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Targeting Protein Synthesis in *Clostridioides difficile* to Develop Antimicrobial Candidate

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TARGETING PROTEIN SYNTHESIS IN *CLOSTRIDIOIDES DIFFICILE* TO DEVELOP ANTIMICROBIAL CANDIDATE

A Thesis

by

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ABSTRACT

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Clostridioides difficile is a gram positive, spore forming, obligate anaerobic bacterium that causes infection known as CDI which results in life threatening diarrhea. Protein synthesis is an essential metabolic process and is a validated target for antibiotics. The translation initiation step is the rate limiting step which includes the three initiation factors (IF1, IF2, and IF3). IF1 is the smallest and associates with the 30S ribosomal subunit. Structural studies using NMR Titrations were conducted with Cd-IF1 to determine key residues involved in binding to the 30S. From these results a short α helical peptide was derived to test its inhibitory affects against bacterial pathogens. Additionally, Cd-IF1 underwent NMR titrations with two microRNAs (miR-155 & miR-146) to determine its RNA binding mode.

DEDICATION

The completion of my master's studies would not have been possible without the support of my parents, and my brother. Without their love and support I wouldn't be where I am today.

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CHAPTER I

INTRODUCTION

Statement of the purpose

Clostridioides difficile, also referred to as *C. difficile*, is a spore forming, gram-positive, toxigenic, anaerobic bacillus bacterium. Production of spores and toxins enables this bacterium in having the ability to create a deadly infection in susceptible people. *Clostridioides difficile* Infection (CDI), is the most common cause of nosocomial infections mediated by the fecal-oral route and is the leading cause of hospital acquired diarrhea and pseudomembranous colitis. This infection has been associated with morbidity and mortality in most hospital settings (Ofosu, 2016). Individuals that are more susceptible at risk for this infection include: old age, recent antibiotic exposure, and hospitalization (Czepiel, 2019). In 2017 a total of 223,000 estimated cases in the United States required treatment for this infection and of those estimated cases, 12,800 people died. Additionally, an estimated 1 billion was attributed to healthcare costs associated with this infection in 2017 (CDC, 2019). Treatment includes antibiotics that create the infection itself and that includes metronidazole, fidaxomicin and vancomycin. In rare instances treatment with these antibiotics has no effect on the infection and in severe cases a Fecal transplant is another form of therapy. Although healthcare-cases have decreased there has been an increase in community-based infections (CDC, 2019). The CDC announced that *C. difficile* is now an urgent threat and there is an increase in community-based cases creating a need for

development of new antimicrobial candidates that have a different mode of action than the ones currently available. CDI has created unmet needs to understand its molecular mechanisms underlying this infection and identifying new molecules than can be considered antimicrobial candidates that can potentially be utilized against *C. difficile*.

Statement of the purpose

Bacterial protein synthesis has been deemed a worthy target for drug discovery and antibiotic development (McCoy et al., 2011). The process of Protein Synthesis begins with the rate limiting step Initiation followed by Elongation, Termination and Recycling. During initiation formation of the initiation complex occurs which consists of the 30S subunit, the three initiation factors (IF1, IF2, & IF3), mRNA and initiator tRNA (Laursen, 2005). Previously in our laboratory, determination of the Initiation Factor 1 protein of *Clostridioides difficile* was elucidated using NMR spectroscopy studies. The Initiation Factor 1 protein of *C. difficile* was found to be composed of one short α helix and five β strands in the following sequential order: β1-β2-β3-α1-β4-β5. Using the NMR chemical shift assignments, a Cd-IF1 model was predicted and submitted to the protein data bank (PDB). A predicted model of Cd-IF1(PDB ID: 6C00) and the 30S subunit from Thermus thermophilus (PDB ID: 1HR0) was constructed using PyMOL.

To identify target molecules that may regulate Cd-IF1 function further studies were conducted. Based on previous structural studies in our laboratory on Pseudomonas aeruginosa, IF1 may have distinct interactions with the 30S ribosomal subunit. Additionally, the short α helix strand may play a role in ribosomal binding to IF1 which also might affect its function. (Valdez, 2021) Antibiotic development studies continued based on Pseudomonas aeruginosa research and its 30S ribosomal subunit. NMR titrations revealed that the residues that had the most significant

chemical shift perturbations were from the α helix (Valdez, 2021). From the short α helix, a short peptide was derived to test against *C. difficile* and other bacterial species to test its inhibitory effects. This peptide is an antimicrobial peptide (AMP) which has been found to have a unique ability of controlling infections while also having a lower chance in acquiring resistance (Liu et al., 2018). AMPs may be an antimicrobial candidate to be used against *C. difficile* and other types of bacterial species. Aside from utilizing components of bacterial pathogens we decided to test molecules from the mammalian immune response, microRNAs, to identify a Cd-IF1 RNA binding mode. MicroRNAs are non-coding RNAs that take part in regulating gene expression and in the case of bacterial infections become upregulated (Eulalio, 2012). The two microRNAs (mir-146-a and mir-155) that play a role in the innate immune response were tested with Cd-IF1 using NMR titrations to identify any interactions or regulations that may occur. Indication of binding can suggest that Cd-IF1 may have an RNA binding mode and microRNAs may be another way to stop protein synthesis. MicroRNAs may be another form of antimicrobial candidates that could potentially be used to treat this infection.

CHAPTER II

REVIEW OF LITERATURE

Microbiology

Clostridioides difficile is a gram-positive, spore forming, anaerobic bacillus bacterium that has the ability in causing infection that may lead to life-threatening diarrhea and pseudomembranous colitis. It was formally known as *Clostridium difficile* but was officially renamed due to its taxonomic differences between other members of the *Clostridium* genus (Lawson, 2016). It is part of the gut microbiome which consists of more than 4,000 bacterial species and is found in the large intestine of most mammals. Additionally, it may be found in the environment such as in the soil and animals (Czepiel, 2019). *C. difficile* strains are a genetically diverse species and can be either toxigenic or nontoxigenic (He, 2010) (Vedantam et al., 2012).

Toxigenic strains are associated with disease by possessing the two virulent toxins A (TcdA) and B (TcdB) which enable this bacterium to cause infection. TcdA and TcdB belong to the family of glucosylating toxins and are composed of a receptor binding domain, a transmembrane domain, and a glucosyl-transferase domain (Davies, 2011). These two toxins target the Rho family of guanosine triphosphatases (Rho GTPases) such as Rac, Rho, and Cdc42 through the enzymatic glucosylation of a theorine residue which leads to an inflammatory

cascade (Kordus et al., 2021). This inflammatory cascade results in tissue damage that leads to life-threatening diarrhea, toxic megacolon, and colitis (Burke, 2014).

Clostridioides difficile Toxins

Once *Clostridioides difficile* spores enter the intestine, this bacterium can switch into its vegetative disease-causing state (Di Bella, 2016). The presence of glycine and cholate derivatives aids germination of *C. difficile* spores leading to disease. As a result of treatment with broad spectrum antibiotics, individuals will lose the additional bacteria that are responsible in processing cholate derivatives (Burns, 2010) (Di Bella, 2016). *Clostridioides difficile* obtains its pathogenetic effects from the production of three major toxins: A (TcdA), B (TcdB) and, in certain strains, the *C. diffile* transferase (CDT) binary toxins. These two toxins are made by the genes tcdA and tcdB which are found in the Pathogenicity loci, also known as PaLoc (Di Bella, 2016). The PaLoc is a 19.3 kilobase DNA sequence and is found in the same site of all toxigenic *C. difficile* strains. All these three belong to the large clostridial glycosylating toxin (LCGT) family (Jank, 2015). The LCGT family contains a receptor binding domain, a transmembrane domain and a glycosyl-transfease domain (Vedantam, 2012). The toxins glycosylate and inactivate host GTPases which lead to alterations in the actin cytoskeleton, disruption of barrier function and cell death (Davies, 2011). Additionally, toxins A and B modify guanine nucleotidebinding proteins of the Rho family leading to inflammation and damage of the gut mucosa (Jank, 2015) (Voth and Ballard, 2005).

Clostridioides difficile Infection (CDI)

Clostridioides difficile Infection (CDI) is a side effect of most Broad-spectrum antibiotic treatments that disrupt the gut microbiota. Once the disruption of the gut microbiome occurs, *C.*

difficile will grow sporadically leading to infection. In a hospital setting, CDI is the leading cause of hospital associated infections with 12.1% followed by Staphylococcus aureus (10.7%), Klebsiella (9.9%), and Escherichia coli (9.3%). (Monegro, 2021). It may also lead to toxic megacolon, colitis, and pseudomembranous colitis.

Risk factors

Risk factors that are mainly associated with *C. difficile* infection include antibiotic exposure, old age, and hospitalization (Czepiel, 2019). Most antibiotics used have been associated with CDI and those frequently associated include the following: ampicillin, amoxicillin, cephalosporins, clindamycin, and fluoroquinones (Leffler, 2015). Surprisingly, even the antibiotics used to treat CDI can also incite the disease. CDI persists in healthcare facilities such as in hospital settings and nursing homes. In both settings, frequent antibiotic use led to infection and environmental contamination due to *C. difficile* spores that persist in the environment. Environmental contamination arises from *C. difficile* spores that stay in the setting due to poor personal hygiene or poor medical professionals not taking proper precautions when sanitizing. Additionally, elderly individuals who are in these healthcare settings are more at risk of infection compared to other individuals. It was found that elderly individuals are 10 times more at risk than other patients. Not only are they more susceptible to infection but the severity of the infection worsens with increase in age (Leffler, 2015). CDI is typically a hospital acquired infection but as of lately, there has been an increase in community acquired infection (CDC, 2019). Community acquired infection of CDI affects younger individuals and that have no exposure to antibiotics.

Current treatment and recurrence

Treatment of this infection includes antibiotic therapy that is tailored to the severity of disease presentation. In mild to moderate cases of infection, the antibiotic usually prescribed can either be metronidazole or vancomycin. Both these antibiotics are the initial treatment for this infection. The recommended prescription for metronidazole is 500 mg between 10-14 days intravenously. If the patient has is intolerant to metronidazole, then vancomycin 125 mg oral can be utilized. In the case of severe or complicated infection, oral vancomycin 125 mg will be immediately prescribed (Ofosu, 2016). Additionally, intravenous metronidazole may be added to therapy for complicated cases of CDI. Fidaxomicin is an alternative to patients with severe infections and have no response to vancomycin. In severe complicated infection, it is best to have a surgical consultation if there is presence of peritoneal signs, severe ileus, and toxic megacolon.

Recurrence of CDI can occur after eight weeks from initial episode completion of treatment due to reactivation of left-over spores in the patient. Risk factors that may affect the recurrence of the infection include the following: old age, additional types of treatment, proton pump inhibitors, and prior cases of CDI recurrence. After the first episode of CDI, there is a 10-20% chance of the infection reoccurring. If there has already been one recurrence, the chance of getting the infection again increases to 40-60% (Ofosu, 2016). Factors that may affect the recurrence include impaired immune response old age, further treatment with antibiotics or chemotherapy, proton pump inhibitors, and previous episodes of recurrence. Data suggests that

there is a significant increase in risk of CDI when associated with proton pump inhibitor (PPI) treatment (Trifan, 2017).

After multiple recurrences of CDI, the use of broad-spectrum antibiotics will eventually lead to a microbe depleted intestine. An intestine without a healthy microbiota flora will have an overgrowth of pathogenic bacteria such as *C. difficile*. In the case of individuals who have multiple recurrences, a Fecal microbiota transplantation may be considered. A Fecal microbiota transplantation (FMT) is a fecal infusion from a healthy donor to a recipient for curative purposes. The FMT is to help re-establish the gut microbiota in the intestine to treat the infection (Czepiel, 2019).

Current targets of antibiotics

Current bacterial targets have been extensively studied in the development of antimicrobial drugs. These targets include the following: cell wall synthesis, protein synthesis, ribonucleic acid synthesis, deoxyribonucleic acid (DNA) synthesis and intermediary metabolism (Hooper, 2001).

Inhibition of DNA replication is commonly done by with treatment that includes the class of antibiotics known as quinolones and fluoroquinones. These antimicrobials interfere with the maintenance of chromosomal topology by targeting DNA gyrase (topoisomerase II) and topoisomerase IV leading to breakage of bacterial chromosomes (Pham, 2019). Both these enzymes are needed in the modulation of chromosomal supercoiling through topoisomerase catalyzed strand breakage and rejoining reactions is required for DNA synthesis, mRNA transcription and cell division. By trapping both DNA gyrase and topoisomerase at the DNA cleavage stage and preventing strand rejoining (Kohanski, 2010). With quinolone topoisomerase-

DNA complex formation, replication machinery of DNA will become arrested at the replication forks which leads to the inhibition of DNA synthesis, leading to bacteriostatic and cell death. Additionally, following the effects of the introduction of double-stranded DNA breaks formed by quinolones induces the DNA stress response (SOS response). The SOS response activates RecA promoting auto-cleave of the LexA repressor protein which induces expression of the SOS-response genes including DNA repair enzymes.

Rifamycins are a class of semi-synthetic bactericidal antibiotics belonging to the ansamycin family that inhibit RNA synthesis. They are a potent means for inducing bacterial cell death and their mode of action is by interfering with RNA synthesis and implicated RNA polymerase (RNAP) as the rifamycin primary target (Adams, 2021) (Campbell, 2001). These drugs inhibit DNA-dependent transcription by stable binding, with high affinity, to the subunit of a DNA-bound and actively transcribing RNA polymerase enzyme (Kohanski, 2010).

Cell wall synthesis inhibition can occur through lytic cell death or non-lytic cell death. Peptidoglycan (PG or Murein) layers enclose the bacterial cell wall to protect against environmental factors. Structural features of PG are linear glycan strands cross linked by short peptides. (Vollmer, 2008) PG is covalently cross-linked polymer matrix that is composed of peptide linked β -(1-4)-N-acetyl hexosamine 54. Lytic cell death can occur with β -lactams and glycopeptides that interfere in steps of homeostatic cell wall biosynthesis. Treatment with successful inhibitors may result in changes to cell shape/size, induce cellular stress responses, and lead to cell lysis (Kohanski, 2012). β lactams inhibit by blocking the cross-link of PG units by inhibiting the peptide bond formation reaction catalyzed by transpeptidases via penicillin-

binding proteins. In certain bacteria, such as S. pneumonia that lack murein, hydrolase activity can be affected by β -lactams at a lower rate (Kohanski, 2010).

Protein synthesis inhibitors include 50S ribosome inhibitors and 30S ribosome inhibitors. The 50S ribosome inhibitors consist of macrolides, lincosamide, streptogramin, amphenicol, and oxazolidinone (Kohanski, 2012). These inhibitors block initiation of protein translation, or translocation of peptidyl-tRNAs, which inhibit the peptidyltransferase reaction that lengthens the peptide chain. In contrast, tetracycline and aminocyclitol antibiotic families make up the inhibitors that target the 30S ribosome. Tetracyclines mechanism of action is by blocking access of aminoacyl-tRNAs to the ribosome. Aminocyclitol includes the spectinomycin and aminoglycoside family of antibiotics which bind the 16S rRNA component of the 30S ribosome subunit leading to protein mistranslation (Kohanski, 2010).

Bacterial protein synthesis

The central dogma begins with replication followed by transcription and ending in the process of translation to make a functioning protein. Translation occurs in the cell's ribosome which is composed primarily of ribosomal RNA and are responsible for translation where they catalyze peptide bond formation. The ribosome is responsible in converting the genetic information given by the messenger RNA (mRNA) into a polypeptide sequence which makes up the cell's proteins and enzymes (Arenz, 2016). Translation occurs in the ribosome and is composed of four phases: initiation, elongation, termination, and ribosome recycling (Laursen, 2005). Initiation is responsible in constructing the large and small ribosomal subunits at the translation initiation region of mRNA. Elongation is where mRNA will slide along the ribosome creating a polypeptide chain with the aide from GTPase factors elongation factor Tu (EF-Tu) and

EF-G (Voorhees, 2013). Once the mRNA reaches a stop codon the polypeptide chain will halt reaching the termination phase. The polypeptide chain will be released from the ribosome and ribosome recycling will occur. The mRNA and ribosomal subunits will then dissociate (Laursen, 2005).

Bacterial translation initiation

Of the four phases involved in Protein Synthesis Initiation is the most highly regulated and is the rate limiting step (Laursen, 2005). Initiation is composed of the following steps: ribosome, aminoacylated and formylated initiator tRNA, mRNA, and the three initiator factor proteins (IF1, IF2, and IF3), a large 50S subunit and a small 30S subunit construct to the complete 70S ribosome. There are three tRNA binding sites aminoacyl (A), peptidyl (P), and exit (E) sites. IF1 enhances IF2 and IF3 activities. When IF3 binds to the small 30S ribosomal subunit dissociation of the ribosome is promoted. IF1 will bind the A site of the 30S subunit which is thought to aid in directing the initiator tRNA to the P site of the ribosome and stopping it from binding to the A site.

Once the dissociation has occurred, IF2, mRNA, and fMet-tRNA will associate with the small 30S ribosomal unit in a random order. The initiation start codon (AUG) as well as other initiation triplets (GUG, UUG, AUU, AUC, and AUA) will be adjusted to the P site when the Shine-Dalgarno sequence interacts with the anti-Shine-Dalgarno sequence from the 16S rRNA (Gualerzi, 2015). Of the three initiation factors, IF3 seems to play a role in adjusting the start codon. Once, the start codon is in the P-site, an initiator tRNA is positioned in the P-site of the 30S. This process, is promoted by IF2 due to its interaction with fMet-tRNA, consists of three steps known as: designated codon- independent binding, codon-dependent binding, and fMet-

tRNA adjustment. Additionally, IF3 is responsible in stabilizing the binding of fMet-tRNA to the P-site of the ribosome.

The 30S ribosomal subunit, three initiation factor proteins (IF1, IF2 & IF3), and mRNA make up the 30S preinitiation complex that is considered unstable (Gualerzi, 2015). The preinitiation complex will then undergo a rate-limiting conformation change which will promote the codon-anticodon interaction and will therefore form a stable 30S initiation complex. Once, a stable 30S complex is made, IF1 and IF3 will be rejected out. The main function of IF2 is to interact with the initiator fMet-tRNA and position it directly in the P site (Marzi, 2003). IF2 will be ejected when the initiator fMet-tRNA is correctly positioned in the P-site, it will also undergo hydrolysis since it is bound to a GTP yielding a GDP and an inorganic phosphate. Once the 50S ribosomal subunit is added onto the 30S complex a 70S initiation complex is created. The 70S complex has a fMet-tRNA as a substrate for the peptidyltransferase of the 50S ribosomal subunit is set for the elongation phase.

Antimicrobial peptides

Emergence of antibiotic resistant bacteria has created a new on-growing threat that may affect public universal health. To combat this new problem new approaches to development of new antibacterial strategies are of need. One of the possible solutions may be in making continuous progress in the research of new antimicrobial agents such as antimicrobial peptides (AMPs) or also known as host defense peptides.

Antimicrobial peptides range 5-50 amino acid residues in length, they are the first line of defense in the innate immune system against pathogenic invasion since they act with microbicidal and immunomodulatory activity (Mwangi, 2019). AMPs may have different

cationicity and hydrophobicity which determine their antimicrobial activity (Liu, 2016). AMPs contain a distribution of basic and hydrophobic residues that align and form structures that are water soluble, hydrophobic, and positively charged (Zhang, 2016). AMPs that fold can be categorized into groups based on their secondary structure: α helical, β sheet and extended AMPs (Zhang, 2016). These antimicrobial agents have been considered a potential alternative to current antibiotics due to their low potential for bacteria to become resistant (Roncˇevic, 2019). These antimicrobial peptides are immune modulation molecules that are derived from host defense peptides which aide against pathogenic bacteria (Liu, 2020). Through various studies AMPs have been shown to have various modes of actions with non-specific targets and pathogenic bacteria are additionally less prone to produce resistance (Mookherjee et al., 2020). These peptides are potential candidates to be used against bacterial pathogens since they have a variety of advantages such as possessing potent microbicidal activity in the micromolar range, rapid bacterial death action as well as having a low resistance selection (Leon-Buitimea, 2020). One of the main mechanisms of action of AMPs is the formation of pores in the bacterial cell membrane (Nuding, 2014) (Brogden, 2004). Additionally, their mode of action is multifunctional since it alters the cell membrane as well as attacking specific targets that are involved intracellular processes such as: inhibition of transcription, translation, protein synthesis and bacterial cell wall formation (Mwangi et al., 2019).

AMPs can either be synthetic or natural peptides. Many AMPs have been synthetically developed in laboratories, but many natural AMPs have been produced by both prokaryotic and eukaryotic organisms. Natural peptides are produced by ribosomal translation of mRNA or non-ribosomal peptide synthesis (Hancock, 1999). Natural peptides have since decreased due to its time-consuming traditional methods to extract, separate, and purify to identify active molecules.

Compared to natural peptides, synthetic AMPs are found to be more effective against antibiotic resistant pathogens due to its computer-assisted de novo design (Liu, 2016). Synthetic AMPs have been shows to be effective against multidrug resistant bacteria alongside combination with conventional drugs (Zharkova, 2019).

Many AMPs have been developed and are reported to be active against Gram-negative and Gram-positive bacteria (Fan, 2016). Their mode of action against bacterial species depends on their ability to interact with bacterial membranes or cell walls (Zhang, 2016). AMPs can also affect broad activity and are able to kill fungi, viruses, and cancer cells (Fan, 2016). These characteristics of AMPs may be the solution to the problem of antibiotic bacterial species. Upon this new development strategy more research is needed to efficiently be utilized and for AMPs to become potential targeted antimicrobial candidates.

MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNAs, 18-23 nucleotides in length that play a part in post transcriptional gene translation and regulate expression of cellular mRNAs. They play a part in the following: proliferation, growth, death, inflammation, and immune response. The miRNA pathway begins with RNA polymerase II initiating transcription of a polyadenylated primary miRNA (pri-mRNA). The pri-mRNA will then be processed by two endonucleases of the RNAse III family. Drosha will process the pri-miRNAs into one mirRNA which will then get processed into intermediates by Dicer. The duplex unwinds, and one strain will be put into RNAinduced silencing complex (RISC) which is composed of Argonaute proteins. A microRNA bound to RISC will bind to a cellular mRNA target site which will result in a repressed translation and promotes degradation of mRNA (Eulalio, 2012). Recent studies have concluded

that these miRNAs are less known in bacterial infections compared to more well-known roles in viral and parasitic infections leading to a new branch of research.

Bacterial infection is detected by Pattern-recognition receptors (PAMPs) such as Toll Like Receptors (TLRs) and NLRs to recognize pathogenic bacteria. PAMPs can sense bacterial pathogens either gram positive or gram negative through specific recognition receptors. Once a bacterial ligand has bonded to a PAMP it will trigger an immune response mediated through adaptor proteins and transcription factors. For instance, in gram-positive bacteria PAMPs consist of bacterial flagellin, Lipoteichoic acid, peptidoglycan, bacterial RNA and bacterial DNA (Eulalio, 2012).

Various studies featuring bacterial pathogens have seen upregulation of both microRNA-155 and microRNA-146. A pioneer study found that miRNA-146a/b and miRNA-155 use the NFKB (Nuclear kappa factor-B) dependent manner and both miRNAs are part of the regulatory mechanisms that aide in inflammatory cytokine production in response to bacterial infection. In various types of bacterial infections including the following had upregulation of the miR-155 family and miR-146a/b family: *Helicobacter pylori*, *Listeria monocytogenes*, *Mycobacterium species* and *Salmonella species* (Eulalio, 2012). Additionally, *Francisella tularensis, Vibrio cholerae,* and *Staphylococcus aureus* all had overexpression of miR-155, which is responsible in modulating immune response, indicating that this microRNA is involved in bacterial infection through the mammal immune response (Mourenza, 2022). Increase in expression of microRNAs depends on the NFKB pathway once the immune system has been triggered leading to regulation of genes that play a factor in adaptive and innate immunity. They also regulate the pathway establishing a negative regulatory loop which may be important in a balanced immune response.

CHAPTER III

METHODOLOGY

Protein expression and purification

Previously in our lab Initiation Factor 1 (IF1) of *Clostridiodides difficile* was predicted. The full backbone and side chain resonance assignments were determined which were used to predict its atomic resolution structure. Cd-IF1 was amplified using polymerase chain reaction (PCR) from genomic DNA (gDNA). IF1 of *C. difficile* is 216 base pairs (bp) and is composed of 72 amino acids. After amplification of Cd-IF1, the PCR Product was inserted into a pET-26b plasmid vector (Novagen) between the following restriction enzyme sites: NheI and XhoI. Transformation of the insert product from PCR Product was then ligated into a NheI-XhoI cut pET-24b Cd-IF1 that contains three additional amino acids (MAS) found at the N-terminal and the C-terminal contained a HIS-TAG with six histidine tag (LEHHHHHH). Once the DNA sequence of the plasmid was confirmed by sequencing results it was then transformed into pET24b vector Dl21 (DE3) *Escherichia coli* competent cells (Novagen) for Cd-IF1 protein expression preparation (Aguilar, 2018).

To have a uniformly labeled ^{15} N sample. An overnight culture of the pET-24b plasmid containing the Cd-IF1 protein was inoculated into 10 mL LB broth and 5 uL kanamycin. The culture was left in the shaker at 37 degrees C and 225 RPM overnight. An autoclaved liter flask of M9 minimal medium was made to cultivate the *E. coli* plasmid vector to express the Cd-IF1

protein. The M9 media contained 1 g of $^{14}NH_4Cl$ and 3 g of dextrose anhydrous. The culture was then allowed to grow until the Optical density (OD) 600 was between 0.5 to 0.8 which was followed by the addition of 500 uL Isopropyl β- d-1-thiogalactopyranoside (IPTG) in order induce the overexpression of the Cd-IF1 protein. After four hours the culture was then harvested and centrifuged to create cell pellets that would then be purified.

Cell pellets were re-suspended in a His-tag binding buffer with the addition of dithiothreitol (DTT) and phenylmethylsulphonyl fluoride (PMSF). After the cell was resuspended, the pellets were sonicated to create cell lysate. After several rounds of sonication, the cell lysate was centrifuged and loaded onto a Ni-NTA (nickel- nitrilotriacetic acid) column. The column and lysate were incubated for binding of His-tag target protein to the Ni-NTA agarose beads. Following incubation, the Ni-NTA (nickel- nitrilotriacetic acid) column with Cd-IF1 bound was washed with both binding and washing buffer to remove impurities. The Cd-IF1 protein was then eluted with a buffer that contained 300 mM imidazole. Once the purified Cd-IF1 protein was eluted the sample went through dialysis to remove high salts. The sample was then loaded onto an AKTA pure column to go through Fast Protein Liquid Chromatography (FPLC) to further purify (Figure 1) and obtain a Cd-IF1 protein to conduct Nuclear Magnetic Resonance (NMR) Spectroscopy studies.

Nuclear magnetic resonance spectroscopy and NMR titration

Cd-IF1 (labeled ^{15}N) underwent buffer exchange to buffer that consists of 20 mM MES at pH 6.0, 50 mM NaCl, 1mM EDTA, 5 mM DTT, and 8% D20. Buffer exchange to buffer used a Millipore Amicon Ultra Centrifugal Filter Ultracel-3K (Millipore #UFC900324, 3 kDa cut off). NMR experiments were conducted at 298 K on a Bruker Ultrashield Plus 600 MHz spectrometer

equipped with a double resonance broad band room temperature probe (BBO). The N15-H1SQC spectrum was recorded using a uniformly labeled ^{15}N-labeled Cd-IF1. Chemical shift assignments were obtained from BMRB Accession No. 27349.

For NMR titration experiments, 30S ribosomal subunit was added into the ^{15}N Cd-IF1 labeled sample at varying increasing concentrations. Calculations of chemical shift perturbations used Equation 1.

Equation 1. $\Delta_{av} = \sqrt{(\Delta H^2 + (\Delta \frac{N^2}{5})/2}$

Complex model of Cd-IF1 and 30S ribosomal subunit

Utilizing PyMOL (Version 2.40a0 Open-Source) PDB IDs from both *C. difficile* Initiation factor 1 (6C00) and the *T. thermophilus* solved 30S ribosomal subunit (1HR0) (Carter, 2001) were aligned to produce an Cd-IF1 bound to a 30S subunit (Figure 5). Additionally, the α helical strand is colored red and the β sheets colored in blue. The red α helical can be seen anchoring to the 30S subunit supported by the NMR titration data (Figure 5).

Complex model of Cd-IF1 Peptide and 30S ribosomal subunit

Utilizing PyMOL (Version 2.40a0 Open-Source) PDB IDs from both *C. difficile* Initiation factor 1 peptide and the *T. thermophilus* solved 30S ribosomal subunit (1HR0) (Carter, 2001) were aligned to produce an Cd-IF1 peptide bound to a 30S subunit (Figure 6).

Minimum Inhibition Concentration (MIC) Assays

Various Minimum Inhibitory Concentration (MIC) assays were conducted to determine if the IF1 peptide obtained antimicrobial activity. Thermo Fisher Scientific 96-Well Microtiter

Microplates were used to test the inhibitory effects of Cd-IF1-derived peptide on bacterial growth. *Staphylococcus epidermidis* (ATCC 12228), *Mycobacterium smegmatis* (ATCC 14468), *Bacillus cereus* (ATCC 14579), *Escherichia coli*, and *P. aeruginosa* (ATCC 47085), were among the bacterial pathogens used in the test. The *E. coli* strain utilized was Invitrogen's BL21(DE3) (One Shot BL21(DE3), cat. no. C600003), which is descended from the *E. coli* B strain and commonly used for high-level recombinant protein expression. *P. vulgaris*, was received from The Microbiology Laboratory at The University of Texas Rio Grande Valley's Department of Biology. From the first well across the plate, the Cd- IF1 peptide was diluted in repeated 2-fold dilutions. The negative and positive controls were DMSO and ampicillin or kanamycin (0.01 g/mL (28.6 mM) in each well, respectively. Results are summarized in Table 1: Minimum Inhibitory Concentration (MIC) Assay Results and can be seen in Figure 7.

Cd-IF1 and microRNAs NMR Titrations

To investigate if Cd-IF1 has an RNA Binding mode and can bind to microRNAs NMR titration spectroscopy experiments were conducted to obtain chemical shift perturbations results. To measure chemical shift perturbations (CSP) which is a sensitive tool that can demonstrate ligand binding to proteins over a range of affinities. (Williamson, 2018) Cd-IF1 (labeled ^{15}N) underwent buffer exchange to buffer that consists of 20 mM MES at pH 6.0, 50 mM NaCl, 1mM EDTA, 5 mM DTT, and 8% D20. Buffer exchange to buffer used a Millipore Amicon Ultra Centrifugal Filter Ultracel-3K (Millipore #UFC900324, 3 kDa cut off). NMR measurements were conducted at 298 K on a Bruker Ultrashield Plus 600 MHz spectrometer equipped with a double resonance broad band room-temperature probe (BBO). The ^{1}H-^{15}NHSQC spectrum was recorded using a uniformly labeled ^{15}N-labeled Cd-IF1 (Figure 7 and 11). Chemical shift

assignments were obtained from BMRB Accession No. 27349. For NMR titration experiments, microRNA-155 (UUAAUGCUAAUCGUGAUAGGGGUU) and microRNA-146-a (UGAGAACUGAAUUCCAUGGGUU) were added into the ^{15} N Cd-IF1 labeled sample at varying increasing concentrations (Figures 8 &12).

Computational docking

Structure prediction of microRNA-155 and microRNA-146-a were generated by RNAComposer the best structure generated can be seen in Figure 9 and 13. To construct a predicted binding structure of the microRNAs and Cd-IF1, binding sites of both macromolecules were identified (Antczak, 2016) (Popenda, 2012). Binding sites of Cd-IF1 were given by the NMR titration data and microRNA binding sites were generated by an online server RBinds (Wang & Zhao, 2020). The binding sites for both Cd-IF1 and the two microRNAs (miR-155 and miR-146-a) were submitted to the HADDOCK server to construct a structural model. The best structure of Cd-IF1 and miR-155 generated by HADDOCK shows the binding interface between both molecules (Figure11). Similarly, the miR-146-a and Cd-IF1 binding interface was generated (Figure 15). Additionally, the key residues seen in Figures 9 and 13 indicate the significant residues that had a change in chemical shift perturbation and were seen to bind to the nucleotides of the microRNAs.

CHAPTER IV

RESULTS AND CONCLUSION

NMR titration results of Cd-IF1 and 30S ribosomal subunit displays a series of amino acid perturbation and changes in peak intensity (Figure 2) Using the ^{1}H, ^{15}N HSQC spectrum from NMR titrations, calculations were computed to determine relative intensity change and chemical shift perturbations. The key amino acid residues that had a significant intensity change were the following: D4, E9, M20, K24, and V54 (Figure 4). Chemical shift perturbations were calculated using Equation 1 and the key residues were found to be in the α -helical section which included I35, L39, R63, R65, and R69 (Figure 3). These results indicate the alpha helix may be important in IF1 binding to the 30S ribosome Additionally, most of the affected residues are basic amino acids which may interact with the 30S due to their positive charge. To further analyze this binding interface a complex model of Cd-IF1 bound 30S ribosome was generated (Figure 5). Therefore, due to the importance of the α -helix to 30S subunit binding a short peptide was derived. The Cd-IF1 peptide could possibly act as a mimic of IF1 to bind to the 30S subunit to inhibit translation initiation. A structure complex of the Cd-IF1 peptide and 30S ribosome was constructed for structural analysis (Figure 6).

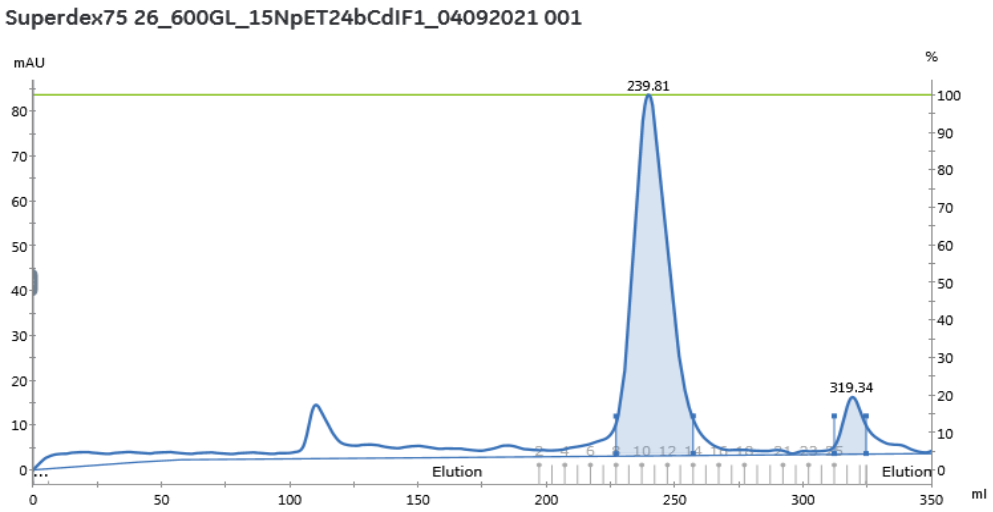

Figure 1. Fast Protein Liquid Chromatography (FPLC) Cd-IF1 sample results

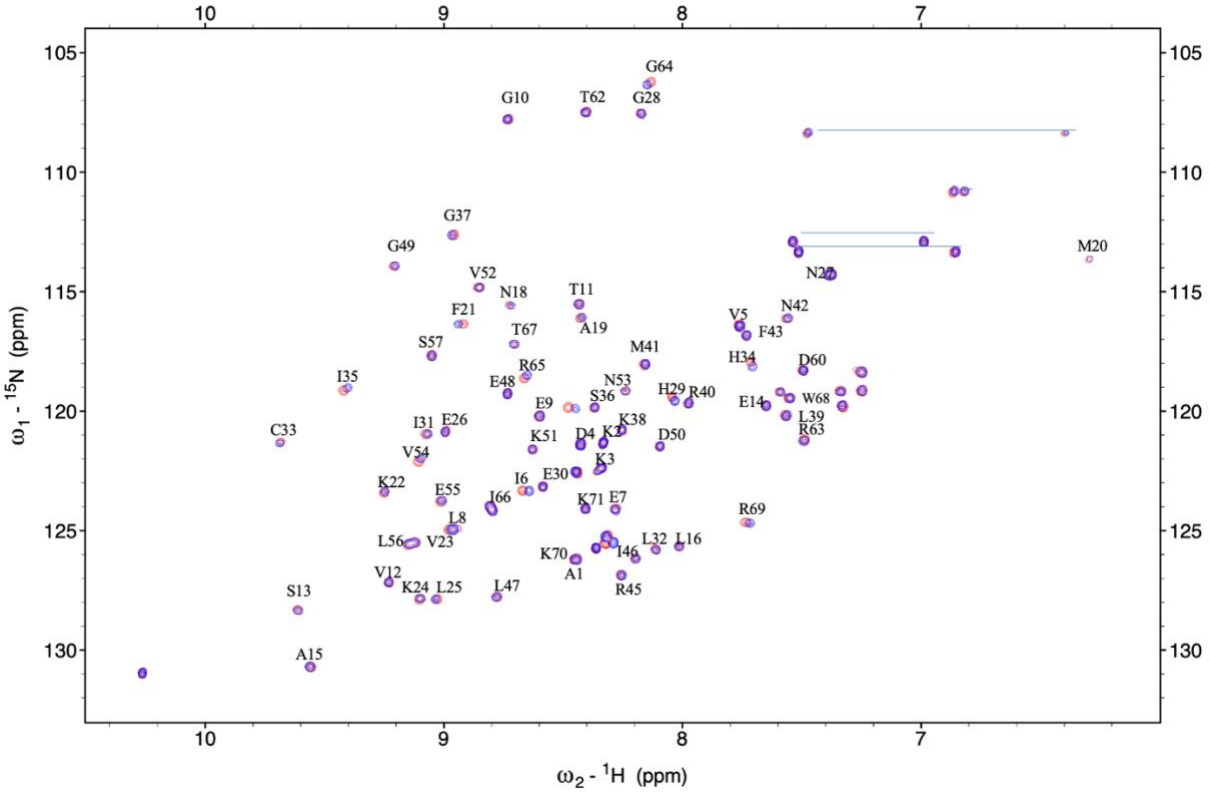

Figure 2: Two-dimensional HSQC spectra of ^{15}N-labeled Cd-IF1and 30S.Cd-IF1 in the absence (blue) and presence (red) of 30S ribosomal subunit.

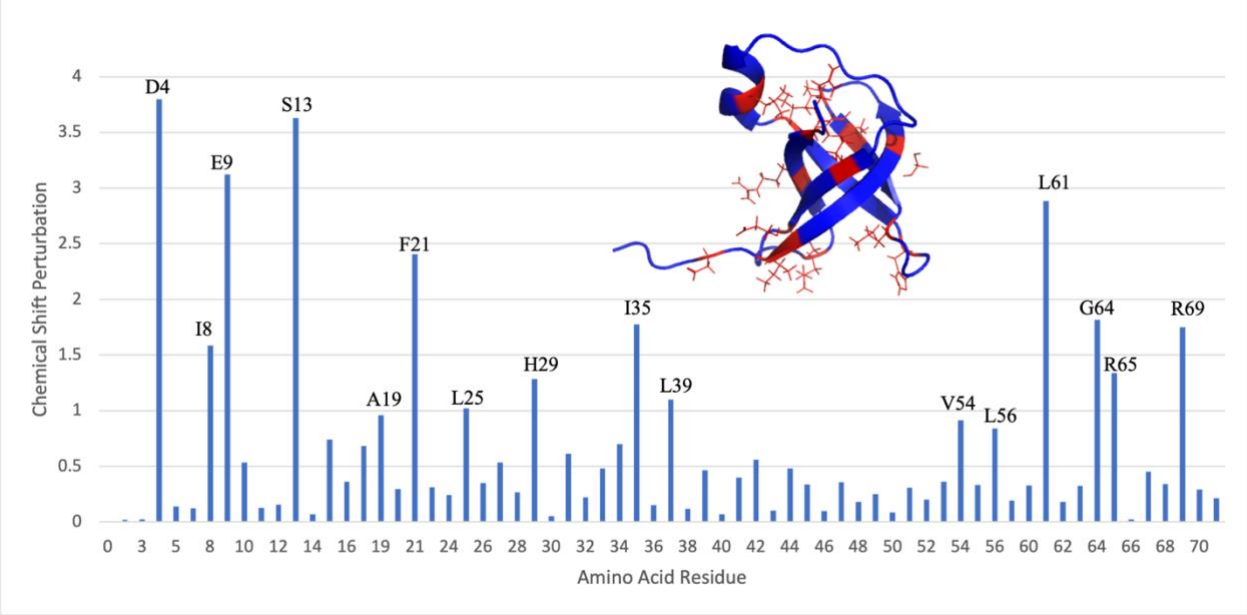

Figure 3: Chemical Shift Perturbations of Cd-IF1 and 30S. Key residues (red) with significant perturbations.

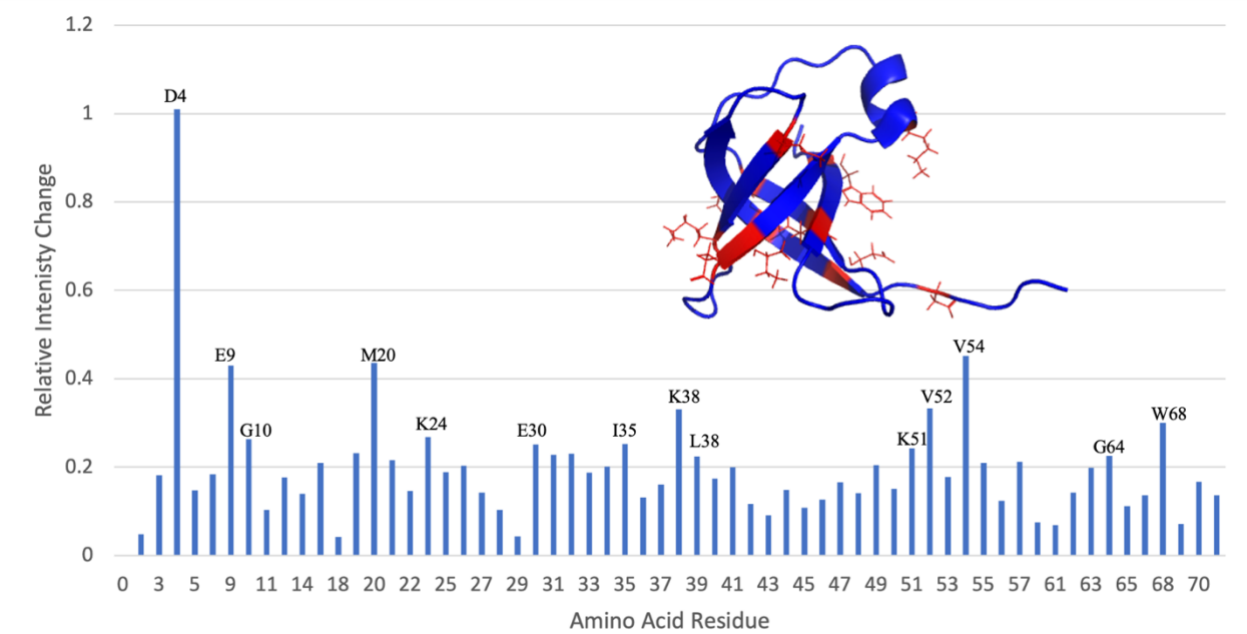

Figure 4: Relative Intensity Changes of Cd-IF1 and 30S. Key residues (red) with significant intensity change.

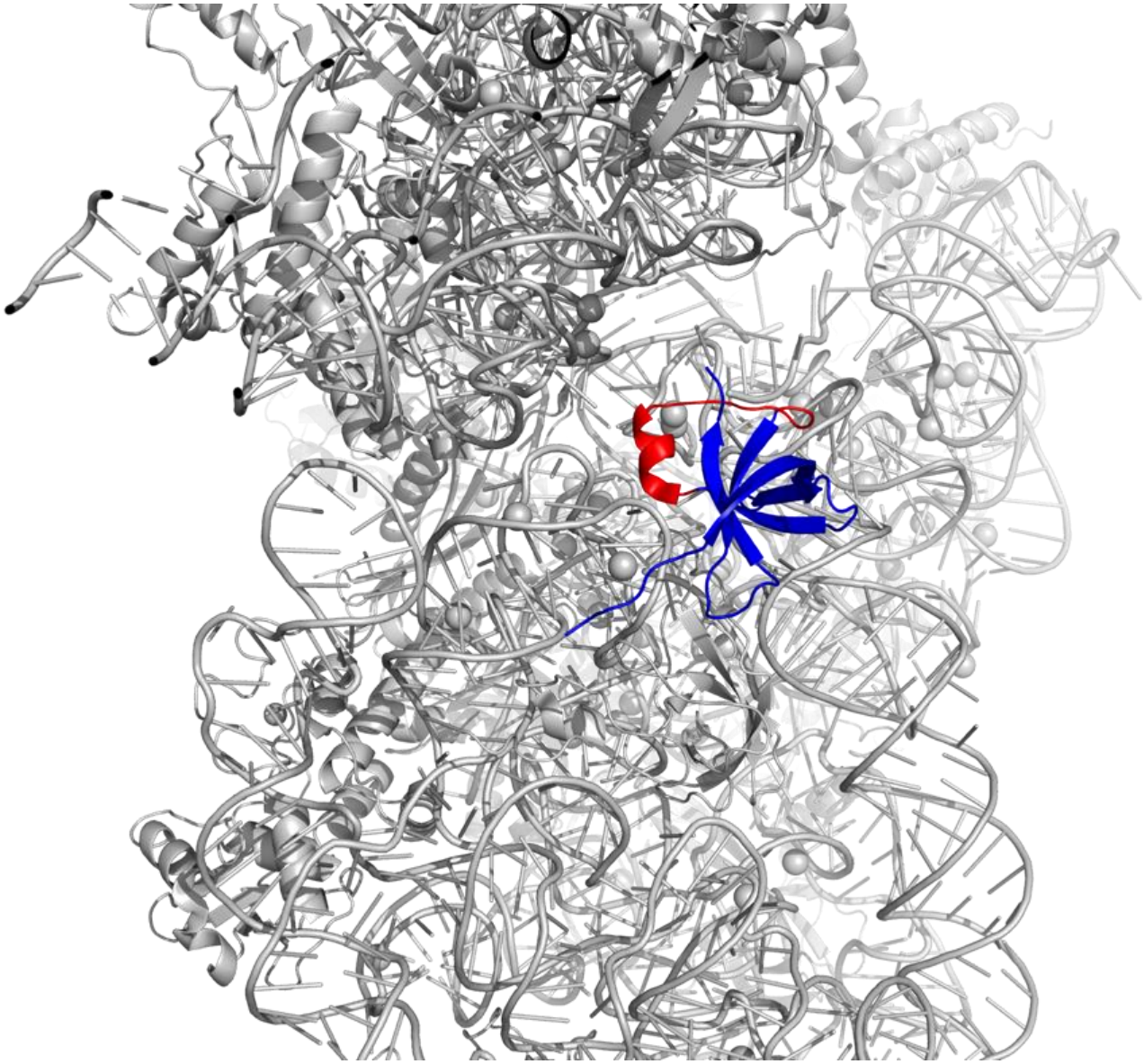

Figure 5. Predicted 30S initiation complex with Cd-IF1 and 30S subunit of *T. thermophilus* PDB ID (6C00) PDB ID (1HR0)

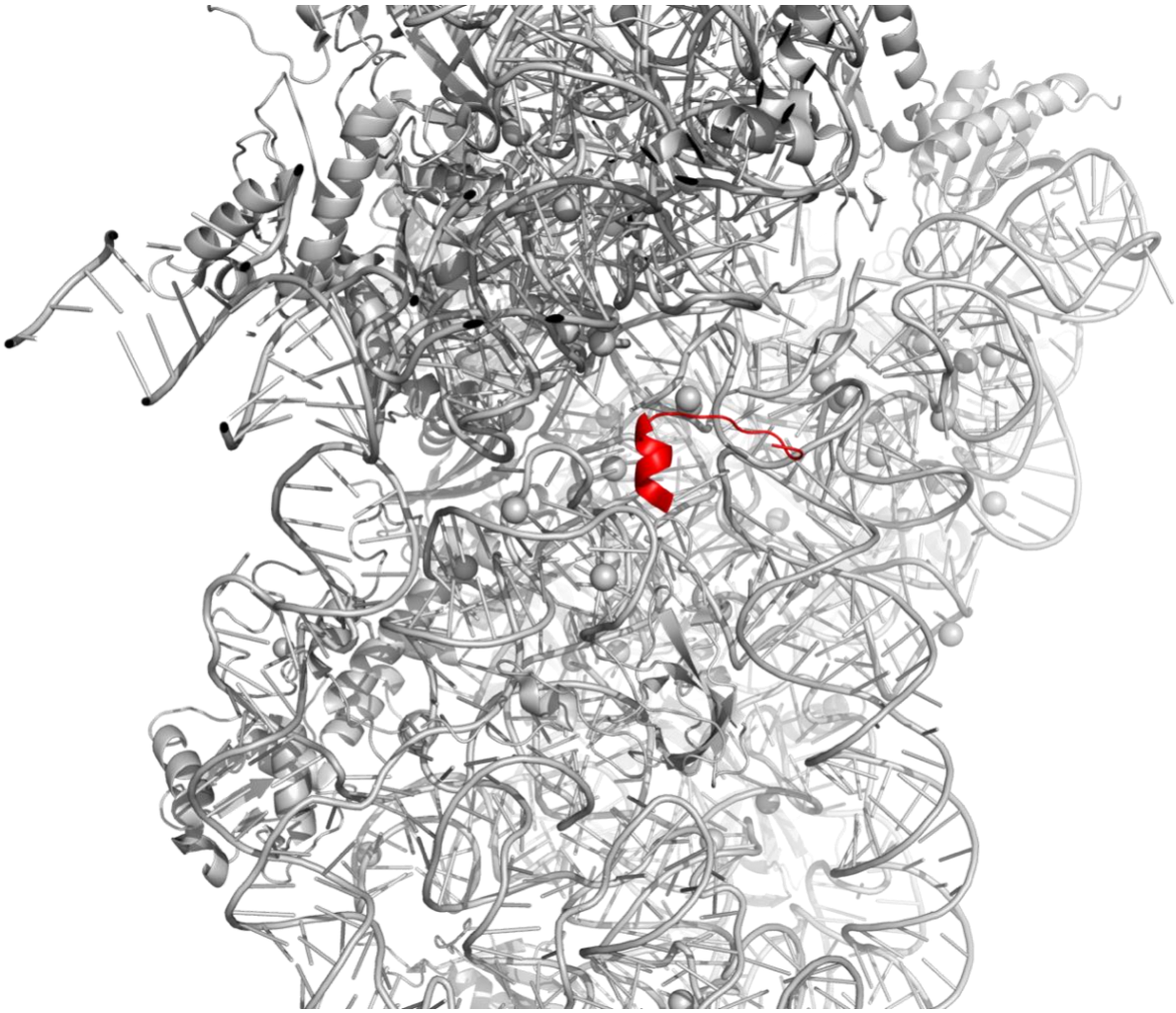

Figure 6. Predicted 30S initiation complex with Cd-IF1 Peptide (red) and the 30S subunit of *T. thermophilus* PDB ID(1HR0).

To determine its antimicrobial activity, the peptide was tested against both Gram-negative and Gram-positive bacteria. The Gram- positive bacterium that were tested were *Staphylococcus epidermidis*, *Mycobacterium Smegmatis* and *Bacillus cereus*. The peptide showed inhibitory activity against all four bacteria and had some inhibited effects against bacterial growth with the lowest concentration of 0.1875 mg/ml against *S. epidermidis*, 0.375 mg/ml against *M. smegmatis*, and the highest concentrations at 1.5 mg/ml against *E. coli*, *P. vulgaris*, and *P. aeruginosa*. The peptide was tested against ampicillin and

kanamycin which are widely used antibiotics as a sort of comparison. The peptide was discovered to have inhibitory efficacy against *S. epidermidis* which was grown in Nutrient broth and *M. smegmatis* grown in Luria Bertani (LB). It was interesting to see how the two best results obtained were from Gram- positive bacteria and therefore the IF1 structures were compared to the IF1 structure of *C. difficile*. Using NCBI Blast the IF1 sequences of both *C. difficile* and *S. epidermidis* it was found that they had 79.17% identity (Altschul, 1997). Indicating the higher the percent identity the less peptide would be needed to inhibit bacterial growth. Modification of the antimicrobial peptide may be considered to inhibit bacterial growth at lower concentrations. Further testing will be done with the α helical peptide including testing it against *C. difficile* itself. Due to different growth parameters the MIC Assay with *C. difficile* was unable to get completed.

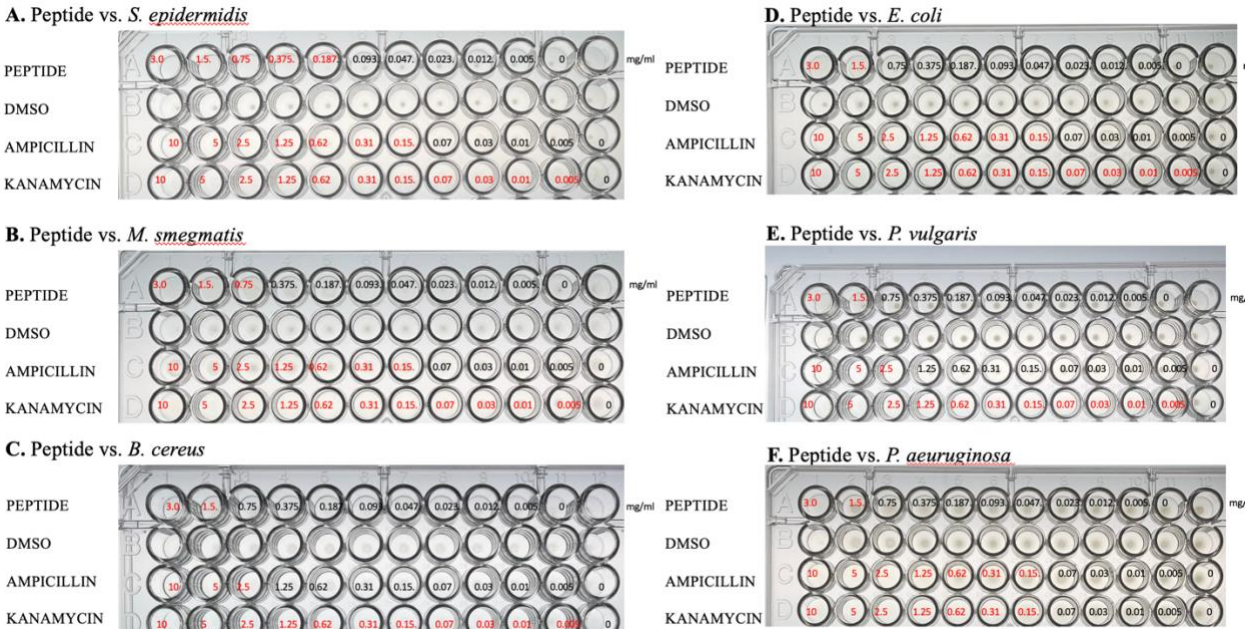

Figure 7. Minimum Inhibitory Concentration (MIC) Assays of various bacterial pathogens. Cd-IF1 peptide inhibits the growth of *S. epidermidis* (Panel A- row A) and *M. smegmatis* (Panel B- row A).

Table 1: Minimum Inhibitory Concentration (MIC) Assay Results

Species Name	**Strain**	**Media**	**Peptide Stock***	**MIC**
Staphylococcus epidermidis	ATCC 12228	Nutrient agar/broth	30 mg/ml	0.1875 mg/ml
Mycobacterium smegmatis	ATCC 14468	LB agar/broth	30 mg/ml	0.375 mg/ml
Bacillus cereus	ATCC 14579	Nutrient agar/broth	30 mg/ml	1.5 mg/ml
Escherichia coli	BL21(DE3)	LB agar/broth	30 mg/ml	1.5 mg/ml
Proteus vulgaris	Microbiology Laboratory UTRGV	LB agar/broth	30 mg/ml	1.5 mg/ml
Pseudomonas aeruginosa	ATCC 47085	LB agar/broth	30 mg/ml	1.5 mg/ml

NMR titration results of Cd-IF1 and both microRNAs (microRNA-155 and microRNA-146-a) suggest that Cd-IF1 may have an RNA binding mode. Based on the two-dimensional HSQC Spectra of Cd-IF1 and MicroRNA-155 relative intensity change, and chemical shift perturbations (Equation 1) were calculated. The key amino acid residues of Cd-IF1 that experienced the most significant perturbations in the presence of microRNA-155 were N18, F21, C33, H34, V52 and K70. These key residues indicate possible polar and hydrophobic interactions between Cd-IF1 and microRNA-155 (Figure 9). Similarly, in the NMR titration data of Cd-IF1 and microRNA-146 the key amino acid residues of Cd-IF1 that experienced the most significant perturbation in the presence of microRNA-146 were S13, A15, L39, N42, F43, R45, I46, and R69 (Figure 14). The residues indicate possible hydrophobic interactions with microRNA-146. Utilizing these key residues structural models of Cd-IF1 and microRNAs were generated via HADDOCK (Figures 9 and 13).

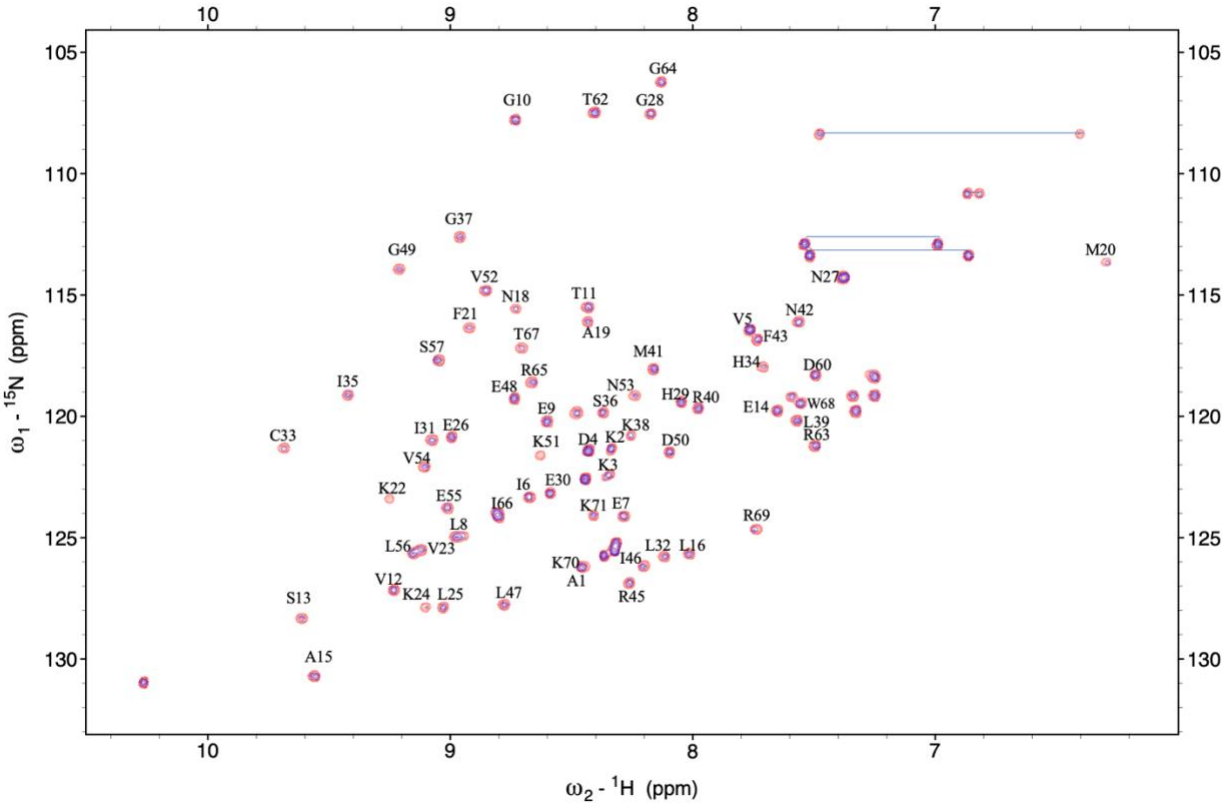

Figure 8: Two-dimensional HSQC spectra of ^{15}N-labeled Cd-IF1in the absence (red) and presence (blue) of microRNA-155.

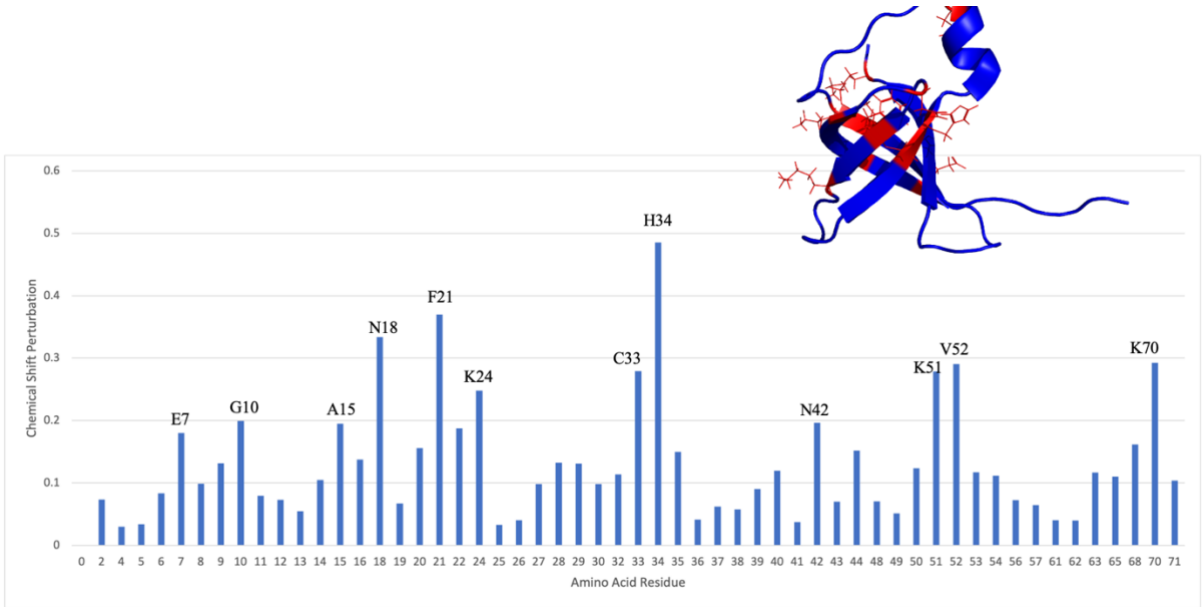

Figure 9: Chemical Shift Perturbations of Cd-IF1 and MicroRNA-155. Key residues (red) with significant perturbations.

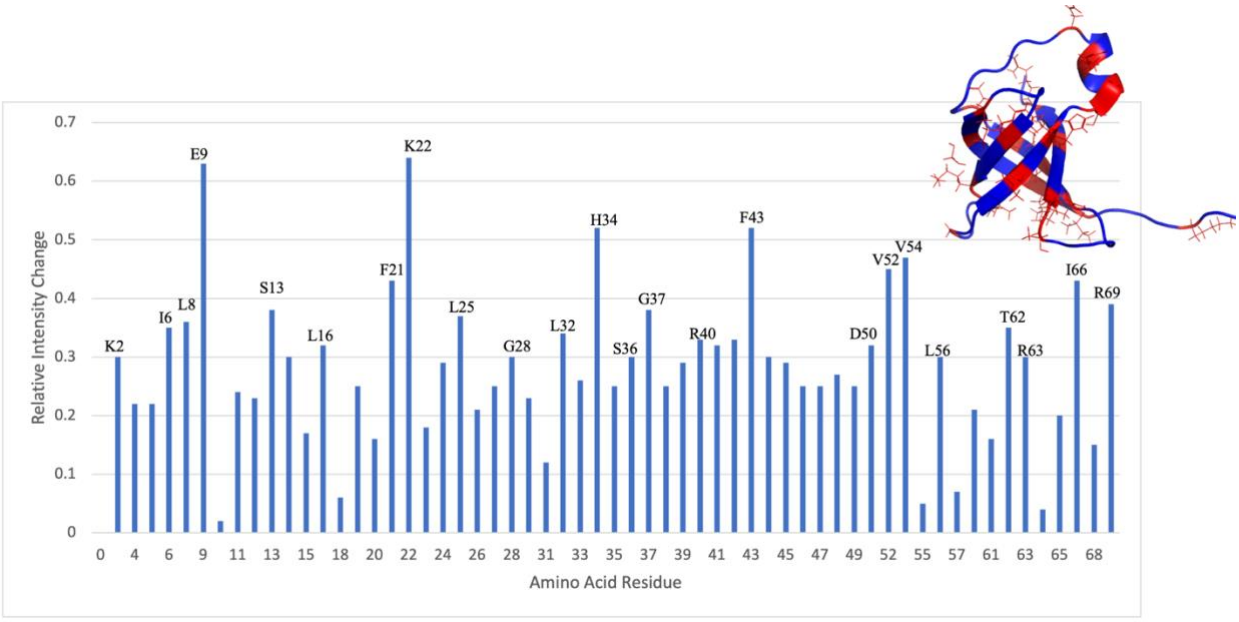

Figure 10: Relative Intensity Changes of Cd-IF1 and MicroRNA-155. Key residues (red) with significant intensity change.

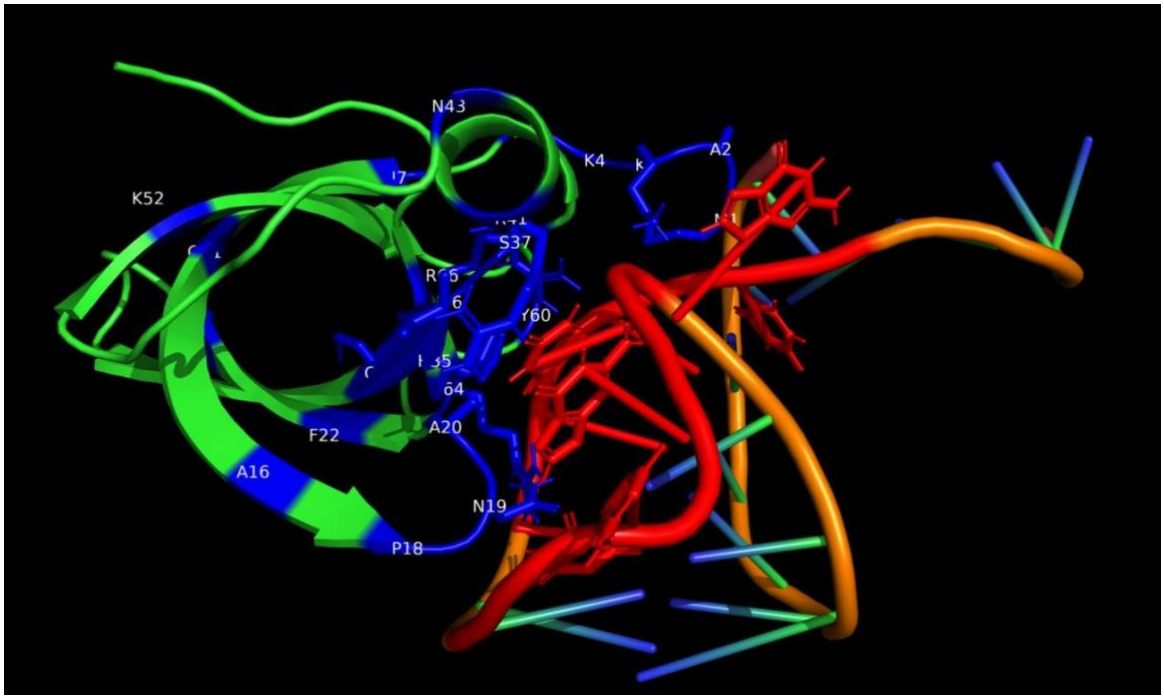

Figure 11. MicroRNA-155 and Cd-IF1 predicted binding interface. Cd-IF1 (green) with key residues (blue) and their side chains binding to MicroRNA-155 (orange, blue and green) nucleotides (red).

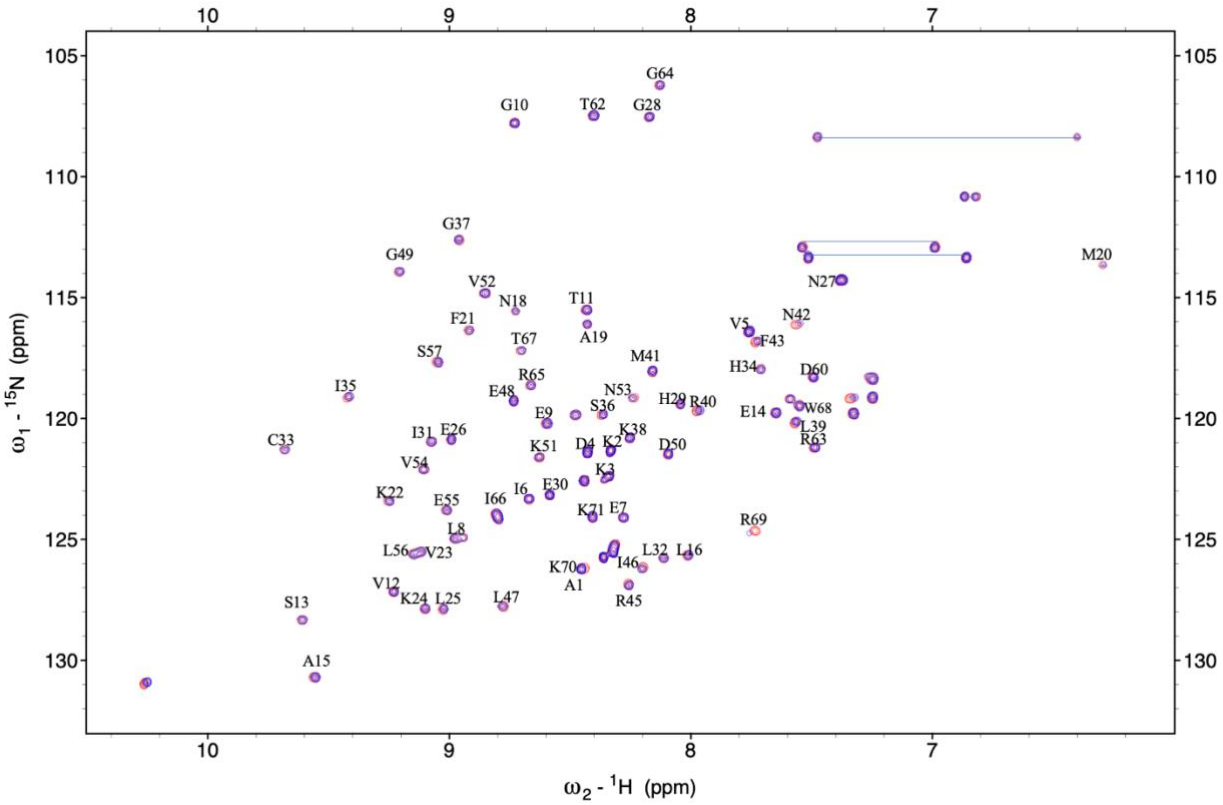

Figure 12: Two-dimensional HSQC spectra of ^{15}N-labeled Cd-IF1in the absence (blue) and presence (red) of microRNA-146-a

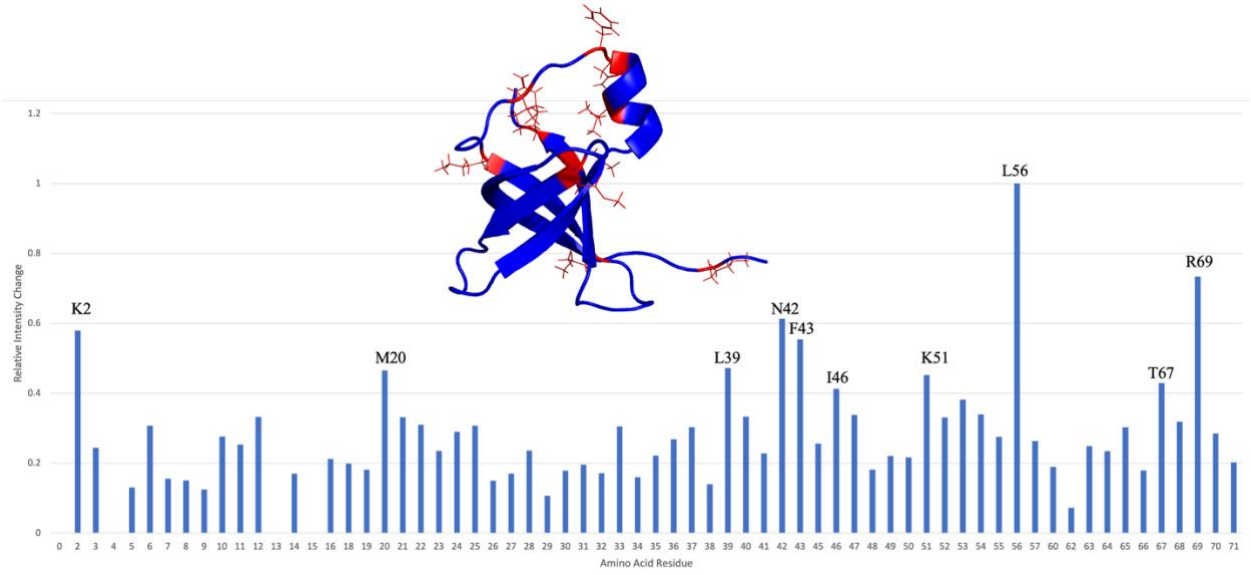

Figure 13: Relative Intensity Change of Cd-IF1 and MicroRNA-146-a. Key residues (red) with significant intensity change.

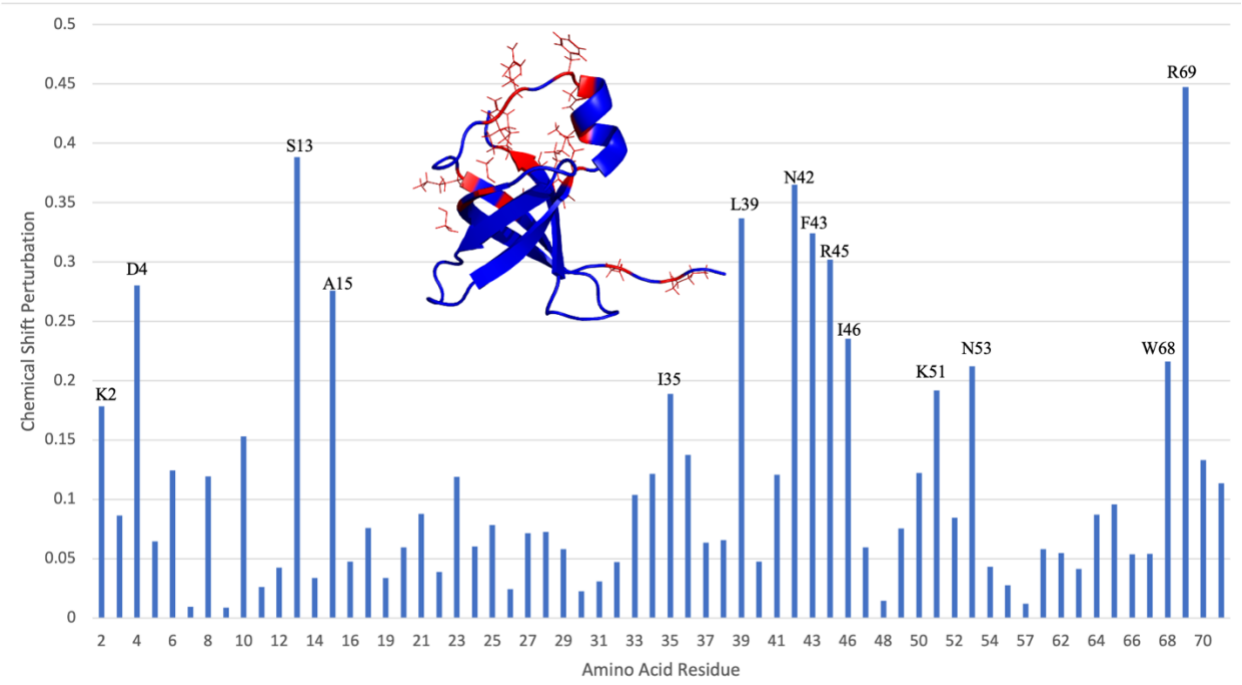

Figure 14: Chemical Shift Perturbations of Cd-IF1 and MicroRNA-146-a titration. Key residues (red) with significant perturbations

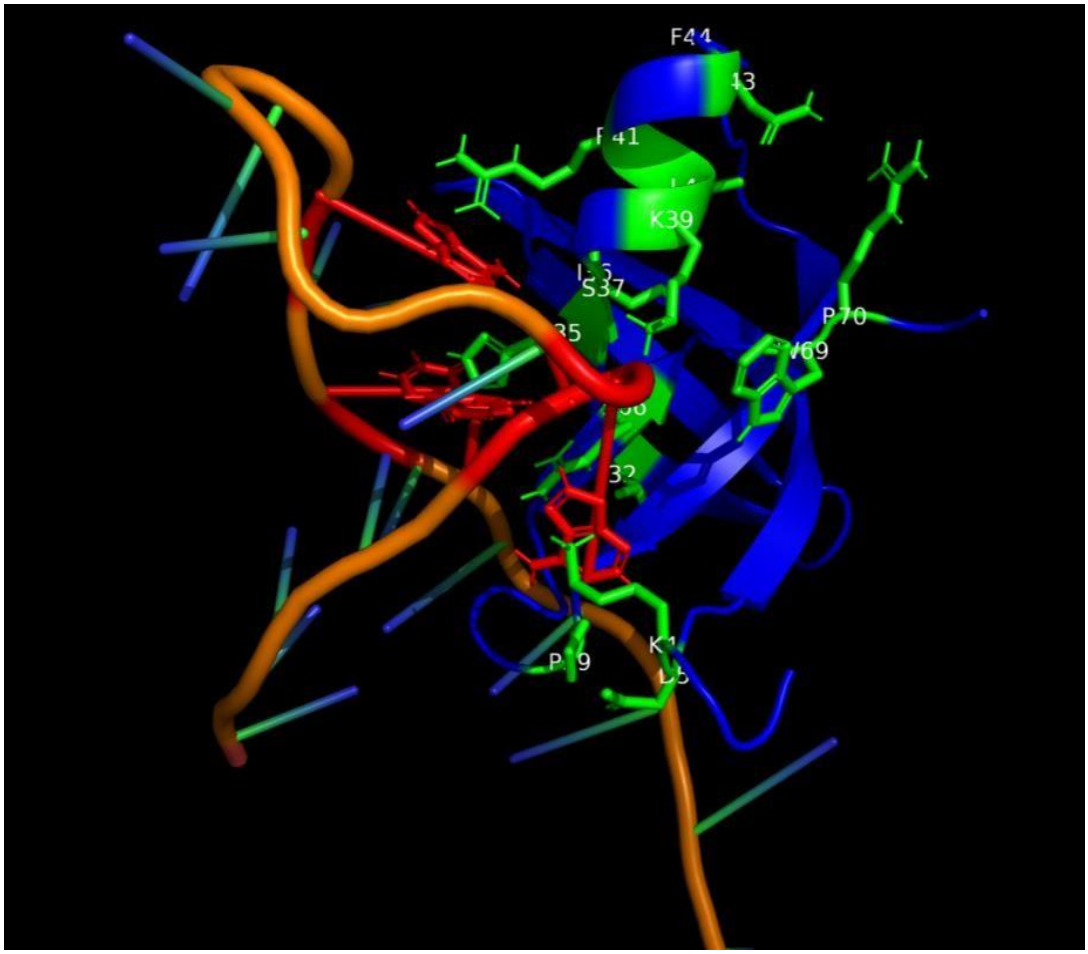

Figure 15. MicroRNA-146-a and Cd-IF1 predicted binding interface Cd-IF1 (green) with key residues (blue) and their side chains binding to MicroRNA-146-a (orange, blue and green) nucleotides (red).

Conclusion

The translation initiation factor 1 of *C. difficile* underwent a variety of structural studies to determine its interaction with target molecules. From these findings key amino acid residues that are involved in binding of Cd-IF1 to the 30S ribosomal subunit were determined. As a result, key amino acid residues were found near the α helical section of the IF1 structure. Due to the importance of this α helical structure a short peptide was derived to test against a variety of bacterial pathogens. Overall, this peptide did have some inhibitory

effects, but further modification might be needed for it to inhibit bacterial growth at smaller concentrations. Additionally, further studies may need to be done using this peptide against C. *difficile.*

From the Cd-IF1 and microRNAs NMR titrations revealed that Cd-IF1 has a suggested RNA binding mode. These results indicate that Cd-IF1 experienced binding to both microRNA-155 and microRNA-146. Further studies include using computer software's to determine binding affinities between Cd-IF1 and the two microRNAs. Additionally, identifying microRNAs that have high binding affinities to Cd-IF1. By identifying these microRNAs with higher binding affinities to Cd-IF1 may suggest that microRNAs can potentially be seen as antimicrobial candidates. Once these microRNAs have been identified they will then be tested for inhibitory effects using minimum inhibitory concentration (MIC) assays.

REFERENCES

Adams, R.A., Leon, G., Miller, N.M. et al. Rifamycin antibiotics and the mechanisms of their failure. J Antibiot 74, 786–798 (2021). https://doi.org/10.1038/s41429-021-00462-x

Aguilar F, Banaei N, Zhang Y. 1H, 13C and 15N resonance assignments and structure prediction of translation initiation factor 1 from Clostridium difficile. Biomol NMR Assign. 2019 Apr;13(1):91-95. doi: 10.1007/s12104-018-9858-8. Epub 2018 Oct 28. PMID: 3037050

Altschul, Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Antczak, M., Popenda, M., Zok, T., Sarzynska, J., Ratajczak, T., Tomczyk, K., Adamiak, R.W., Szachniuk, M. New functionality of RNAComposer: an application to shape the axis of miR160 precursor structure, Acta Biochimica Polonica, 2016, 63(4):737-744 (doi:10.18388/abp.2016_1329).

Arenz S, Wilson DN. Bacterial Protein Synthesis as a Target for Antibiotic Inhibition. Cold Spring Harb Perspect Med. 2016 Sep 1;6(9):a025361. doi: 10.1101/cshperspect.a025361. PMID: 27481773; PMCID: PMC5008061.

Brogden, K. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. Nat Rev Microbiol 3, 238–250 (2005). https://doi.org/10.1038/nrmicro1098

Burns DA, Heap JT, Minton NP. Clostridium difficile spore germination: an update. Res Microbiol. 2010 Nov;161(9):730-4. doi: 10.1016/j.resmic.2010.09.007. Epub 2010 Sep 21. PMID: 20863888.

Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. Structural mechanism for rifampicin inhibition of bacterial rna polymerase. Cell. 2001 Mar 23;104(6):901-12. doi: 10.1016/s0092-8674(01)00286-0. PMID: 11290327.

Carter AP, Clemons WM Jr, Brodersen DE, Morgan-Warren RJ, Hartsch T, Wimberly BT, Ramakrishnan V. Crystal structure of an initiation factor bound to the 30S ribosomal subunit. Science. 2001 Jan 19;291(5503):498-501. doi: 10.1126/science.1057766. PMID: 11228145.

CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.

Czepiel, J., Dróżdż, M., Pituch, H., Kuijper, E. J., Perucki, W., Mielimonka, A., Goldman, S., Wultańska, D., Garlicki, A., & Biesiada, G. (2019). Clostridium difficile infection: review. European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology, 38(7), 1211–1221.

Dahlquist KD, Puglisi JD. Interaction of translation initiation factor IF1 with the E. coli ribosomal A site. J Mol Biol. 2000 May 26;299(1):1-15. doi: 10.1006/jmbi.2000.3672. PMID: 10860719.

Davies AH, Roberts AK, Shone CC, Acharya KR. Super toxins from a super bug: structure and function of Clostridium difficile toxins. Biochem J. 2011 Jun 15;436(3):517-26. doi: 10.1042/BJ20110106. PMID: 21615333.

Di Bella, S., Ascenzi, P., Siarakas, S., Petrosillo, N., & di Masi, A. (2016). Clostridium difficile Toxins A and B: Insights into Pathogenic Properties and Extraintestinal Effects. Toxins, 8(5), 134.

Edwards, A. N., & McBride, S. M. (2014). Initiation of sporulation in Clostridium difficile: a twist on the classic model. FEMS microbiology letters, 358(2), 110–118.

Eulalio, Leon Schulte & Jörg Vogel (2012) The mammalian microRNA response to bacterial infections, RNA Biology, 9:6, 742-750, DOI: 10.4161/rna.20018

Eze, P., Balsells, E., Kyaw, M. H., & Nair, H. (2017). Risk factors for Clostridium difficile infections - an overview of the evidence base and challenges in data synthesis. Journal of global health, 7(1), 010417.

Gualerzi CO, Pon CL. Initiation of mRNA translation in bacteria: structural and dynamic aspects. Cell Mol Life Sci. 2015 Nov;72(22):4341-67. doi: 10.1007/s00018-015-2010-3. Epub 2015 Aug 11. PMID: 26259514; PMCID: PMC4611024.

Hancock RE. Peptide antibiotics. Lancet. 1997 Feb 8;349(9049):418-22. doi: 10.1016/S0140-6736(97)80051-7. PMID: 9033483.

He M, Sebaihia M, Lawley TD, Stabler RA, Dawson LF, Martin MJ, Holt KE, Seth-Smith HM, Quail MA, Rance R, Brooks K, Churcher C, Harris D, Bentley SD, Burrows C, Clark L, Corton C, Murray V, Rose G, Thurston S, van Tonder A, Walker D, Wren BW, Dougan G, Parkhill J. Evolutionary dynamics of Clostridium difficile over short and long time scales. Proc Natl Acad Sci U S A. 2010 Apr 20;107(16):7527-32. doi: 10.1073/pnas.0914322107. Epub 2010 Apr 5. PMID: 20368420; PMCID: PMC2867753.

Hooper DC. Mechanisms of action of antimicrobials: focus on fluoroquinolones. Clin Infect Dis. 2001 Mar 15;32 Suppl 1:S9-S15. doi: 10.1086/319370. PMID: 11249823

Jank T, Belyi Y, Aktories K. Bacterial glycosyltransferase toxins. Cell Microbiol. 2015 Dec;17(12):1752-65. doi: 10.1111/cmi.12533. Epub 2015 Nov 4. PMID: 26445410.

Kohanski MA, Dwyer DJ, Collins JJ. How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol. 2010 Jun;8(6):423-35. doi: 10.1038/nrmicro2333. Epub 2010 May 4. PMID: 20440275; PMCID: PMC2896384.

Kordus SL, Thomas AK, Lacy DB. Clostridioides difficile toxins: mechanisms of action and antitoxin therapeutics. Nat Rev Microbiol. 2021 Nov 26. doi: 10.1038/s41579-021-00660-2. Epub ahead of print. PMID: 34837014.

Laursen, B. S., Sørensen, H. P., Mortensen, K. K., & Sperling-Petersen, H. U. (2005). Initiation of protein synthesis in bacteria. Microbiology and molecular biology reviews: MMBR, 69(1), 101–123.

Lawson PA, Citron DM, Tyrrell KL, Finegold SM. Reclassification of Clostridium difficile as Clostridioides difficile (Hall and O'Toole 1935) Prévot 1938. Anaerobe. 2016 Aug;40:95-9. doi: 10.1016/j.anaerobe.2016.06.008. Epub 2016 Jun 28. PMID: 27370902.

León-Buitimea A, Garza-Cárdenas CR, Garza-Cervantes JA, Lerma-Escalera JA, Morones-Ramírez JR. The Demand for New Antibiotics: Antimicrobial Peptides, Nanoparticles, and Combinatorial Therapies as Future Strategies in Antibacterial Agent Design. Front Microbiol. 2020 Jul 24;11:1669. doi: 10.3389/fmicb.2020.01669. PMID: 32793156; PMCID: PMC7393301.

Liu Y, Shi J, Tong Z, Jia Y, Yang B, Wang Z. The revitalization of antimicrobial peptides in the resistance era. Pharmacol Res. 2021 Jan;163:105276. doi: 10.1016/j.phrs.2020.105276. Epub 2020 Nov 5. PMID: 33161137.

Marzi S, Knight W, Brandi L, Caserta E, Soboleva N, Hill WE, Gualerzi CO, Lodmell JS. Ribosomal localization of translation initiation factor IF2. RNA. 2003 Aug;9(8):958-69. doi: 10.1261/rna.2116303. PMID: 12869707; PMCID: PMC1370462.

McCoy LS, Xie Y, Tor Y. Antibiotics that target protein synthesis. Wiley Interdiscip Rev RNA. 2011 Mar-Apr;2(2):209-32. doi: 10.1002/wrna.60. Epub 2010 Nov 22. PMID: 21957007.

Monegro AF, Muppidi V, Regunath H. Hospital Acquired Infections. [Updated 2021 Aug 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441857/

Mookherjee, N., Anderson, M.A., Haagsman, H.P. et al. Antimicrobial host defence peptides: functions and clinical potential. Nat Rev Drug Discov 19, 311–332 (2020). https://doi.org/10.1038/s41573-019-0058-8

Mourenza, Á., Lorente-Torres, B., Durante, E., Llano-Verdeja, J., Aparicio, J. F., Fernández-López, A., Gil, J. A., Mateos, L. M., & Letek, M. (2022). Understanding microRNAs in the Context of Infection to Find New Treatments against Human Bacterial Pathogens. Antibiotics (Basel, Switzerland), 11(3), 356. https://doi.org/10.3390/antibiotics11030356

Mwangi J, Hao X, Lai R, Zhang ZY. Antimicrobial peptides: new hope in the war against multidrug resistance. Zool Res. 2019 Nov 18;40(6):488-505. doi: 10.24272/j.issn.2095-8137.2019.062. PMID: 31592585; PMCID: PMC6822926.

Nuding S, Frasch T, Schaller M, Stange EF, Zabel LT. Synergistic effects of antimicrobial peptides and antibiotics against Clostridium difficile. Antimicrob Agents Chemother. 2014 Oct;58(10):5719-25. doi: 10.1128/AAC.02542-14. Epub 2014 Jul 14. PMID: 25022581; PMCID: PMC4187972.

Ofosu A. Clostridium difficile infection: a review of current and emerging therapies. Ann Gastroenterol. 2016 Apr-Jun;29(2):147-54. doi: 10.20524/aog.2016.0006. PMID: 27065726; PMCID: PMC4805733.

Pham TDM, Ziora ZM, Blaskovich MAT. Quinolone antibiotics. Medchemcomm. 2019 Jun 28;10(10):1719-1739. doi: 10.1039/c9md00120d. PMID: 31803393; PMCID: PMC6836748.

Popenda, M., Szachniuk, M., Antczak, M., Purzycka, K.J., Lukasiak, P., Bartol, N., Blazewicz, J., Adamiak, R.W. Automated 3D structure composition for large RNAs, Nucleic Acids Research, 2012, 40(14):e112 (doi:10.1093/nar/gks339).

Rončević T, Puizina J, Tossi A. Antimicrobial Peptides as Anti-Infective Agents in Pre-Post-Antibiotic Era? International Journal of Molecular Sciences. 2019; 20(22):5713. https://doi.org/10.3390/ijms20225713

Trifan, A., Stanciu, C., Girleanu, I., Stoica, O. C., Singeap, A. M., Maxim, R., Chiriac, S. A., Ciobica, A., & Boiculese, L. (2017). Proton pump inhibitors therapy and risk of Clostridium difficile infection: Systematic review and meta-analysis. World journal of gastroenterology, 23(35), 6500–6515.

Valdez N, Hughes C, Palmer SO, Sepulveda A, Dean FB, Escamilla Y, et al. Rational design of an antimicrobial peptide based on structural insight into the interaction of pseudomonas aeruginosa initiation factor 1 with its cognate 30S ribosomal subunit. ACS Infectious Diseases. 2021;7(12):3161–7.

Vedantam G, Clark A, Chu M, McQuade R, Mallozzi M, Viswanathan VK. Clostridium difficile infection: toxins and non-toxin virulence factors, and their contributions to disease establishment and host response. Gut Microbes. 2012 Mar-Apr;3(2):121-34. doi: 10.4161/gmic.19399. Epub 2012 Mar 1. PMID: 22555464; PMCID: PMC3370945

Vollmer, Didier Blanot, Miguel A. De Pedro, Peptidoglycan structure and architecture, FEMS Microbiology Reviews, Volume 32, Issue 2, March 2008, Pages 149–167, https://doi.org/10.1111/j.1574-6976.2007.00094.x

Voorhees RM, Ramakrishnan V. Structural basis of the translational elongation cycle. Annu Rev Biochem. 2013;82:203-36. doi: 10.1146/annurev-biochem-113009-092313. PMID: 23746255

Voth DE, Ballard JD. Clostridium difficile toxins: mechanism of action and role in disease. Clin Microbiol Rev. 2005 Apr;18(2):247-63. doi: 10.1128/CMR.18.2.247-263.2005. PMID: 15831824; PMCID: PMC1082799.

Wang H, Zhao Y. RBinds: A user-friendly server for RNA binding site prediction. Comput Struct Biotechnol J. 2020 Nov 24;18:3762-3765. doi: 10.1016/j.csbj.2020.10.043. PMID: 34136090; PMCID: PMC8164131.

Williamson M.P. (2018) Chemical Shift Perturbation. In: Webb G. (eds) Modern Magnetic Resonance. Springer, Cham. https://doi.org/10.1007/978-3-319-28388-3_76

Zharkova MS, Orlov DS, Golubeva OY, Chakchir OB, Eliseev IE, Grinchuk TM, Shamova OV. Application of Antimicrobial Peptides of the Innate Immune System in Combination With Conventional Antibiotics-A Novel Way to Combat Antibiotic Resistance? Front Cell Infect Microbiol. 2019 Apr 30;9:128. doi: 10.3389/fcimb.2019.00128. PMID: 31114762; PMCID: PMC6503114.

Zhang LJ, Gallo RL. Antimicrobial peptides. Curr Biol. 2016 Jan 11;26(1):R14-9. doi: 10.1016/j.cub.2015.11.017. PMID: 26766224.

Zhou, X., Li, X. & Wu, M. miRNAs reshape immunity and inflammatory responses in bacterial infection. Sig Transduct Target Ther 3, 14 (2018). https://doi.org/10.1038/s41392-018-0006-9

BIOGRAPHICAL SKETCH

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