THE PENNSYLVANIA STATE UNIVERSITY

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DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Differences in Flavivirus NS1 Phenotype

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Abstract

The family of viruses known as *Flaviviridae* includes several well-known human pathogens that are primarily spread through arthropod vectors, and includes the dengue, West-Nile, deer tick, and Zika viruses among others. These viruses have been responsible for disease outbreaks around the globe, and with warmer temperatures resulting in a wider distribution of vectors, understanding the pathogenesis of these viruses is more important than ever. Flavivirus genomes are small, encoding a variety of structural (C, prM and E) and nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) that are essential to the replication and survival of the virus. Among these proteins is NS1, which plays a role in both replication and immune evasion. This protein is extremely highly conserved amongst flaviviruses, with few differences in amino acid composition between different species. This work examines differences in cell phenotype upon expression of NS1 from different flaviviruses, including a previously unknown behavior of the Zika NS1 protein. Copies of four different viral NS1 were expressed from a mammalian expression plasmid pcDNA 3.1 + form with an affixed fluorescent tag to allow for cell imaging upon transfection. Chimeric NS1 proteins were also produced in order to determine the regions responsible for differing NS1 behaviors. Results indicate that specific regions in the N-terminal region of Zika NS1 differentiate it from the NS1 of other flaviviruses and allow it to induce actin remodeling upon expression in a host cell.

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Acknowledgements

I would like to thank Dr. Joyce Jose and the entire Jose lab for their support and guidance throughout this project. I would also like to thank my advisor Dr. Lorraine Santy and the entirety of the Schreyer Faculty which made this research possible. I would also like to give a special thanks to Dr. Anoop Narayanan for teaching me so much about molecular biology and instructing me in laboratory technique.

Chapter 1: Introduction

Flaviviruses are a group of viruses belonging to the family Flaviviridae, and are primarily spread through arthropod vectors such as mosquitoes and ticks. Among these viruses, several are known to cause disease inside human hosts. Some of the most well-known flaviviruses are Zika virus (ZIKV), dengue virus (DENV) and West-Nile virus (WNV), all of which are spread via mosquito.[1] Deer tick virus (DTV) is transmitted through tick bite and can be found throughout in United States, particularly in the Northeast.[2] Arbovirus spread is of growing concern for much of the world, with increasing temperature and humidity being observed as a result of climate change, the arthropods that spread these viruses are experiencing increased geographic range. Some estimates predict DENV transmission in the United Kingdom by the end of the century.[3] With increased transmission comes an increase in pressure to better understand and treat the human diseases caused by these viruses, and to that end, an understanding of viral proteins and their interactions offers potential solutions for both drug and vaccine development. Flaviviruses have a single stranded, positive sense RNA genome that encodes three structural proteins and seven nonstructural proteins. Of the nonstructural proteins, nonstructural protein 1 (NS1) is highly conserved among all flaviviruses and varies in mass between 46-55 kDa depending upon its degree of glycosylation. NS1 exists as a monomer that plays a role in viral replication, a dimer that sits on the cell membrane, and a hexamer that is secreted to the outside of the cell.[4] The monomeric form of the protein interacts with several components of the replication complex as a cofactor and colocalizes with double stranded RNA, yet the exact role this form of the protein plays in replication is still poorly understood [5, 6, 7]. The secreted hexamer variant is responsible for several interactions with the host immune system, and is known to be involved in evading the complement

system (4, 8, 9). The role of the membrane bound, dimerized NS1 is the least understood, although its structure is known. The NS1 dimer is able to sit upon the cell membrane via interactions between its wing and β-roll domains and the surface of the cell (Figure 1).

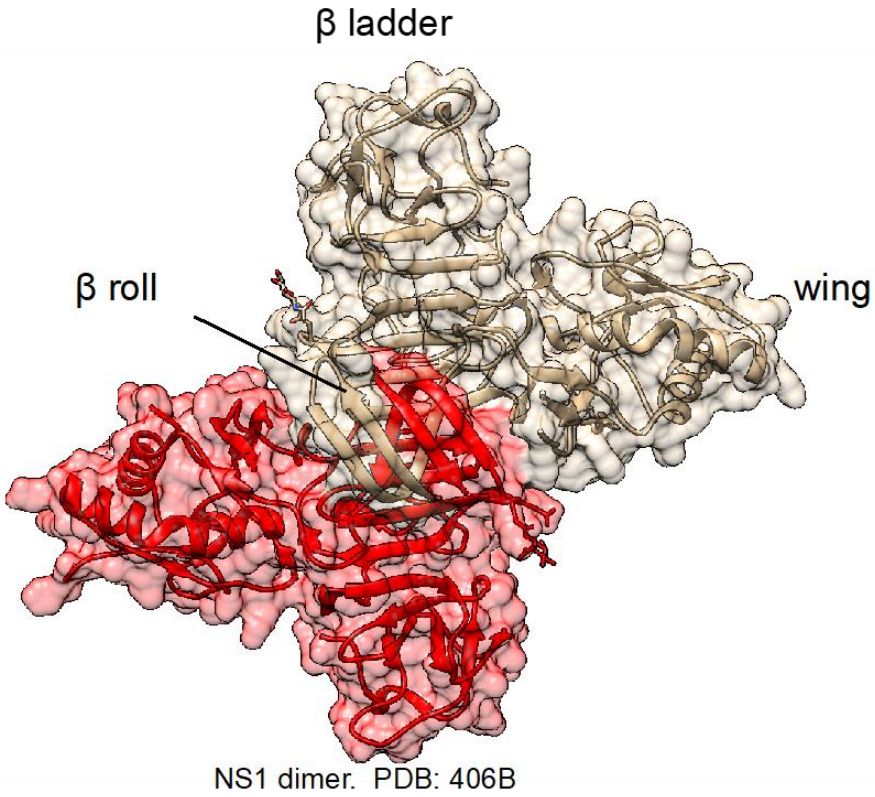

Figure 1: NS1 Dimer[10].

Previously, it has been shown that actin remodeling occurs in many types of cells upon flavivirus infection, although until now, only in WNV has the expression of NS1 alone proven sufficient to induce this remodeling. Upon expression of WNV NS1 in Vero E6 cells, extensive actin remodeling was shown to occur and projections similar to tunneling nanotubes (TNTs) formed. Inside of these projections, NS1 was found, and when the projections resulted from infection with a complete virus instead of just NS1 expression, both NS1 and viral E protein were located within

them[11]. The benefit to the virus of these structures is not yet known, although many possibilities exist. The evidence that certain viral proteins are contained within the projections suggests that they may serve as either a method of trafficking virions into neighboring cells, or of releasing virions from the cell and into the surrounding environment. Another possibility is that these projections aid in immune evasion, as certain tumors have been observed using TNTs to hijack mitochondria from approaching immune cells in order to weaken them[12]. The Jose Laboratory at the Pennsylvania State University has done extensive research on both flaviviruses and flavivirus NS1, and while the exact benefit to the virus provided by NS1 localized to the plasma membrane is not yet known, this work has revealed distinctive phenotypic differences upon the expression of different types of NS1 in cells.

Prior to this work, ZIKV NS1 was not known to be capable of inducing actin remodeling upon expression, yet here we demonstrate that ZIKV NS1 does indeed display actin remodeling upon expression in some human cell lines. Using an mCherry fluorescent protein tag and live cell imaging, my work has allowed us to observe the effects of expressing NS1 from different flavivirus species in varying cell lines. Furthermore, the fluorescent tag has allowed for confirmation that the protein behaves as it should, localized to the outside of the plasma membrane and being secreted into the media surrounding expressing cells.

Chapter 2: Methodology

Determining Localization of Expressed ZIKV NS1

The first step of the project was to confirm that the mCherry-tagged ZIKV NS1 was behaving in the same fashion as the untagged protein upon expression in mammalian cells. In order to determine whether or not the ZIKV NS1 was localizing to the outside of the cell membrane, pH changes in the cell media were performed in order to exploit the tendency of mCherry to lose its fluorescent properties below a pH of 4.5[13]. Huh 7.5 cells were transfected with plasmid encoding mCherry-tagged wild type (WT) ZIKV NS1 and GFP-SEC61 ER marker as described by the protocol in appendix A. After one day, the cells were examined using fluorescent microscopy and had the media replaced with media adjusted to a pH of 4.2. The cells were examined immediately after the acidification to determine whether or not the mCherry-tagged NS1 was localizing to the exterior of the cell.

Determining Secretion of Expressed ZIKV NS1

In order to determine if the expressed NS1 was being secreted from transfected cells, beads coated in an mCherry nanobody were added to the media of Huh 7.5 cells that had been transfected with mCherry-tagged WT ZIKV NS1 and allowed to grow for a day. After 15 minutes, the cells were imaged using fluorescence microscopy.

Determining Observed Structures are Actin

In order to determine whether or not the structures seen upon ZIKV NS1 expression are a result of actin remodeling, mCherry-tagged ZIKV NS1 was transfected alongside GFP-tagged actin into one group of Huh 7.5 cells, while another group had GFP-tagged tubulin transfected alongside NS1.

Generation of DTV NS1, DENV NS1, and ZIKV NS1 Without Intron Clones

Following the initial experiments with ZIKV NS1, the decision was made to explore DTV and DENV NS1 behavior in cells in order to compare the behavior of NS1 from all three species. Because the original full-length ZIKV cDNA clone possessed an intron in NS1 region, a clone without the intron needed to be made to ensure that the intron in the original clone was not affecting protein behavior. To generate ZIKV NS1 without an intron, reverse transcription (RT) PCR was carried out from viral RNA as discussed in Appendix A, with the PCR product being joined with mCherry via overlap PCR, the protocol for which can be found in Appendix A. The product of this reaction was then cloned into pCDNA 3.1 vector after both insert and vector had been cut via restriction enzymes. The digested insert and vector DNA were joined via ligation and transformed into competent *E. coli* cells as described in Appendix A. Following plasmid uptake and cell growth, the intact plasmid product was reisolated using the mini-prep protocol found in Appendix A. The DTV and DENV NS1 inserts were created with viral RNA from cells infected with DTV and DENV. All RT-PCR products were purified by gel elution, as described

in Appendix A. DENV and DTV NS1 clones were prepared using ligation independent cloning (LIC), with the protocol for this procedure being described in Appendix A.

Comparison of Phenotype for DTV NS1, DENV NS1, ZIKV NS1, and ZIKV NS1 Without Intron Upon Expression in Mammalian Cells.

Both ZIKV NS1 clones, along with the DTV and DENV NS1 clones, were transfected into Huh 7.5 cells. ZIKV NS1 was also transfected into HEK293T and A549 cells using the protocol described in Appendix A. All cells were then imaged via confocal microscopy, and the phenotypes were compared.

Generation of Chimeric ZIKV NS1 and Evaluation of Phenotype

Chimeric versions of ZIKV NS1 were created using overlap and site-directed mutagenesis (SDM) PCR, as described in Appendix A. The ZIKV NS1 had both the first and last 50 amino acids in its sequence replaced by the corresponding sequence from DENV NS1 via overlap PCR, and the mutants were then transfected into Huh 7.5 cells. Following results from this experiment, the first 50 amino acids of the ZIKV NS1 N-terminus were divided into sections of 12-13 amino acids and systematically replaced with the corresponding DENV sequence using SDM in order to further evaluate which region was responsible for the unique phenotype of expressed ZIKV NS1. All inserts from the chimeric protein experiments were inserted into vectors using ligation independent cloning (LIC).

Chapter 3: Results and Discussion

Given that NS1 is known to aid in viral replication as a monomer, localize to the cell membrane as a dimer, and be secreted as a hexamer to aid in immune evasion, it was of some concern that the mCherry-tagged variant might fail to behave as the unmodified form. Our results indicate that not only does our mCherry-tagged NS1 retain its functionality, and that ZIKV NS1 induces actin remodeling upon expression while DTV and DENV NS1 do not. Previously, such behavior has only been seen in WNV NS1, making it relatively unique amongst flaviviruses. It is possible that these structures serve to transmit viral material from infected cells to their healthy neighbors, or even that they aid in immune evasion via mitochondrial hijacking. While the exact purpose of these projections is not known, NS1 is the only viral protein required for ZIKV to induce their appearance.

Localization and Secretion of mCherry-Tagged ZIKV NS1

The results of the acidification assay can be seen in Figure 2. Media acidification resulted in almost immediate loss of mCherry fluorescence, indicating that the mCherry tagged NS1 was indeed localizing to the outside of the cell. Note that amidst the GFP-tagged SEC61, red flecks of mCherry fluorescence persist, meaning that the interior of the cell had not been acidified.

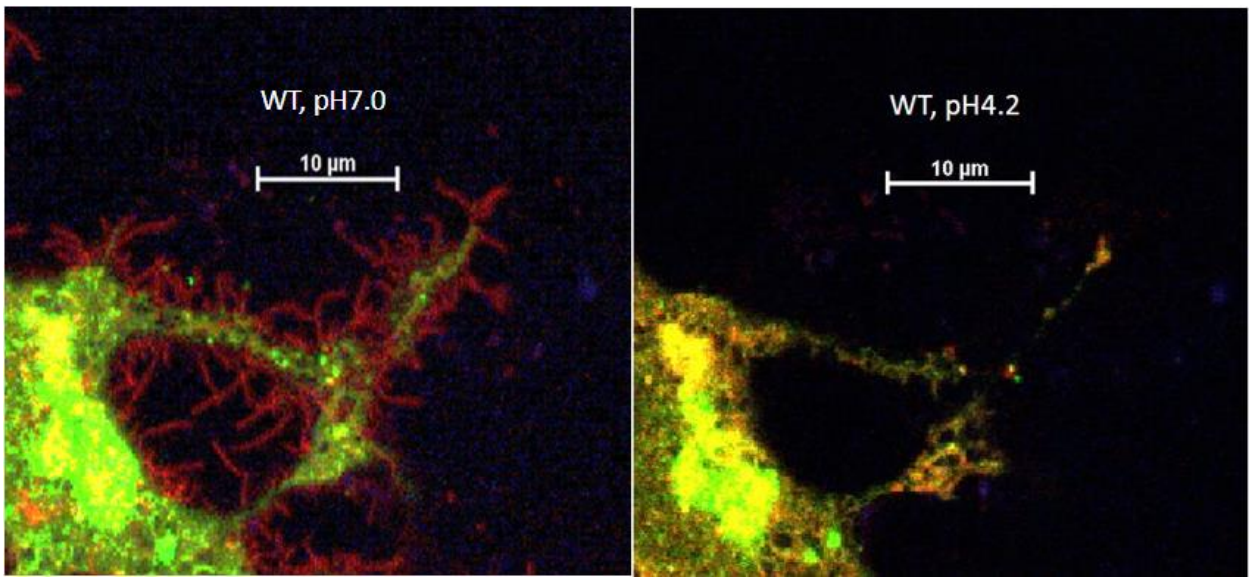

Figure 2: Huh 7.5 cells expressing mCherry-tagged WT ZIKV NS1 and ER marker GFP-tagged SEC61 in both neutral and acidic media.

The results of the secretion assay are shown in Figure 3. The fluorescence of the nanobody beads demonstrates mCherry binding, indicating that the tagged NS1 is successfully able to be secreted by cells.

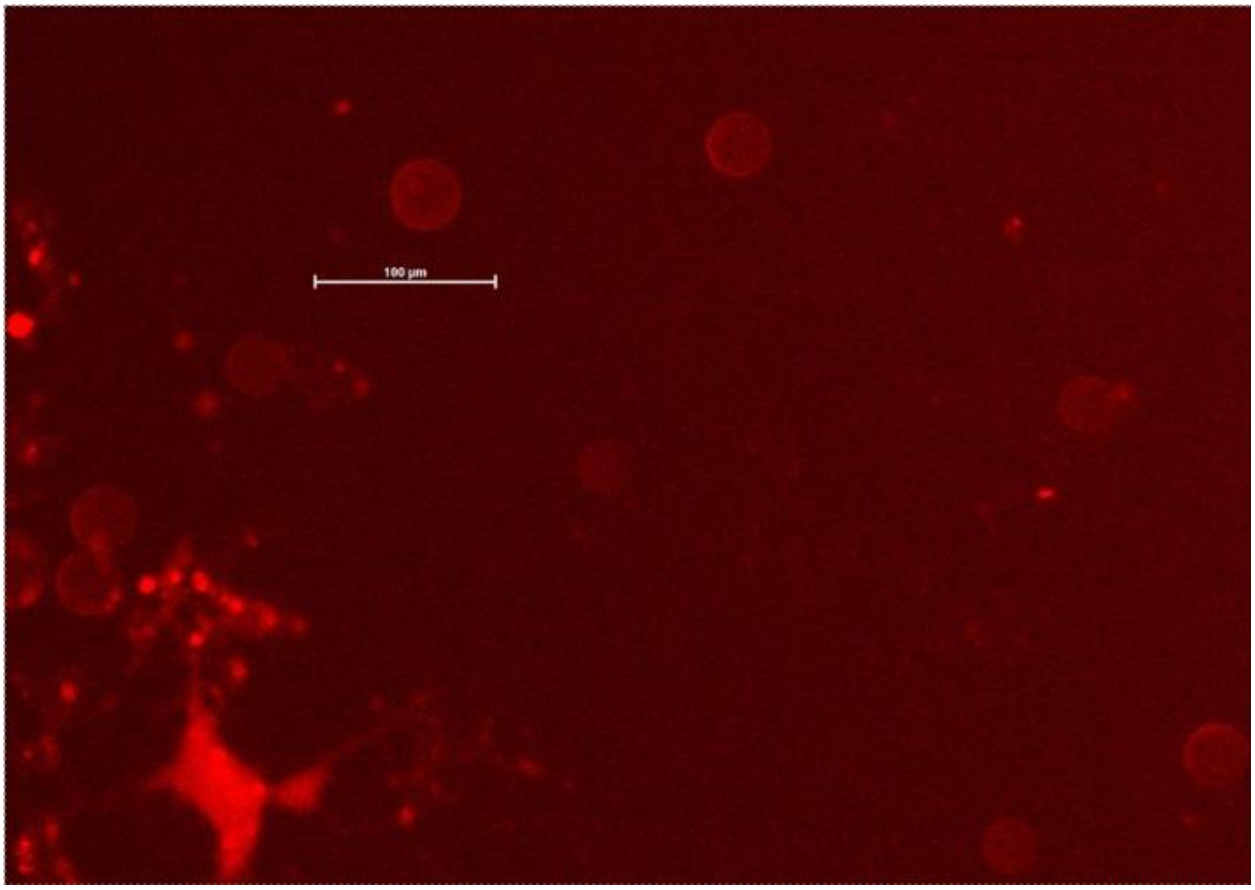

Figure 3: Beads coated in nanobodies that bind mCherry in the media of Huh 7.5 cells expressing tagged WT ZIKV NS1.

Composition of Observed Structures

The cells that were transfected with mCherry-tagged ZIKV NS1 and GFP-tagged actin showed actin to be present in the extended structures that formed, whereas the GFP-tagged tubulin cells showed reduced tubulin presence in the extensions (Figures 4-5). This confirms that the structures seen upon ZIKV NS1 expression are a result of actin remodeling. However, an Immunofluorescence assay (IFA) would be required to rule out the presence of tubulin entirely in these structures.

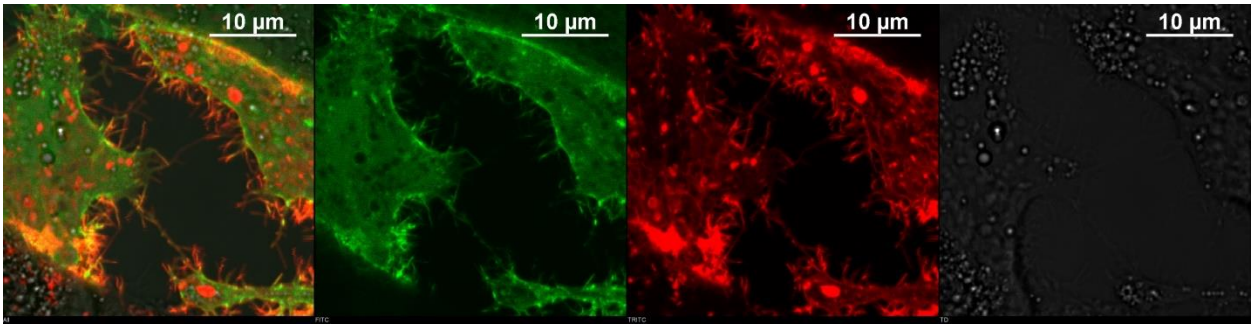

Figure 4: mCherry-tagged ZIKV NS1 co-expressed with GFP-tagged actin in Huh 7.5 cells.

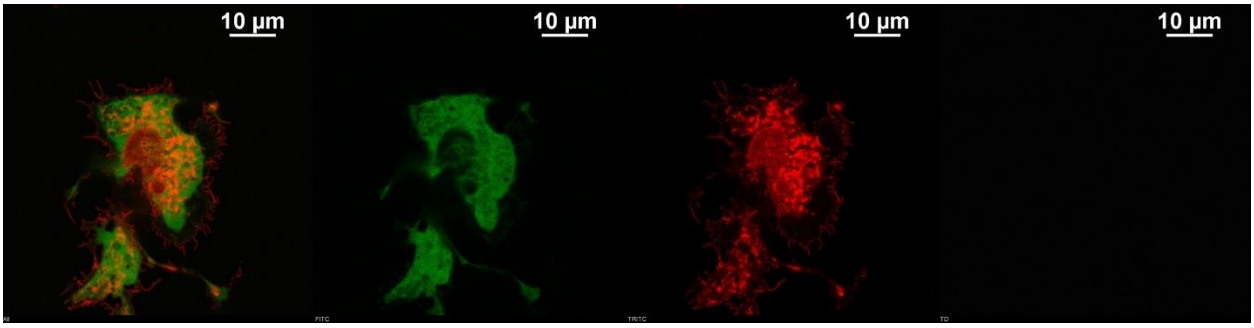

Figure 5: mCherry-tagged ZIKV NS1 co-expressed with GFP-tagged tubulin in Huh 7.5 cells.

Comparison of NS1 phenotypes

The results of the transfection assays can be seen in Figures 6-7. Upon transfection into Huh 7.5, only ZIKV NS1 showed actin remodeling, with neither DENV nor DTV NS1 demonstrating filopodia or TNT formation. ZIKV NS1 with and without the intron both behaved identically, meaning the presence of the intron in the original clone had no effect on the protein. Transfection into HEK293T did not result in actin remodeling for ZIKV NS1 (Figure 8), and so in order to ensure that the ZIKV NS1 remodeling was not unique to Huh 7.5 in human cells, ZIKV NS1 was transfected into A549 cells. Transfection into A549 resulted in actin remodeling (Figure 9), indicating that ZIKV NS1 is capable of inducing actin remodeling in multiple human cell lines.

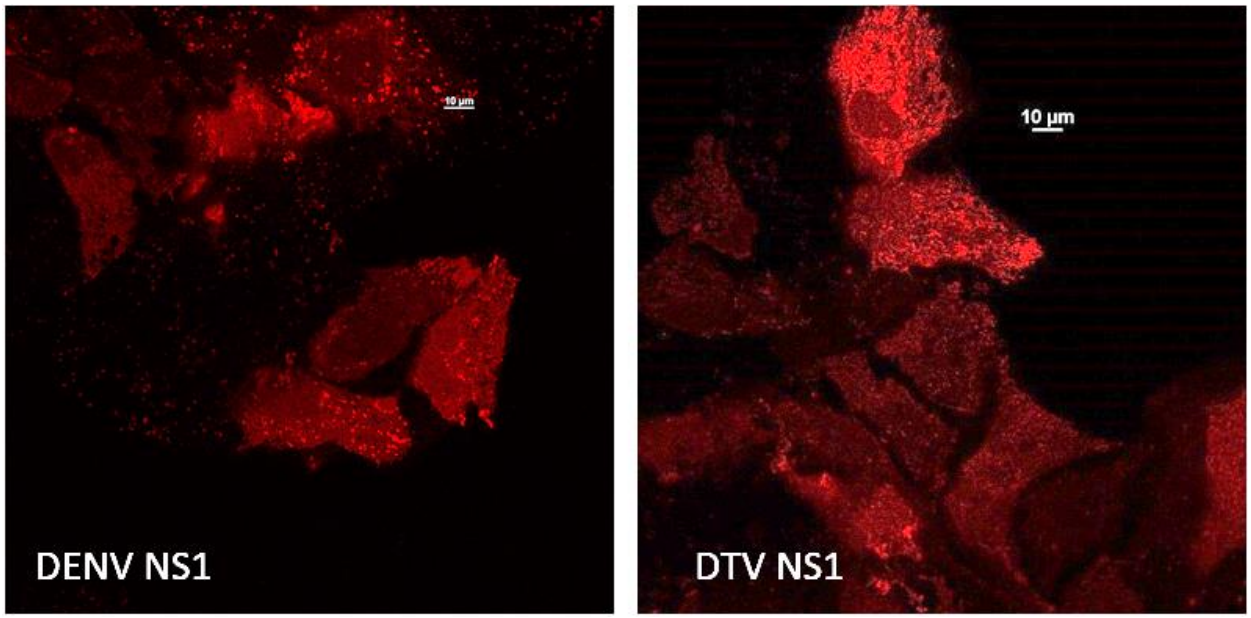

Figure 6: mCherry-tagged DENV and DTV NS1 expressed in Huh 7.5 cells.

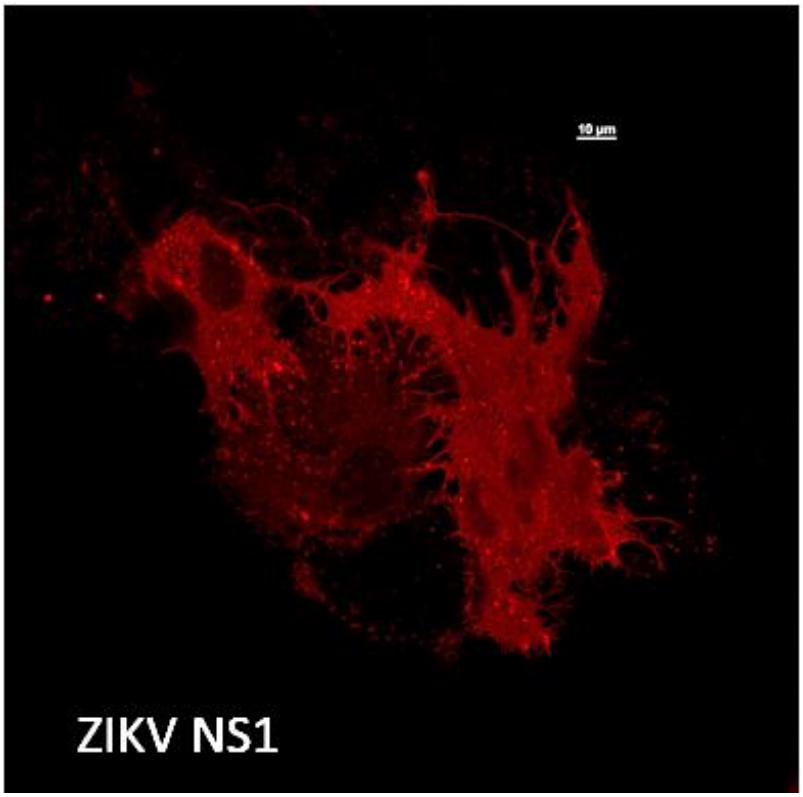

Figure 7: mCherry-tagged ZIKV NS1 expressed in Huh 7.5 cells.

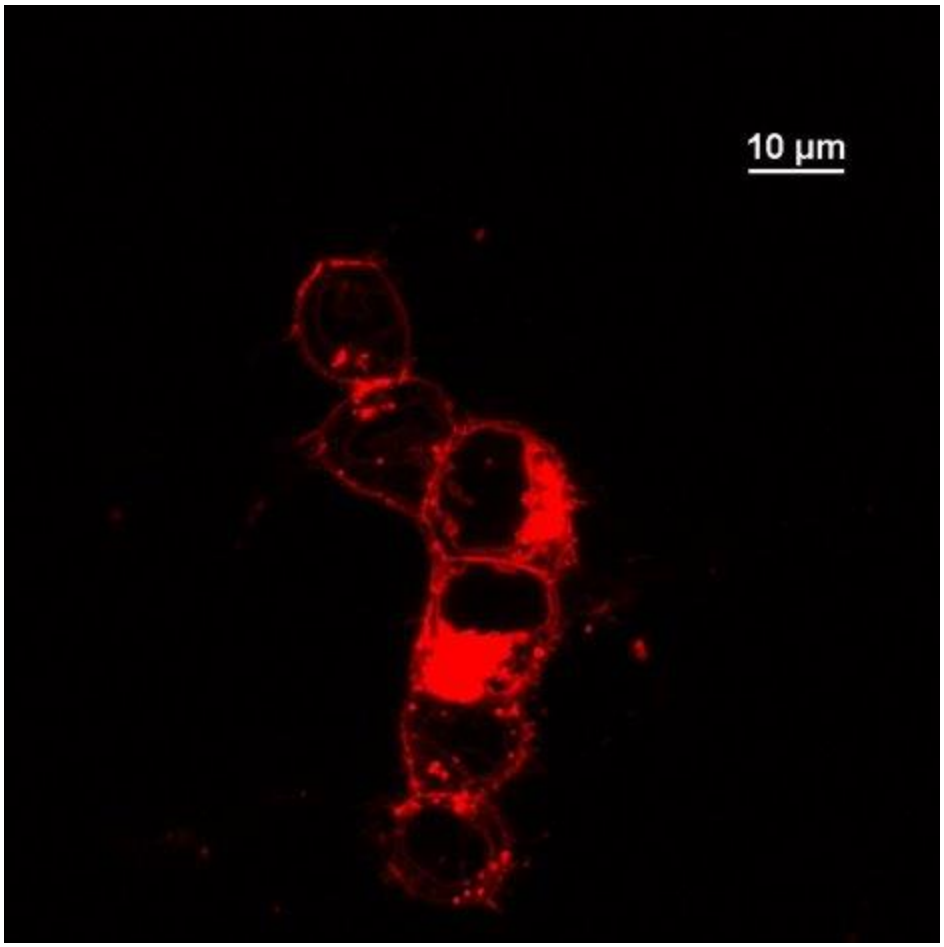

Figure 8: mCherry-tagged ZIKV NS1 expressed in HEK293T cells.

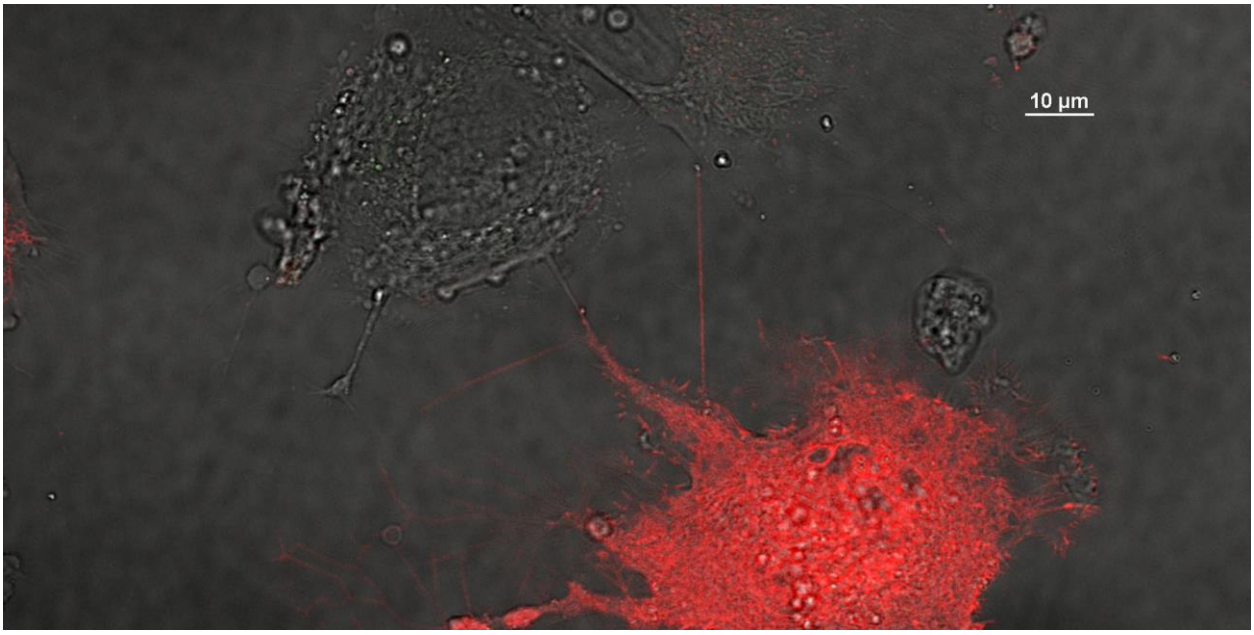

Figure 9: mCherry-tagged ZIKV NS1 in A549 cells.

Results from Chimeric ZIKV NS1 and Evaluation of Chimeric Phenotypes

The results of the chimeric ZIKV NS1 being expressed in Huh 7.5 cells are shown in Figures 9-13. When the first 50 amino acids of the N-terminal ZIKV NS1 sequence were substituted with the first 50 amino acids of DENV NS1, a complete cessation of actin remodeling activity was observed (Figure 10). However, on mutation of the last 50 amino acids of the ZIKV NS1 C-terminal sequence showed no impact on NS1 phenotype, with the same localization and actin remodeling behavior observed in the WT ZIKV NS1 also being observed in this chimeric protein (Figure 11). Furthermore, when any of the 12-13 amino acid sections from the first 40 amino acids were mutated, no membrane localization or actin remodeling was observed (Figure 12), yet when the last 12 were mutated, a variant was obtained that was able to localize to the membrane without inducing actin remodeling (Figure 13). It is known that the first 50 amino acids of NS1 are located within the crucial wing/β-roll domains, and thus tampering with them would logically have an impact on membrane interactions. As such, these results are not particularly surprising, but do help to further narrow down the precise residues responsible for the phenotypic differences observed in ZIKV NS1.

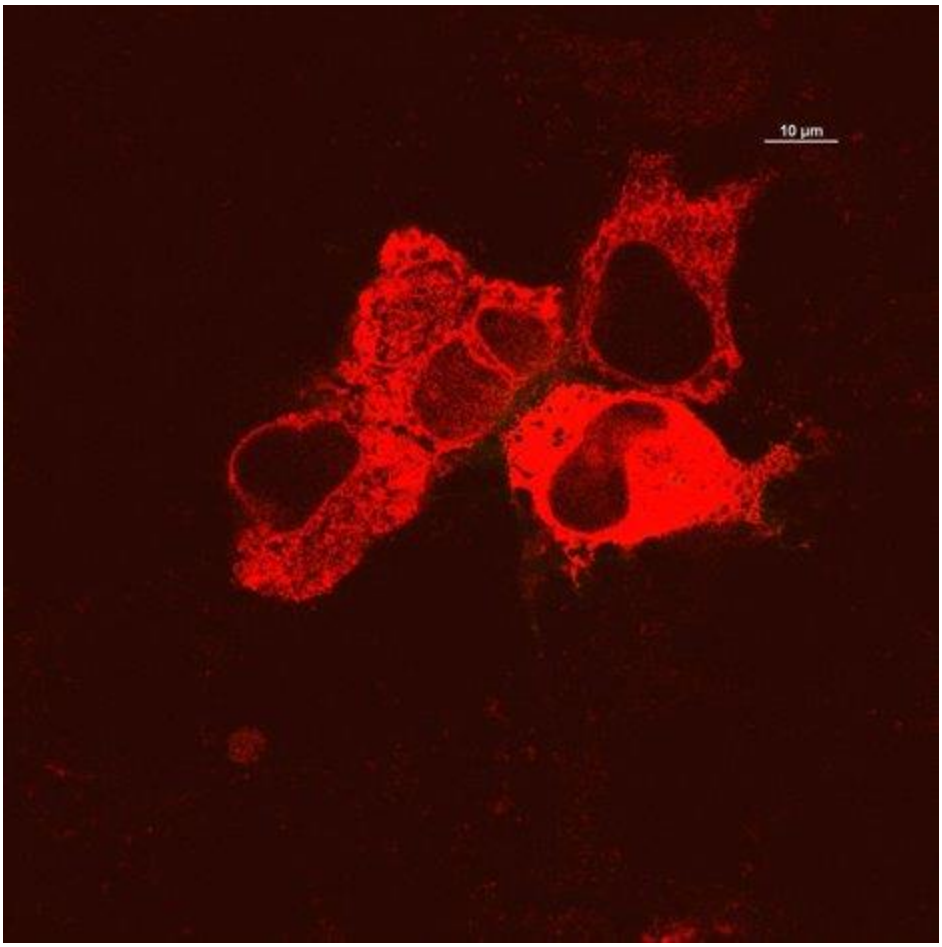

Figure 10: Chimeric mCherry-tagged ZIKV NS1 (last 50 amino acids switched to DENV sequence) being expressed in Huh 7.5

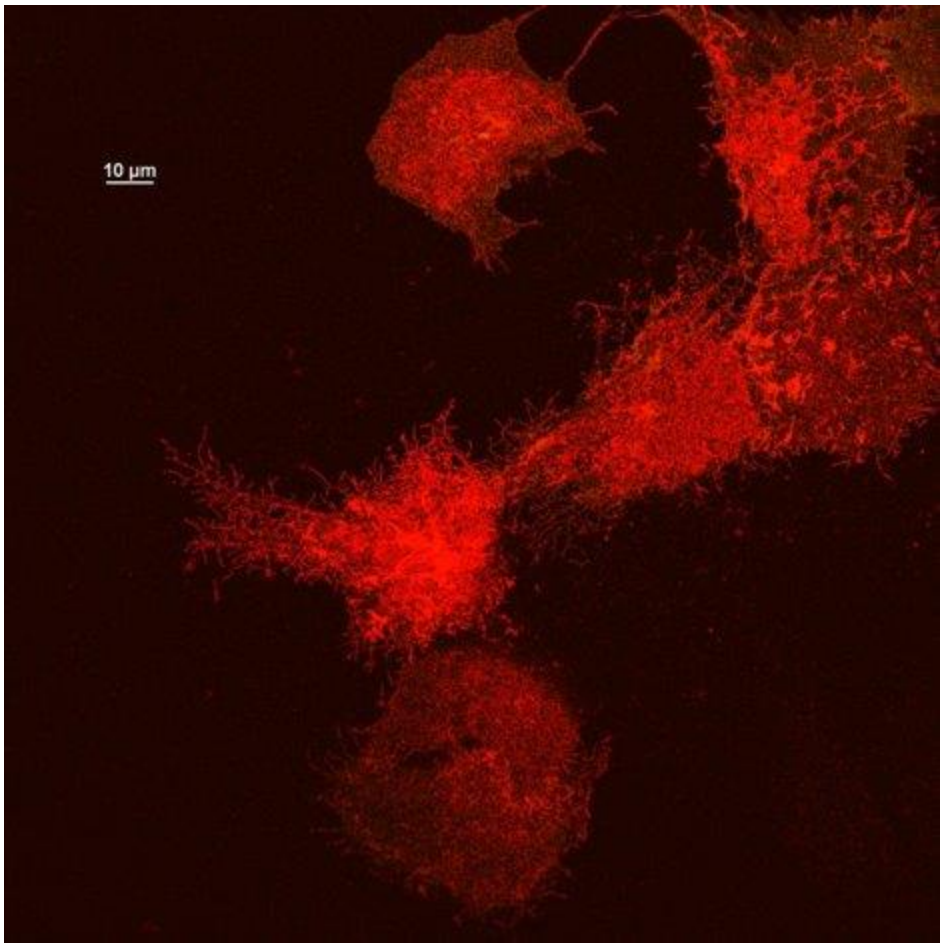

Figure 11:Chimeric mCherry-tagged ZIKV NS1 (last 50 amino acids replaced with DENV sequence) expressed in Huh 7.5.

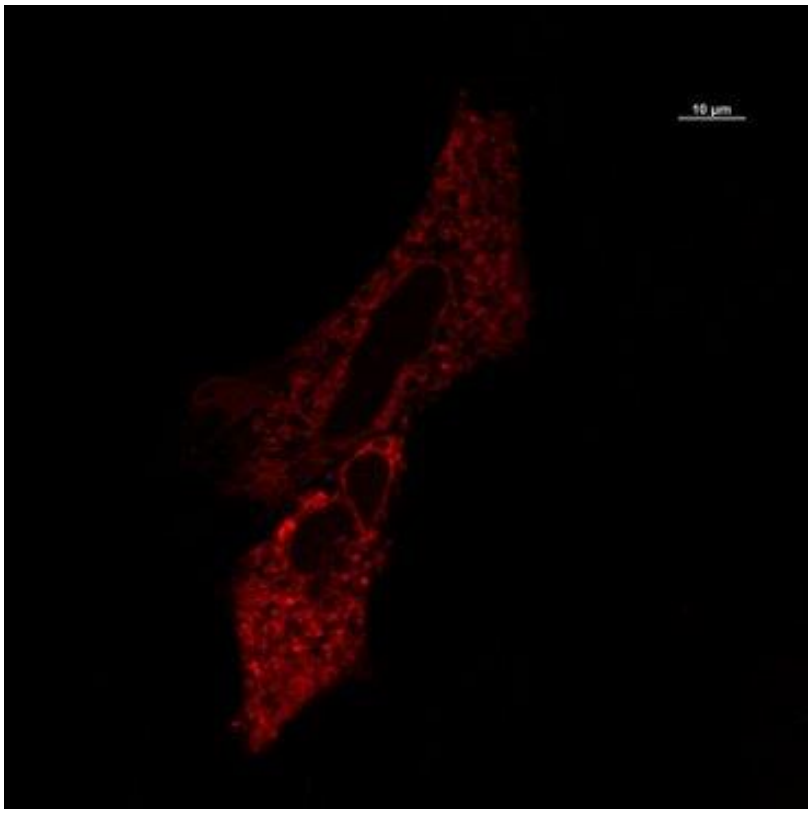

Figure 12: Chimeric mCherry-tagged ZIKV NS1 (amino acids 1-12 mutated to DENV sequence) expressed in Huh 7.5. All of the chimeric proteins prior to residues 40-52 resulted in this phenotype.

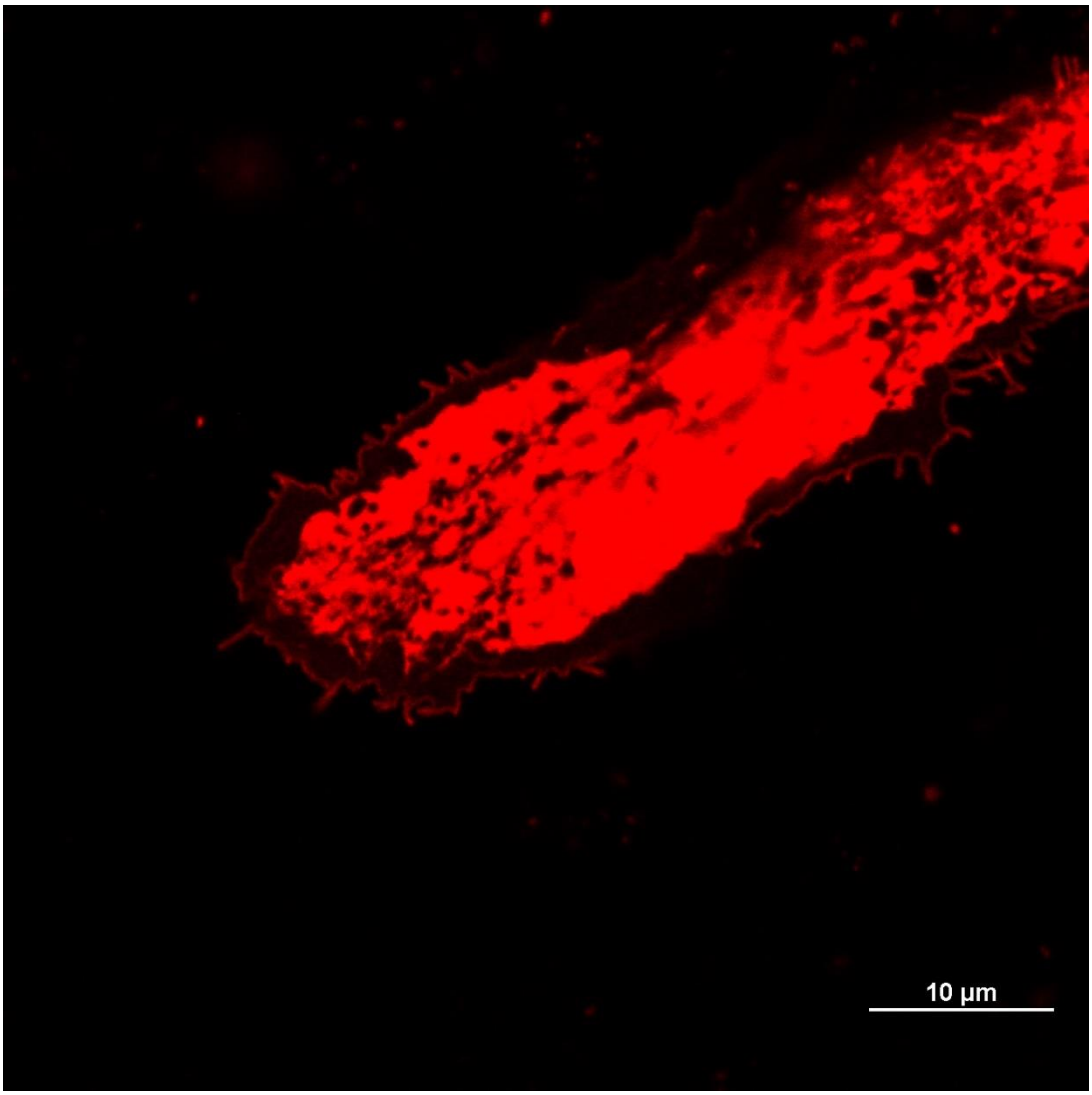

Figure 13: Chimeric mCherry-tagged ZIKV NS1 (amino acids 40-52 changed to DENV sequence) expressed in Huh 7.5

Chapter 4: Conclusion and Future Work

NS1 is a key nonstructural protein in the flavivirus life cycle, and it fulfills a variety of roles, ranging from interactions with the host immune system to assisting in viral replication. Here, I demonstrate that ZIKV NS1 appears to be capable of behavior that has previously only been reported in WNV NS1, whereas DENV and DTV NS1 do not appear to be capable of doing so. Furthermore, the residues of ZIKV NS1 responsible for this difference in behavior appear to be located within the first 52 amino acids of the NS1 sequence. Altering these residues to make them identical to their DENV counterpart resulted in loss of the distinctive phenotype, whereas altering small segments of N-terminal sequence resulted in chimeric protein that also showed this lack of actin remodeling, with many also failing to localize to the cell membrane. Replacing amino acids 40 to 52 with the DENV sequence resulted in a protein that localized to the membrane but was unable to induce actin remodeling, which combined with results from the chimera that failed to localize to the membrane, indicates that residues found within the first 40 amino acid of ZIKV NS1 are necessary but not sufficient to enable actin remodeling in expressing cells. There is more work yet to be done on this project, especially in regards to creating even more specific mutants/chimeras to determine the exact residues that cause ZIKV NS1 to behave so uniquely. In addition to the generation of these mutants, it will be important to run a permeability assay on placental cells in order to determine whether or not these structures aid in the ability of ZIKV to cross the placenta. Another experiment that will be conducted in the near future is a quantitative pulldown assay using the chimeric protein that localizes to the membrane but does not induce actin remodeling as a control in order to determine which host proteins play into the actin remodeling process. With that being said, the work conducted here

has laid a fine groundwork for future experimentation with ZIKV NS1 and has documented a phenomenon previously thought to be restricted to only WNV in a ZIKV protein.

Appendix A: Protocols

Cloning Protocol for ZIKV Without Intron (Ligation Dependent)

After creation of the ZIKV mCherry-tagged NS1 without intron insert, a vector containing a Cytomegalovirus (CMV) promoter was cut via appropriate restriction enzymes, as was the insert. In the case of ZIKV without intron, both the insert and vector were prepared with EcoR1 and XHO1 restriction enzymes, incubated for one hour at 37°C. Three µl of the treated vector and four µl of the treated insert were then mixed with 1 µl T4 DNA ligase, 1.5 µl DEPC-treated water, and 0.5 µl T4 DNA ligase. The mixture sat at room temperature for 15 minutes before being placed on ice overnight and transformed.

Cloning Protocol for DTV, DENV, and Chimeric ZIKV NS1 (Ligation Independent)

DTV NS1, DENV NS1, and mutant ZIKV NS1 inserts were purified via gel elution. The purified inserts then underwent T4 treatment with the following mixture:

17 µl DNA

2 µl buffer 2.1

0.5 µl DTT,

0.5 ul dCTP

0.5 µl T4 polymerase

The mixture was then run on a T4 treatment thermocycler protocol. 2.5 µl of the T4 treated inserts from each NS1 were then added to 1.5 µl linearized vector, spun, and kept at room

temperature for 20 minutes, before 0.5 µl EDTA was added. The mixture was kept for an additional five minutes before 3 µl was transformed into *E. coli*.

Gel Elution Protocol

PCR products were purified via gel elution. After being run on 0.8% agarose gel, bands of the desired size were cut out and added to 700 µl capture buffer type 3, which was then incubated at 52°C for 10 minutes, being inverted every two minutes. The melted gel was then spun for one minute at 9000 RPM and pipetted into an extraction column, taking care to avoid pipetting the gel at the bottom of the tube in case any solid remained. The column was spun at 9000 RPM and the flow through discarded, before having 700 µl wash buffer added and being spun for one minute. The flow through was then discarded again, and the column was spun two minutes in order to ensure it was dry. The column was then transferred to a clean 2.5 mL Eppendorf tube, and 50 µl of elution buffer that had been preheated to 52°C was added. The column was allowed to stand for one minute before being spun for another minute, and the eluted DNA was kept.

Overlap PCR Protocol

For the generation of mCherry-tagged ZIKV NS1 without intron and the generation of the 50 amino acid ZIKV-DENV NS1 chimeras, the PCR products to be joined underwent an overlap PCR reaction. In the case of the ZIKV without intron NS1, these products were ZIKV NS1 and mCherry. In the case of the ZIKV/DENV chimeras, these were sections from both ZIKV and DENV NS1. After elution of both PCR products for purification, one µl of each PCR product was mixed with 2.5 µl of a forward primer (10 µM) and 2.5 µl of a reverse primer (10 µM). 18 µl of DEPC-water was also added, and 25 µl of CloneAmp™ HiFi PCR master mix was also added. The reaction was then run on a thermocycler protocol designed for CloneAmp™ PCR reactions.

Plasmid Mini-Prep Protocol

Cells were spun down at 9000 RPM for ten minutes before having media drained off and being resuspended in 250 µl of P1 buffer. 250 µl of P2 lysis buffer was then added and thoroughly mixed via inversion, before being allowed to stand for approximately two minutes. 350 µl N3 neutralization buffer was then added and mixed via inversion, and the entire mixture was spun at 13,000 RPM for ten minutes. Following spindown, 800 µl of liquid was removed via pipette, carefully so as not to disturb the pelleted solid. This 800 µl was placed in a QIAprep® column and spun at 13,000 RPM for 60 seconds. The flow through was discarded and 500 µl buffer PB was added to the column, which was then spun at 13,000 RPM for another 60 seconds. Flow through was discarded again and 750 µl buffer PE was added, before being spun at 13,000 RPM for another 60 seconds. Flow through was discarded and the sample was then spun for another two minutes to remove any remaining liquid, which was then discarded. Finally, the column was

removed and placed in a clean 2.5 mL Eppendorf tube. The DNA was then eluted via the addition of 60 µl elution buffer, which was allowed to sit for one minute before being spun for another minute. The column was discarded and the eluted DNA was kept.

RT PCR Protocol

2.5 µl of RNA from infected cells was mixed with 1.5 µl of a forward primer and 1.5 µl (10 µM) of a reverse primer, along with 6 µl of DEPC-treated water and 12.5 µl OneTaq® One-Step Reaction Mix along with 1 µl OneTaq® enzyme mix. The entire solution was mixed via low speed spin and then run on a thermocycler protocol designed for RT-PCR.

Sequencing

All clones were sent to the Genomics Core Facility at the Pennsylvania State University for Sanger sequencing to ensure the desired product was obtained.

SDM PCR

SDM was carried out using:

4 µl of 5X PFU HF buffer

0.4 µl 10MM DNTP

0.6 µl DMSO

12.5 µl DPEC treated water

0.2 µl PFU polymerase

2 µl forward primer

2 µl reverse primer

1 µl template

The PCR was run on an SDM thermocycler protocol.

Transfection Protocol

48 µl of Opti-MEM® media was mixed with 2 µl of Lipofectamine® (for Huh 7.5 transfection) or PEI MAX® (for HEK293T/Vero E6) transfection reagent. This mixture was then combined with 45 µl Opti-MEM® and 5 µl of the DNA to be transfected, mixed 25 times via 100 µl pipette, and allowed to sit for 20 minutes at room temperature. The entire mixture was then added to cells that had just had their media changed to 400 µl Opti-MEM®. After approximately 6 hours, the cell media was changed to DMEM 10% FBS.

Transformation Protocol

2.5 µl of DNA was added to 25 µl of NEB® Stable Competent *E. coli* and allowed to sit on ice for 30 minutes. The cells were then heat shocked in a 42 °C water bath for one minute, before being placed on ice again for another five minutes. The cells were then added to 250 µl of Lysogeny broth (LB) media and allowed to incubate for approximately one hour before being plated on LB agar containing ampicillin and incubating for 16 hours at 32°C.

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Academic Vitae of Shay Toner

RESEARCH INTERESTS

Infectious disease, plastic degrading microorganisms, biological and chemical defense. I am fascinated by microorganisms and how they impact the world around them. I am also interested in the mechanisms of action for various toxic substances.

EDUCATION

Bachelor of Microbiology, May 2022 – The Pennsylvania State University, University Park, PA (Expected)

High school diploma, June 2018 – North Penn-Mansfield Junior Senior High School, Mansfield, PA

EXPERIENCE

Undergraduate Research

Zika Virus NS1, **Penn State University Park**, January 2020 – Present

- Evaluating the function of the NS1 protein
- Identifying where the protein localizes within cells/on cell membrane, investigating how it interacts with the virus in both infected cells and cells that have been modified to express the protein
- So far, the project has shown promising results in identifying NS1 interactions with host cells, and several key pieces of data have already been collected. Zika NS1 expressing HuH-7.5 cells show filopodia where Dengue, Powassan, and West Nile NS1 expressing cells do not. Cells expressing Zika NS1 also showed far greater vulnerability to Zika infection than cells that were not.
- Stable cell lines expressing Zika NS1 have already been created, with Dengue and DTV NS1 expressing lines currently being created.
- Created Chimeric NS1 proteins, replacing sections of the Zika protein with their Dengue and West Nile equivalents in order to determine section of protein responsible for filopodia formation.
- Future steps will center around identifying the host protein interacted with via pull down assay and evaluating the impact of NS1 expression on vascular permeability via an endothelial cell monolayer and a permeability assay.

SARS-CoV-2, **Penn State University Park,** Fall 2020 – Present

- Aided in writing a paper evaluating several antiviral compounds ("Identification and structural characterization of SARS-CoV-2 inhibitors targeting Mpro and PLpro using in-cell-protease assays").
- These compounds were chosen specifically for their ability to inhibit SARS-CoV-2 proteases
- Paper submitted for review.
- Aided in writing a literature review regarding SARS-CoV-2 protease inhibitor design and repurposing, also submitted for review ("Structure based inhibitor design and repurposing clinical drugs to target SARS-CoV-2 proteases").
- Will continue working with SARS-CoV-2 proteins periodically, although my main focus right now is flavivirus NS1.

Additional Experience

Emergency Room Intern, **Soldiers & Sailors Memorial Hospital**, Wellsboro PA, July 2017 – August 2017

- Participated in patient care
- Spent time in the microbiology lab learning basic technique
- Went on numerous calls with EMS personnel

SKILLS

- Basic robotics
- Basic Spanish
- Chemistry lab techniques
- Data analysis
- Microbiology lab techniques
- Microsoft Office
- Molecular biology lab techniques
- Public speaking
- Scientific writing

HONORS / AWARDS

2021 Mel Chrostowski Endowed Scholarship, 2021
2021 Stiles Scholarship in the Biological Sciences, 2021
2021 Student Undergraduate Research Fellowship, 2021
2021 Dan Tershak Memorial Scholarship in Biology, 2021
2020 Poole Family Honors Scholarship, 2020
2020 President's Sparks Award, 2020
2019 President's Freshman Award, 2019

MEMBERSHIPS / AFFILIATIONS

American Society for Microbiology (PSU chapter)
Penn State Fencing Club
Schreyer Honors College
Society of Toxicology

Publications: Narayanan, A., Toner, S. A., & Jose, J. (2021). Structure-based inhibitor design and repurposing clinical drugs to target SARS-COV-2 proteases. *Biochemical Society Transactions*, *50*(1), 151–165. https://doi.org/10.1042/bst20211180

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