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A Bio-Synthetic Interface for Discovery of Viral Entry Mechanisms

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A Bio-Synthetic Interface for Discover of Viral Entry Mechanisms

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Abstract

Understanding and defending against pathogenic viruses is an important public health and biodefense challenge. The focus of our LDRD project has been is uncover the mechanisms enveloped viruses use to identify and invade host cells. We have constructed interfaces between viral particles and synthetic lipid bilayers. This approach provides a minimal setting for investigating the initial events of host-virus interaction – (i) recognition of, and (ii) entry into the host via membrane fusion. This understanding could enable rational design of therapeutics that block viral entry as well as future construction of synthetic, non-proliferating sensors that detect live virus in the environment. We have observed fusion between synthetic lipid vesicles and Vesicular Stomatitis virus particles, and we have observed interactions between Nipah virus-like particles and supported lipid bilayers and giant unilamellar vesicles.

CONTENTS

NOMENCLATURE

GUV	giant unilamellar vesicle
NiV	Nipah virus
SLB	supported lipid bilayer
SUV	small unilamellar vesicle
TIRFM	total internal reflection fluorescence microscopy
VLP	virus-like particle
VSV	Vesicular Stomatitis Virus

1. INTRODUCTION

1.1. Problem

The initial events of infection by enveloped viruses include attachment to the host cell lipid membrane by recognition of cell surface receptors followed by entry into host cells via fusion of the host and viral lipid membranes. The precise mechanisms of these early events are of great interest for development of assays and therapeutics because they occur externally to the cell and therefore could be disrupted by therapeutics, preventing infection (**Figure 1**). However, the inherently complex, interconnected, and redundant nature of cellular processes in the host has made the details of viral entry difficult to study [1]. What are the minimal cell surface markers and physical properties of host cells that viruses require for recognition and fusion? Such questions are hard to fully answer because key factors including lipid composition and membrane protein content, cannot be isolated in the study of living host cells.

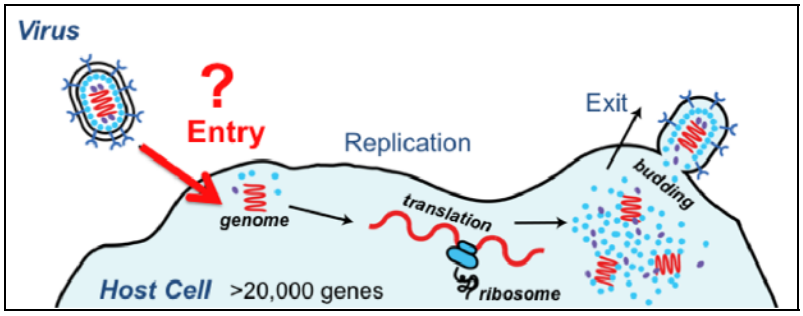

Figure 1. Entry, the first step of viral infection, deposits the viral contents inside the host cell. Mechanistic understanding of this step would address many important questions in biophysics and point to strategies for effective defense.

1.2. Innovation

In the study of host-pathogen interactions, traditional top-down assays on host cells have been very useful but are limited by the stochastic, redundant nature of cellular processes and the need to maintain viability. An emerging complementary approach, enabled by increasing knowledge from top-down studies and critical technical advances, is to build cell-like systems component-by-component from the bottom up. By reconstituting specific biochemical and synthetic components in a cell-like environment, this strategy could capture the essential functionality of cellular processes in a simplified and controllable setting.

To examine the fundamental biochemical and environmental requirements necessary for viral entry into host cells, this project seeks to construct a bio-synthetic interface. This interface can recreate the essential early events in the host-virus interaction – viral attachment and envelope fusion - in a controlled minimal setting (**Figure 2**). Using this strategy, hypotheses emerging from top-down studies, such as lipid mixtures and membrane proteins implicated in entry, can be tested, revealing the necessary and sufficient conditions for host entry by viruses. Specifically, the bio-synthetic interface consists of a synthetic lipid membrane, for which the lipid and protein content are precisely controlled to mimic the lipid membrane of a host cell. Interactions between this membrane and enveloped virus were monitored using advanced optical methods to reveal changes in membrane organization and examine fusion events.

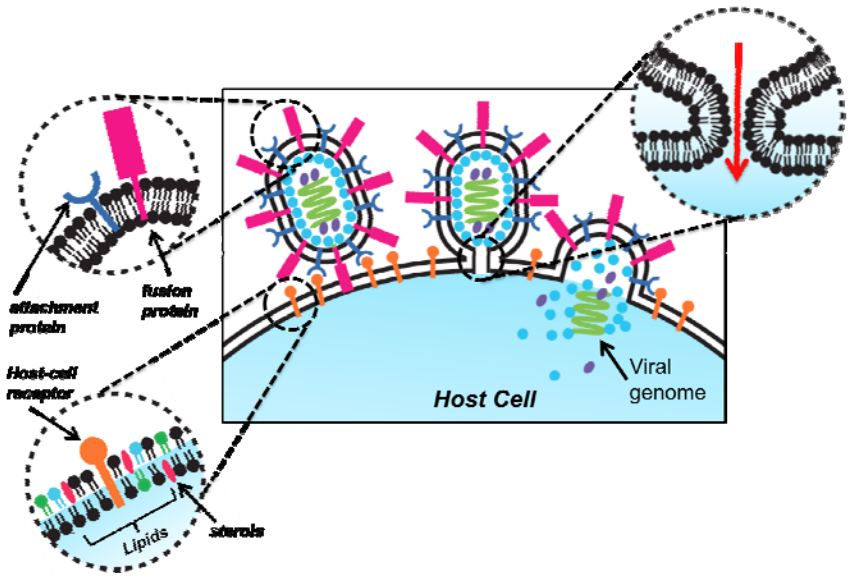

Figure 2. Entry of enveloped viruses is a physical process that requires fusion of the viral and host lipid membranes in order to transfer the viral contents across the host cell membrane. This process depends on the lipid and protein composition of the two membranes, but the basic biochemical and biophysical requirements for membrane fusion are poorly understood.

1.3. Approach

Interactions between cell-like synthetic lipid membranes and model viruses were monitored using fluorescence spectroscopy and microscopy. Synthetic membranes were produced in several forms including small unilamellar vesicles, giant unilamellar vesicles, and supported lipid bilayers. Model viruses including Vesicular Stomatits virus and Nipah virus were produced in several formats including native virus particles (VSV only), pseudovirus, and virus-like particles. To observe fusion events, fluorescence emission spectra were measured and fluorescence microcopy methods including epi-fluorescence, and total internal reflection fluorescence were utilized.

Literature reports have implicated a variety of lipid species in viral fusion including phosphatidylcholines, charged lipids, sphingomyelin, cholesterol, and glycolipids [2]. The membrane content of these components as well as the required membrane receptors (EphrinB2 for NiV model virus), were varied to determine their effect on viral fusion with synthetic lipid bilayers.

2. METHODS

2.1. Lipid Bilayer Substrates

In order to observe fusion between a synthetic lipid bilayer and a virus particle, the synthetic lipid bilayer must be carefully chosen for compatibility with the experimental design. A variety of synthetic bilayer formulations are available to meet the needs of different types of fusion experiments (**Figure 3**). Small unilamellar vesicles (SUVs) have diameters less than one micron, similar in size to virus particles. They can be prepared in bulk at high concentrations (milli-molar) and are relatively rugged. Owing to their small size, SUV membranes have high curvature, likely increasing their capacity for membrane fusion. These properties make SUVs well suited to bulk assays of fusion such as the fluorimeter assay described in section 3.2 [3]. SUVs are near or below the optical diffraction limit, making them generally incompatible with optical microscopy studies. Giant unilamellar vesicles (GUVs) have diameters ranging from several microns to about 100 microns. These larger vesicles are more fragile than SUVs, but large enough for optical microscopy studies. Their membranes have low curvature and are highly fluid, making them a better physical mimic of the cellular plasma membrane. Section 3.3 describes interactions between GUVs and virus particles. When quantitative observation of single fusion events is desired, supported lipid bilayers (SLBs) are an attractive substrate [4]. SLBs are lipid bilayers formed on the surface of solid substrates such as glass. While these substrates are ideal for imaging, interaction between the lipids and the substrates sometimes reduces membrane fluidity and capacity for reorganization, possibly interfering with membrane fusion. A strategy for eliminating these effects in the supported bilayer formulation is to create cushioned bilayers, which are supported by solid substrates conjugated with a polymer layer. The following sections describe how each of these types of lipid membrane substrates is formed.

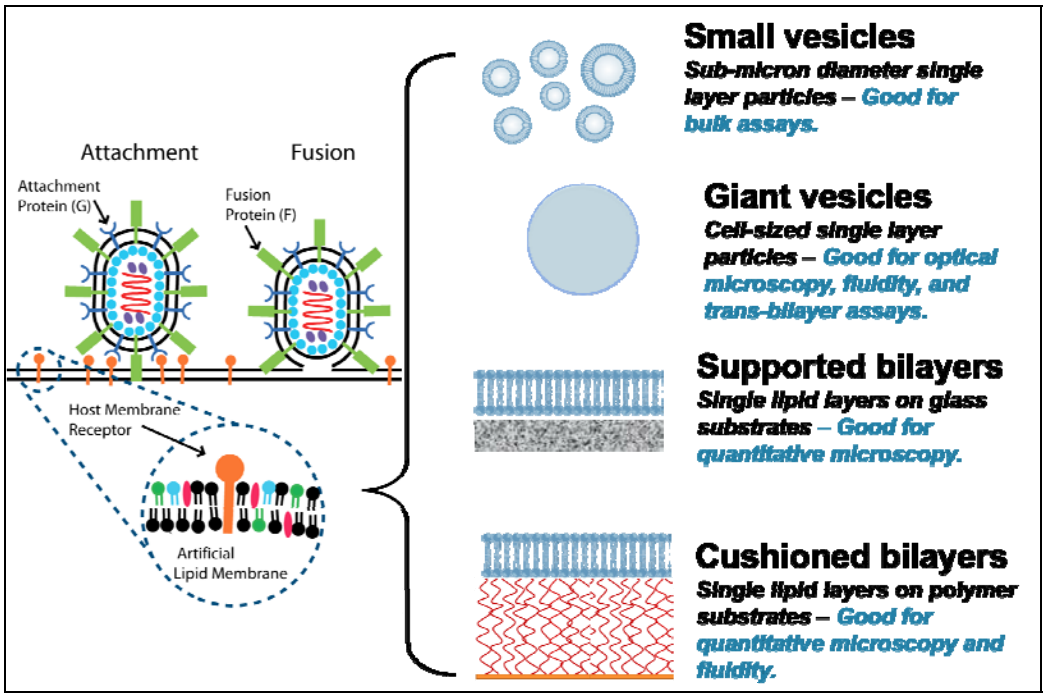

Figure 3. Several model membrane systems are available for fusion studies including (i) small vesicles of sub-micron diameter, (ii) giant vesicles similar in size to cells, (iii) supported bilayers on hard substrates such as glass, and (iv) cushioned bilayers on polymer coated surfaces.

2.1.1. Materials

Lipid molecules including DOPC (18:1 (Δ9) Cis phosphocholine), DOPE (18:1 (Δ9) Cis phosphoethanolamine), cholesterol, and egg sphingomyelin were purchased from Avanti Polar Lipids (Alabaster, AL). DSIDA (Distearylglycero triethyleneglycyl iminodiacetic acid) and DOIDA (Dioleylglycero triethyleneglycyl iminodiacetic acid) were synthesized according to previously reported protocols [5, 6]. OptiMEM media was purchased from Invitrogen. Ephrin-B2 Fc Chimera protein was purchased from R&D Systems. R18 lipid probes (rhodamine B octadecyl ester perchlorate) were purchased from Sigma Aldrich.

2.1.2. Small Unilamellar Vesicles (SUVs)

Small unilamellar vesicles were formed by dissolving desired lipids in chloroform to a final concentration of about 2 mM. In a conical flask, the chloroform solution was evaporated under rotary evaporation, leaving behind a smooth lipid film. The film was further dried under vacuum overnight and then rehydrated in 2-5 mL of OptiMEM media for at least 15 minutes. The rehydrated film was vigorously swirled to fully suspend the lipid film. The lipid solution was then sonicated with a tip sonicator at a power of 10 watts under a constant flow of nitrogen for a total of 12 minutes (3 segments, 4 minutes long each, separated by 2 minute rest periods). The sonicated solution was then centrifuged (14000 rpm, 20 minutes) and the supernatant filtered through a 0.2 μm GHP filter. This protocol produces SUVs with a diameter less than 50 nm, which are ideal for construction of supported lipid bilayers (section 2.1.3). In contrast, fusion assays directly between viral particles and liposomes work best using SUVs with 100-200 nm diameter. To form these SUVs, the rehydrated lipid solution is extruded through filters with the

desired pore size (100 nm pores used in section 3.2). Filters were purchased from Whatman Millipore.

2.1.3. Supported Lipid Bilayers (SLBs)

Following formation of SUVs, supported lipid bilayers (SLBs) were prepared by incubating a 120 μL solution of small unilamellar vesicles prepared by within a 1 cm diameter hole in a silicon gasket fixed against a glass cover slip. Cover slips were cleaned by immersion in piranha solution (25% (30% aqueous H_2O_2)/H_2SO_4). [Caution, piranha is extremely corrosive and dangerous to the skin, eyes, and lungs.] Following incubation, the sample was liberally rinsed leaving 120 μL of fresh OptiMEM media on the coverslip. The lipid bilayer was then incubated in OptiMEM media containing 10 μM $CuCl_2$ in order to activate the DOIDA binding sites on the membrane. After incubation the sample was liberally rinsed leaving 120 μL of fresh OptiMEM media on the coverslip. Next, the sample was incubated with OptiMEM media containing 100 nM of the Ephrin B2 Fc Chimera protein for 15 minutes. After incubation the sample was liberally rinsed leaving 120 μL of fresh OptiMEM media on the coverslip. Finally the bilayers were observed using epi-fluorescence microscopy. When TMR-labeled Ephrin B2 was used, it was observed to distribute evenly over the bilayer surface and was free of holes and static regions.

2.1.4. Dextran-supported bilayers

For our studies of the detailed mechanisms of virus fusion, the lipid bilayer experimental system is critical. The membrane must be very fluid to facilitate fusion, which is often not the case for membranes supported directly on glass. Ideally ample fluid space should be present under the membrane to allow for completion of fusion events with the discharge of vesicle contents. Finally the membrane should accommodate intact functional membrane proteins without interference from the support. All these requirements can be met by creating lipid membranes on hydrated dextran layers. We have developed the technique for making dextran-supported bilayers on glass coverslips. The glass is first functionalized with a glycidoxysilane that can react with OH groups on the dextran. Dextran with an average molecular weight of 500000 is covalently bound to the glycidoxysilane to form a dense layer. SUVs of the desired lipid composition are then made to fuse into a bilayer on the dextran surface. Video imaging illustrated the fluidity and essentially perfect coverage of a dye labeled bilayer composed of a mixture of POPC and DOIDA. Currently we are working to determine the thickness and diffusion characteristics of the dextran layer. Another important feature of the dextran-supported bilayers is that they allow fusion studies using the sensitive and surface specific TIRFM method.

2.1.4. Giant Unilamellar Vesicles (GUVs)

Giant unilamellar vesicles (GUVs) were formed by the electroformation procedure according to published protocols [7]. We performed electroformation at approximately 60 °C to exceed the highest expected melting temperature of lipid mixtures. Vesicles were electroformed in sucrose solution (~350 mOsm).

2.2. Virus Particles

Several types of virus, virus-derived, and virus-inspired particles are available for studies of fusion with synthetic lipid membranes. When Biosafety level 2 viruses are used, it is possible to work directly with native virus, when has the maximum fusion potential. When higher Biosafety level viruses are desired, virus-like particles (VLPs) can be used. These particles, which consist of viral matrix and envelope proteins only, are capable of membrane fusion, but likely fuse with reduced efficiency in comparison to native viruses. In this work, native virus was used to study fusion of Vesicular Stomatitis Virus and virus-like particles were used to study fusion of Nipah virus.

2.1.1 Preparation of Vesicular Stomatitis Virus (VSV) Particles

We propagated Vesicular Stomatitis virus (VSV) in HEK293T cells, which produced yields between $10^8 – 10^9$ plaque forming units (pfu) per ml. We obtained these titers after 48 hr by inoculating monolayers a multiplicity of infection (MOI) of 0.01 pfu/cell. The supernatants were collected and clarified before pelleting the virus at 110,000 *g* through a 20% sucrose (in PBS) cushion followed by resuspension in OptiMEM media. Final concentration of virus was typically 10^10 pfu/ml.

2.1.2 Preparation of Nipah virus (NiV) virus-like particles (VLPs)

Nipah virus is highly pathogenic and requires Biosafety level 4 (BSL-4) containment when performing live virus assays. To circumvent the need for high-level containment when studying only the characteristics of Nipah virus entry, we have developed a virus-like particle (VLP) system. VLPs can be produced via expression of Nipah virus matrix protein (NiV-M) alone or in combination with its fusion protein (NiV-F) and receptor-binding protein (NiV-G). VLPs do not encapsulate any of the virus genome and thus are not infectious. The matrix protein directs budding of virions from the surface of cells and interacts with the cytoplasmic tails of the envelope proteins, ultimately assisting in viral assembly. Despite many functionalities of the matrix protein, none appear to be significantly disrupted by fusing large reporter proteins to the N-terminus of NiV-M. Thus, we sought to exploit this property by fusing the fluorescent reporters GFP and mCherry to the N-terminus of NiV-M in an effort to create viral entry assay for fluorescent imaging studies in less than BSL-2 containment.

Fluorescently labeled NiV-M VLPs were made by transfecting the GFP-*M or* mCherry-*M* expression plasmids (25 μg) with NiV-*F* and *G* (10 μg each) expression plasmids into 10 cm dishes of 293T. At 24 h post-transfection, the expression of the NiV-M protein was visualized by fluorescent microscopy while the expression of NiV-G and NiV-F proteins were verified by the presence of cell-to-cell fusion. The supernatants were then collected and clarified before pelleting the VLPs at 110,000 *g* through a 20% sucrose (in PBS) cushion followed by resuspension in OptiMEM media.

2.3. Imaging

2.3.1 Epi-Fluorescence Microscopy

Interactions between viral particles and giant vesicles or supported bilayers were observed using epi fluorescence microscopy. All studies were performed using either a 63X or 100X oil immersion objective. Two-color epi fluorescent images were taken on an Olympus inverted microscope using an Andor cooled CCD camera.

2.3.2 Total Internal Reflection Fluorescence Microscopy (TIRFM)

For studies of protein driven virus fusion mechanisms, the total internal reflection fluorescence microscopy technique offers several advantages. The technique has single molecule sensitivity and the evanescent wave illumination has been shown to reduce photobleaching, allowing for long time observations. Additionally, the illumination to a depth of ~200 nm is well matched to our supported bilayers. The limited illumination depth is critical for observation of reactions on the membrane surface without fluorescence interference from labeled proteins or vesicles in the bulk solution. Our implementation uses TIRF illumination through the microscope objective. This approach simplifies the interface to samples. In addition it is readily switched to widefield illumination for observation deeper into the samples. Focusing the input laser on the edge of a high NA objective produces the total internal reflection condition. The laser exits the objective in a collimated beam at a sharp angle that exceeds the critical angle for total reflection as it hits the coverslip surface. The laser beam is reflected back through the objective. Fluorescence is collected in the usual fashion and imaged onto a CCD camera.

3. RESULTS AND DISCUSSION

3.1. Model Viruses

Fusion with synthetic lipid membranes can be used to investigate the entry requirements of all enveloped viruses, which make up the majority of priority pathogen viruses. These viruses are "enveloped" by a lipid membrane and therefore enter host cells by inducing fusion of their membrane with the lipid membranes of the host cell. Enveloped viruses enter host cells according to two distinct membrane fusion strategies: (i) direct fusion to the cytoplasmic membrane (e.g. Nipah virus, HIV, Vesicular Stomatitis virus (VSV)) and (ii) endocytosis followed by escape from the endosome via fusion at low pH (e.g. Lassa Fever, Rift Valley Fever, Ebola viruses). To represent these mechanisms we have chosen two well-studied model viruses (**Figure 4**). Nipah (NiV) virus (priority pathogen) models direct fusion, while VSV models endosomal fusion. NiV requires the Ephrin B2 receptor in the host cell membrane [8]. To mimic NiV attachment and fusion suitable for studies at biosafety level 2 (BSL-2), we will develop a non-pathogenic virus-like particle system. Since our artificial host cell does not have an actin cytoskeleton, it is not capable of endocytosis. Therefore, a low-pH environment outside the host cell can simulate the environment inside an endosome, leading to VSV entry by membrane fusion [3].

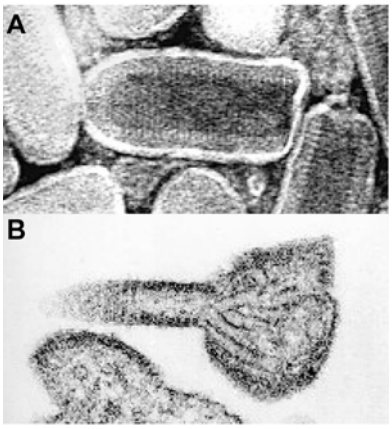

Figure 4. Our model virus systems include (A) Vesicular Stomatits Virus (VSV), which fuses to protein-free membranes at low pH, and (B) Nipah Virus (NiV) which fuses to the plasma membrane and requires the membrane protein receptor, Ephrin B2.

3.2. Bulk Assays of Vesicular Stomatitis Virus Fusion

Virus fusion with liposomes was monitored using the fluorescent probe octadecyl rhodamine B (R18). R18 associates with membranes rapidly and can be used to label intact viral membranes. At high concentrations, R18 is self-quenching and as viral fusion with a target membrane progresses, R18 becomes dequenched and leads to an increase in fluorescent signal (**Figure 5**). For our experiments, purified Vesicular stomatitis virus (VSV) at the concentration of 10^{10} pfu/100μl was labeled with 3 μl of R18 (1.4 mM in ethanol) for 2 hours on ice and subsequently pelleted through a sucrose cushion. The R18 labeled VSV was then resuspended in 100 μl of HMSS buffer (5 mM HEPES, 5 mM MES, 5 mM sodium succinate 150 mM NaCl) at pH 7.2. To monitor the shift in fluorescent signal change simply due to pH change, 10 μl of R18 labeled VSV was diluted with 700 μl of HMSS buffer at pH 7.2 and the fluorescence emission spectrum was monitored from 540 nm to 600 nm using an excitation of 520 nm. Acid addition (3 μl of 1N HCl) induced a drop to pH 5.8 and the fluorescence emission spectrum was again taken. Lastly, to determine a maximum level of R18 dequenching, 0.1% final concentration of Triton-X 100 was injected in to the sample. The fluorescent signal increased dramatically upon the addition of the Triton X lysis buffer and very minimally due to pH change.

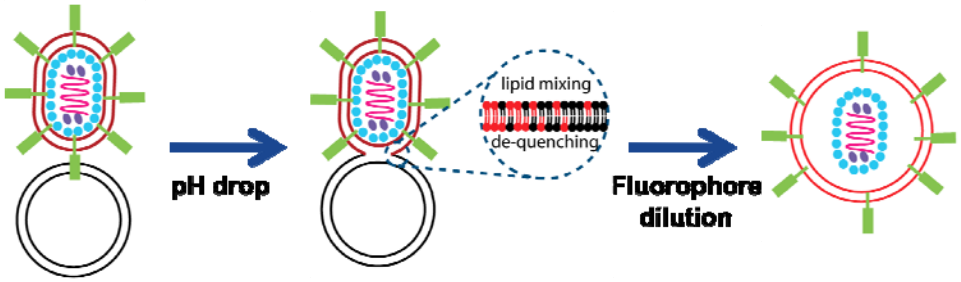

Figure 5. Fusion of virus particles to liposomes can be accomplished in bulk using a fluorescence de-quenching assay. VSV particles saturated with fluorescent probes are brought into contact with liposomes. When the pH is reduced, fusion events can occur, diluting the fluorescent probes into the liposome membrane. This dilution de-quenches the fluorophores, increasing the fluorescence signal.

To monitor VSV fusion with target membranes, liposomes of two different compositions were formed. The first population of liposomes consisted of 100% DOPC lipids. This composition is not expected to be sufficient for fusion with VSV. The second population of liposomes consisted of 35% DOPC, 30% DOPE, 15% egg sphingomyelin, and 20% cholesterol. This composition has been reported to be ideal for membrane fusion [9]. We measured a fluorescent emission spectrum before and after the addition of 3µl of 1N HCl to lower the pH of the buffer to 5.8. The acid addition was used to induce fusion. Finally, the Triton-X lysis buffer was added to the solution. The greatest change in fluorescent signal due to the pH changed occurred when the virus was in the presence of liposome of mixed composition (**Figure 6**).

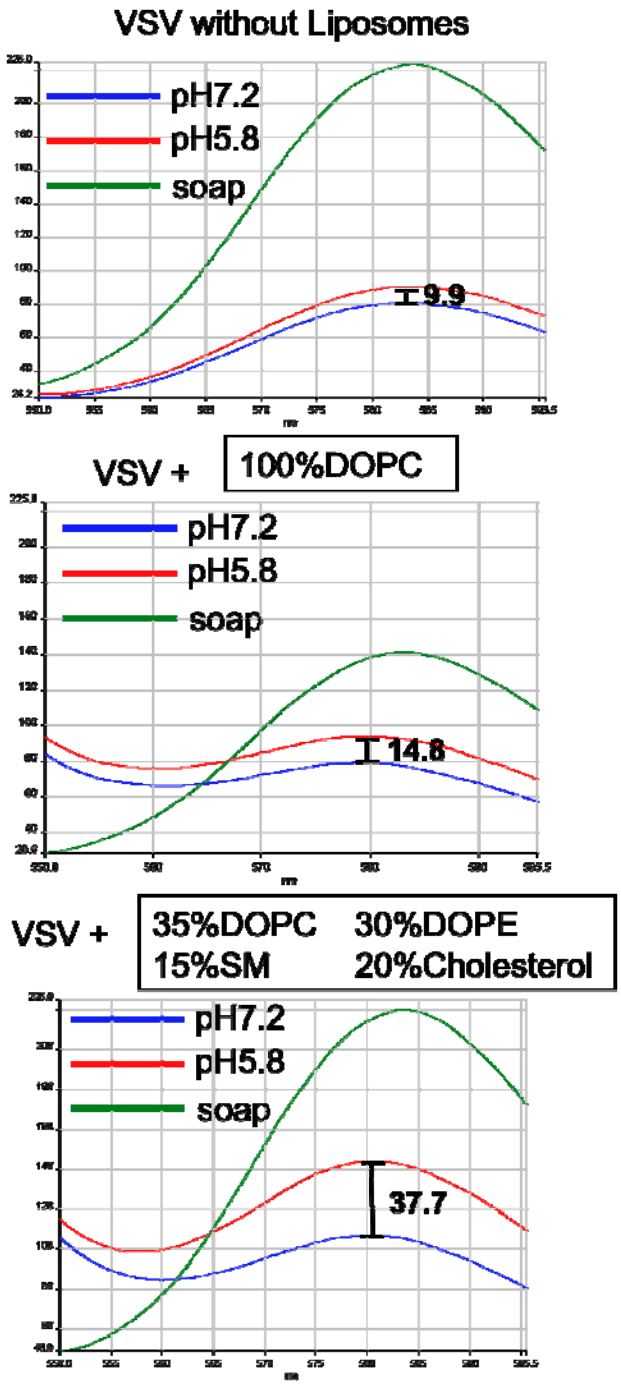

Figure 6. Bulk fusion assays between liposomes and R18 labeled VSV virus particles show the dependency of fusion on lipid composition. Each graph compares the fluorescence emission spectrum of the sample at pH 7.2 (blue), pH 5.8 (red), and with 0.1% Triton X-100 lysis buffer (green). The three conditions were (top) virus particles without liposomes, (center) virus particles mixed with 100% DOPC liposomes, and (bottom) virus particles mixed with 35%DOPC/30%DOPE/15%SM/20%Cholesterol liposomes.

3.3. GUV Interaction with Vesicular Stomatitis Virus

In order to study the interaction of VSV particles with lipid substrates using optical microscopy, we created GUVs on the mixed compostion: 35% DOPC, 30% DOPE, 15% egg sphingomyelin, 20% cholesterol with 0.03% Bodipy 530/550. Interestingly, when we observed these vesicles using fluorescence microscopy, we found that the fluorescent probe was not evenly distributed. Circular regions on the GUV surface that excluded the fluorophore (appear dark) were observed. We also observed phase separation on the surfaces of SLBs created from the mixed lipid composition (**Figure 7**).

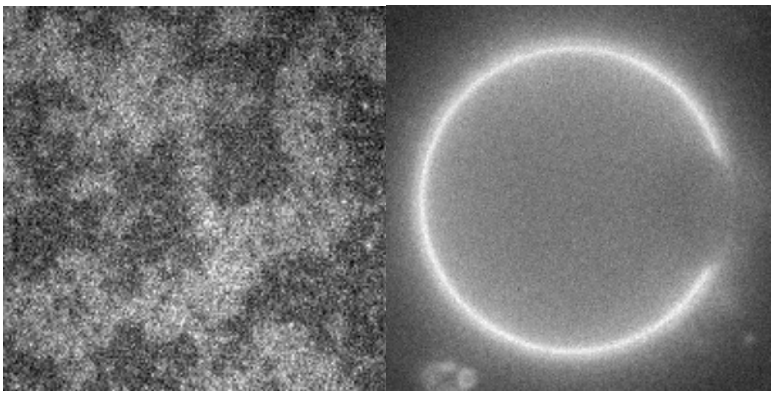

Figure 7. Supported lipid bilayers (left) and Giant unilamellar vesicles (right) with mixed composition (35%DOPC / 30%DOPE /15%SM / 20%Cholesterol) displayed an un-even distribution of the fluorescent probe Bodipy 530/550 (0.3%) that is indicative of phase separation. Each image is about 20 microns wide.

This morphology is indicative of liquid-liquid phase separation [10]. Here the vesicle surface separates into fluid disordered regions, which are rich in fluid phase lipids such as DOPC and DOPE and fluid ordered regions, which are rich in cholesterol and sphingomyelin. The phase behavior of our mixed lipid composition has not been specifically investigated in the literature. However, the phase behavior of the DOPC, sphingomyelin, and cholesterol system has been reported [10]. If we assume that the DOPE lipids will partition with the DOPC lipids, we estimate that our mixed lipid composition should undergo liquid-liquid phase separation at room temperature, as marked in **Figure 8**.

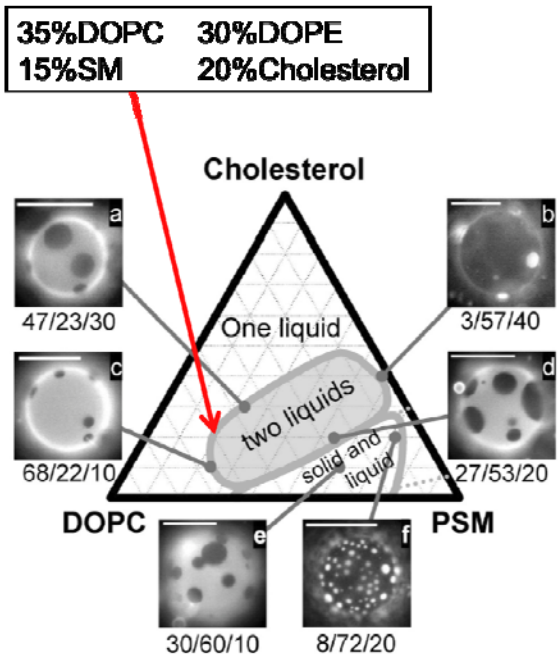

Figure 8. Mixtures of unsaturated lipids, cholesterol, and sphingomyelin are known to undergo phase separation into liquied ordered and liquid disordered regions. Diagram taken from Veatch SL, Keller SL, "Miscibility Phase Diagrams of Giant Vesicles Containing Sphingomyelin," PRL, 2005, 94, 148101. The red arrow marks the approximate location on the diagram of the mixed vesicle composition used in our fusion assays, if the DOPE content is assumed to partition with the DOPC content.

We mixed the GUVs with VSV particles and observed the sample using phase and fluorescence microscopy (**Figure 9**). We observed clusters of brightly labeled virus particles surrounding spherical GUVs. In this assay, fusion of viral particles with GUVs is expected to transfer fluorescent probes to the GUV membrane. It was difficult for us to determine whether this transfer occurred because the viral particle clusters were too bright to enable sufficient contrast for imaging the single-bilayer wall of the GUV. Future studies should seek to disperse or wash away the clusters of virus particles.

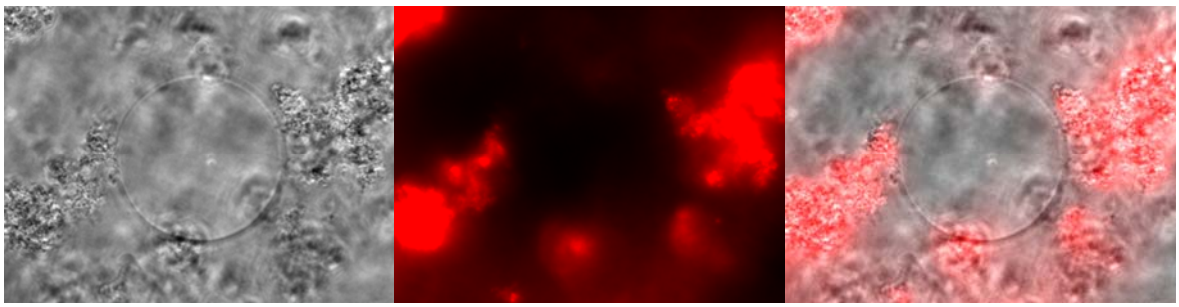

Figure 9. Giant unilmellar vesicles were mixed with VSV virus particles and imaged using phase and fluorescence microscopy. The vesicles contained 35%DOPC / 30%DOPE / 15%SM / 20%Cholesterol. The virus particles were labeled with R18 as described above for the bulk liposome fusion assays. (Left) Phase contrast image, (Center) epi fluorescent image of R18, (Right) merge.

3.4. Supported Bilayer Interaction with Nipah Virus

We next examined the interaction between Nipah virus VLPs and supported lipid bilayers functionalized with the Ephrin B2 receptor, which is known to be necessary for Nipah virus fusion (**Figure 10**) [8]. VLPs contained a GFP-labeled matrix protein. After production, their membranes were labeled using R18 lipid probes dissolved in ethanol mixed at 3% by volume with the VLP solution in OptiMEM media. Excess R18 was removed using size exclusion chromatography spin columns purchased from Fisher Scientific.

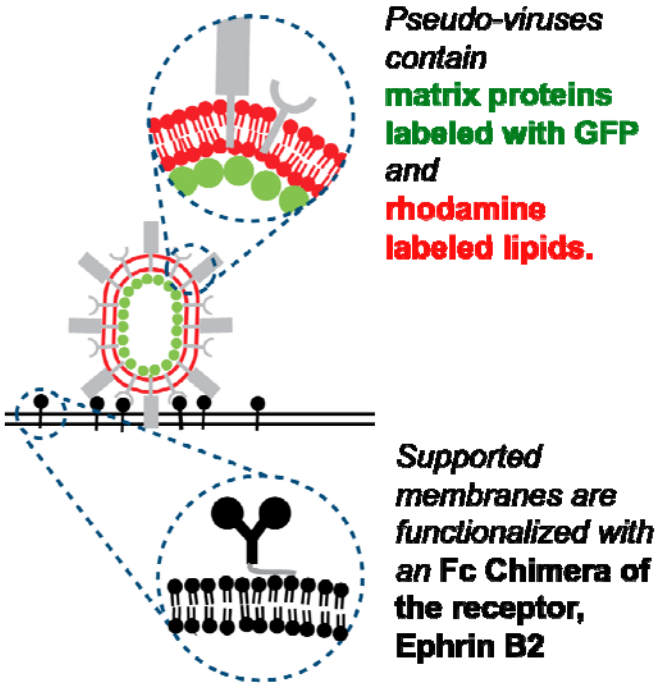

Figure 10. Virus-like particles of NiV were engineered to contain a GFP-labeled matrix protein and and R18-labeled membrane. Supported lipid membranes contained DOIDA lipids that bound a 6-his tagged Fc Chimera of Ephrin B2, a known receptor of NiV.

Following production and labeling, VLPs were imaged by fluorescence microscopy. Sub-micron particles labeled in both the green and red channels were observed to diffuse freely in solution (**Figure 11**). Many particles labeled only in the red channel were also observed. These particles were likely composed of cell debris created during VLP production. Particles labeled only in the green channel were not observed.

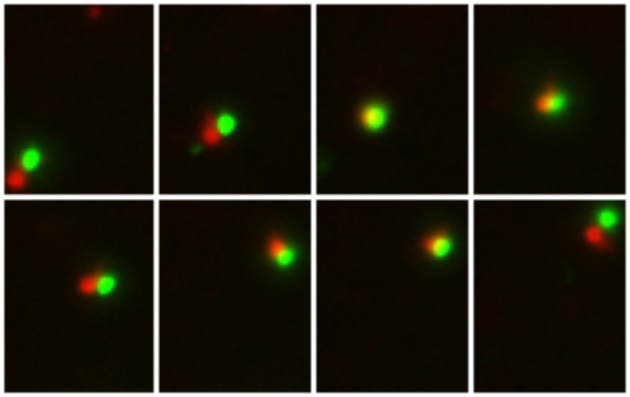

Figure 11. NiV virus-like particles diffusing in solution. Frames were taken sequentially from left to right, top to bottom, where each frame is separated by 2 seconds. Red (R-18-membrane) and green (GFP-matrix protein) signals are co-localized.

SLBs consisting of 25% DOPC, 30% DOPE, 15% egg sphingomyelin, 20% cholesterol, and 10% DOIDA were formed. These bilayers were conjugated with Ephrin B2 protein as described in the methods section.

Labeled VLPs were added to Ephrin B2 conjugated SLBs. After a few minutes, many VLPs were observed to localize to the membrane surface. Some particles were fixed firmly in place, while others diffused in two dimensions on the surface (**Figure 11**). Fusion of the VLPs with the SLB should result in a loss of red channel fluorescence for the fused particles. We did not observe this effect, and we therefore conclude that fusion did not occur. There are several possible explanations for the lack of fusion between VLPs and the SLB surface. One possibility is that the Ephrin-B2, which existed in the form of an Fc Chimera, was not sufficient for fusion. To overcome this possibility, full-length recombinant Ephrin B2 or his-tagged Ephrin B2 should be used in future studies. Another possibility is that the SLB surface was not adequately fluid to permit the membrane reorganizations that are necessary for fusion. To overcome this possibility, future studies should employ a substrate with greater fluidity such as a cushioned lipid bilayer or GUV. Still another possibility is that either the lipid or protein composition of the SLB is lacking in one or more components that are necessary for Nipah virus fusion.

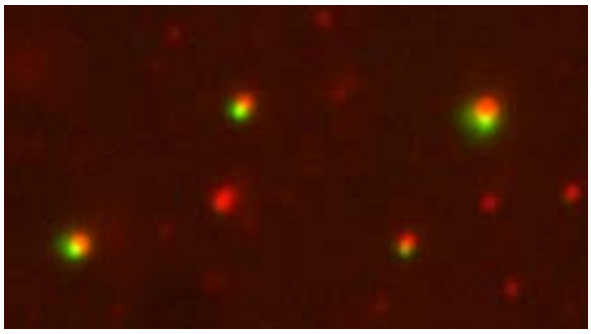

Figure 5. NiV virus-lke particles attached to a membrane conjugated with a 6-his tagged Fc Chimera of Ephrin B2, a known receptor of NiV.

4. CONCLUSIONS

We have built a variety of model systems for virus particles and lipid membranes to reconstitute the interaction between enveloped viruses and host cell membranes. We have demonstrated fusion between VSV particles and synthetic vesicles, making VSV a good model system to study. We have discovered that the preferred vesicle composition for VSV fusion contains liquid-liquid phase separations when reconstituted in giant vesicles. Future studies could investigate whether VSV fuses preferentially to one liquid phase over the other. We have reconstituted NiV virus-like particles as well as Ephrin-B2 functionalized membranes. Expression and purification of our own recombinant Ephrin B2 is likely needed to observe fusion in this system. We have developed TIRM microscopy and cushioned lipid bilayers to address the challenges we encountered with quantitative imaging and substrate fluidity, respectively.

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