

Effect of Ceramide in Model Raft Membranes: A TIRF Study

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Lipid rafts are membrane microdomains enriched in tightly packed cholesterol and sphingolipids. They provide a liquid ordered phase microenvironment for membrane proteins in an otherwise fluid cell membrane. They are implicated in important cell signaling events. Their small size and dynamic nature make it difficult to visualize rafts directly in cell membranes. Enzymatic hydrolysis of raft Sphingolipid to give ceramide is thought to modulate their properties and functions.

We followed the effect of in situ ceramide formation in model Sphingomyelin and cholesterol rich rafts using Total Internal Reflection Fluorescence (TIRF) microscopy and Atomic Force Microscopy (AFM). Fluorescent lipids were used to label the rafts and/or the surrounding fluid phase and ceramide was generated via the action of Sphingomyelinase. Fluorescence images indicate some restructuring in the bilayer and finer details from AFM images reveal increased heterogeneity in the individual rafts.