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Tethered Supported Lipid Bilayers

Understanding Viral Entry

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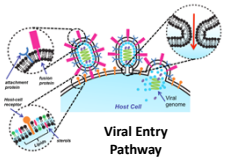
Background

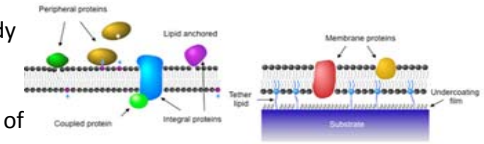
that makes up living organisms. It acts as a selectively permeable barrier responsible for regulating all substances that pass between the interior of the cell and the external environment. A major role of the cell membrane is to protect the cell from outside forces. However, the membrane is susceptible to viral entry.



Viral Entry Pathway

Pathogenic viruses are a serious threat to human health and national security. Our goal is to develop platforms for detecting and identifying viral particles, and understand pathways for infection. Many biodefense priority viruses enter host cells by lipid membrane fusion. We are working on the development of cell membrane mimetics that can be used to detect and evaluate viral particle binding and fusion.

In order to study the binding and fusion process, simplified models of the cell membrane called supported lipid bilayers (SLBs) can be used. SLBs are phospholipid membranes adsorbed to a planar substrate. The presence of the substrate can cause unwanted interactions with the bilayer, so we are developing tethered SLBs (t-SLBs) to separate the SLB from the substrate.

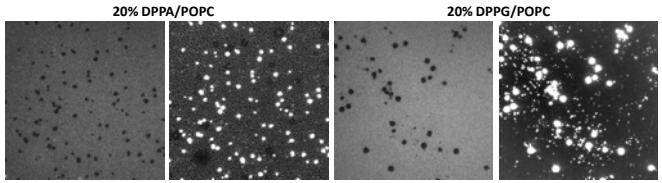


Cell Membrane | Tethered SLB

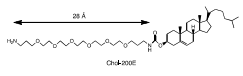
Results

Vesicle Fusion Pathway

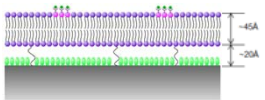
We formed SLBs on glass through vesicle fusion and adhesion, and demonstrated the formation of functionalized domains by selectively binding cationic polymers.



We are now working on constructing t-SLBs in order to improve membrane fluidity, protein function, and stability. A typical SLB on glass has a 10 Å water cushion, compared to a 20 Å cushion in our t-SLBs.

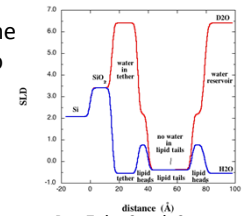


Cholesterol-PEG Tether



t-SLB Structure

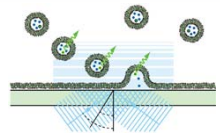
In order to study and characterize the t-SLBs, we used neutron reflectivity to observe their structure. Pure tether samples have been studied; we are in the process of studying t-SLBs with a cushioned substrate.



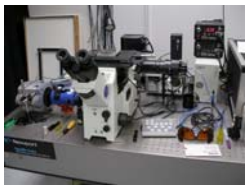
Pure Tether Sample Structure

Conclusion

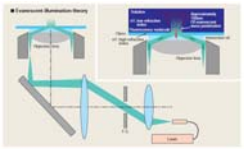
We will be using total internal reflection fluorescence (TIRF) microscopy to observe and study the fusion of viral particles to the membrane.



TIRF Microscopy of Fusion

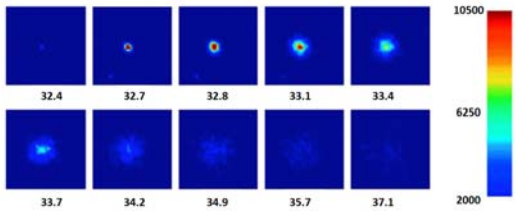


TIRF Microscope



Evanescent Illumination Theory

Previous work (below) has been done studying viral particle binding and fusion using typical SLBs. Our research will be taking this a step further and observing this binding and fusion on t-SLBs, which would more accurately mimic the cell membrane.



Single Particle (Sindbis) Hemifusion/Fusion Event on Supported Membrane

Wessels L., Elting M.W., Scimeca D., Weninger K., *Biophys. J.* (2007) 93: 526-538.

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