCCF function distribution after removal of raft components generally happens concomitantly with an increase in diffusion rate, measured by confocal FCS. Cytoskeletal disruption alters diffusion to a greater extent than cholesterol removal does. However, the opposite is true for anisotropy: cytoskeletal disruption homogenizes the membrane *less* than lipid disruption. Therefore membrane anisotropy is not solely an outcome of cytoskeletal organization. Additionally, preliminary results suggest that membrane heterogeneities in liquid-disordered phase lipids such as DiI appear after treatments that change the expected lipid composition (e.g. increasing ceramide content). Surprisingly, these heterogeneities are not reflected in the diffusion rate, suggesting again that heterogeneity expressed as ΔCCF tells us about a fundamentally different level of membrane organization from that of simple diffusion. We therefore hypothesize that membrane organization is determined at two or more levels, by both lipid composition, and cytoskeletal barriers, and that both of these contribute to restricted diffusion behavior.

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