

# UC Riverside

## UC Riverside Electronic Theses and Dissertations

### Title

Development of Molecular Host-Centric Methodology Seeking to Pinpoint Reservoirs of Nairoviruses by Interferon-Stimulated Gene Product 15

### Permalink

<https://escholarship.org/uc/item/90g4s2tq>

### Author

Jalf, Amrit Singh

### Publication Date

2023

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA  
RIVERSIDE

Development of Molecular Host-Centric Methodology Seeking to Pinpoint Reservoirs of  
Nairoviruses by Interferon-Stimulated Gene Product 15

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

Master of Science

in

Biomedical Sciences

by

Amrit Singh Jalf

September 2023

Thesis Committee:

Dr. Scott Pegan, Chairperson  
Dr. Adam Godzik  
Dr. Marcus Kaul

Copyright by  
Amrit Singh Jalf  
2023

The Thesis of Amrit Singh Jalf is approved:

---

---

---

Committee Chairperson

University of California, Riverside

## ACKNOWLEDGEMENTS

I'd like to express my sincere appreciation and acknowledgements to those who made it possible to obtain my Master's degree.

Thank you to my friends and family, for providing me with support, encouragement, nurture, and resources throughout my time as a student.

Thank you to past and present lab members, for providing any sort of assistance whenever I was struggling.

Thank you to Jack McGuire, for teaching me the fundamentals and techniques that I used throughout my experiments.

Thank you to Dr. Vanessa Moresco and David Gonzalez, for being great teammates, as we partook in this journey in science together.

Thank you to Dr. Scott Pegan, for being the best mentor I could ask for. No matter the experimental outcome, he never once doubted me succeeding. I always had his full support and guidance in endeavors both in and out of research.

Finally, thank you to science, for teaching me resilience. I came to learn that there is always a way.

## ABSTRACT OF THE THESIS

Development of Host-Centric Methodology Seeking to Pinpoint Reservoirs of  
Nairoviruses by Interferon-Stimulated Gene Product 15

by

Amrit Singh Jalf

Master of Science, Graduate Program in Biomedical Sciences  
University of California, Riverside, September 2023  
Dr. Scott Pegan, Chairperson

Nairoviruses, tick-borne viruses from the *Bunyaviridae* family, pose persistent and significant threats to human health. While the most notable is Crimean-Congo hemorrhagic fever virus (CCHFV), which possesses a fatality rate pushing 30%, other nairoviruses are establishing themselves in our viromes including Pacific Coast tick nairovirus (PCTN) and Songling virus (SGLV). To thwart host antiviral immune response, specifically reversing the post-translational modifications of interferon-stimulated gene product 15 (ISG15) after infection, nairoviruses encode for and utilize an ovarian tumor domain protease (OTU) capable of deISGylation. The fact that ISG15 structure and sequence is hardly conserved between species, coupled with previous studies demonstrating diverse OTU preferences for certain ISG15s, pinpoints these two as key subjects of disease prevention due to possible variations in interaction. To gain a broader understanding of nairovirus OTU-ISG15 interface and identify possible host

carriers, a high-throughput method of obtaining binding kinetic data was developed which employed bio-layer interferometry (BLI). ~72 interactions between vOTUs and ISG15 were examined, which revealed significant differences for nairovirus OTU binding preferences. CCHF and PCTN vOTUs put on robust displays of binding, while SGLV vOTU showcased more specificity for which ISG15s it bound to. These insights serve to be progress and contributions towards building a larger biosurveillance tool ultimately unveiling pivotal animal reservoirs of nairoviruses.

INDEX WORDS: Biosurveillance, Nairovirus, vOTU, ISG15, CCHF, PCTN, SGLV, BLI, Binding

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	iv
ABSTRACT OF THE THESIS.....	v
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
CHAPTER	
1 INTRODUCTION.....	1
The nairovirus that sparks initiative.....	2
ISG15 in the antiviral innate immune response.....	4
Researching CCHFV and nairoviruses alike.....	6
2 DEVELOPMENT OF MOLECULAR HOST-CENTRIC METHODOLOGY SEEKING TO PINPOINT RESERVOIRS OF NAIROVIRUSES BY INTERFERON-STIMULATED GENE PRODUCT 15.....	11
Results.....	12
Discussion.....	26
Materials and Methods.....	32
References.....	39
3 SUMMARY & CONCLUSION.....	44
The need for a biosurveillance tool.....	44
Unveiling vOTU-ISG15 interface.....	45
Impact of nairoviral research.....	46



## LIST OF TABLES

Table 2.1: Summary of nairovirus OTU binding against mature ISG15s.....	22
Table 2.2: CCHF vOTU vs ISG15 binding kinetics dataset.....	23
Table 2.3: PCTN vOTU vs ISG15 binding kinetics dataset.....	24
Table 2.4: SGLV vOTU vs ISG15 binding kinetics dataset.....	25

## LIST OF FIGURES

Figure 1.1: ISGylation process.....	9
Figure 1.2: Streptavidin bio-layer Interferometry.....	10
Figure 2.1: Nairovirus OTU phylogenetic diversity tree.....	14
Figure 2.2: ISG15 phylogenetic diversity tree.....	15
Figure 2.3: 96-well assay plate setup.....	38

## CHAPTER 1

### INTRODUCTION

The impact of viruses is profound and widespread. Since the beginning of time, viruses have done us both good and evil. Viruses are beneficial in host life cycles, protect organisms from infection and disease, and provide crucial mutualisms [1]. On the other hand, viruses also hijack host cells, causing life-threatening disease. They have been haunting us for centuries with their malicious effects. This is highlighted by examples such as the Spanish flu pandemic responsible for about 21 million lives in 1918, and the Rabies virus which dates back 4000 years - presenting with almost a 99% fatality rate once clinical symptoms arise (still today). The recent events have highlighted that more such pandemics could occur in our era of modern medicine. Specifically, the impact of coronavirus disease 2019 (caused by SARS-Cov-2) has led to 770 million cases and the death of 7 million people up to date [2]. This recent pandemic has made a convincing case to the world of the need for increased resilient and prepared communities through adapting the way we view modern pandemic preparedness [3]. This has brought to light the critical need to research and prepare therapeutics and tools to mitigate the future viral outbreak candidates.

Such threats include emerging nairoviruses, which are a genus of negative sense single stranded RNA tick-borne viruses in the family *Bunyaviridae*, linked to often-fatal human and animal disease [4]. Nairovirus presentation in humans can consist of high fever, headache, joint pain, abdominal pain, or vomiting. Some of these viruses seem to be appearing unexpectedly, in a response to possible habitat changes and increased

contacts with disease carriers [5]. In a planet that is currently experiencing climate change, global warming could mean expansion of host tick ranges and ultimately increases of disease incidence [6].

*The nairovirus that sparks initiative*

Although there are a multitude of viruses classified as nairovirus, Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is viewed as one of the key prototype nairoviruses [7]. CCHF is one of the top diseases on the Blueprint List of Priority Diseases from the World Health Organization (WHO). CCHFV presents symptoms in humans in a similar fashion to other nairoviruses, with fatality rates reaching up to 80% in some countries [8]. Disease from CCHFV has the ability to clinically progress and become hemorrhagic in about 1 week after infection, causing epistaxis, melena, hematuria, and eventually shock and death [9]. Considered as a biosafety level 4 (BSL4) human pathogen, therapies and treatments for the disease are scarce. Currently, there is no FDA-approved vaccine or efficacious antiviral therapy available [9, 10]. Further research on the virus is called for and imperative.

In a similar fashion as other nairoviruses, CCHFV transmits through tick vectors, specifically *Hyalomma* [10]. Once an infected tick has successfully bit their host, CCHFV penetrates the basolateral membrane of epithelial cells and spreads into the bloodstream, where it is able to delay induction of interferons, suppress antibody response, lead to lymphocyte apoptosis, and eventually allow for its uncontrolled replication in different organs of the body [11]. In addition to tick bites, transmission may also occur through direct contact with the blood or tissues of infected humans, animals, or

ticks. This opens possibilities of transmission via inappropriate sterilization and/or contamination of medical equipment at hospitals, the butchering of infected animals at farms, and travel to endemic areas. Key populations at risk include healthcare workers, militaries, and livestock workers.

At a molecular level, nairoviruses encode viral ovarian tumor (vOTU) proteases in an attempt to bypass host innate immune defenses and antiviral responses to enable efficient replication. These proteases can target and regulate proteins with crucial roles in the immune response, such as Ubiquitin (Ub) and interferon-stimulated gene (ISG) products. Ubiquitin is a highly conserved protein in eukaryotes known to regulate cell signaling, DNA damage response, cell cycle control, and programmed cell death by a process coined “ubiquitination”, through a cascade utilizing activating (E1), conjugating (E2), and ligating (E3) enzymes [12]. Interferon-stimulated gene products act in a similar fashion, impairing assembly of viral proteins and preventing host translational shutoff through binding of target proteins, termed “ISGylation” [13]. CCHF vOTU specifically is known to have both deubiquitinase and deISGylase activity, which subdues type 1 interferon responses and removes ISG conjugated proteins respectively [4]. By possessing these functions to reverse post-translational modifications by Ubiquitin and ISGs, nairoviruses hold a pivotal virulence factor in their vOTUs. These vOTU proteases are essential for nairoviruses to replicate and allow for adaptation to hosts.

Several new nairoviruses have begun to emerge that implicate human consequence, which provide a prompt motive to further research nairovirus vOTU function and preference for host proteins [14-18]. Notable viruses include Pacific Coast

tick nairovirus (PCTN) which was recently screened in ticks in Mendocino County, California [14], and Songling virus (SGLV), identified in hospitalized patients in northeastern China [15]. From the Tamdy genera of nairoviruses, they have potential for catastrophic outcomes and severe human pathogenesis as witnessed from CCHFV, and thus comparisons among their vOTU proteases are called for. This stems from a particular reason, being that there is a high degree of vOTU sequence variation among all nairoviruses [4]. Possibilities arise for differences in nairovirus vOTU structural features and additionally, preferences for host proteins. In a planet filled with millions of animal species, potential animal reservoirs for nairoviruses may exist that were never considered before.

#### *ISG15 in the antiviral innate immune response*

A key antiviral protein under the efforts of research is interferon-stimulated gene product 15 (ISG15). Once pathogen-typical molecules are detected by host pattern-recognition receptors such as RIG-I and MDA5, type I interferons are released which ultimately cause ISG15 (along with its conjugating enzymes) to be strongly upregulated and expressed. ISG15 holds functions such as conjugating to both viral and host target proteins, and acting as an extracellular cytokine. Denominated “ISGylation”, ISG15 conjugates intracellularly to lysine (K) residues through an enzymatic cascade similar to Ubiquitin - although ISG15 only conjugates as a monomer via a finite batch of enzymes including Ube1L, UbcH8, and Herc5 [13] (Figure 1.1). It can conjugate to host proteins such as pattern-recognition receptors to limit interferon induction, and viral proteins such

as nucleoproteins to impair and inhibit viral replication and assembly [13, 19].

Conjugation can also be reversed, by Ubiquitin Specific Peptidase 18 (USP18).

Although ISG15 has been investigated for decades, the protein is still not fully understood due to its fluctuation in nature. ISG15 is designated “Ubiquitin-like” due to its post-translational modification ability in cells and structural homology to Ubiquitin, but it is not so “Ubiquitin-like” in the sense that it is very diverse between host species in contrast to Ubiquitin’s high conservation. ISG15 structure is characterized generally by two Ubiquitin-like domains (N-terminal and C-terminal domains), which are connected via a “hinge” [13, 20]. ISG15 amino acid sequence terminates with LRLRGG in the C-terminus, and conjugation would not be possible without this. Aside from the regions necessary for protein interaction via conjugation, ISG15 has stark differentiation in sequence and structure from species to species, and therefore differences in functions in the immune response against pathogens [13]. This can be inferred with previous studies that show ISG15-deficient mice take a hit on their resistance to multiple prominent viruses, whereas on the contrary ISG15-deficient humans are shown to actually be better protected against viral infection - possibly due to distinctions in how these species utilize their ISG15s to interact with USP18 [21].

Because of their high diversity and possibility for protein interaction variance, ISG15s are an important target of antiviral research. Previous mutational and structural research identified at least seven ISG15 amino acid residues that nairovirus OTU proteases interact with, which are the driving force for species-specific vOTU-ISG15 interfaces [22]. As these specific residues are not conserved from species to species, this

allows for vOTUs to build a preference for activity against ISG15s. For reference, cleavage assay studies show CCHFV activity against both human and sheep ISG15s, while Kupe virus (KUPEV) only shows activity against sheep ISG15 and not human ISG15 [22]. Leveraging this advancement, we are prompted to build a model that can gauge vOTU deISGylase activity from multiple nairoviruses (and eventually viruses encoding ISG15 interacting proteins in general) against ISG15s from a myriad of species to predict future viral consequences.

#### *Researching CCHFV and nairoviruses alike*

CCHFV is known to infect a multitude of animal species, thus opening the possibility of various animal reservoirs. Being a zoonotic virus, identifying new reservoirs can aid in the prevention of animal to human transmission. As of now, progression from infection to disease is limited to humans, which complicates identification of these animal reservoirs. Viremic periods of CCHFV in animals are generally brief [23], and further understanding them would aid in preventing unintended viral contact. As of this day and age, identification of reservoirs for CCHFV depends predominantly on seroepidemiological studies [24]. Correspondingly, this can be quite costly and time-consuming. Previous methods of surveillance for disease, such as using biological markers in blood samples, have also been shown to be unrepresentative of the population of interest, and can pose ethical issues [25]. Taking these factors into account, the need for an efficient method of nairovirus surveillance was apparent and was the drive behind the creation of a high-throughput tool. The determined method of action was to deploy bio-layer interferometry (BLI) and take advantage of biotin-streptavidin



interactions to run high-throughput assays (Figure 1.2). This allowed a biomolecular approach in determining the drivers of nairovirus vOTU protease and ISG15 interaction, in order to measure host possibility.

Not just limited to CCHFV, previous studies have proven evolutionary pressure continues to drive nairovirus vOTU preference for deISGylation of ISG15s from certain species [22]. Our previous research has had success in qualitatively defining the impact of ISG15 biodiversity on nairovirus vOTUs, by assessing activities of 14 nairovirus OTUs and ISG15s of 12 varying species through cleavage activity assays [22]. This study capitalizes on these findings and fills in a gap, by providing quantitative data. Dealing with viruses that have capability for severe human consequence, this study provides new insights into engagement of viral proteins on host immune responses. We are able to get a broad picture of vOTU-ISG15 interface, and a deeper understanding of how sequence and structure differences correlate with vOTU deISGylation capability. Methods from this study can also be implemented to research other prominent viral proteases, such as SARS-CoV-2 papain-like protease.

This study aims to develop and implement a tool that defines the influence of ISG15 biodiversity on nairovirus vOTUs. Nairoviral threats are looming, and without the evolution of new methods to track them, we are destined to deal with the health concerns, financial burdens, and societal hardships that may come with them. Here, we present an opportunity to gain deeper knowledge of how evolutionary pressure drives viral protein campaigns in different hosts. Coupled with a future bioinformatics approach, we strive to

create a biosurveillance mechanism to monitor vOTU-ISG15 interactions that aims to limit nairovirus disease introduction and progression in humans.

Figure 1.1. ISGylation process

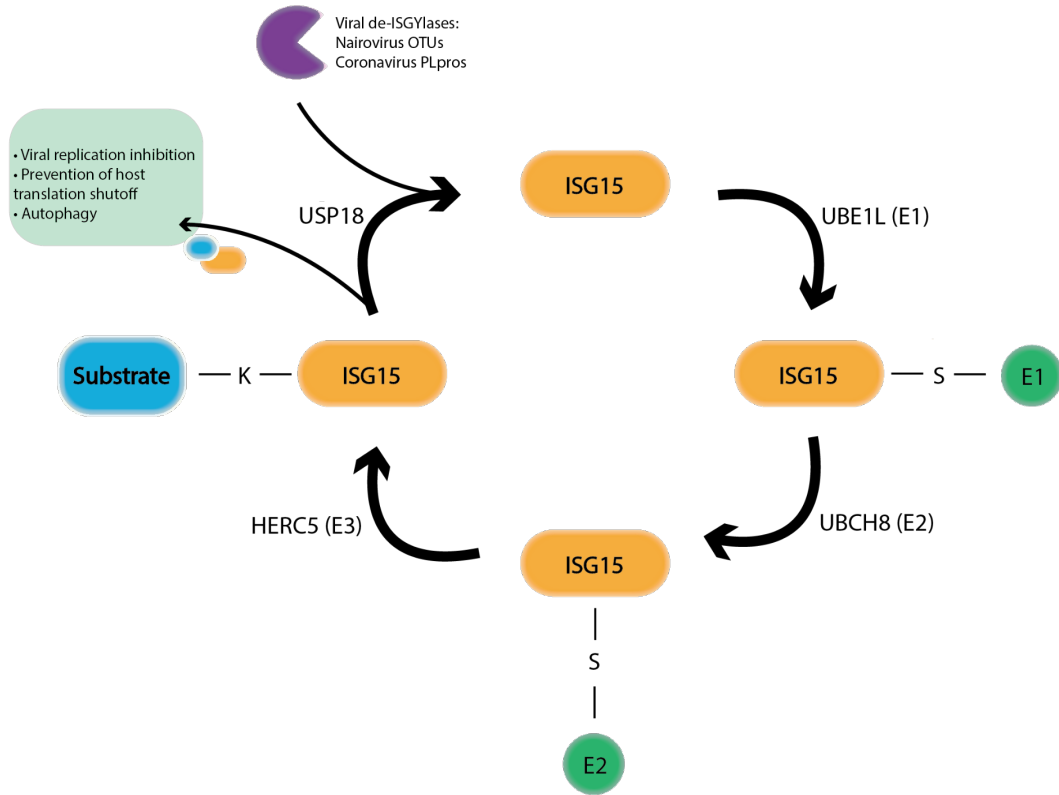
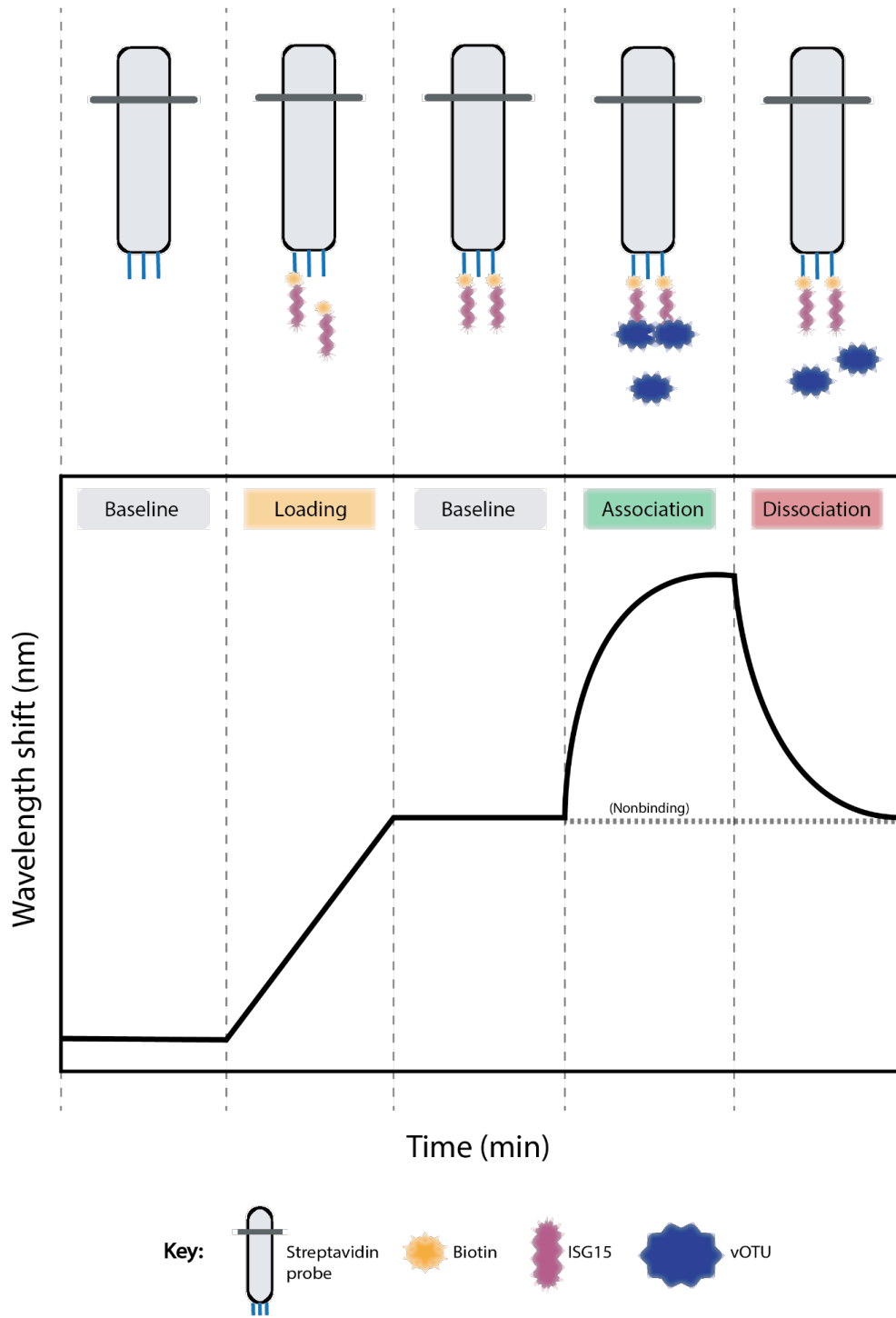


Figure 1.2. Streptavidin bio-layer interferometry



## CHAPTER 2

### Development of Molecular Host-Centric Methodology Seeking to Pinpoint Reservoirs of Nairoviruses by Interferon-Stimulated Gene Product 15

---

To be submitted to scientific journal

## Results

### *Determining an approach considering nairovirus and ISG15 diversity*

Previous methods of interpreting nairovirus OTU-ISG15 interactions including aminomethylcoumarin (AMC) and cleavage assays were presented to be costly and time-intensive. Nairovirus OTUs are known to have less than 25% amino acid identities [4], and considering this diversity, previous studies are limited by the fact that the activity of only a small subset of the viruses were represented. Additionally, a copious amount of ISG15s have recently been sequenced, leaving questions on how far the previous results can be applied to understanding full OTU-ISG15 interface. Although it is impractical to experiment with the hundreds of identified ISG15s, here we take a subset of ISG15s with determined specificity residues that span and represent species originating from different facets of the planet. We know that, at the minimum, seven amino acid specificity residues exist that interact with nairovirus OTUs [22]. Hence, we chose 24 ISG15s displaying ~15 diverse specificity residue combinations, which covers a large quantity of those possible in nature. In order to efficiently screen all these ISG15s against a specific vOTU, bio-layer interferometry was applied to rapidly obtain association rate constant ( $K_{on}$ ), dissociation rate constant ( $K_{off}$ ), and equilibrium dissociation constant ( $K_D$ ) values from their interactions. To reveal the trends of binding preferences for ISG15s of different species by nairovirus OTUs, this was necessary to efficiently screen and analyze ~72 interactions between ISG15 and vOTU. This proved to be an equivalent to previous enzymological studies that would be unviable to utilize in this scheme, unveiling novel

insights into vOTU-ISG15 interface and becoming a model to emulate in future surveillance applications.

Figure 2.1. Nairovirus OTU phylogenetic diversity tree. Indicated in the ovals are current species groupings classified with color. vOTUs included in this study are signified with red lettering (CCHFV, PCTN, SGLV). vOTUs with known activity towards ISG15s [22] are indicated with orange diamonds.

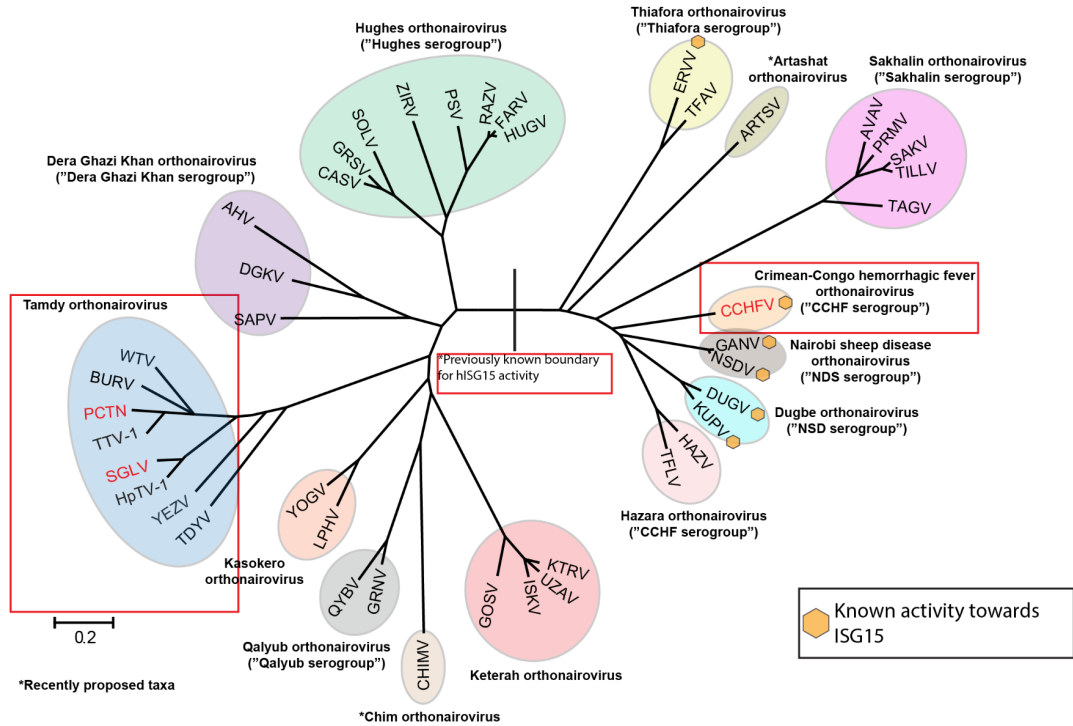
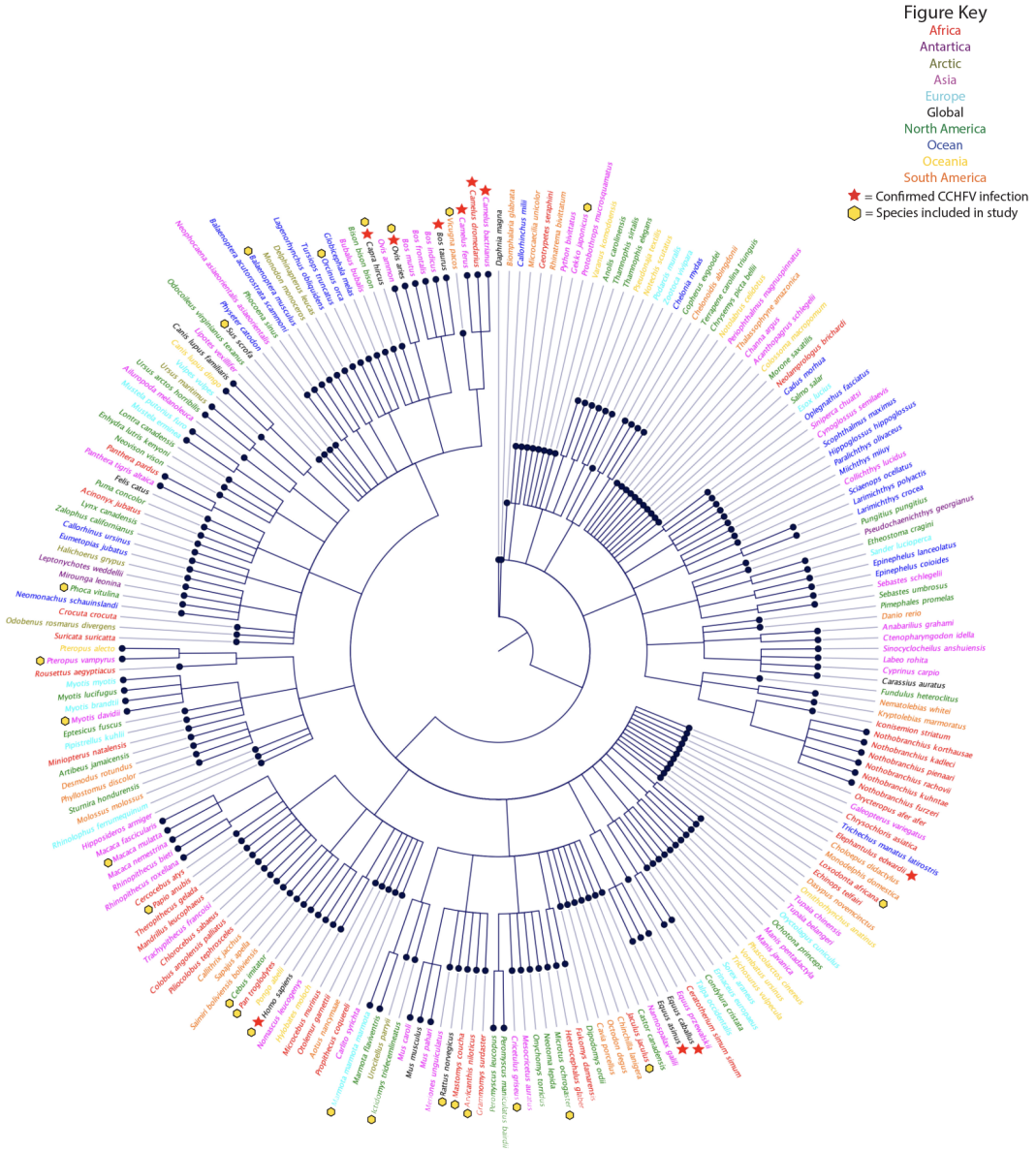




Figure 2.2. ISG15 phylogenetic diversity tree. ISG15s are color-coded for region of origin. Species with known CCHFV infection denoted with red stars. Species included in this study are signified with yellow hexagons.



*Nairovirus OTU binding preference for mature ISG15s*

Mature ISG15 substrates were derived from 24 species, which comprised of human (hISG15), chimpanzee (cISG15), Chinese hamster (chISG15), blue whale (wISG15), sheep (shISG15), goat (gISG15), alpaca (aISG15), pig (pISG15), capuchin monkey (cmISG15), Egyptian jerboa (ejISG15), ground squirrel (sqISG15), alpine marmot (amISG15), rhesus monkey (rmISG15), olive baboon (obISG15), prairie vole (pvISG15), Schlegel's Japanese gecko (gISG15), vesper bat (bISG15), African grass rat (grISG15), harbor seal (hsISG15), flying fox (ffISG15), killer whale (kwISG15), southern mouse (smISG15), elephant (eISG15), and brown rat (brISG15). Alongside CCHF vOTU, vOTUs from the Tamdy genera of nairoviruses including PCTN and SGLV were tested against this comprehensive array of ISG15s.

Enticingly, the vOTUs displayed distinctive fashions of binding towards the ISG15s compared to one another. Although, this does not come as a complete surprise as previous enzymatic studies presented a contrasting cleavage profile between proISG15s and nairovirus OTUs [22]. CCHF vOTU displayed binding to ISG15s of 17/24 species, which revealed a more balanced pattern of binding preference in contrast to PCTN and SGLV vOTUs. This also complements the previous enzymatic study showing CCHF vOTU cleavage preference being dispersed more evenly among ISG15s from 12 species [22]. PCTN vOTU displayed binding to the entirety of the 24 ISG15 species tested, exhibiting principally no specific preference for binding attraction. SGLV vOTU was more particular in the ISG15s it bound to, displaying binding to only 3/24 of ISG15 species tested. Both PCTN and SGLV being from the Tamdy genera of nairovirus but

having drastically different binding profiles to ISG15s, suggests that even minor evolutionary divergence could have a role in determining drivers for species-specific binding.

One luring species SGLV vOTU bound to was kwISG15, and of the 24 diverse ISG15s investigated, only kwISG15 had an identical binding result against all 3 vOTUs. Interestingly, the other ISG15s SGLV vOTU bound to were gISG15 and eISG15, which were 2/7 of the ISG15s CCHF vOTU did not showcase binding to. This is a stark contrast to PCTN vOTU, which bound all 7 of the ISG15s CCHF vOTU did not bind to. Compellingly, SGLV vOTU had a stronger binding affinity to both gISG15 and kwISG15 when compared to PCTN vOTU.

Diving further into the quantitative sector of the data, CCHF vOTU demonstrates stronger binding ( $K_D$  values) to all the ISG15s it bound to, when compared to the interactions between the same ISG15s and PCTN vOTU. In return, PCTN vOTU generally displays slower  $K_{on}$  values and faster  $K_{off}$  values than CCHF vOTU. An important finding to point out is that all 3 vOTUs had the same ISG15 it bound strongest to (kwISG15), but had varying ISG15s they bound the weakest to. CCHF vOTU bound the weakest to obISG15, PCTN vOTU bound the weakest to grISG15, and SGLV vOTU bound the weakest to gISG15.

#### *CCHF vOTU Displays A Diverse Binding Profile*

CCHF vOTU has been shown to have stark variety in both cleavage preference and strength to Ubiquitin and differing ISG15s [4, 22, 26]. This trend is continued with this study, as it exhibited binding to 71% of ISG15 species tested, with differing

strengths. Notably, CCHF vOTU bound to all ISG15s from species that have been confirmed to be infected with CCHFV, including human, sheep, and goat [27-29]. This gives us confidence and implication that binding is somewhat connected to deISGylation capability by vOTU and therefore infectivity by the virus.

An important detection is that all binding/non-bindings between CCHF vOTU and ISG15 occurred in the same fashion within the same sequence specificity residue combinations. ISG15s that bound to CCHF vOTU came from the specificity residues of DQPEFMN, DQPDFMN, DLPEYMN, NLPEFMN, DLPEFLN, DLPEFMN, DQPELMN, DQPDQMN, AQPEIMN, DLPDFMN, and NQPEFMN. Specificity residues of ISG15s that did not bind to CCHF vOTU consisted of DQPEYMN, DQSEYLN, and EQPEHMN. Additionally, if multiple ISG15s were tested against CCHF vOTU in a certain residue combination and exhibited binding, they had similar binding ( $K_D$ ) strengths. These findings demonstrate the importance of evolutionary progression in amino acid sequences in species-specific innate immunity towards vexing viral threats.

Of the 17 species of ISG15s CCHF vOTU bound to, the strongest binding came with kwISG15 at a  $K_D$  of  $2.11E-07$  M, and the weakest with obISG15 at a  $K_D$  of  $4.25E-04$  M. Inherently, binding with kwISG15 had the fastest  $K_{on}$  value, but surprisingly did not have the slowest  $K_{off}$  value - which was held by wISG15. Luringly, binding with hISG15 showcased the slowest  $K_{on}$  value, which may suggest direct correlations to previous research showing CCHFV actually had one of the lower activity turnover rates towards human ISG15-AMC compared to other vOTUs [4, 26]. When surveying for the fastest

$K_{\text{off}}$  value, it was obtained from the obISG15-CCHF vOTU interaction. This does not come at a surprise, as it held the weakest binding strength out of all the binding ISG15s.

A drawing trend that is observed is that generally, the loss of a phenylalanine (F) group in ISG15 leads to a loss of binding to CCHF vOTU. This is apparent in ISG15s from the residue groups of DQPEYMN, DQSEYLN, and EQPEHMN. ISG15s that do not follow this trend are cmISG15 and ejISG15, which consist of the DLPEYMN specificity residue combination. For these ISG15s, it is important to note that they also substitute glutamine (Q) for leucine (L), as opposed to the other groups that lose phenylalanine. This suggests that some sort of polar-nonpolar amino acid balance plays a part in determining binding to CCHF vOTU. The substitution of phenylalanine for tyrosine (non-polar to polar) halts binding, but the substitution of glutamine for leucine (polar to non-polar) somehow reverses this. Further proving this point, this counter is also seen in grISG15, where it loses a phenylalanine, but incorporates alanine (another non-polar amino acid) and retains binding activity. It is evident that the multitude to which viruses infect hosts varies widely, which can be specifically traced to differences in host protein sequences [30]. The data presented in this study suggests that although CCHF vOTU has the capability of variegated and strong binding to ISG15s, there lie some vulnerabilities that can prevent this binding, and possibly cleavage.

#### *PCTN vOTU Demonstrates Substantial Binding To ISG15s*

In order to determine the drivers behind nairovirus OTU binding preference with ISG15s, it was obvious to include other vOTUs from the nairovirus family. PCTN, being recently screened in ticks across the coast of California [14], presented another viral

deISGylating threat to the immunity of hosts. After being put to the test against the same array of ISG15s as CCHFV, it fascinatingly bound to every species of ISG15. This made it clear that PCTN vOTU had no regard for ISG15 diversity when it came to choosing which proteins it bound to, as binding was observed in every ISG15 specificity residue combination.

PCTN vOTU had the strongest binding affinity to kwISG15, at 1.91E-06 M, and the weakest binding affinity to grISG15 at 1.04E-03 M. Again, binding with kwISG15 shows the greatest  $K_D$  value in this dataset. Binding with kwISG15 produced the fastest on-rate of the dataset, while binding with grISG15 generated the slowest. This supports the notion that association rates are the driving forces in determining binding strength between ligand and analyte. Binding with grISG15 not only showed the lowest  $K_D$  value in this dataset, but it was the lowest binding affinity in all binding interactions from the three vOTUs tested. Although, this impingement in binding strength seems to come from PCTN vOTU's on-rate with grISG15, as this pairing's off-rate is one of the slowest out of all 24 ISG15s. The fastest off-rate is held by the hISG15-PCTN vOTU interaction, which also seems to be outbalanced by its on-rate, as the interaction's binding affinity is not in the lower end of the dataset.

#### *SGLV vOTU Engages In Limited Binding with ISG15s*

To further uncover the influence of ISG15 diversity on nairovirus OTU preference, and potential for differing roles in antiviral response, another OTU was employed in this study from the Tamdy genera of nairoviruses. SGLV, recently found to be associated with human febrile illness in China [15], seems to have already participated

in zoonotic transmission. Consequently, this virus poses a prominent threat that requires prompt biosurveillance. Hence, the inclusion of SGLV vOTU in this study was patented, and yielded interesting results. SGLV vOTU only bound to 3/24 ISG15s, including kwISG15, gISG15, and eISG15. Preference and specificity was especially evident for this vOTU, suggesting barriers certain ISG15s hold towards binding.

Although kwISG15 was found to bind strongest to SGLV vOTU, similar to the previous two vOTUs tested, another strong binding interaction occurred with gISG15 at  $6.38E-06$  M. Intriguingly, this ISG15 was the only ISG15 tested with a serine (S) amino acid group in its specificity residues. This leads to the belief that possible underpinnings lie which increase SGLV vOTU's attraction (or accessibility towards binding) for this specific amino acid group. Not only is this binding interaction stronger than 96% of those PCTN vOTU puts forward, but it is only weaker than six of those CCHF vOTU possesses.

Table 2.1. Summary of nairovirus OTU binding against mature ISG15s. ISG15s from 24 species listed, with respective amino acid specificity residues. Green represents binding with ISG15. Red represents no binding detected with ISG15.

Specificity Residue	Species	CCHFV	PCTN	SGLV
NLPEFMN	Human ( <i>H. sapiens</i> )	Green	Green	Red
	Chimpanzee ( <i>P. troglodytes</i> )	Green	Green	Red
DQPDFMN	Sheep ( <i>O. aries</i> )	Green	Green	Red
	Goat ( <i>C. hircus</i> )	Green	Green	Red
DQPEFMN	Chinese hamster ( <i>C. griseus</i> )	Green	Green	Red
	Blue whale ( <i>B. musculus</i> )	Green	Green	Red
DQPEYMN	Alpaca ( <i>V. pacos</i> )	Red	Green	Red
	Pig ( <i>S. scrofa</i> )	Red	Green	Red
DLPEYMN	Capuchin ( <i>C. imitator</i> )	Green	Green	Red
	Egyptian jerboa ( <i>J. jaculus</i> )	Green	Green	Red
DLPEFLN	Squirrel ( <i>I. tridecemlineatus</i> )	Green	Green	Red
	Alpine marmot ( <i>M. marmota</i> )	Green	Green	Red
DLPEFMN	Rhesus monkey ( <i>M. mulatta</i> )	Green	Green	Red
	Olive baboon ( <i>P. anubis</i> )	Green	Green	Red
DQPELMN	Prairie vole ( <i>M. ochrogaster</i> )	Green	Green	Red
DQSEYLN	Gecko ( <i>G. japonicus</i> )	Red	Green	Green
DQPDQMN	Vesper bat ( <i>M. davidii</i> )	Green	Green	Red
AQPEIMN	African grass rat ( <i>A. niloticus</i> )	Green	Green	Red
DLPDFMN	Harbor seal ( <i>P. vitulina</i> )	Green	Green	Red
EQPEHMN	Flying fox ( <i>P. vampyrus</i> )	Red	Green	Red
NQPEFMN	Killer whale/Orca ( <i>O. orca</i> )	Green	Green	Green
Unique	Southern mouse ( <i>M. coucha</i> )	Red	Green	Red
	Elephant ( <i>L. africana</i> )	Red	Green	Green
	Brown rat ( <i>R. norvegicus</i> )	Red	Green	Red



Table 2.2. CCHF vOTU vs ISG15 binding kinetics dataset. Included are  $R^2$ ,  $K_{on}$ ,  $K_{off}$ , and  $K_D$  values obtained from CCHF vOTU and ISG15 BLI assays. ISG15s that did not bind to CCHF vOTU are denoted by DNB.

					CCHFV
Specificity Residue	Species	$R^2$	$K_{on}$ (1/Ms)	$K_{off}$ (1/s)	$K_D$ (M)
NLPEFMN	Human ( <i>H. sapiens</i> )	0.99	5.23E+02	2.99E-02	5.71E-05
	Chimpanzee ( <i>P. troglodytes</i> )	0.9939	3.40E+04	4.73E-01	1.39E-05
DQPDFMN	Sheep ( <i>O. aries</i> )	0.9902	3.99E+04	2.96E-01	7.42E-06
	Goat ( <i>C. hircus</i> )	0.9902	8.97E+04	2.81E-01	3.13E-06
DQPEFMN	Chinese hamster ( <i>C. griseus</i> )	0.9924	5.44E+04	3.11E-01	5.72E-06
	Blue whale ( <i>B. musculus</i> )	0.9904	2.20E+03	1.72E-02	7.82E-06
DQPEYMN	Alpaca ( <i>V. pacos</i> )	-	-	-	DNB
	Pig ( <i>S. scrofa</i> )	-	-	-	DNB
DLPEYMN	Capuchin ( <i>C. imitator</i> )	0.9903	8.37E+02	2.16E-02	2.59E-05
	Egyptian jerboa ( <i>J. jaculus</i> )	0.9921	3.29E+04	2.45E-01	7.45E-06
DLPEFLN	Squirrel ( <i>I. tridecemlineatus</i> )	0.9906	6.04E+04	3.02E-01	5.00E-06
	Alpine marmot ( <i>M. marmota</i> )	0.992	5.00E+04	3.21E-01	5.72E-06
DLPEFMN	Rhesus monkey ( <i>M. mulatta</i> )	0.991	1.51E+04	3.39E-01	2.24E-05
	Olive baboon ( <i>P. anubis</i> )	0.9902	1.12E+03	4.75E-01	4.25E-04
DQPELMN	Prairie vole ( <i>M. ochrogaster</i> )	0.9925	1.41E+04	4.66E-01	3.31E-05
DQSEYLN	Gecko ( <i>G. japonicus</i> )	-	-	-	DNB
DQPDQMN	Vesper bat ( <i>M. davidii</i> )	0.99	1.54E+03	4.46E-01	2.89E-04
AQPEIMN	African grass rat ( <i>A. niloticus</i> )	0.9912	8.64E+04	3.07E-01	3.56E-06
DLPDFMN	Harbor seal ( <i>P. vitulina</i> )	0.9918	4.83E+04	3.10E-01	6.43E-06
EQPEHMN	Flying fox ( <i>P. vampyrus</i> )	-	-	-	DNB
NQPEFMN	Killer whale/Orca ( <i>O. orca</i> )	0.99	1.69E+05	3.57E-02	2.11E-07
Unique	Southern mouse ( <i>M. coucha</i> )	-	-	-	DNB
	Elephant ( <i>L. africana</i> )	-	-	-	DNB
	Brown rat ( <i>R. norvegicus</i> )	-	-	-	DNB

Table 2.3. PCTN vOTU vs ISG15 binding kinetics dataset. Included are  $R^2$ ,  $K_{on}$ ,  $K_{off}$ , and  $K_D$  values obtained from PCTN vOTU and ISG15 BLI assays. Present, but unmeasurable binding is denoted by B.U.

Specificity Residue	Species	$R^2$	$K_{on}$ (1/Ms)	$K_{off}$ (1/s)	PCTN
					$K_D$ (M)
NLPEFMN	Human ( <i>H. sapiens</i> )	0.9975	7.51E+03	5.17E-01	6.88E-05
	Chimpanzee ( <i>P. troglodytes</i> )	0.9945	9.51E+03	3.46E-01	3.64E-05
DQPDMN	Sheep ( <i>O. aries</i> )	0.9981	1.79E+04	4.00E-01	2.23E-05
	Goat ( <i>C. hircus</i> )	0.9983	1.93E+04	2.21E-03	2.20E-05
DQPEFMN	Chinese hamster ( <i>C. griseus</i> )	0.9932	1.08E+04	2.87E-01	2.66E-05
	Blue whale ( <i>B. musculus</i> )	B.U.	B.U.	B.U.	B.U.
DQPEYMN	Alpaca ( <i>V. pacos</i> )	B.U.	B.U.	B.U.	B.U.
	Pig ( <i>S. scrofa</i> )	0.9909	7.14E+03	4.15E-01	5.81E-05
DLPEYMN	Capuchin ( <i>C. imitator</i> )	0.9907	6.54E+02	3.89E-01	5.95E-04
	Egyptian jerboa ( <i>J. jaculus</i> )	0.9904	3.55E+03	3.45E-01	9.71E-05
DLPEFLN	Squirrel ( <i>I. tridecemlineatus</i> )	0.9975	2.11E+04	2.85E-01	1.35E-05
	Alpine marmot ( <i>M. marmota</i> )	0.9958	6.76E+03	4.06E-01	6.00E-05
DLPEFMN	Rhesus monkey ( <i>M. mulatta</i> )	0.9902	1.07E+04	2.70E-01	2.52E-05
	Olive baboon ( <i>P. anubis</i> )	0.9931	9.83E+03	3.02E-01	3.08E-05
DQPELMN	Prairie vole ( <i>M. ochrogaster</i> )	0.9922	3.68E+03	4.96E-01	1.35E-04
DQSEYLN	Gecko ( <i>G. japonicus</i> )	0.9902	6.03E+03	3.56E-01	5.90E-05
DQPDMN	Vesper bat ( <i>M. davidii</i> )	0.9912	2.86E+03	3.89E-01	1.36E-04
AQPEIMN	African grass rat ( <i>A. niloticus</i> )	0.9901	3.98E+02	5.53E-03	1.04E-03
DLPDFMN	Harbor seal ( <i>P. vitulina</i> )	0.9901	9.73E+03	3.45E-01	3.55E-05
EQPEHMN	Flying fox ( <i>P. vampyrus</i> )	0.9913	7.53E+03	2.83E-01	3.76E-05
NQPEFMN	Killer whale/Orca ( <i>O. orca</i> )	0.9901	4.31E+04	8.25E-02	1.91E-06
Unique	Southern mouse ( <i>M. coucha</i> )	0.9914	6.49E+03	3.24E-01	4.99E-05
	Elephant ( <i>L. africana</i> )	0.99	3.91E+03	3.66E-01	9.35E-05
	Brown rat ( <i>R. norvegicus</i> )	0.9907	6.46E+02	4.78E-01	7.39E-04

Table 2.4. SGLV vOTU vs ISG15 binding kinetics dataset. Included are  $R^2$ ,  $K_{on}$ ,  $K_{off}$ , and  $K_D$  values obtained from SGLV vOTU and ISG15 BLI assays. Present, but unmeasurable binding is denoted by B.U. ISG15s that did not bind to SGLV vOTU are denoted by DNB.

					SGLV
Specificity Residue	Species	$R^2$	$K_{on}$ (1/Ms)	$K_{off}$ (1/s)	$K_D$ (M)
NLPEFMN	Human ( <i>H. sapiens</i> )	-	-	-	DNB
	Chimpanzee ( <i>P. troglodytes</i> )	-	-	-	DNB
DQPDFMN	Sheep ( <i>O. aries</i> )	-	-	-	DNB
	Goat ( <i>C. hircus</i> )	-	-	-	DNB
DQPEFMN	Chinese hamster ( <i>C. griseus</i> )	-	-	-	DNB
	Blue whale ( <i>B. musculus</i> )	-	-	-	DNB
DQPEYMN	Alpaca ( <i>V. pacos</i> )	-	-	-	DNB
	Pig ( <i>S. scrofa</i> )	-	-	-	DNB
DLPEYMN	Capuchin ( <i>C. imitator</i> )	-	-	-	DNB
	Egyptian jerboa ( <i>J. jaculus</i> )	-	-	-	DNB
DLPEFLN	Squirrel ( <i>I. tridecemlineatus</i> )	-	-	-	DNB
	Alpine marmot ( <i>M. marmota</i> )	-	-	-	DNB
DLPEFMN	Rhesus monkey ( <i>M. mulatta</i> )	-	-	-	DNB
	Olive baboon ( <i>P. anubis</i> )	-	-	-	DNB
DQPELMN	Prairie vole ( <i>M. ochrogaster</i> )	-	-	-	DNB
DQSEYLN	Gecko ( <i>G. japonicus</i> )	0.9962	4.62E+04	2.94E-01	6.38E-06
DQPDQMN	Vesper bat ( <i>M. davidii</i> )	-	-	-	DNB
AQPEIMN	African grass rat ( <i>A. niloticus</i> )	-	-	-	DNB
DLPDFMN	Harbor seal ( <i>P. vitulina</i> )	-	-	-	DNB
EQPEHMN	Flying fox ( <i>P. vampyrus</i> )	-	-	-	DNB
NQPEFMN	Killer whale/Orca ( <i>O. orca</i> )	0.9922	4.50E-03	8.07E-03	1.79E-06
Unique	Southern mouse ( <i>M. coucha</i> )	-	-	-	DNB
	Elephant ( <i>L. africana</i> )	B.U.	B.U.	B.U.	B.U.
	Brown rat ( <i>R. norvegicus</i> )	-	-	-	DNB

## Discussion

### *Developing a Biosurveillance Tool for Nairoviruses*

Inefficient disease data acquisition and surveillance pose major impairments to response measures, hindering the efficiency of healthcare and treatment that comes with them [31]. This is an inherent issue that affects low-income countries, where case fatality rates by CCHFV are the highest and disease burden is pronounced [32]. Adequate methods of biosurveillance against disease threats are always necessitated to protect vulnerable populations, and through the monitoring of viral OTU and host ISG15 interactions, we gained a comprehensive understanding of the pathways and host options nairoviruses may have to increase their prevalence in at-risk areas. With this information, we now have definitive insights into the host tropism of nairoviruses, paving the path for a honed-in practical approach and application of therapeutic development and treatment strategies.

It is made obvious that the capacity of an OTU to bind to a specific ISG15 protein holds correlations to cleavage ability, as ISG15s from the same species tested previously (human, sheep, vesper bat) that were cleaved by CCHF vOTU [22], also bound to each other in this study. Moreover, seroprevalence of CCHFV is found around the world in many species whose ISG15 bound to its OTU [24]. Although CCHFV (due to its increased prevalence) possesses the strongest background research out of the three nairoviruses in this study, from this framework, we have exposed a myriad of possible animal hosts for all of them which were not considered before. If transmission from one of these nairoviruses to a certain animal species has not already occurred in nature, this

tool at hand acts as a predictive measure and forces the unveiling of hosts through binding interaction experiments to display if deISGylation is a possibility. There are certain species in which there is no seroprevalence of CCHFV found, yet, but their ISG15s exhibited binding to its OTU. In addition, with the short supply of seroprevalence studies for PCTN and SGLV as of now, results from this study show multiple possible vertebrate-nairovirus interactions that could or may be occurring in nature.

*Impact of ISG15 Biodiversity on Nairovirus Host Preference*

As ISG15 holds substantial interspecies diversity, we have a key component of the host immune response upon which vOTUs act on differently, allowing us to reveal host ranges of these nairoviruses. In result, binding studies with ISG15s from species spanning the entirety of the planet allows us to differentiate and gauge possibilities of outbreaks or zoonotic transmission by these nairoviruses. To begin with, when comparing the possible host ranges of the three nairoviruses in regard to binding results, they vary drastically. It is now discernible that PCTN vOTU has the ability to interact with any and all presented ISG15s, while SGLV vOTU is very limited and selective in its interactions. CCHF vOTU, on the other hand, binds to most ISG15s but still experiences restraints to some from certain specificity residue groups. There are multiple possibilities for this divergence in ISG15 preference.

First, this could be a byproduct of differences in, or lack thereof, coevolution between nairovirus and ISG15s. What is unknown is how far PCTN and SGLV have come in terms of co-adapting to host immune responses, due to limited reports of their incidence. From one point of view, as there are a scarce amount of reported infections

from either virus, it can be suggested they are relatively young viruses. This is quite plausible, as their vOTUs present relatively one-sided binding profiles to ISG15s in comparison to CCHFV, a virus that has great intricacy in its evolutionary history [33]. What also can make sense, is that SGLV has not been circulating in animals long enough for it to adapt to their immune responses, while PCTN has been spreading for quite some time as explained by its abilities to interact with a multitude of ISG15s.

Secondly, another reasonable possibility for vOTU-specific preference for ISG15s within the binding realm is differences in structural characteristics between the vOTUs. As structure commands biochemical function [34], it is not out of the question that SGLV vOTU possesses some sorts of steric hindrances limiting access to binding and deISGylation to a small number of species in comparison to CCHF and PCTN vOTUs. The small amount of ISG15 species that do interact with SGLV vOTU, may just be the “keys fitting the lock”. This intriguingly invokes a path for future SGLV vOTU-ISG15 complex solving for interacting ISG15s.

Third, we cannot disregard the possibility that these nairovirus OTUs may just be significantly different in their efficiency as deISGylating proteases. PCTN vOTU, by taking a hit on binding strength, could have compensated with its capability of interacting with a broader range of hosts. SGLV vOTU on the other hand, may be a poor deISGylase, and could be targeting other innate immune candidates such as ubiquitin, which could explain its lack of capacity to bind to ISG15s. This correlates with the fact that SGLV vOTU did not bind to hISG15, but was still found to cause human febrile

illness [15]. Of course, these relatively small amounts of reports are inadequate to make proper conclusions about SGLV vOTU's role against the immune response.

Lastly, it is entirely possible that the differences arising in vOTU binding with ISG15s may just be a consequence of variances of mammalian host ranges within ticks carrying the nairoviruses. *Dermacentor occidentalis*, the tick species flagged with presence of PCTN, is widely distributed in California [35]. It is possible that animal species inhabiting these regions feature broad diversity of ISG15, resulting in the extensive PCTN vOTU-ISG15 binding profile. Previous work has suggested that a majority of possible host species, lying within the biogeographical regions ticks are restricted to, are utilized [36].

#### *Identifying Key Animal Species of Nairovirus Desire*

The circulation of viruses in animals before zoonotic transmission to humans serves as a precursor to impending outbreaks. Not only recognizing which hosts may be carriers of nairoviruses, but gauging which ones could contribute to transmission the most, may help us pinpoint which populations experience the greatest risk to their public health. To begin, interestingly, the emergence of killer whale/orca (kwISG15) in this study as not only the single ISG15 to bind to all vOTUS but the strongest binder, sheds a light on the increased susceptibility of marine birds and animals to agents from potential viral threats. Of course, this result can also be attributed to kwISG15 having an extremely preferable specificity residue combination (NQPEFMN) to the nairoviruses by chance, which can be confirmed with future studies centering on ISG15s possessing the same residues. Nevertheless, these results suggest these animals do not have range overlaps

with ticks that carry nairoviruses. Thus, in the case of orca, its susceptibility to viral threats may be escalated, and adaptation of its host proteins to a certain viral threat is never really in question. Unsurprisingly, marine animals carry the risk of obtaining an entourage of zoonotic agents as a result of sewage, agricultural, and medical waste runoff [37], but the implications of RNA viruses in the ocean are still not fully understood [38]. Considering there are scores of marine species that migrate seasonally around the world, they are an intriguing and keen subset of possibly vulnerable host carriers to be investigated when monitoring viral spread.

Although SGLV vOTU only bound to 3 species, one being orca, it's tempting to entertain the idea that we are in the early stages of detection of the virus. Recalling the idea of limited co-adaptation and spread between animal host and SGLV, it may only have been introduced in an extremely minor number of species as of currently. The recent identification of the virus in humans [15] provokes the question of whether one of these species (orca, gecko, or elephant) carrying SGLV came into contact with these patients by chance. This calls for testing between SGLV vOTU and other ISG15s holding the same specificity residues as these species, to get a deeper pinpoint where transmission may have exactly occurred.

#### *Paving the Path for Future Studies*

Results from this study present exciting opportunities for future work to further sharpen this biosurveillance tool and detect host reservoirs of nairoviruses in an attempt to limit threats to public health. To start, an unknown factor is how much of a relation binding affinity strength has on robustness of cleavage and deISGylase activity. Although



we suggest that detection of binding has correlations to ability of cleavage by OTU, it would be clarifying to figure out if a stronger binding affinity also means a more robust act of cleavage of an ISG15. From there, steps can be taken in an attempt to discover animal species that actually develop disease or illness from viral infection, rather than just act as carriers. This opens the door to track and implement new animal models in research for these nairoviruses. This would be most beneficial for CCHFV, as animal models for this virus are limited to mutant mice [7], due to the fact that progression to disease as of now is only found in humans. Animal models would be of great value to PCTN and SGLV as well, to capture a clearer picture of these emerging viruses' pathogenesis.

This study also unearths the possible impact mutational studies may have on nairovirus-host research. Could alterations in codons leading to changes in amino acid sequence allow/prevent certain ISG15s to bind to specific vOTUs? If so, could manipulation of sequence lead to changes in ISGylation ability by nairovirus OTU, and therefore success of infection? Considering that ISG15s aren't the only stimulated defense against pathogens, a key inquiry to pursue includes the effect of access to ISG15, and how it could affect infection outcome as a whole. This would be most notable to look into with SGLV, as it seems to not have bound with hISG15 but still infect humans, suggesting that it may use some sort of alternative pathway to bypass host immune response. These are intriguing questions that could be answered with research focused on the role of mutant ISG15s in the innate immune response in comparison to their wildtypes.

Research that utilizes bio-layer interferometry also comes with the notion that many of the results we acquired can be predecessors to future drug or therapeutic discovery. A scheme that comes to mind is the use of “anti-binding” drugs; possibly developing an antagonistic-like protein that is derived from amino acid residues most attracted by nairovirus OTUs, developed from ISG15s with the strongest binding affinities. This would act as a sort of placebo pathway for the vOTU in viral infection, allowing the body’s natural ISG15s to carry on with their functions and proceed with ISGylation. Dealing with viruses who either currently have little to no available therapeutics or who have not been studied enough to begin development of treatments, emphasizes the notion that any sort of possibilities must be explored.

### Materials and Methods

#### *Constructs, Expression, and Purification of vOTUs and mature ISG15s*

The OTU of CCHFV was constructed, expressed, and purified as previously described [39]. The OTUs of PCTN and SGLV were constructed, expressed, and purified in a similar manner, to ensure consistency and replicability. To recapitulate, T7 Express Competent *E. coli* cells bearing vOTU constructs were grown in 3 L of LB broth containing 100 µg/mL ampicillin at 37°C until an optical density at 600 nm (OD<sub>600</sub>) of 0.6 to 0.8 was reached. Expression was next induced with the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and cultures were set to grow overnight at 18°C. Cells were then pelleted by centrifugation at 5000 x g for 10 min and stored at -80°C until purification. To begin purification, cell pellets were lysed in buffer A (500 mM NaCl, 50 mM Tris [pH 8.0]) containing 5 mg of lysozyme for 30 min at 4°C. The

solution was then sonicated on ice at 50% power with 5-s pulse increments for 3 cycles, at 2 min each. Cell debris was removed by centrifugation at 26,000 x g for 45 min, and the supernatant was filtered with a 0.80- $\mu$ m filter. Following filtration, the supernatant was flowed over high-density nickel agarose beads (GoldBio), which were equilibrated with buffer A. The protein column was subsequently washed with buffer A supplemented with 10 mM imidazole, and then eluted with buffer A supplemented with 250 mM imidazole. Proteins were then dialyzed overnight at 4°C in buffer B (100 mM NaCl, 5 mM HEPES [pH 7.4], 2 mM dithiothreitol [DTT]). Nairovirus OTUs were then purified by size exclusion chromatography using a Superdex S200 column (GE Healthcare, Piscataway, NJ), and fractions containing vOTU were concentrated to ~8-10 mg/mL for storage at 4°C until BLI assays.

For ISG15s, mature forms from human (*Homo sapiens*; Accession: AAH09507.1), sheep (*Ovis aries*; Accession: AF152103.1), and vesper bat (*Myotis davidii*; Accession: ELK23605.1) were constructed, expressed, and purified as previously described [40]. In a similar fashion to previously reported mature ISG15s, the constructs of mature ISG15s originating from chinese hamster (*Cricetulus griseus*; Accession: XP\_027254434), blue whale (*Balaenoptera musculus*; Accession: XP\_036723871.1), goat (*Capra hircus*; Accession: XP\_005690852.1), alpaca (*Vicugna pacos*; Accession: XP\_015093270.1), pig (*Sus scrofa*; Accession: NP\_001121941.2), Panamanian white-faced capuchin (*Cebus capucinus imitator*; Accession: XP\_017398013.1), lesser Egyptian jerboa (*Jaculus jaculus*; Accession: XP\_045006261.1), chimpanzee (*Pan troglodytes*; Accession: XP\_016814061.1), thirteen-lined ground squirrel (*Ictidomys*

*tridecemlineatus*; Accession: XP\_005339329.1), alpine marmot (*Marmota marmota marmota*; Accession: XP\_015357044.1), rhesus monkey (*Macaca mulatta*; Accession: NP\_001253735.1), Olive baboon (*Papio anubis*; Accession: XP\_031519322.1), prairie vole (*Microtus ochrogaster*; Accession: XP\_005368768.1), Schlegel's japanese gecko (*Gekko japonicus*; Accession: XP\_015280624.1), African grass rat (*Arvicanthis niloticus*; Accession: XP\_034359250.1), harbor seal (*Phoca vitulina*; Accession: XP\_032251720.1), large flying fox (*Pteropus vampyrus*; Accession: XP\_011384344.1), killer whale (*Orcinus orca*; Accession: XP\_012389452.1), southern multimammate mouse (*Mastomys coucha*; Accession: XP\_031232864.1), African savanna elephant (*Loxodonta africana*; Accession: XP\_003413282.2), and Brown rat (*Rattus norvegicus*; Accession: NP\_001100170.1) were identified by sequence homology. Mature ISG15s were created with the implementation of a stop codon after the C-terminal RLRGG motif of pro versions from these ISG15s to prevent appendation of the pro GTEPGGRSGHHHHHH motif. Constructs were placed into pET-15B plasmids using NdeI/BamHI restriction sites, and gene synthesis was performed by GenScript (Piscataway, NJ). Expression and purification of these mature ISG15s was performed by reproducing previously described mature ISG15 methods [40]. In summary, T7 Express Competent *E. coli* cells containing these mature ISG15 constructs were grown in 3 L of LB broth containing 100 µg/mL ampicillin at 37°C until an OD<sub>600</sub> of 0.6 to 0.8 was attained. Expression was then induced by the addition of 1 mM IPTG, and cultures were grown overnight at 18°C. Cells were next pelleted by centrifugation at 5000 x g for 10 min and stored at -80°C until purification. Upon purification, cell pellets were lysed in

buffer A and 5 mg of lysozyme for 30 min at 4°C. The solution was then sonicated on ice at 50% power with 5-s pulse increments for 3 cycles, for a total of 6 min. Insoluble cell debris was removed by centrifugation at 48,000 x g for 30 min, and the supernatant was filtered with a 0.80-µm filter. Succeeding filtration, the supernatant was flowed over high-density nickel agarose beads (GoldBio), which were equilibrated with buffer A. The protein column was thereafter washed with buffer A supplemented with 30 mM imidazole, and then eluted with buffer A supplemented with 300 mM imidazole. Proteins were then dialyzed and thrombin cleaved overnight at 4°C in buffer B. Mature ISG15s were then purified by size exclusion chromatography using a Superdex S200 column (GE Healthcare, Piscataway, NJ), and fractions containing ISG15 were concentrated to ~8-10 mg/mL for storage at 4°C until biotinylation.

#### *Biotinylation of mature ISG15s*

Mature ISG15s were biotinylated for protein immobilization on streptavidin (SA) bio-layer interferometry probes using a predetermined biotinylation protocol (Gator Bio, CA, USA). In summary, proteins were dialyzed in PBS buffer (138 mM NaCl, 2.7 mM KCl [pH 7.4]) for 3 hrs at room temperature, and then overnight at 4°C. Subsequently, proteins were removed from dialysis and a 20 mM solution of biotin (EZ-Link NHS-PEG4-Biotin) was added after equilibration to room temperature. Biotinylation was allowed to proceed for 30 minutes, and reaction was terminated using desalting columns (Zeba Spin) to remove free biotin. Biotinylated proteins were then stored at 4°C until kinetics assays were performed.

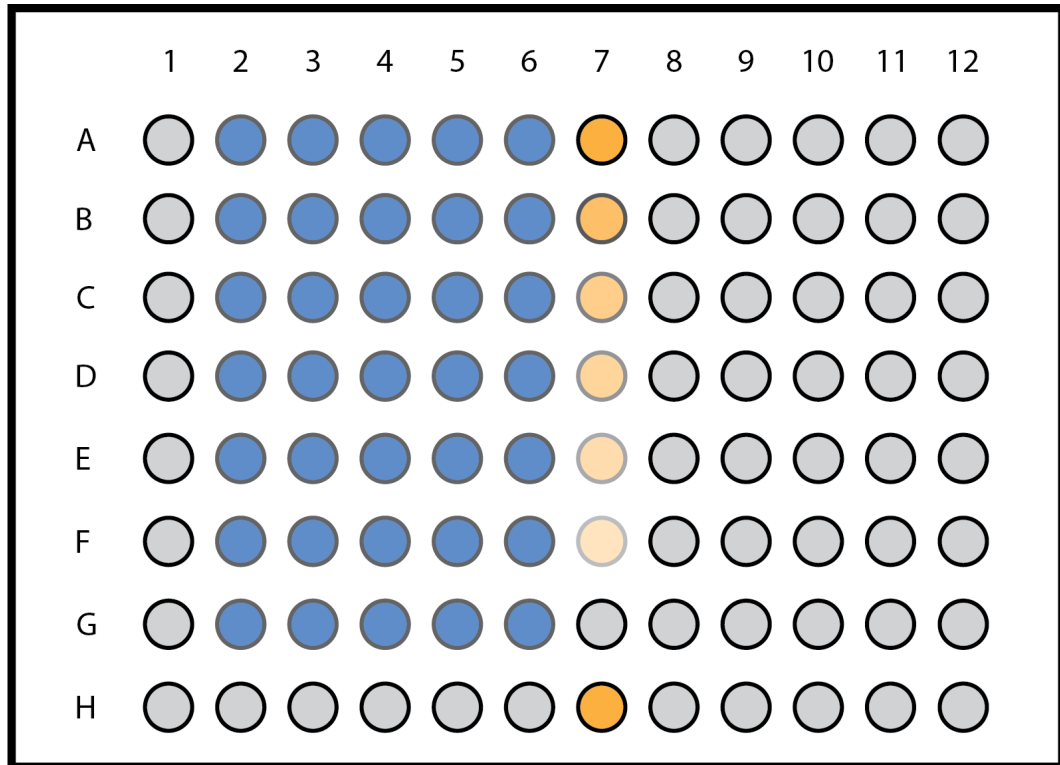
### *BLI kinetics assays*

All kinetics assay experiments were performed using bio-layer interferometry on the GatorPrime system, utilizing Flex SA Kit probes and reagents (Gator Bio, CA, USA). All buffers and samples were equilibrated to room temperature before conducting any assay. Biotinylated ISG15s were diluted to 25 µg/mL for optimal 50%-80% probe loading capacity. CCHF and SGLV vOTUs were diluted to a 6-fold concentration series of 6000 nM, 3000 nM, 1500 nM, 750 nM, 325 nM, and 175 nM. PCTN vOTU was diluted to a 6-fold concentration series of 48000 nM, 24000 nM, 12000 nM, 6000 nM, 3000 nM, and 1500 nM. All dilutions took place in K Buffer (1:10 dilution of Q Buffer [Cat #120010] in PBS).

Max plates (Cat #130062) were prepared by adding 250 µL/well of Flex SA Priming Reagent to columns 3-5 and 7-10, 250 µL/well of Flex SA Reactivation Reagent to column 1, 250 µL/well of Flex SA Capture Reagent to column 6, and 250 µL/well of 100% dimethyl sulfoxide (DMSO) to column 2. Fresh Flex SA probes were placed in column 10 of the Max plate and allowed to hydrate for at least 10 min before beginning assay. 96-well plates (Cat #130118) for kinetic assays were prepared by adding 200 µL/well of K Buffer to columns 1 and 8-12, 200 µL/well of ISG15 #1 to column 3, 200 µL/well of ISG15 #2 to column 4, 200 µL/well of ISG15 #3 to column 5, 200 µL/well of ISG15 #4 to column 6, and 200 µL/well of the 6-fold serial dilution of vOTU to column 7. Positive control ISG15s were added to column 2 of plates in the same 200 µL/well fashion: *Capra hircus* ISG15 for CCHFV, *Homo sapiens* ISG15 for PCTN, and *Orcinus orca* ISG15 for SGLV.

Upon running the assays, Flex SA probes are first plunged in K Buffer for 120 s to obtain a baseline signal. Probes are then loaded with an ISG15 for 100 s. Thereafter, probes are once again dipped in K buffer for 120 s as a wash step before being immersed in the serial dilution of vOTU for an association step of 60 s. This is followed by a dissociation step in K buffer for 120 s. Kinetic assays were run at 30°C, with data collected at 5-10 Hz. Binding affinity values were obtained with GatorPrime software (Gator Bio) using association and dissociation curves, with a minimum inclusion of 3 serial dilution concentrations. Baseline drift was subtracted with probes loaded with ISG15 and dipped into a 0 nM concentration of vOTU. Nonspecific binding was ruled out with non-loaded probes dipped into a well with the highest concentration of vOTU. Any interaction showing an association-nonspecific binding response ratio of less than 3:1 was considered not binding. All binding affinity values ( $K_{on}$ ,  $K_{off}$ , and  $K_D$ ) were obtained with a minimum of 0.99 full  $R^2$  coefficient value.

Figure 2.3. 96-well assay plate setup. Indicated are the specific wells used for binding affinity experiments. Gray wells represent K Buffer. Blue wells represent biotinylated ISG15s (total of 5). Orange wells (column 7) represent vOTU serial dilution, with nonspecific binding (highest concentration) control in well 7H.





## REFERENCES

1. Roossinck, M. J. (2011). The good viruses: Viral mutualistic symbioses. *Nature Reviews Microbiology*, 9(2), 99–108. <https://doi.org/10.1038/nrmicro2491>
2. World Health Organization. (n.d.). *Who coronavirus (COVID-19) dashboard*. World Health Organization. <https://covid19.who.int/>
3. Rockström, J., Norström, A. V., Matthews, N., Biggs, R., Folke, C., Harikishun, A., Huq, S., Krishnan, N., Warszawski, L., & Nel, D. (2023). Shaping a resilient future in response to COVID-19. *Nature Sustainability*, 6(8), 897–907. <https://doi.org/10.1038/s41893-023-01105-9>
4. Dzimianski, J. V., Beldon, B. S., Daczkowski, C. M., Goodwin, O. Y., Scholte, F. E., Bergeron, É., & Pegan, S. D. (2019). Probing the impact of Nairovirus genomic diversity on viral ovarian tumor domain protease (vOTU) structure and deubiquitinase activity. *PLOS Pathogens*, 15(1). <https://doi.org/10.1371/journal.ppat.1007515>
5. Lasecka, L., & Baron, M. D. (2013). The molecular biology of Nairoviruses, an emerging group of tick-borne arboviruses. *Archives of Virology*, 159(6), 1249–1265. <https://doi.org/10.1007/s00705-013-1940-z>
6. Bouchard, C., Dibernardo, A., Koffi, J., Wood, H., Leighton, P., & Lindsay, L. (2019). Increased risk of tick-borne diseases with climate and environmental changes. *Canada Communicable Disease Report*, 45(4), 83–89. <https://doi.org/10.14745/ccdr.v45i04a02>
7. Tipih, T., & Burt, F. J. (2020). Crimean–Congo hemorrhagic fever virus: Advances in vaccine development. *BioResearch Open Access*, 9(1), 137–150. <https://doi.org/10.1089/biores.2019.0057>
8. Spengler, J. R., Bente, D. A., Bray, M., Burt, F., Hewson, R., Korukluoglu, G., Mirazimi, A., Weber, F., & Papa, A. (2018). Second international conference on crimean-congo hemorrhagic fever. *Antiviral Research*, 150, 137–147. <https://doi.org/10.1016/j.antiviral.2017.11.019>
9. Hawman, D. W., & Feldmann, H. (2023). Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology*, 21(7), 463–477. <https://doi.org/10.1038/s41579-023-00871-9>

10. Jeeva, S., Mir, S., Velasquez, A., Ragan, J., Leka, A., Wu, S., Sevarany, A. T., Royster, A. D., Almeida, N. A., Chan, F., O'Brien, L., & Mir, M. A. (2019). Crimean-Congo Hemorrhagic fever virus nucleocapsid protein harbors distinct RNA-binding sites in the stalk and head domains. *Journal of Biological Chemistry*, 294(13), 5023–5037. <https://doi.org/10.1074/jbc.ra118.004976>
11. Akıncı, E., Bodur, H., & Leblebicioglu, H. (2013). Pathogenesis of Crimean-Congo Hemorrhagic fever. *Vector-Borne and Zoonotic Diseases*, 13(7), 429–437. <https://doi.org/10.1089/vbz.2012.1061>
12. Damgaard, R. B. (2021). The ubiquitin system: From cell signalling to disease biology and New Therapeutic Opportunities. *Cell Death & Differentiation*, 28(2), 423–426. <https://doi.org/10.1038/s41418-020-00703-w>
13. Dzimianski, J. V., Scholte, F. E. M., Bergeron, É., & Pegan, S. D. (2019). ISG15: It's complicated. *Journal of Molecular Biology*, 431(21), 4203–4216. <https://doi.org/10.1016/j.jmb.2019.03.013>
14. Bouquet, J., Melgar, M., Swei, A., Delwart, E., Lane, R. S., & Chiu, C. Y. (2017). Metagenomic-based surveillance of Pacific coast tick *Dermacentor occidentalis* identifies two novel bunyaviruses and an emerging human rickettsial pathogen. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-12047-6>
15. Ma, J., Lv, X.-L., Zhang, X., Han, S.-Z., Wang, Z.-D., Li, L., Sun, H.-T., Ma, L.-X., Cheng, Z.-L., Shao, J.-W., Chen, C., Zhao, Y.-H., Sui, L., Liu, L.-N., Qian, J., Wang, W., & Liu, Q. (2021). Identification of a new orthonairovirus associated with human febrile illness in China. *Nature Medicine*, 27(3), 434–439. <https://doi.org/10.1038/s41591-020-01228-y>
16. Kodama, F., Yamaguchi, H., Park, E., Tatemoto, K., Sashika, M., Nakao, R., Terauchi, Y., Mizuma, K., Orba, Y., Kariwa, H., Hagiwara, K., Okazaki, K., Goto, A., Komagome, R., Miyoshi, M., Ito, T., Yamano, K., Yoshii, K., Funaki, C., ... Matsuno, K. (2021). A novel nairovirus associated with acute febrile illness in Hokkaido, Japan. *Nature Communications*, 12(1). <https://doi.org/10.1038/s41467-021-25857-0>
17. Walker, P. J., Widen, S. G., Wood, T. G., Guzman, H., Tesh, R. B., & Vasilakis, N. (2016). A global genomic characterization of Nairoviruses identifies nine discrete genogroups with distinctive structural characteristics and host-vector associations. *The American Journal of Tropical Medicine and Hygiene*, 94(5), 1107–1122. <https://doi.org/10.4269/ajtmh.15-0917>

18. Cai, X., Cai, X., Xu, Y., Shao, Y., Fu, L., Men, X., & Zhu, Y. (2023). Virome analysis of ticks and tick-borne viruses in Heilongjiang and Jilin provinces, China. *Virus Research*, 323, 199006. <https://doi.org/10.1016/j.virusres.2022.199006>
19. Perng, Y.-C., & Lenschow, D. J. (2018). ISG15 in antiviral immunity and beyond. *Nature Reviews Microbiology*, 16(7), 423–439. <https://doi.org/10.1038/s41579-018-0020-5>
20. Narasimhan, J., Wang, M., Fu, Z., Klein, J. M., Haas, A. L., & Kim, J.-J. P. (2005). Crystal structure of the interferon-induced ubiquitin-like protein ISG15. *Journal of Biological Chemistry*, 280(29), 27356–27365. <https://doi.org/10.1074/jbc.m502814200>
21. Speer, S. D., Li, Z., Buta, S., Payelle-Brogard, B., Qian, L., Vigant, F., Rubino, E., Gardner, T. J., Wedeking, T., Hermann, M., Duehr, J., Sanal, O., Tezcan, I., Mansouri, N., Tabarsi, P., Mansouri, D., Francois-Newton, V., Daussy, C. F., Rodriguez, M. R., ... Bogunovic, D. (2016). ISG15 deficiency and increased viral resistance in humans but not mice. *Nature Communications*, 7(1). <https://doi.org/10.1038/ncomms11496>
22. Dzimianski, J. V., Scholte, F. E., Williams, I. L., Langley, C., Freitas, B. T., Spengler, J. R., Bergeron, É., & Pegan, S. D. (2019). Determining the molecular drivers of species-specific interferon-stimulated gene product 15 interactions with nairovirus ovarian tumor domain proteases. *PLOS ONE*, 14(12). <https://doi.org/10.1371/journal.pone.0226415>
23. Spengler, J. R., Bergeron, É., & Spiropoulou, C. F. (2019). Crimean-Congo hemorrhagic fever and expansion from endemic regions. *Current Opinion in Virology*, 34, 70–78. <https://doi.org/10.1016/j.coviro.2018.12.002>
24. Spengler, J. R., Bergeron, É., & Rollin, P. E. (2016a). Seroepidemiological studies of Crimean-Congo hemorrhagic fever virus in domestic and wild animals. *PLOS Neglected Tropical Diseases*, 10(1). <https://doi.org/10.1371/journal.pntd.0004210>
25. Jiles, R., Klevens, M., & Hughes, E. (2014a). Surveillance and Seroepidemiology. *Viral Infections of Humans*, 63–79. [https://doi.org/10.1007/978-1-4899-7448-8\\_4](https://doi.org/10.1007/978-1-4899-7448-8_4)
26. Capodagli, G. C., & Pegan, S. D. (2013). Diversity of Ubiquitin and ISG15 Specificity amongst Nairoviruses Ovarian Tumor Domain Proteases. <https://doi.org/10.2210/pdb4hxd/pdb>

27. Zohaib, A., Saqib, M., Athar, M. A., Hussain, M. H., Sial, A.-R., Tayyab, M. H., Batool, M., Sadia, H., Taj, Z., Tahir, U., Jakhrani, M. Y., Tayyab, J., Kakar, M. A., Shahid, M. F., Yaqub, T., Zhang, J., Wu, Q., Deng, F., Corman, V. M., ... Shi, Z.-L. (2020). Crimean-Congo hemorrhagic fever virus in humans and livestock, Pakistan, 2015–2017. *Emerging Infectious Diseases*, 26(4), 773–777. <https://doi.org/10.3201/eid2604.191154>
28. Gonzalez, J.-P., Camicas, J.-L., Cornet, J.-P., & Wilson, M. L. (1998). Biological and clinical responses of West African sheep to Crimean-Congo haemorrhagic fever virus experimental infection. *Research in Virology*, 149(6), 445–455. [https://doi.org/10.1016/s0923-2516\(99\)80013-2](https://doi.org/10.1016/s0923-2516(99)80013-2)
29. Wangchuk, S., Pelden, S., Dorji, T., Tenzin, S., Thapa, B., Zangmo, S., Gurung, R., Dukpa, K., & Tenzin, T. (2016). Crimean-Congo hemorrhagic fever virus IGG in goats, Bhutan. *Emerging Infectious Diseases*, 22(5), 919–920. <https://doi.org/10.3201/eid2205.151777>
30. Bahir, I., Fromer, M., Prat, Y., & Linal, M. (2009). Viral adaptation to host: A proteome-based analysis of codon usage and amino acid preferences. *Molecular Systems Biology*, 5(1). <https://doi.org/10.1038/msb.2009.71>
31. Akinsulie, O. C., Adesola, R. O., Aliyu, V., Oladapo, I. P., & Hamzat, A. (2023). *Epidemiology and Transmission Dynamics of Viral Encephalitis in West Africa*. <https://doi.org/10.20944/preprints202306.0162.v1>
32. Belobo, J. T., Kenmoe, S., Kengne-Nde, C., Emoh, C. P., Bowo-Ngandji, A., Tchatchouang, S., Sowe Wobessi, J. N., Mbongue Mikangue, C. A., Tazokong, H. R., Kingue Bebey, S. R., Atembeh Noura, E., Ka'e, A. C., Guiamdjo Simo, R. E., Modiyinji, A. F., Ngongang, D. T., Che, E., Kenfack, S., Nzukui, N. D., Amvongo Adjia, N., ... Njouom, R. (2021). Worldwide Epidemiology of crimean-congo hemorrhagic fever virus in humans, ticks and other animal species, a systematic review and meta-analysis. *PLOS Neglected Tropical Diseases*, 15(4). <https://doi.org/10.1371/journal.pntd.0009299>
33. Anagnostou, V., & Papa, A. (2009). Evolution of crimean-congo hemorrhagic fever virus. *Infection, Genetics and Evolution*, 9(5), 948–954. <https://doi.org/10.1016/j.meegid.2009.06.018>
34. Alberts, B., Bray, D., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2013). Protein structure and function. *Essential Cell Biolog*, 121–170. <https://doi.org/10.1201/9781315815015-4>

35. Gurfield, N., Grewal, S., Cua, L. S., Torres, P. J., & Kelley, S. T. (2017). Endosymbiont interference and microbial diversity of the pacific coast tick, *dermacentor occidentalis*, in San Diego County, California. *PeerJ*, 5. <https://doi.org/10.7717/peerj.3202>
36. McCoy, K. D., Léger, E., & Dietrich, M. (2013). Host specialization in ticks and transmission of tick-borne diseases: A Review. *Frontiers in Cellular and Infection Microbiology*, 3. <https://doi.org/10.3389/fcimb.2013.00057>
37. Bogomolni, A., Gast, R., Ellis, J., Dennett, M., Pugliares, K., Lentell, B., & Moore, M. (2008). Victims or vectors: A survey of marine vertebrate zoonoses from coastal waters of the Northwest Atlantic. *Diseases of Aquatic Organisms*, 81, 13–38. <https://doi.org/10.3354/dao01936>
38. Lang, A. S., Rise, M. L., Culley, A. I., & Steward, G. F. (2009). RNA viruses in the sea. *FEMS Microbiology Reviews*, 33(2), 295–323. <https://doi.org/10.1111/j.1574-6976.2008.00132.x>
39. Capodagli, G. C., McKercher, M. A., Baker, E. A., Masters, E. M., Brunzelle, J. S., & Pegan, S. D. (2011). *Structural Analysis of a Viral OTU Domain Protease from the Crimean-Congo Hemorrhagic Fever Virus in Complex with Human Ubiquitin*. <https://doi.org/10.2210/pdb3prm/pdb>
40. Deaton, M. K., Dzimianski, J. V., Daczkowski, C. M., Whitney, G. K., Mank, N. J., Parham, M. M., Bergeron, E., & Pegan, S. D. (2016). Biochemical and structural insights into the preference of nairoviral deisgylases for interferon-stimulated gene product 15 originating from certain species. *Journal of Virology*, 90(18), 8314–8327. <https://doi.org/10.1128/jvi.00975-16>

## CHAPTER 3

### SUMMARY AND CONCLUSION

#### *The Need for a Biosurveillance Tool*

Viruses declared their presence on this planet billions of years prior to humans. Subsequently, it is not surprising that there has been a constant arms-race between us throughout history, striving towards a common biological goal: survival. It is often forgotten how imperative it is to take the lead of this competition, but viral threats remind us time and time again, as seen with the Coronavirus Disease 2019 (COVID-19) pandemic. It is remarkable how what started as an unknown pneumonia in Wuhan, China, led to the shutdown of societies in the entirety of the planet, keeping us in our houses for what felt like ages. What this proved, although, was the vital need to study and prepare for infectious threats of human consequence. Considering the fatality rate and pathogenesis of CCHFV, it poses a said infectious threat. It is important to note that until an efficacious vaccination was developed for COVID-19, the outbreak was almost unmanageable. CCHFV currently does not have any viable therapeutics, which magnifies the need for its prompt monitoring. In addition, it is believed that the outbreak of COVID-19 began from a spillover event from an animal carrying the virus. Thus, to fully understand the future path CCHFV will take in disease spread and progression, surveillance and identification of animals hosts for the virus was required. Developing and applying a tool created for biosurveillance of this virus using host ISG15 protein and viral OTU protease, and applying it to other unfolding nairoviruses in PCTN and SGLV, yielded key insights into the drivers behind nairovirus preference for host proteins. With

the assistance of previously identified ISG15 specificity residues partaking in interactions with nairoviruses, intriguing trends were discovered within the realm of host protein-viral protease interactions.

### *Unveiling vOTU-ISG15 Interface*

To expand on previous studies and better understand how nairoviruses choose to interact with host ISG15 proteins, bio-layer interferometry was utilized to screen a large quantity of ISG15s against the OTUs of CCHFV, PCTN, and SGLV. Application of streptavidin probes made it possible to run multiple ISG15 proteins, of differing species, at once against the OTUs by tagging them with biotin. Through these experiments, it was found that the three vOTUs had drastically different binding profiles and preferences. Interestingly, in comparison to CCHF vOTU, both PCTN and SGLV vOTUs had one-sided interactions. PCTN vOTU bound to every single ISG15 tested, while SGLV vOTU only bound to three. Although CCHFV was expected to have a mixed binding profile to different ISG15s, it came as a surprise that PCTN and SGLV, both being from the Tamdy genera of nairovirus, had almost complete opposite results of binding to each other. Comparing the binding results amongst the vOTUs, brought to light the substantial effects divergent evolution could have on species-specific engagement of ISG15s. Evolution may have induced differences in structure of these OTUs, which could affect their binding ability to ISG15s due to possible presence, or lack, of steric hindrance. Additionally, we cannot ignore the possibility of these variations being a result of differences in functions within OTUs. SGLV vOTU may just be acting upon a different member of the host innate immune response like ubiquitin, while PCTN vOTU's main

barrier to viral replication is indeed ISG15, explaining why there would be an absence or enhanced need of engaging with ISG15 respectively. Enticingly, key species that may pose as reservoirs for these viruses were identified, with orca standing out from its strong binding to all three OTUs. What remains unknown, however, is the correlations between binding affinity strength and cleavage robustness. Although, clues from this study have presented that the engagement in binding is linked to the ability of deISGylation, and therefore infection. Thus, unprecedented opportunities to discover animal reservoirs are put forward with findings from this study.

#### *Impact of Nairoviral Research*

It is often difficult to visualize the bigger picture of research studies when they may be focused on small biological molecules like proteins. However, in the context of this research, we need to consider what the outcomes would be of developing a tool to predict whether a virus would engage with the IGS15 in particular hosts. To begin with, pinpointing certain hosts where these viruses may circulate in would take the pressure off testing our luck with random sampling and metagenomics, and allow us to shift our focus on certain species that serve as hotspots, to better isolate viral spread. *In silico* research, as presented here, presents avenues towards time and cost-effective methods to limit the introduction of these pathogens in society. Furthermore, these revelations may help with area-based tracking of nairoviral outbreaks. It wouldn't be a surprise that animals from certain regions of the planet are more susceptible to infection by nairovirus than others, as a result of close evolutionary relations. Moreover, not only does nairoviral research impact human public health, but also may have strong influences on wildlife



conservation. Although CCHFV symptomatic disease progression is limited to humans, we do not know that for other emerging nairoviruses. For all we know, they could bring fatal outcomes to wildlife as well. This will only be solved with further research on these imminent pathogens, and the hosts that carry them.

At the end of the day, it is important to recognize one of our goals as scientists focusing on disease prevention, which is protecting the most vulnerable. It's made clear that the populations most at risk from harm by CCHFV include soldiers, healthcare workers, and those from low-income countries. Implementing the message received from this virus' outcomes, we obtained a key mission as researchers, which is taking steps towards prevention of harmful threats for populations most jeopardized. It is a great privilege to be a part of working towards providing adequate health for all, through the detection and monitoring of nairoviruses.