UNIVERSITY OF CALIFORNIA SAN DIEGO

Does HIV-1 Envelope Trimer Conformation Determine Sensitivity to Host-Protein SERINC5?

A Thesis submitted in partial satisfaction of the requirements for the degree

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in

Biology

by

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The Thesis of Aaron Otto Angerstein is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California San Diego

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* These are figures I did not make but used as a visual aid to assist in my explanation of various topics.

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Abstract of the Thesis

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As scientists work towards elucidating the various mechanisms HIV uses for infection and evasion of the immune system, a significant focus has been on the HIV-1 protein Envelope (Env). Env is essential for the cellular attachment and entry of virions and is the target of neutralizing antibodies. The host-protein SERINC5 inhibits HIV infectivity of some but not all Envs. Our goal was to understand the relationship between Env and SERINC5.

The Env-SERINC5 relationship was discovered by investigating how the HIV-1 accessory protein Nef increases viral infectivity. Nef enhances infectivity by removing the host-protein SERINC5 from the plasma membrane, preventing SERINC5 from inhibiting viral infectivity at the level of entry. However, Nef's ability to increase virion infectivity appears to be Env dependent.

We hypothesized that the conformation of the Env trimer determined the relative sensitivity to SERINC5. We used the monoclonal antibody 447-52D as a marker of trimer "openness," and assessed if trimer "openness" and SERINC5 sensitivity were related. Using a luciferase infectivity assay to measure the infectivity of cell-free virions, we found that Envs that were sensitive to the antibody 447-52D were also relatively more sensitive to SERINC5. We also used a specific mutation in the V2 loop of the gp120 subunit of Env to "open" the trimer; this increased the sensitivity to SERINC5.

Our findings support the hypothesis that trimer conformation is associated with SERINC5 sensitivity. However, the findings also suggest that trimer "openness" is not the sole factor determining SERINC5 sensitivity.

Introduction

Background

Nearly 40 years ago, doctors were baffled by unusual infections in young, previously healthy, gay men across the United States. These patients all had severely deficient immune systems along with rare infections or cancers (Avert 2017, CDC 1981). These reports marked the beginning of what would later be known as the AIDS epidemic. An epidemiologic and phylogenetic analysis of the human immunodeficiency virus (HIV) shows that HIV was introduced into the human population around 1920-1940 (Seitz 2016). HIV preferentially infects activated $CD4^+$ T cells that are a part of the immune system, resulting in a decline of $CD4^+$ T cells. The progressive failure of the immune system leads to the acquired immunodeficiency syndrome (AIDS), where patients become susceptible to opportunistic diseases (Avert 2017). According to the World Health Organization, approximately 37 million people are currently living with HIV, and about 35 million people have died from an AIDS-related illness as of 2018.

HIV Overview

As medications and treatments for HIV advance, researchers are working to understand the mechanisms that take place in each step of the HIV replication cycle (Fig. 1). One area of focus is on the lipid membrane that encapsulates the viral genome. This membrane originates from the host target cell's plasma membrane through a process called "budding", in which the viral RNA exits the host cell inside viral particles (Fig.1, Fig. 2, Rubbert 2011). The viral membrane protects the viral genome from degradation and contains an HIV protein, Envelope (Env), which plays a crucial role in HIV infectivity (Rubbert 2011).

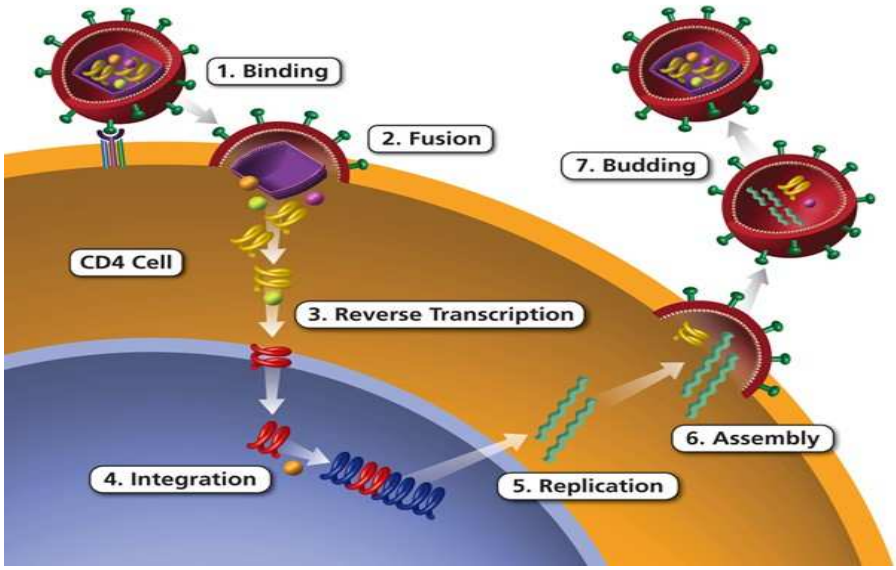


Figure 1. HIV Replication Cycle. (AIDSinfo.NIH, 2018)

HIV Envelope (Env)

Embedded into the viral membrane is the HIV protein Env, which consists of the two viral glycoproteins, gp120 and gp41. The viral gene env encodes the Env glycoprotein precursor gp160. Proteolytic cleavage of gp160 leads to the formation of Env spikes comprising trimeric heterodimers of gp120 and gp41 (Fig. 2, Rubbert 2011).

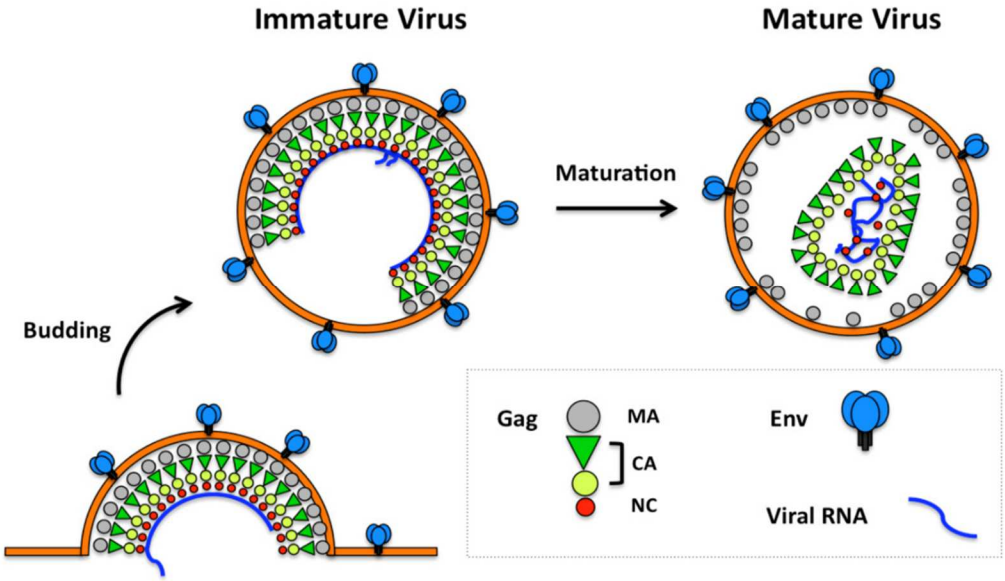


Figure 2. HIV Virion Structure. (Zhang 2015)

Env protrudes through the viral membrane and is responsible for binding to and fusion of mature infectious virus particles (virions) to target cells (Fig.1, Fig. 2, Brandenberg 2015). HIV entry is a multistep process that requires both subunits, gp120 and gp41 (Fig. 3, Hope 2000, Rubbert 2011).

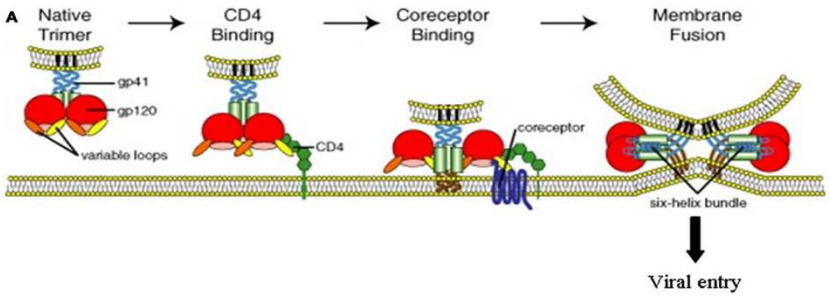


Figure 3. Env Attachment and Fusion. (Connell, 2013

The binding of gp120 to a $CD4^+$ receptor found on the target cell causes a conformational change in gp120 (Fig. 3). This change allows hypervariable regions in gp120 to bind to a co-receptor on the target cell, either the chemokine receptor CXCR4 or CCR5 (Fig. 3, Berger 1999, Rubbert 2011). In gp120, the third hypervariable region (V3) determines which chemokine receptor the HIV virion binds (Fig. 3, Rubbert 2011). The binding of gp120 to CXCR4 or CCR5 induces a second conformational change in the Env trimer, this time in gp41 (Berger 1999, Hope 2000). This conformational change in gp41 exposes a hydrophobic region of gp41 to the aqueous surroundings, and this hydrophobic region harpoons itself into the target cell plasma membrane (Hope 2000, Seitz 2016). Once gp41 has penetrated the target cell plasma membrane, it begins to form a pore that allows the viral and host cell membranes to fuse and the HIV capsid to enter the target cell (Fig. 3, Brandenberg 2015, Klasse 2012).

Env Trimer Conformation

Envs can differ in structure. They can have a closed, partially open, or open trimer conformation (Fig. 4). Envs with an open trimer conformation are usually laboratory-adapted

strains that are not under constant selective pressure from the immune system (Fig. 4, Brandenberg 2015). On the other hand, primary Env isolates derived from HIV patients typically have a closed trimer conformation. (Fig. 4, Brandenberg 2015, Cai 2017).

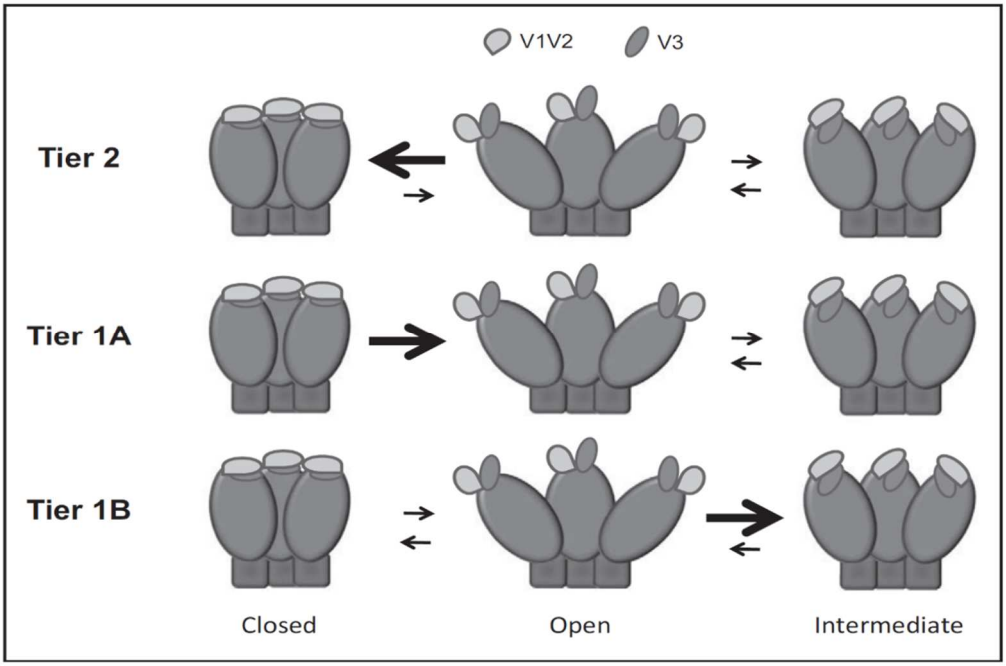


Figure 4. Envs are Categorized into Tiers Based on Their Trimer Conformation. (Montefiori 2018)

Neutralizing Antibodies

When people become infected with HIV, an immune response is evoked in which antibodies are generated against epitopes (specific regions on an antigen to which antibodies bind) on gp120 and gp41 (Fig. 5, Richman 2003, Rubbert 2011). Once bound, the antibodies can potentially neutralize the HIV virion (Seitz 2016). One shortcoming of this immune response is that these antibodies are usually strain-specific (Seitz 2016). Because HIV is continually undergoing mutations, and new variants occur rapidly in a given individual, a person's immune

system is not able to create neutralizing antibodies of sufficient potency and breadth of activity in time to defend against or clear the infection (Richman 2003, Rubbert 2011).

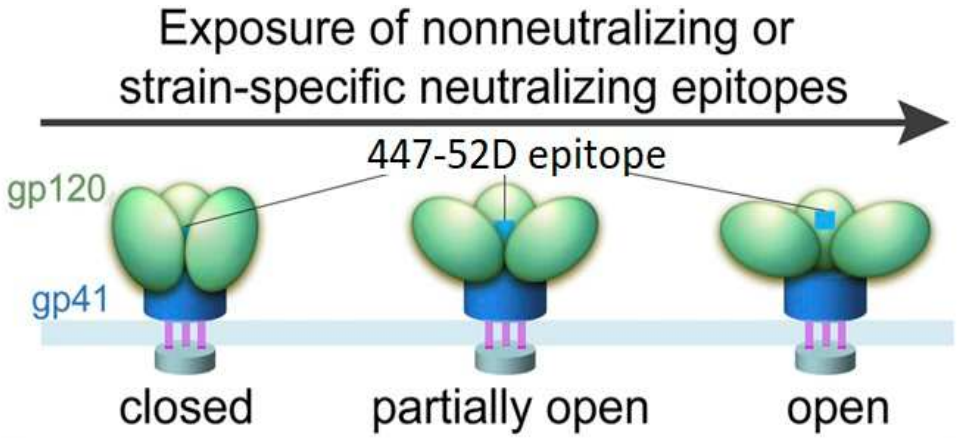


Figure 5. Trimer "Openness" can be Discerned by Sensitivity to 447-52D Antibody. (Cai 2017).

To understand how neutralizing antibodies target Env, Seaman and colleagues, assembled a panel of over 100 HIV-1 Envs drawing from all major circulating genetic subtypes of HIV-1. In this panel, they ranked the Envs according to sensitivity to antibody-mediated neutralization (Seaman 2010). They found that the antibody neutralization sensitivity of Envs widely varied between HIV strains and suggested that antibody neutralization may be related to an open configuration of the Env trimer (Fig. 4, Fig. 5). This may make the epitopes of certain HIV-1 Envs more accessible to the antibodies (Seaman 2010). The antibody 447-52D was used as a marker for trimer "openness". To further understand HIV-1 infectivity, researchers have also focused on another HIV protein – the accessory protein Nef, which renders cell-free virions more infectious in an Env-specific manner.

HIV Nef

HIV-1 Nef is uniquely expressed by primate lentiviruses like HIV and increases the infectivity of cell-free virions (Fig. 6, Chowers 1994). After HIV-1 infection, Nef is the first viral

protein to accumulate to detectable levels in host CD4 T-cells. Nef is not required for viral replication, but it contributes to pathogenesis via several mechanisms (Hope 2000, Usami 2013).

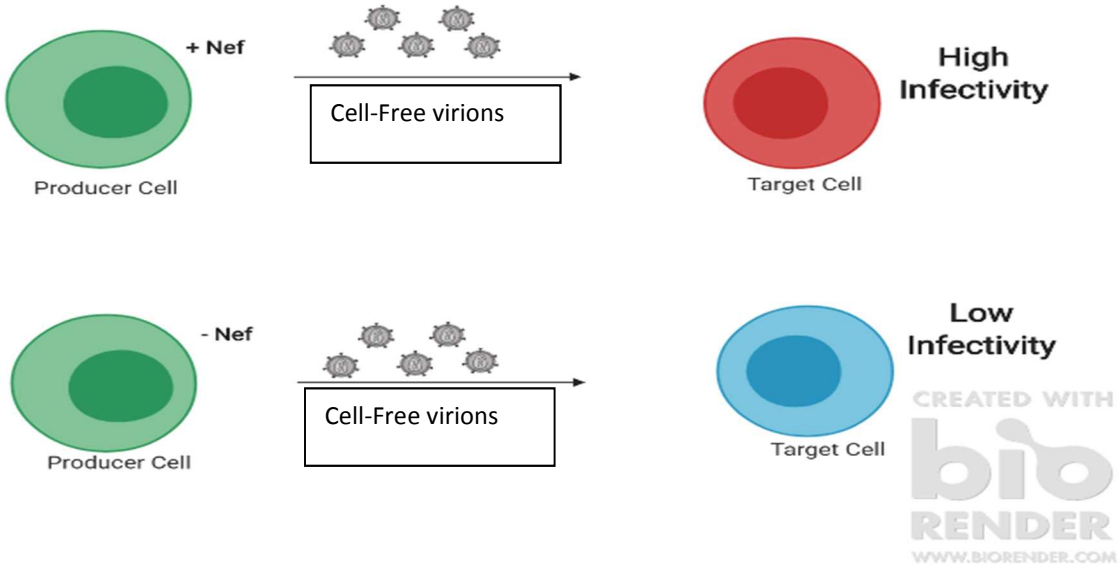


Figure 6. HIV Nef Enhances the Infectivity of Cell-Free Virions.

Nef downregulates several host cell receptors that are responsible for eliciting an immune response; for example, Nef downregulates MHC class-I and MHC class-II molecules (Willey 1992, Sugden 2016). These two cell surface receptors present viral antigens and play a key role in aspects of adaptive immunity (Willey 1992, Sugden 2016). Although Nef plays a prominent role in downregulating certain receptors on $CD4^+$ T cells, including CD4 itself, it was unclear exactly how Nef increases viral infectivity (Fig. 6). To explore how Nef increases the infectivity of cell-free virions, Usami and colleagues examined two Envs that either had an open (SF162) or closed (JRFL) trimer conformation. They used these Envs because previous work suggested that the *nef* gene was important for infectivity in many settings, but not when the Env from the isolate JRFL was used (Lai 2011).

Usami and colleagues confirmed that the infectivity of SF162 is significantly higher when Nef is expressed in the virion-producer cell in comparison to the absence of Nef. However, in the

case of JRFL, the presence or absence of Nef did not markedly affect the level of infectivity (Fig. 7). The group then showed that the increase in cell-free virion infectivity in the presence of Nef was determined by the V2 region of gp120 (Fig. 7). If the Env had the V2 from SF162 (Fig. 7; JR (SF V2)), then not only did it support a significant Nef-effect, but it was also sensitive to the 447-52D antibody, and presumably had an open trimer (Fig. 7). Conversely, if the Env had the V2 region of JRFL (Fig. 7; SF (JR V2)), then it did not support a Nef-effect, and it was not susceptible to the 447-52D, and presumably had a closed trimer (Fig. 7). By switching the V2 regions of an Env that is sensitive to antibody neutralization (SF162) with the V2 region of an Env that is resistant to antibody neutralization (JRFL), Usami and colleagues show that their responsiveness to Nef would also switch (Fig. 7). These findings confirmed that Nef's ability to increase infectivity was Env-dependent and further showed that this phenotype could be modulated by V2 sequences in a manner that correlated with neutralization-sensitivity (Usami 2013). Nonetheless, the actual mechanism Nef used to increase infectivity remained elusive.

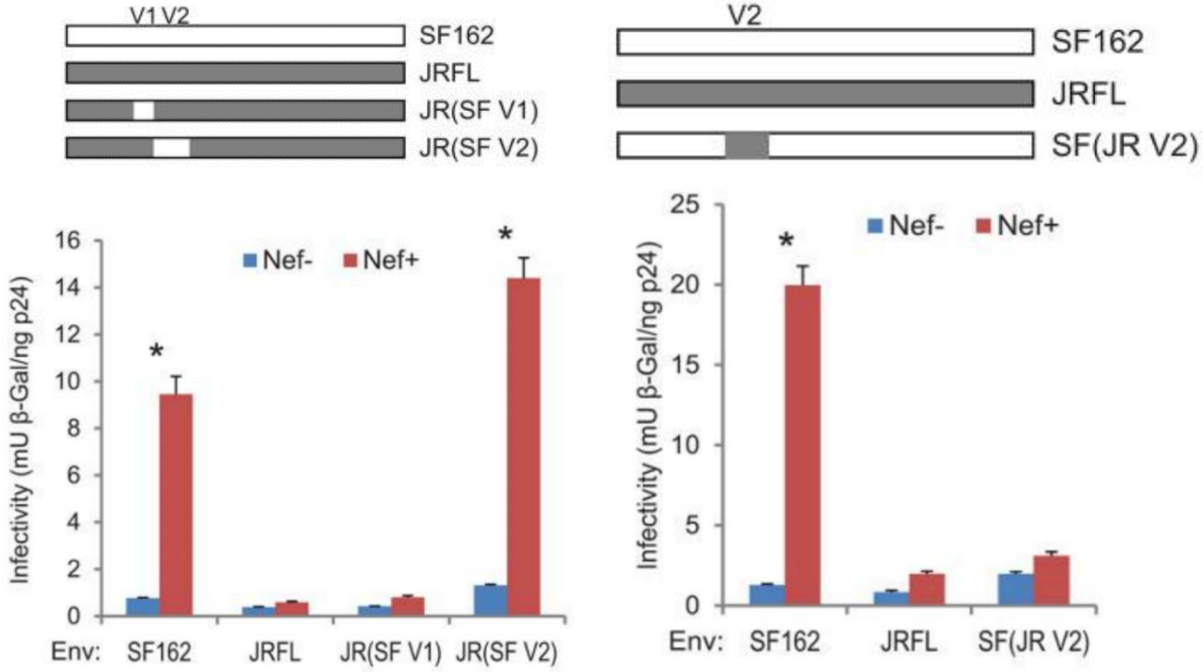


Figure 7. Increase in HIV Infectivity by Nef is Env Dependent (Usami, 2013)

Host-Protein SERINC5

Usami and colleagues, as well as Rosa and colleagues, independently identified a mechanism by which Nef increases the infectivity of HIV-1 virions - both groups identified the host transmembrane protein, SERINC5, and to a much lesser extent, SERINC3, as inhibitors of HIV-1 infectivity (The rest of this thesis will be focused on SERINC5) (Usami 2015, Rosa 2015). The key finding was that Nef counteracts the effects of SERINC5, explaining the basis of the infectivity-effect.

The Antagonistic Relationship Between Nef and SERINC

To better understand how SERINC5 inhibited HIV infectivity, these two groups studied its role in HIV virions (Usami 2015, Rosa 2015). The SERINC transmembrane protein family consists of five proteins (1 to 5) which are present in all eukaryotes. One previously described function is the mediation of serine incorporation (SERINC) into the plasma membrane in the brain, but their physiologic function remains largely unknown (Inuzuka 2005). In the absence of Nef, SERINC5 is incorporated into the membrane of budding virions and impairs virion infectivity (Fig. 8, Rosa 2015, Usami 2015).

When Nef is present, SERINC5 is redirected to an endosomal compartment, and prevented from incorporating into the virion membranes (Fig. 8, Rosa 2015). When Nef is absent, SERINC5 is incorporated into the virion membranes and prevents or delays fusion between virions and $CD4^+$ T cells through an unknown mechanism (Fig. 8, Aiken 2015). The function of SERINC5 as an HIV-1 inhibitor thus accounts for Nef's ability to increase HIV-1 infectivity. Moreover, these authors suggested that SERINC5 might impair fusion pore enlargement and increase the thermodynamic energy required for infection (Fig. 8, Usami 2015). Alternatively, SERINC5 might

indirectly inhibit fusion by changing the fluidity or lipid composition of the viral membrane (Aiken 2015).

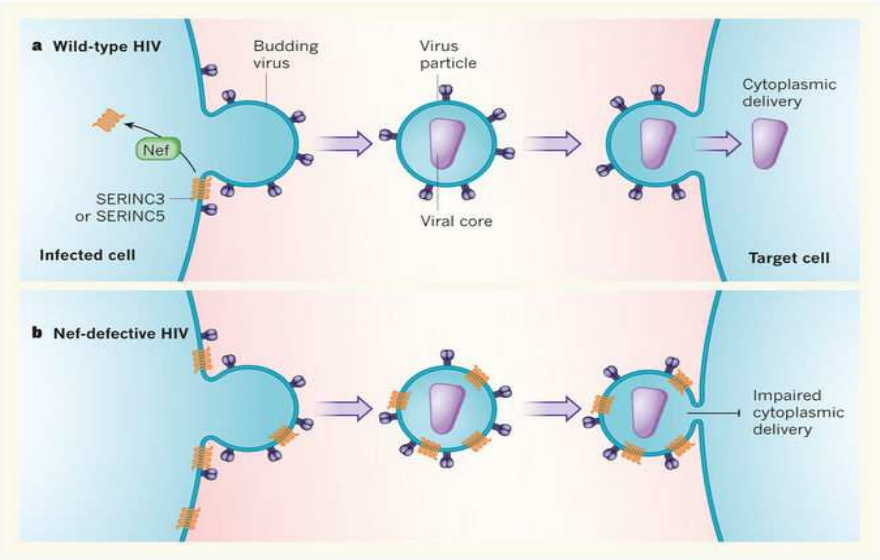


Figure 8. The Antagonistic Relationship Between Nef and SERINC5 (Aiken, 2015).

Beitari and colleagues showed that unlike Nef, Env does not prevent the incorporation of SERINC5 into the virion membranes. Nevertheless, certain Envs seem to resist its inhibitory effect (Beitari 2017). They also suggested that SERINC5 might cause a conformational change in the Env trimer (Fig. 5), not only reducing infectivity but also rendering certain epitopes in gp120 and gp41 more accessible to neutralizing antibodies (Beitari 2017).

Goals and Hypothesis

This thesis aimed to study the relationship between Env and SERINC5 (when Nef is absent) and to shed light on the following questions: Why are some Envs susceptible to SERINC5 while others were not? Is this difference a binary one, or is SERINC5-sensitivity instead a continuum? Is trimer "openness" a determinant of SERINC5-sensitivity across many Envs, and is it the only determinant of SERINC5-sensitivity?

The hypervariable regions V1/V2 and V3 in gp120 seem to play vital roles in HIV infectivity (Fig. 3, Rubbert 2011, Brandenberg 2015). Relevant to the work herein, these loops, particularly V2, influence the ability of Nef to enhance infectivity and therefore likely influence the sensitivity to SERINC5 (Fig. 7).

We hypothesized that the conformation of the Env trimer determines its sensitivity to SERINC5. To test this, we acquired a panel of HIV-1 Envs that have open, intermediate, or closed trimer conformations. Based on the literature, we divided these Envs into two categories – sensitive or resistant to the antibody 447-52D (Seaman 2010). A subset of our panel of Envs were then tested for their sensitivity to SERINC5 using an infectivity assay. If our hypothesis is correct, then Envs that have an open trimer conformation and are sensitive to the antibody 447-52D will also be relatively sensitive to SERINC5. Conversely, Envs that have a closed trimer conformation and are resistant to the antibody 447-52D will also be relatively resistant to SERINC5. We also hypothesized that opening the trimer mutationally would increase the sensitivity to SERINC5; we tested this using previously characterized mutations in the V2 loop.

Materials and Methods

Molecular Cloning

A panel of 14 HIV-1 Env molecular clones were obtained through the NIH AIDS Reagents Program, Division of AIDS NIAID, NIH. The HIV-1 Envs are denoted as: SF162.LS, MN/H9, BaL.26, HxB2.DG, 6536.3, ss1196.1, 1012-11.TC21.3257, 1056-10.TA11.1826, TRO.11, 1006-11.C3.1601, AC10.029, CAAN. 5342.A2, 1054-07.TC4.1499, and PVO.4. Envs with an ID_{50} titer less than 50μg/mL were categorized as sensitive to the antibody 447-52D. Envs with an ID_{50} titer greater than 50μg/mL were categorized as resistant to the antibody 447-52D.

Table 1. A Panel of HIV-1 Env Molecular Clones. Sensitivity to Antibody 447-52D Determined by ID_{50} titer. Tier of Each Env Provided. (Seaman, 2010).

HIV-1 Isolate	Tier	Sensitivity to 447-52D	ID_{50} titer (μg/mL)
SF162.LS	1A	Sensitive	<0.02
MN/H9	1A	Sensitive	<0.02
BaL.26	1B	Sensitive	0.04
HxB2.DG	1B	Sensitive	<0.02
6535.3	1B	Sensitive	0.1
SS1196.1	1B	Sensitive	0.4
1012-11.TC21.3257	1B	Resistant	>50
1056-10.TA11.1826	1B	Resistant	>50
TRO.11	2	Resistant	>50
1006-11.C3.1601	2	Resistant	>50
AC10.029	2	Resistant	>50
CAAN5342.A2	2	Resistant	>50
1054-07.TC4.1499	2	Resistant	>50
PVO.4	3	Resistant	>50

The clones were verified through Sanger sequencing. Once verified, they were propagated in E. coli competent cells in Luria Broth with ampicillin and the plasmid DNA was extracted with the Wizard Plus SV Minipreps DNA Purification System (Promega) or QIAGEN Plasmid Midi kit, according to manufacturer's instructions.

Cell Culture

HEK293T cells and TZM-bl cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and penicillin-streptomycin (P/S).

Transfection

HEK293T cells were plated in 12-well plates with 1.5 x 10^5 cells per well. Each well contained 1mL of DMEM that contained 10% FBS and P/S. A transfection mix for Lipofectamine (ThermoFisher) was made according to the manufacturer's instructions. Next, we created a master mix for each Env that contained a total of 1µg of DNA; 625ng of a lab clone of HIV that lacks both HIV-1 Env and HIV-1 Nef (DHIV-ΔnefΔenv) and 375ng of the Env plasmid. An additional plasmid, pBJ5-SERINC5-HA (a kind gift from Dr. Heinrich Gottlinger) was added at 0, 3ng, 10ng, 30ng or 100ng to the designated master mixes. The master mix was then added to the diluted Lipofectamine 3000 and incubated at room temperature for 10-15 minutes. 100µL of the transfection mix was added per well in the 12-well plate containing the HEK293Ts. The plates were then swirled by hand to ensure the transfection mix was well distributed, and the plates were then incubated for 72 hours at 37°C.

The supernatant was then collected from each well and centrifuged for five minutes at 300 x g to ensure any cells that may have been collected were pelleted. 1mL of the supernatant was then layered on top of 200µL 20% sucrose and centrifuged at 23,500 x g for 1 hour at 4°C. After

the virions were pelleted through the sucrose cushion, the supernatant was aspirated, and the virion pellets were resuspended in 600µL of DMEM that contained 10% FBS and P/S. 1:3 dilutions and 1:9 dilutions were made for each virion that had been pelleted.

The HEK293T cells in the 12-well plates were harvested for Western Blot Analysis.

Infectivity Assay

In a 96-well black wall plate, 20,000 TZM-bl cells in 100µL of DMEM that contained 10% FBS and P/S were seeded to each well. 100µL of the resuspended and diluted virus was added to the wells, in triplicate. Once the virions had been added to the TZM-bl cells, the 96-well plates were incubated for 48 hours at 37°C.

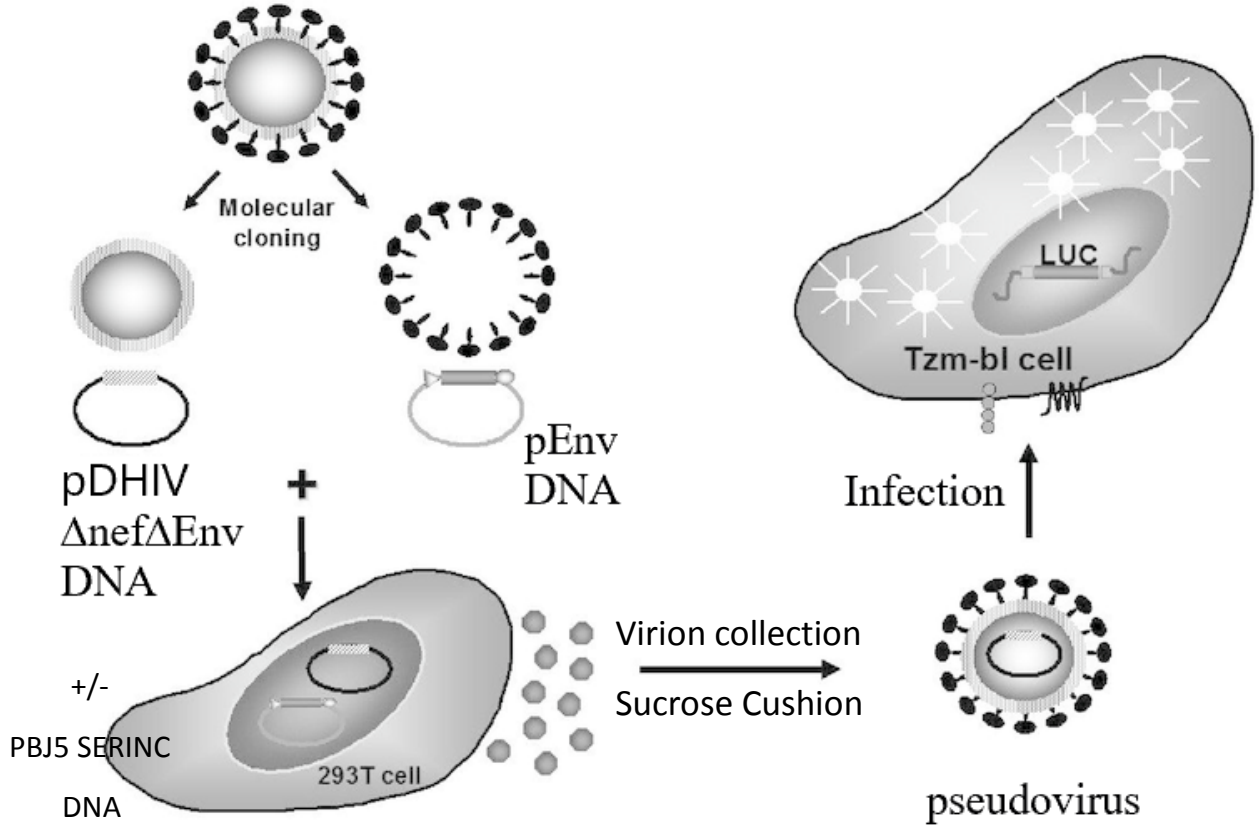


Figure 9. Assay Set-Up. Molecular cloning, transfection, and infectivity assay. (Ozaki, 2012).

After 48 hours the supernatant was removed, and 70µL of 0.5% Triton-X was added per well, and 70µL of Brite Lite luciferase reagent (Perkin Elmer) was added per well. The luciferase activity was measured using a Perkin Elmer plate reader and the number of relative light units (RLUs) in each well were recorded, according to the manufacturer's instructions.

Statistical Analysis

Normalization of infectivity assay data and the generation of graphs and error bars along with Welch's t-test were done using GraphPad Prism 8.

Chapter 1: Determining the Sensitivity to SERINC5 for Envs That Are Sensitive or Resistant to the Antibody 447-52D.

For our first experimental design, we analyzed the sensitivity of five Envs to SERINC5 (Fig. 10). Two of the Envs, LAI and SF162, are sensitive to the antibody 447-52D and have an open trimer conformation. JRFL, AC10 and 1012 Envs, have a relatively closed trimer conformation and are resistant to the antibody 447-52D. Each Env was expressed *in trans* by transfection along with an HIV plasmid (containing all of the HIV proteins except Env and Nef), and various amounts of a plasmid expressing SERINC5 into HEK293T cells. After 72 hours, the produced pseudoviruses were collected and used to infected TZM-bl reporter cells, which express luciferase when infected. Luciferase activity, expressed as relative light units (RLUs), was measured in lysates of the TZM-bl cells 48 hours post-infection. For each Env tested, the data were normalized, to the 0 ng SERINC5 sample, which was set to 100% for each Env. The relative infectivity of the pseudoviruses, produced at each amount of SERINC5 expression plasmid (3ng, 10ng, or 30ng), were then compared (Fig. 10).

In Figure 10, we see that each Env is impacted by SERINC5. As the amount of the transfected SERINC5 expression-plasmid increases, the amount of infectious virus (as measured by RLUs [%]) detected decreases. Interestingly SF162, which has an open conformation and is sensitive to the antibody 447-52D, resembles JRFL at 3ng and 10ng of SERINC5 but behaves like LAI at 30ng SERINC5 (Fig. 10). AC10 and 1012, which are resistant to the antibody 447-52D behave as expected (resembling JRFL and being relatively resistant to SERINC5) at the 3ng and 10ng amounts of SERINC5 (Fig. 10). Because the results were relative and not binary (i.e., Envs are not “sensitive” or “resistant” to SERINC5, but rather display quantitative relationships that changed with the amounts of SERINC5 expressed), it was challenging to make definitive conclusions when the data were assessed in this way.

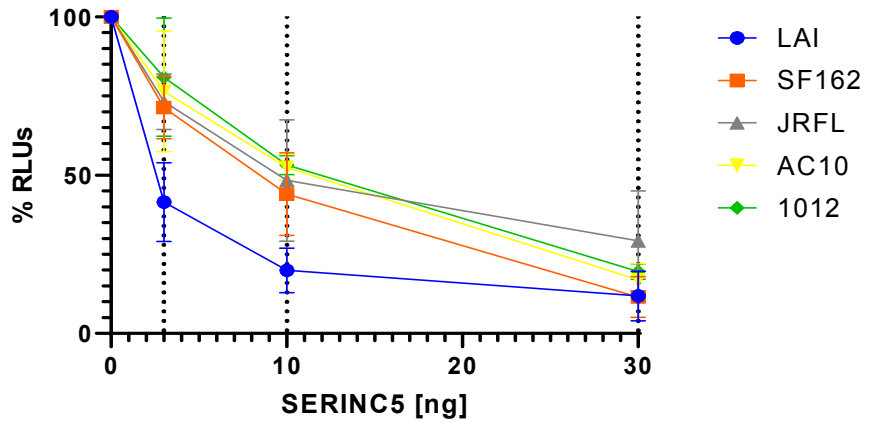


Figure 10. Relative Sensitivity of Different Envs to SERINC5. LAI is the control for sensitivity to both 447-52D and SERINC5. JRFL is the control for resistance (not sensitive) to both 447-52D and SERINC5. The amount of SERINC5 added was either 0ng, 3ng, 10ng or 30ng. Each assay was run in triplicate, and the graph represents three independent experiments. Each Env was normalized to 0ng SERINC5 added (0ng SERINC5 = 100%). The y-axis is percentages of the RLUs detected after data has been normalized to 0ng SERINC5.

We also analyzed every Env at each amount of SERINC5 expression plasmid (Fig. 11). Each Env was then compared to LAI, which was our control for maximal sensitivity to both the antibody 447-52D and SERINC5. The results represent three independent infectivity assays (Fig. 10, 11), each done in triplicate.

To confirm that the apparent differences were significant between Envs regarding their sensitivity to SERINC5, we conducted several statistical tests that looked into detail at the different conditions (levels) of SERINC5 expression. We found that at 3ng and 10ng of SERINC5 plasmid, LAI was significantly different from the other four Envs, including SF162 (Fig. 11). At 30ng of SERINC5, each Env was inhibited, and only LAI and JRFL were significantly different (Fig. 11).

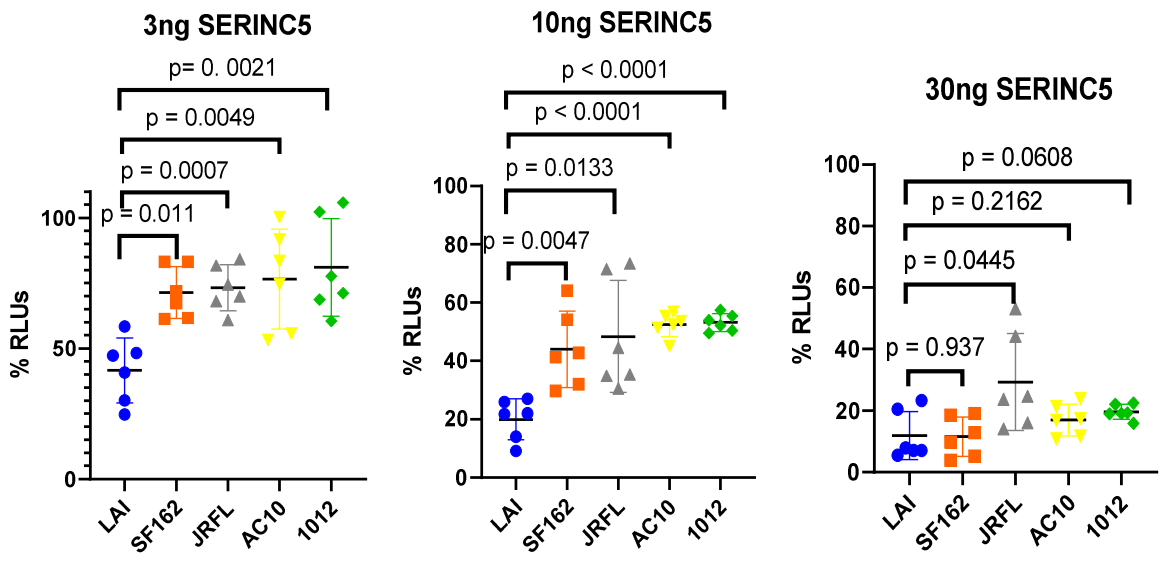


Figure 11. Statistical Analysis of Pseudoviral Sensitivity to SERINC5 at Specific Amounts. Each analysis compared the HIV Env LAI to every other HIV Env tested for its sensitivity to SERINC5. Welch's t-test was used to determine p values, $\alpha = 0.05$.

For further comparison, we combined the Envs into two different groups and compared these two groups: Envs sensitive to the 447-52D antibody, and Envs resistant to the 447-52D antibody. The data were again normalized to 0ng of SERINC5 for each Env. The two groups (Sensitive vs. Resistant), were then compared to one another at each amount of SERINC5 (Fig. 12). Grouping the Envs by this characteristic, we show that at each amount of SERINC5 there is a statistically significant difference in SERINC5-sensitivity between the Envs that are sensitive to 447-52D, and the Envs that are resistant to 447-52D (Fig. 12). Thus, we show that there is an association between Env sensitivity to the antibody and its sensitivity to SERINC5.

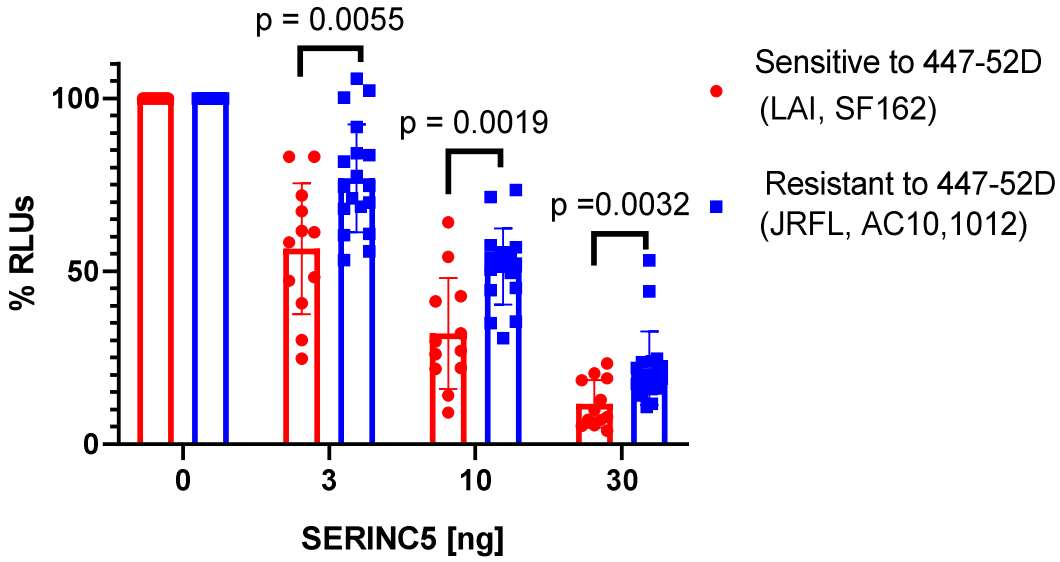
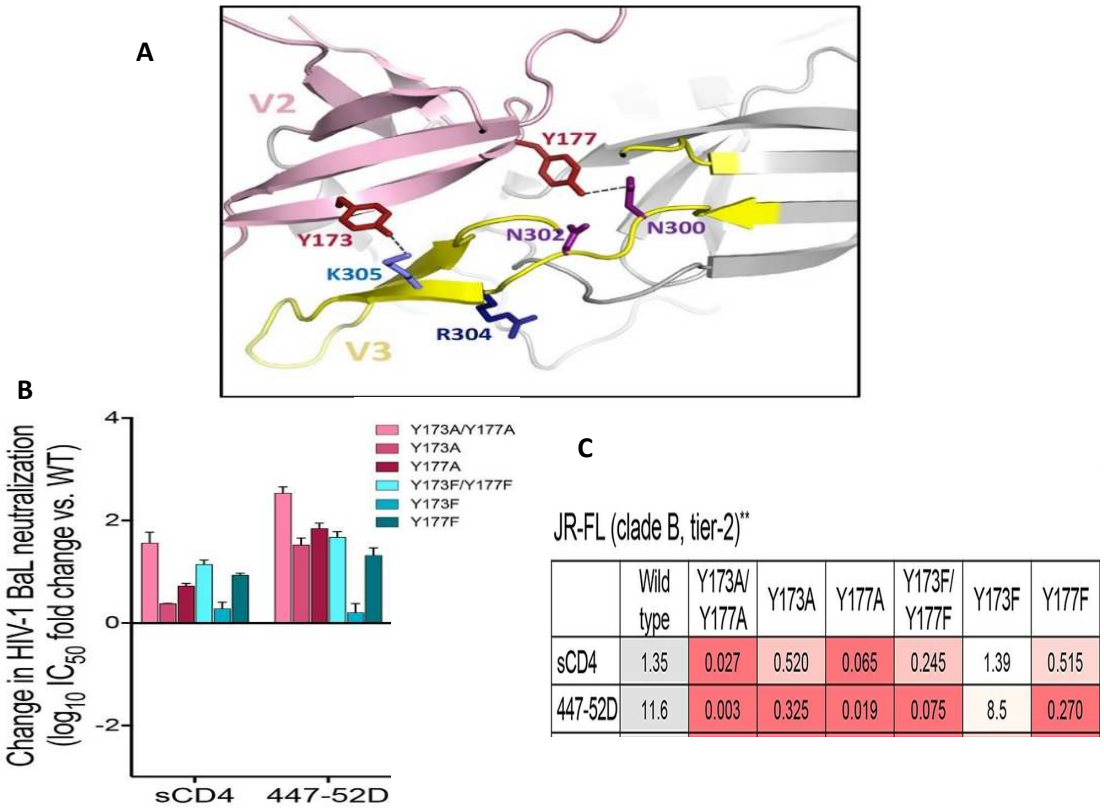


Figure 12. Grouping Pseudoviruses on Their Sensitivity to 447-52D Shows a Correlation Between Env Sensitivity to 447-52D and Env Sensitivity to SERINC5. Welch's t-test was used to determine p values, $\alpha = 0.05$.

Chapter 2: Does Opening the Env Trimer Mutationally Increase its Sensitivity to SERINC5?

For our second experimental design, we obtained a set of HIV-1 Env plasmids (a kind gift from Dr. Paolo Lusso) to test our hypothesis that trimer conformation determines the Env sensitivity to SERINC5 in a different way: by using mutations to open the trimer rather than by comparing different Envs. The Lusso group had made several mutations to the tyrosine at position 173 and/or 177 in variable loop 2 in the gp120 protein (Fig. 13A). Dr. Lusso and colleagues then tested these Envs for neutralization sensitivity, comparing the wild-type to the mutants they had made. In Figure 13B, the Lusso group tested the BaL Env, which has an opened trimer conformation. The mutants appeared to be significantly more sensitive to the antibody 447-52D. In Figure 13C, the Lusso group tested another Env, JRFL, which has a closed trimer conformation. They showed that these mutations (changing the tyrosine to phenylalanine or alanine) made JRFL much more susceptible to the antibody 447-52D.

Our goal was to use these mutants to support our findings from experimental design 1 and show that trimer conformation influences Env sensitivity to SERINC5. We used four of the Env plasmids the Lusso group had provided to compare the wild-type to the mutant FF (in which both tyrosines [173 and 177] were changed to phenylalanines) for both BaL and JRFL. The RLUs measured by our luciferase-based infectivity assay were normalized to 0ng SERINC5, as described before (Fig. 14).



	Wild type	Y173A/ Y177A	Y173A	Y177A	Y173F/ Y177F	Y173F	Y177F
sCD4	1.35	0.027	0.520	0.065	0.245	1.39	0.515
447-52D	11.6	0.003	0.325	0.019	0.075	8.5	0.270

Figure 13. Mutations to Y173 and Y177 Open the Trimer Conformation of Env, and the Mutants Are More Sensitive to 447-52D Antibody Compared to Wild-Type. (Guzzo, 2018). A) mutating the tyrosines at positions 173 and 177 between variable loops 2 and 3 in gp120 open the trimer conformation. B) BaL is a tier 1B HIV Env that is sensitive to the antibody 447-52D. When the tyrosines at 173 or 177 are changed to phenylalanines or alanines, they make BaL even more sensitive to 447-52D. C) JRFL is a tier 2 HIV Env that is resistant to 447-52D. When its tyrosines are mutated, it becomes more sensitive to the antibody 447-52D.

In Figure 14, we show that BaL (tier 1B, sensitive to 447-52D antibody) is intrinsically more sensitive to SERINC5 compared to JRFL (tier 2, resistant to 447-52D antibody) – a result that is consistent with our results comparing several different Envs presented in chapter 1. The mutation of the tyrosines at positions 173 and 177 to phenylalanine made BaL more sensitive to SERINC5 compared to the wild-type (Fig. 14). Also consistent with our central hypothesis, the JRFL FF mutant was slightly more sensitive to SERINC5 than the JRFL WT at 100ng of SERINC5 plasmid (Fig. 14).

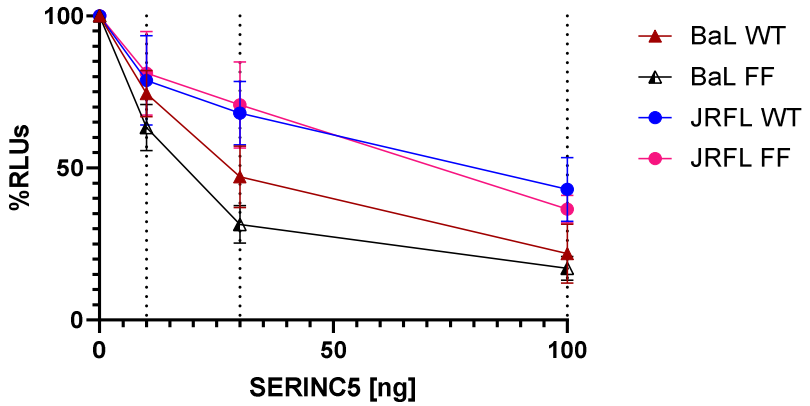


Figure 14. Effect of V2 Tyrosine Substitutions on Sensitivity to SERINC5. Both BaL and JRFL wild-type Envs were compared to the mutants which had both 173, and 177 tyrosines changed to phenylalanine. The amount of SERINC5 added was either 0ng, 10ng, 30ng or 100ng. Each assay was run in triplicate, and the graph represents four independent experiments. Each Env was normalized to 0ng SERINC5 added (0ng SERINC5 = 100%). The y-axis is percentages of the RLUs detected after data has been normalized to 0ng SERINC5.

Similar to our first experimental design, we then conducted a statistical analysis at each amount of SERINC5 (Fig. 15). The wild-type was compared to our FF mutant for both BaL and JRFL (Fig. 15). When we performed a detailed analysis of both BaL and JRFL at the specific amounts of the transfected SERINC5 expression plasmid, we observed statistically significant differences between BaL WT and BaL FF at the 10ng and 30ng amounts of SERINC5 (Fig. 15). At 100ng of SERINC5, both BaL WT and BaL FF infectivity were suppressed to roughly 20% compared to the 0ng SERINC5 of BaL WT and BaL FF (Fig. 15). While the differences between the JRFL WT and JRFL FF mutant at all three amounts of SERINC5 plasmid did not reach statistical significance, the average values at the 100ng plasmid amount suggested that the FF mutant might be slightly more sensitive than the WT (Fig. 15).

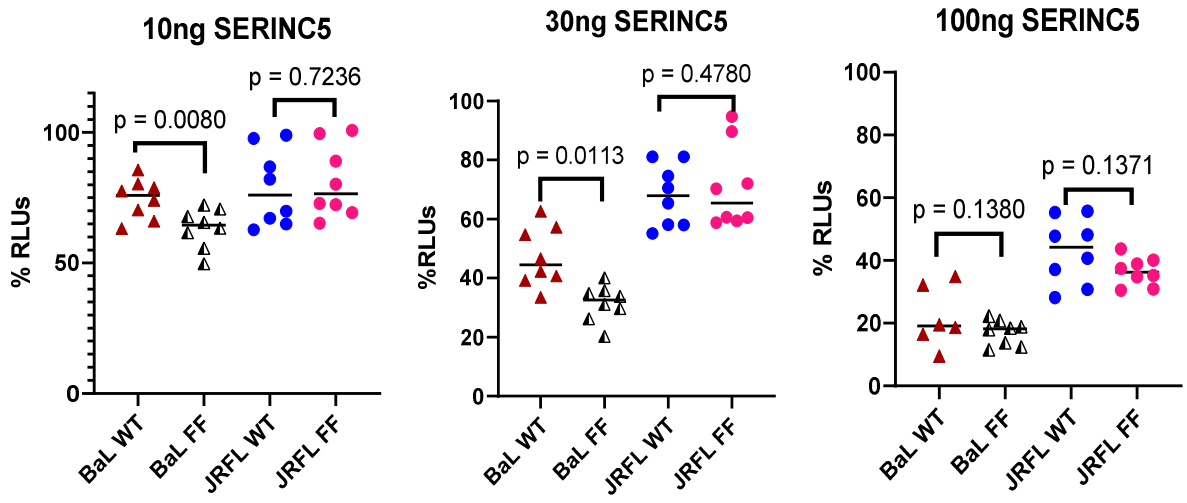


Figure 15. Comparing Env Sensitivity to SERINC5 at Specific Amounts of SERINC5. BaL wild-type is compared to BaL FF mutant, and JRFL wild-type is compared to JRFL FF mutant at 10ng, 30ng, and 100ng of SERINC5. Welch's t-test was used to determine p values, $\alpha = 0.05$.

Lastly, we created a bar graph of the Envs to assess the differences between the wild-type and mutant. The bar graph represents five independent experiments all done in triplicate. Additional independent infectivity assays of BaL and JRFL were added to create this data set. We observed that the trends in the expanded data remained largely unchanged (Fig. 16). Consistent with the data of Figure 15, the increased sensitivity of the BaL FF mutant relative to BaL WT was statistically significant at each amount of the SERINC5 plasmid (Fig. 16). Also consistent with Figure 15, we did not observe a statistically significant difference between JRFL WT and the JRFL FF mutant at 10ng or 30ng amounts of SERINC5. However, we did observe a statistically significant difference at 100ng of the SERINC5 plasmid: the FF mutant was slightly more sensitive to SERINC5. This suggested that at high levels of SERINC5 expression, trimer opening can increase sensitivity to SERINC5 even in the setting of an Env (here JRFL) that is intrinsically relatively resistant.

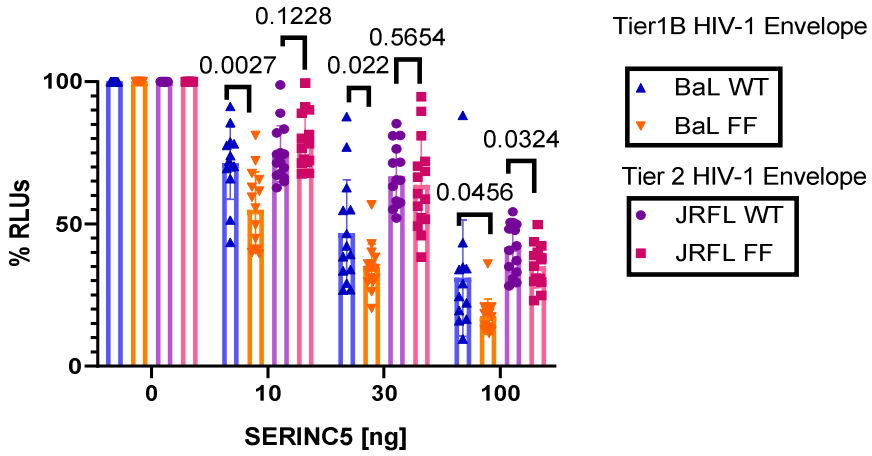


Figure 16. Bar Graph of Env Sensitivity to SERINC5 and Statistical Analyses Comparing the Wild-Type to the Mutant for Both BaL and JRFL. The amount of SERINC5 added was either 0ng, 10ng, 30ng or 100ng. Each assay was run in triplicate, and the graph represents five independent experiments. Each Env was normalized to 0ng SERINC5 added (0ng SERINC = 100%). The y-axis is percentages of the RLUs detected after data has been normalized to 0ng SERINC5. Welch's t-test was used to determine p values, $\alpha = 0.05$.

Conclusions and Future Directions

In our first experimental design, we were able to support the hypothesis that Env trimer "openness" relates to Env sensitivity to SERINC5. In Figure 12, we show that the group of Envs sensitive to the antibody 447-52D (LAI and SF162) are also relatively sensitive to SERINC5. Conversely, the group of Envs resistant to 447-52D (JRFL, AC10 and 1012) are also relatively resistant to SERINC5 (Fig. 12). One limitation of our first experimental design was that when comparing different Envs, we could not account for multiple potential variables that might contribute to sensitivity to SERINC5. By comparing different Envs to one another based on their sensitivity to the antibody 447-52D, we were only accounting for one variable, trimer "openness," which might not be the only property that contributes to sensitivity to SERINC5.

To address this, we used mutations to change the HIV Env trimer to a more open conformation, thus isolating only on this engineered difference. We tested whether these mutations, which presumably opened the trimer, would increase the sensitivity of the Env to SERINC5. This approach allowed us to compare the Envs to themselves (with or without the trimer opening mutations) with the only difference being the trimer conformation.

From our second experimental design, we conclude that mutations that open the trimer cause an increase in sensitivity to SERINC5 in the case of BaL-Env, but do so to a much lesser extent in the case of JRFL-Env. We propose, based on these data that relative trimer "openness" might be necessary for high SERINC5 sensitivity, but that trimer "openness" alone is not sufficient for SERINC5 sensitivity. We suggest that other factors must play a role in the sensitivity to SERINC5 in addition to Env trimer "openness".

The Lusso group showed that a specific change in trimer conformation caused by mutational substitution of two tyrosines in the V2 loop made the Envs more susceptible to the

antibody 447-52D (Fig. 13). Similarly, in Figure 7, Usami and colleagues show that by switching the V2 regions of an Env that is resistant to antibody neutralization (JRFL) with the V2 region of an Env that is sensitive to antibody neutralization (SF162), their need for Nef also switched. Usami and colleagues conducted a detailed mapping of the V2 region to understand the Nef responsiveness – the specific residues Usami's group identified were only a few positions away from the tyrosines that the Lusso group showed affected Env trimer conformation. It could be that the residues responsible for Nef responsiveness are very similar in function to the tyrosines and help hold the trimer in a more closed conformation, making the Env less susceptible to antibody neutralization and SERINC5 inhibition.

Beitari and colleagues tested a panel of Envs from different HIV strains to observe their susceptibility to SERINC5 (Beitari 2017). Using SERINC1 and SERINC5, the group was able to compare SERINC's overall impact on Env (Beitari 2017). Through several experiments, they showed that the hypervariable regions of gp120, determine the SERINC5 sensitivity of HIV Envs similar to the Lusso and Usami groups (Beitari 2017). However, Beitari and colleagues concluded that the V3 loop is the key determinant in Env confers resistance to SERINC5 (Beitari, 2017). The mutational substitutions made by the Lusso group disrupted the tyrosines that bound the V2 loop to the V3 loop – the interactions between the V2 and V3 loop could play a prominent role in trimer "openness" and SERINC5 sensitivity. Mutating the residues in the V3 loop that interact with the V2 tyrosines and seeing if the same phenotype occurs (increased sensitivity to the antibody 447-52D and SERINC5), would be informative regarding hypervariable loop interactions and how those interactions protect HIV virions.

Beitari and colleagues suggested the majority of primary Envs are "resistant" to SERINC5, which is consistent with their tier-categorization and presumed relatively closed trimers (Fig. 4,

Fig 5). However, Zhang and colleagues showed that the expression of CD4 could cause such Envs to become relatively sensitive to SERINC5 (Zhang 2019). The results of our second experimental setup, where we open the trimer mutationally, are consistent with their findings. Along these lines, we could test whether CD4 renders "resistant" Envs more sensitive to the antibody 447-52D, similar to the results Zhang and colleagues showed with CD4 and SERINC5.

In this thesis, we show that there is an association between trimer "openness" and SERINC5 sensitivity. The next steps are to analyze this relationship further and determine what other factors might be playing a role. Is there a direct interaction between Env and SERINC5 or does SERINC5 indirectly inhibit viral infectivity? Do Envs with an open trimer conformation bind "better" to SERINC5 and this attachment prevents proper pore formation? Or does SERINC5 delay virion-cell fusion such that open trimers come apart and no longer support infectivity? Lastly, and related to the last question, is trimer conformation directly correlated with trimer stability?

As we gain further insight into the mechanisms Env uses, we can use these findings to ultimately exploit the virus and develop further therapies for treatment and prevention.

This thesis contains material coauthored with Aaron Angerstein, Thomas Vollbrecht, and John Guatelli.

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