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SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

IDENTIFICATION OF INHIBITORS OF THE σ^E/HFQ PATHWAY IN *E. COLI*

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ABSTRACT

Antibiotic resistance has rapidly emerged and spread, which has rendered many common antibiotics ineffective against several diseases. New antibiotics and combination therapies are necessary to combat this ever-growing problem. One potential novel target for new drugs is the σ^E factor in *E. coli*. σ^E is involved in a stress response pathway important for the viability of *E. coli* and the pathogenicity of other species of bacteria. Inhibiting σ^E prevents cells from properly repairing the outer membrane proteins, causing cells to lyse. In order to discover new compounds to inhibit this factor, a high-throughput screening of chemical libraries was completed. The screening uses a luciferase-based assay, which allowed for inhibitors of the σ^E/Hfq pathway to cause bacterial cells to luminesce. In order to find σ^E-specific inhibitors, secondary assays were performed to characterize inhibitors as inhibiting σ^E or Hfq, an RNA chaperone that can pair sRNAs transcribed by σ^E to their mRNA target. The σ^E-specific assay uses the fluorescence produced by transcription of a fluorescent protein under the control of a σ^E-dependent reporter. KKL-17131 is a promising inhibitor of σ^E identified through the initial high-throughput screening. It was further specified as a σ^E-specific inhibitor through the results obtained with the σ^E-specific screen and through the presence of an MIC.

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Chapter 1

Introduction

1.1 – Antibiotic Resistance Crisis

Antibiotic resistance is a global crisis that has slowly grown since the discovery of antibiotics. The development of antibiotics led to a revolution in the treatment of bacterial infections that previously were often left untreated. Many of the antibiotics that have been developed since their discovery have been derived from natural products from bacteria, fungi, plants, etc. However, many of these once-revolutionary antibiotics have been rendered ineffective due to the emergence of resistance strains of bacteria.

The emergence of resistant strains occurs naturally as a response to natural selection and competition against other organisms present in their environment. However, the overuse and misuse of antibiotics from the time of discovery and implementation into healthcare has exacerbated this problem. This misuse stems from insufficient evidence of a bacterial infection before a physician prescribes an antibiotic to treat a patient's infection. Commonly, this lack of evidence leads to antibiotic prescription to treat a viral infection (Ventola, 2015). Other sources of antibiotic resistance are from the extensive use of these drugs in agriculture. Antibiotics are added to livestock feed, which can transfer to humans during consumption or to the environment through water sources when used on produce (Ventola, 2015). Without the ability to develop antibiotics to match the rate of resistance development, the crisis continues to worsen.

The Centers for Disease Control and Prevention has determined several bacteria that pose a more severe threat to public safety due to their resistance profile (ANTIBIOTIC RESISTANCE THREATS in the United States, 2013). One of the most prominent resistant strains, especially in hospital settings, is methicillin-resistant *Staphylococcus aureus* (MRSA) (Ventola, 2015). Other bacteria of concern with dangerous levels of resistance include *Mycobacterium tuberculosis, Streptococcus pneumoniae, Pseudomonas aeruginosa, Escherichia coli,* and *Neisseria gonorrhoeae* (Ventola, 2015). These bacteria make up the majority of problematic strains due to single or multiple drug resistance, however other species have also developed resistant strains. These resistance infections have created an economic burden in the United States, totaling up to $20 million in 2015 for health care costs of patients with these infections (Ventola, 2015). With this crisis of antibiotic resistance and minimal number of effective antibiotics, there is urgency to develop antibiotics with novel targets. One such novel target is an alternative sigma factor in *E. coli*, σ^E.

1.2 – σ^E Pathway in *Escherichia coli*

σ^E is one of several alternative sigma factors present in *E. coli* and is specifically involved in cell envelope maintenance and cell stress response pathways (Nicoloff et al., 2017). This sigma factor is also necessary for cell survival in *E. coli* and plays a role in pathogenicity of other bacterial strains (Kazmierczak et al., 2005). Due to both the absence of sigma factors in humans and the presence and role of σ^E in *E. coli* and other bacterial strains, σ^E is a favorable novel target for antibiotic development.

σ^E is encoded by the *rpoE* gene in *E. coli* and is also referred to as RpoE or σ^{24} (Raina et al., 1995). σ^E becomes activated when the folding of outer membrane proteins is disrupted and RseA, an inner membrane antisigma factor protein, is degraded as a result of a signaling pathway triggered by envelope stress (Ades, 2008). RseA sequesters σ^E and prevents its binding to RNA polymerase (Ades, 2008). RseA is degraded by inner membrane proteases and a cytoplasmic protease (Ades, 2008). DegS, one of the inner membrane proteases, cuts RseA, which releases the periplasmic domain (Ades, 2008). The other inner membrane protease, RseP, also cleaves RseA to release the cytoplasmic domain still attached σ^E (Ades, 2008). This cleavage allows this cytoplasmic domain to be degraded by ClpXP, a cytoplasmic protease (Ades, 2008). The degradation of RseA releases σ^E and allows it to interact with RNA polymerase and transcribe genes encoding proteases and chaperones that are involved in outer membrane protein folding and other cell envelope proteins (Hayden and Ades, 2008)(Figure 1). Some of the small RNAs that are transcribed by σ^E function to reduce the expression of outer membrane porins (OMPs), which become misfolded and accumulate during cell stress (Hayden and Ades, 2008). Other genes transcribed by σ^E act to degrade or refold these misfolded OMPs in addition to the reduced expression of these proteins (Ades, 2004). These all function to maintain the viability of the cell envelope both in the presence and absence of stress that may be due to antibiotics or environmental changes. σ^E is necessary for *E. coli* cell survival with loss resulting in inability of cells to live, but it also can be lethal to the cell when there is overexpression of σ^E (Nicoloff et al., 2017).

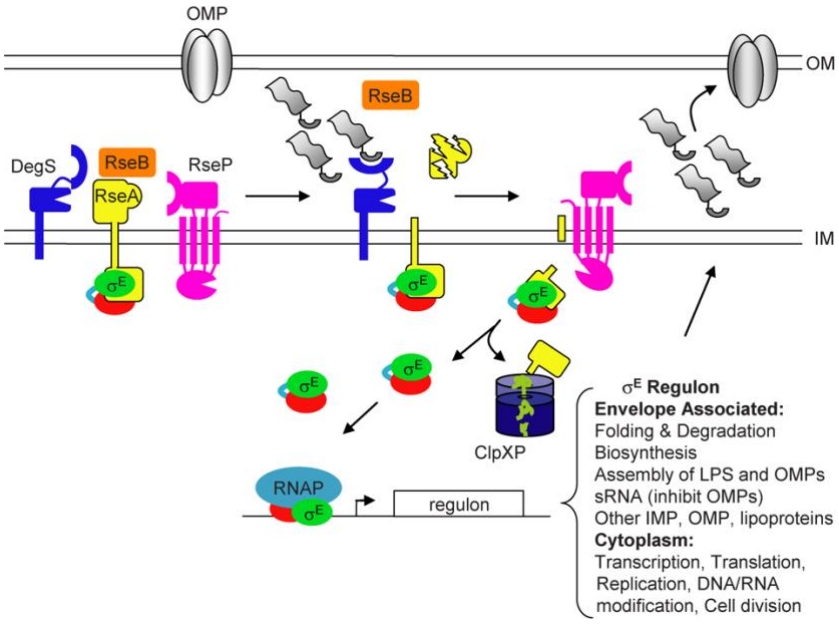

Figure 1. σ^E Pathway in *E. coli*. This demonstrates the pathway in which σ^E becomes activated through a cascade triggered by misfolded or unfolded outer membrane proteins in the cell. These OMPs bind and activate DegS leading to cleavage of the periplasmic RseA domain, the anti-sigma factor of σ^E. RseP cleaves the cytoplasmic domain of RseA allowing for ClpXP to degrade this domain leading to the release of σ^E. σ^E binds core polymerase to transcribe the σ^E regulon. This regulon encodes for proteins that aid in cell envelope repair and maintenance. [Figure taken from Hayden, Ades (2008)(Hayden and Ades, 2008)]

1.3 – Role of Hfq in σ^E Pathway in *E. coli*

A secondary protein involved in the σ^E pathway is the Hfq protein. This protein acts as an RNA chaperone by facilitating the pairing of an sRNA with its target mRNA(De Lay et al., 2013). When the sRNA is paired with its target, there are multiple possible outcomes that may occur for the mRNA. Some of these possibilities have inhibitory effects while other can lead to activation of other molecules. One method an sRNA prevents translation of its target mRNA is through association at the ribosome-binding site (De Lay et al., 2013). This can lead to gene regulation through blocking ribosome binding and subsequently blocking translation. This can target the mRNA for degradation. This pathway is involved with the σ^E-pathway in *E. coli* due σ^E-regulated

transcription of sRNAs that can be identified by Hfq (El-Mowafi et al., 2015). These sRNAs are then bound by Hfq and paired with a target mRNA (De Lay et al., 2013). For this pathway, it often results in the prevention of translation of the mRNA by blocking the binding of the 30S ribosomal subunit and degradation of the mRNA by RNaseE cleavage (Figure 2) (El-Mowafi et al., 2014; De Lay et al., 2013).

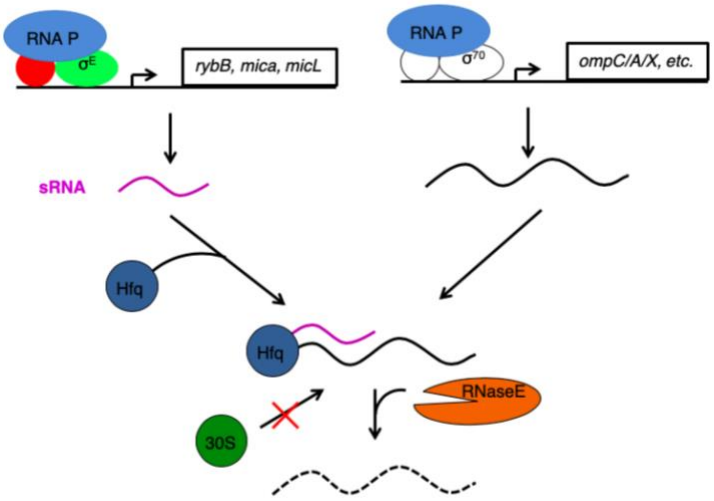

Figure 2. Hfq Protein Mechanism of Action in *E. coli.* σ^E-dependent transcription of the *rybB* gene generates the *RybB* sRNA that is recognized by Hfq, along with other sRNAs. Hfq pairs this sRNA with its target mRNA, which blocks the 30s subunit of the ribosome from binding to this mRNA. This inhibits translation of the target mRNA, which becomes degraded by RNaseE [Figure adapted from (El-Mowafi et al., 2015, 2014)].

1.4 – Known Inhibitors of the σ^E Pathway

There are several compounds that have been characterized as inhibitors of σ^E or other molecules involved in this pathway. Some of these compounds were discovered using a high-throughput screen of a cyclic peptide library (El-Mowafi et al., 2015). The SI24 cyclic peptide was determined to be the best inhibitor of the σ^E-pathway from this library through inhibition of RNA

polymerase holoenzyme formation and thus transcription *in vitro* (El-Mowafi et al., 2015). However, this cyclic peptide was unable to inhibit σ^E activity *in vivo* when added exogenously to cells, so it would not make a viable drug due to the absence of activity in living cells (El-Mowafi et al., 2015). The inability of SI24 to enter *E. coli* cells would suggest that the use of cyclic peptides as inhibitors of the σ^E-pathway is not a feasible class for drug development.

Another inhibitor of the σ^E-pathway that has recently been characterized is Batimastat, which was shown to inhibit RseP (Konovalova et al., 2018). This compound was discovered from a library of small molecules screened for inhibition of the σ^E-pathway (Konovalova et al., 2018). Batimastat inhibition of RseP leads to reduction of σ^E activity due to the role RseP plays in cleaving the anti-sigma factor, RseA (Konovalova et al., 2018). This compound shows promise for future drug development but does not inhibit σ^E directly. The success of this small molecule inhibitor provides evidence of potential success of other small molecule compounds to enter the cell and inhibit σ^E.

Due to the lack of success of the cyclic peptide inhibitors for cell entry, small molecules were determined to be a potentially success class for inhibitors of σ^E. Due to the small size of these molecules, there is a greater chance for successful entry into a living cell. With this in mind, a library of small molecules was obtained from Calibr to screen for inhibitors of the σ^E-Hfq pathway in *E. coli*.

1.5 – σ^E/Hfq Inhibitor Screening Method

The primary screening assay utilized was designed as a screen to select for small molecule inhibitors of the σ^E/Hfq pathway (Figure 3). This screen was adapted from a high-throughput

screening assay developed for discovering inhibitors of the σ^E/Hfq pathway from a cyclic peptide library (El-Mowafi et al., 2015). The basis of the screen to detect inhibitors of both σ^E and Hfq is due to interconnection of the two proteins in their pathways in *E. coli*, and it allows for a broader range for potential antibiotic development. The assay was also developed to follow the naturally occurring σ^E-pathway in *E. coli* cells. It incorporates the σ^E-directed transcription of the *rybB* gene to produce the *RybB* sRNA along with Hfq blocking translation of the *RybB* target mRNA leading to degradation. Following the naturally occurring stress pathway in cells allows for a more realistic and thorough screen for inhibitors of the pathway.

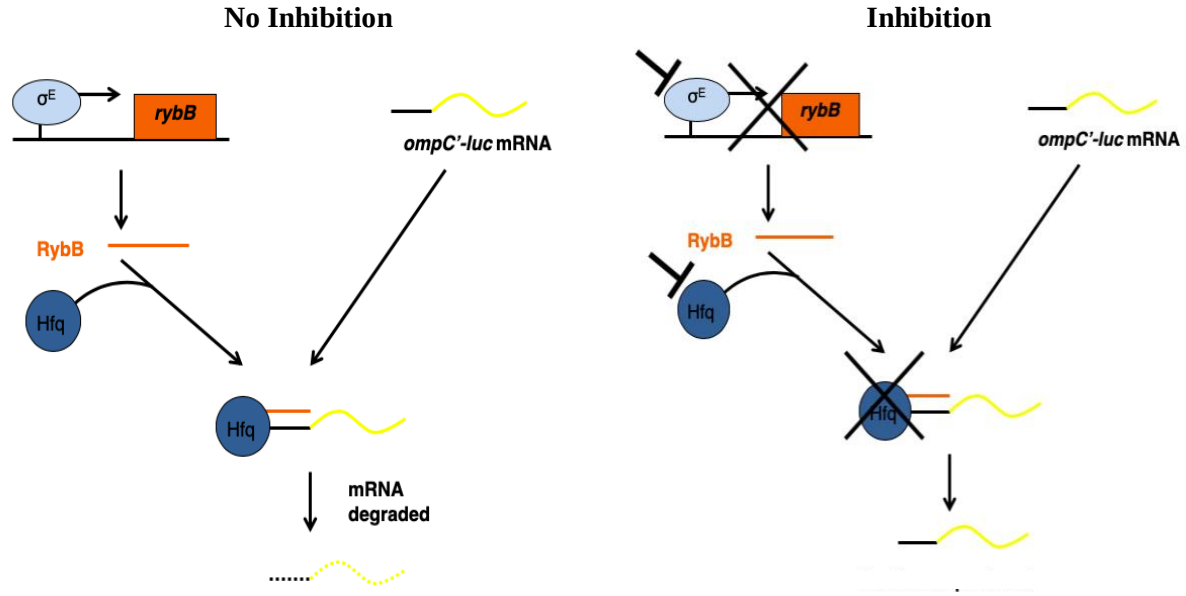

Figure 3. Model of mechanism of σ^E/Hfq Small Molecular Inhibitor Screening Assay. In the absence of an inhibitor, σ^E-dependent transcription of *rybB* occurs and leads to pairing of the *RybB* sRNA to the *ompC'-luc* mRNA by Hfq. This blocks translation and the mRNA is degraded leading to no luciferase production. In the presence of an inhibitor, either *rybB* transcription can be blocked by σ^E inhibition or *RybB* pairing with the *ompC'-luc* mRNA by Hfq inhibition. This allows for the translation of the *ompC'-luc* mRNA leading to luciferase production.

The next step in the screening process was to determine the specific target of the compounds determined as hits from this primary assay. The goal was to determine if the hits are either σ^E-specific inhibitor or Hfq-specific inhibitors. This leads into a set of secondary assays that select for either σ^E or Hfq-specific inhibitors. Testing the hits in both secondary screening assays not only helps to determine the target of the compound, but also allows for the determination of hits that may not specifically inhibit σ^E or Hfq. Compounds that show activity in both assays would not be ideal for future drug development due to the presence of unknown targets. However, a compound that shows activity in only one assay can be characterized as specific inhibitor.

The σ^E-specific secondary assay uses a σ^E-inducible promoter of a fluorescent protein in order to measure σ^E activity (Figure 4). By controlling σ^E expression and thus the expression of a fluorescent protein on a plasmid, higher levels of σ^E expression and activity can be obtained. This allows for a greater range for the measurement of σ^E activity and subsequently inhibition. Inhibition of σ^E is characterized by reduction in fluorescence due to the inability of σ^E to transcribe the *gfp* gene and produce the fluorescent protein.

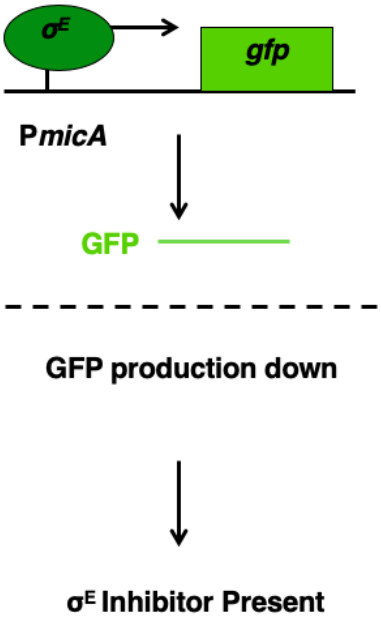

Figure 4. Model of inhibition by small molecule σ^E-specific inhibitor in Fluorescence Inhibition Assay. *rpoE* is present on a plasmid with σ^E expression induced by IPTG. The gene encoding green fluorescent protein (GFP) is controlled by a σ^E-dependent promoter. The inhibition of σ^E leads to a decrease in GFP expression and less measurable fluorescence.

To determine hits as Hfq-specific inhibitors, a Hfq/sRNA secondary screening assay uses an approach independent of sRNAs transcribed by σ^E. Transcription of both the *RybB* sRNA and *ompC'-luc* mRNA are dependent on σ^{70} alone (Figure 5). This *ompC'-luc* mRNA functions in a similar manner to the primary screening assay. The absence of an inhibitor allows for pairing of the *RybB* sRNA with the target *ompC'-luc* mRNA by Hfq, ultimately resulting in the degradation of the mRNA. However, a Hfq-specific inhibitor prevents this pairing leading to the translation of the mRNA. This will lead to increased luminescence through the production of luciferase, which oxidizes it substrate to release light (Figure 5). This assay is currently being reoptimized for use for the compounds tested in this project.

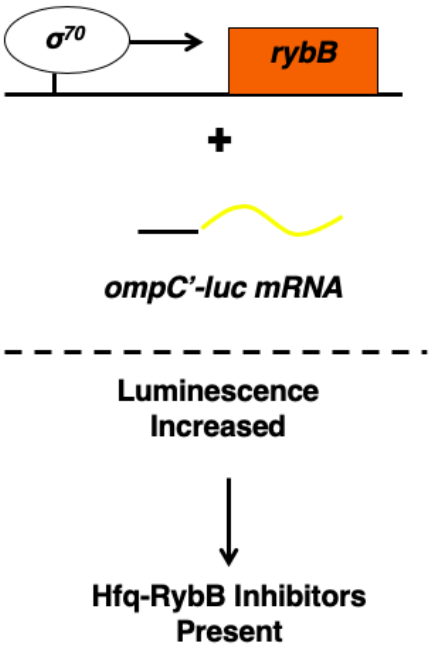

Figure 5. Hfq/sRNA secondary screening assay. *rybB* transcription is dependent on σ^{70}, and Hfq facilitates the pairing of this sRNA with the *ompC'-luc* mRNA target. Upon this pairing, Hfq and the sRNA block the ribosomal binding site of the *ompC'-luc* mRNA. This prevents translation of the mRNA, and the mRNA is degraded. An inhibitor of Hfq prevents pairing of the

RybB sRNA with the *ompC'-luc* mRNA, so the mRNA is translated. This produces luciferase, which oxidizes the luciferin substrate releasing light via luminescence.

After testing with the secondary screening assays, other experiments may be performed for better target validation and drug property characterization of the hits from the primary and secondary screening assays. Multiple growth assays such as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and MIC checkerboard characterize the growth inhibition properties of the compound both alone and with other antibiotics. The presence of an MIC is important for σ^E-specific inhibitors due to the essentiality of σ^E to the viability of *E. coli* (Kazmierczak et al., 2005). With the role of σ^E in maintaining cell envelope integrity, assays that induce cell envelope stress would also lead to better characterization of σ^E-specific inhibitors (Nicoloff et al., 2017).

Although the Hfq-specific inhibitors may be characterized with the same growth inhibition assays, an MIC would not be expected because Hfq is not essential in *E. coli* (Niba et al., 2007). Instead, testing the co-antibiotic property of Hfq and common antibiotics with an MIC checkerboard would be more applicable. In addition, characterizing these compounds with a biofilm production assay would lead to greater target validation. Hfq has been shown to play a critical role in the biofilm formation of *E. coli* cells (Hu et al., 2010). In order to determine if a compound inhibits Hfq function, the effect on biofilm formation may be tested. This assay compares a potential inhibitor's effect on biofilm formation to a strain with a deletion of *hfq* from the genome, which is not lethal to the cells (Niba et al., 2007). Comparison of biofilm formation by the wild type strain with the compound to the Δ*hfq* strain is indicative of the level of inhibition

of Hfq. These post-secondary screening assays are a method of further validating the target of the compounds along with understanding the drug properties of them as well.

The goal of my research project was to identify small molecule inhibitors of the σ^E/Hfq pathway in *E. coli.* We were able to identify 5 hits from the primary screening assay as potential inhibitors of the σ^E/Hfq pathway after screening the 13,000 compound library from Calibr. One of these hits is KKL-17131, which I have determined to be a promising inhibitor of σ^E from performing the secondary σ^E-specific assay along with growth inhibition assays. In addition to KKL-17131, KKL-2170 is another promising inhibitor of σ^E. This compound was previously characterized as a σ^E inhibitor from a high-throughput screening of a chemical library from GSK.

Chapter 2

Materials and Methods

2.1 – Materials

Lysogeny Broth (LB) media consisted of 5 g/L NaCl, 5 g/L yeast extract, and 10 g/L tryptone. TEKnova MOPS EZ Rich Defined Media was made according to manufacturer's instructions. Kanamycin working concentration used was 20 μg/mL, and ampicillin working concentration was 100 μg/mL.

2.2 – Strains

Table 1. List of Strains

Strain	Description	Genotype
CAG45113	Wild Type	WT MG1655
SEA216	Wild Type with TolC deletion	MG1655 Δ*tolC*
SEA212	Wild Type with RpoE suppressor and TolC deletion	MG1655 Δ*ydcQ* Δ*tolC*
SEA222	Wild Type with RpoE suppressor mutation	MC1061 *prlF1*
SEA223	Wild Type with RpoE suppressor mutation and TolC deletion	MC1061 *prlF1* Δ*tolC*
SEA281	σ^E-Hfq Luciferase Screening Strain	MC1061 *prlF1* p*ompC-luc*, p*rpoE-rybB*
SEA282	σ^E-Hfq Luciferase Control Strain	MC1061 *prlF1* p*ompC-luc*, p*Trc99a*
SAE051	σ^E Fluorescence Screening Strain	MG1655 Δ*ydcQ*, p*SRE*, p*LC245*
SAE052	σ^E Fluorescence Control Strain	MG1655 Δ*ydcQ*, p*SRE*, p*Trc99a*
LMW001	σ^E Fluorescence Screening Strain	MG1655 Δ*ydcQ*, Δ*tolC*, p*SRE*, p*LC245*
LMW002	σ^E Fluorescence Control Strain	MG1655 Δ*ydcQ*, Δ*tolC*, p*SRE*, p*Trc99a*
SEA761	Hfq deletion strain	MG1655 Δ*hfq*
SEA286	Potential σ^E Fluorescence Screening Strain	MC1061 *prlF1* HK022 p*micA*-cfp
SEA287	Potential σ^E Fluorescence Control Strain	MC1061 *prlF1* HK022 p*micA*-cfp Δ*rseA*
SEA288	Potential σ^E Fluorescence Screening Strain	MC1061 *prlF1* p*micA*-yfp
SEA289	Potential σ^E Fluorescence Control Strain	MC1061 *prlF1* p*micA*-yfp Δ*rseA*
SEA5171	Potential σ^E Fluorescence Screening Strain	MG1655 Δ*ydcQ* HKp*micA*-cfp
SEA5174	Potential σ^E Fluorescence Control Strain	MG1655 Δ*ydcQ* HKp*micA*-cfp Δ*rseA*

2.3 – Plasmid Purification and Transformation

2.3.1 – Bacterial Plasmid Purification

The *E. coli* strain containing the plasmid of interest was grown in 3 mL of LB with appropriate antibiotics for selected plasmid for 16-18 hours at 30℃ or 37℃ in a roller drum. The bacterial culture was pelleted by centrifugation at 8000 rpm for 3 minutes at room temperature. Plasmids were isolated with the QIAprep® Spin Miniprep Kit following manufacturing instructions.

2.3.2 – Making Chemically Competent Cells

The desired *E. coli* strain to transform was inoculated in 3 mL of LB and grown for 16-18 hours at 30℃ or 37℃ in a roller drum. The bacterial culture was diluted in a 1:100 ratio in LB in a flask. This flask was incubated in an air shaker at 37℃ with shaking until an optical density (600 nm) of 0.5-0.6 was reached. The culture was placed into a centrifuge tube and centrifuged for 10 minutes at 7500 rpm at 4℃. The supernatant was discarded. The pellet was resuspended in 1/10 of their original volume of cold TSS Buffer (10% PEG, 10% glycerol, 20 mM $MgCl_2$).

2.3.3 – Transformation of Chemically Competent Cells

1 μL of the desired plasmid was added to 100 μL of chemically competent *E. coli* cells in an eppendorf tube. This solution was incubated on ice for 30 minutes, then heat shocked in a water bath at 42℃ for 45 seconds. The culture was then put back on ice for 5 minutes. The cells were

diluted with 1 mL LB and incubated at 37℃ for 30-60 minutes in a roller drum. The cells were pelleted in a centrifuge for 1 minute. Supernatant was removed except for 100 µL, and cells were resuspended in the remaining 100 µL of supernatant. The total cell suspension was plated onto LB plates containing selective antibiotics and incubated at 37℃ for 16-18 hrs.

2.4 – Inhibition Assays

2.4.1 – Sigma E/Hfq Pathway-Inhibitor Luminescence Assay

Frozen stocks of SEA 281 and SEA 282 strains were inoculated in 3 mL LB containing ampicillin and kanamycin and grown for 16-18 hours in a roller drum at 30℃. The absorbance (600 nm) was measured, and both cultures were diluted to an optical density (600 nm) of 0.02 in LB containing ampicillin and kanamycin. The cultures were incubated at 37℃ in an air shaker with shaking until an absorbance of 0.3-0.5 was reached (~2.5 hrs). To induce expression of *rpoE* in SEA 281, 1M IPTG was added to each culture to a final concentration of 1 mM. White Grenier-Bio-One F-bottom, Lumitrac 96-well plates containing the sample compounds were thawed and 50 µL of SEA 281 were added to sample wells. 50 µL of SEA 282 were added to control wells. Plates were sealed with EXCEL Scientific, Inc., ThermalSeals and incubated at 37℃ for 45 minutes. 5 µL of 1 mg/mL AHT was diluted in 5 mL of LB, and 5 µL of this solution was added to each well to induce expression of reporter gene. The plate was resealed and incubated for 1 hour at 37℃. B-PER reagent was mixed in a 1:1 ratio with Bright-Glo. 50 µL of this solution was added to each well, and the plate was sealed with a new seal. The plate was vortexed for 30 seconds, centrifuged for 1 min at 1000 rpm, and incubated for 20 minutes at room temperature. The plates

were read on a SpectraMax i3 Microplate Reader to measure endpoint luminescence (560 nm, emission 15 nm) for the entire plate.

2.4.2 – Sigma E Inhibitor Fluorescence Assay

SAE 051 and SAE 052 or LMW 001 and LMW 002 frozen stocks were inoculated in 3 mL of EZ Rich Defined medium containing ampicillin and kanamycin and incubated at 37℃ in a roller drum for 16-18 hours. The absorbance (600 nm) was measured, and the cultures were diluted to an absorbance (600 nm) of 0.02 in fresh EZ Rich medium. The cultures were incubated at 37℃ in an air shaker until an absorbance (600 nm) of 0.1-0.2 was reached. Cells were induced with 1M IPTG to a final concentration of 1 mM IPTG. 5 mL aliquots were made of each culture for the desired tests. These contained a control that did not contain any additional compounds and a DMSO control. The GFP fluorescence (λ_{ex}468 nm/λ_{em}510 nm) and the absorbance (600 nm) were measured at the time of induction using SpectraMax i3 Microplate Reader. To measure fluorescence and absorbance, 100 μL of the culture was pipetted into a 96-well Costar black/clear bottom plate in duplicate at indicated time points. The cells were incubated in a shaking water bath at 37℃. The fluorescence and absorbance were measured every hour for four hours. The fluorescence was normalized by calculating the fluorescence divided by the absorbance.

2.4.3 – Minimum Inhibitory Concentration (MIC) and IC_{50}

The *E. coli* strain of interest was inoculated in 3 mL of LB from a frozen stock and incubated at 30℃ or 37℃ for 16-18 hours in a roller drum. For a 2-fold serial dilution series of the compound of interest, 200 μL of LB was added to the first column of the plate, and 100 μL was

added to the remaining columns in a 96-well plate. The compound of interest was added to all wells except 2 of the first column to desired concentration, and an equal amount of DMSO was added to the remaining 2 wells. A 2-fold serial dilution was performed by removing 100 µL from column one and transferring to the next using a multichannel pipette, and 100 µL was discarded from the last column resulting in a fixed volume of 100 µL for all wells. 5 µL of the overnight culture was diluted in 5 mL of LB, and 10 µL of the cell dilution was added to all wells except for negative control wells in the last column. The second to last column did not have any added compounds as a positive control for cell growth. The plate was sealed with an EXCEL Scientific, Inc., ThermalSeal and incubated for 18-24 hours at 37℃. The MIC of a compound was determined to be the lowest concentration in which there was no visible bacterial growth. To determine the IC_{50}, the absorbance (600 nm) of each well was measured on a SpectraMax i3 Microplate Reader.

2.4.4 – Minimum Bactericidal Concentration (MBC)

For the MBC, 5 µL from each well from the MIC plate were spotted on a LB plate. The spots were allowed to dry before incubation at 37℃ for 24 hours. The MBC is determined as the lowest concentration of compound at which there was no bacterial growth on the LB plates.

2.4.5 – MIC Checkerboard Assay

The *E. coli* strain of interest from a frozen stock was inoculated in 3 mL of LB and incubated at 30℃ or 37℃ for 16-18 hours in a roller drum. Using a 96-well plate, 200 µL of LB was added to the first column and first row of the plate, and 100 µL were added to the remaining wells. The compound of interest was added to all wells except the top of the first column to desired

concentration. A 2-fold serial dilution was performed by removing 100 µL and transferring to the next column, and 100 µL was discarded from the last column. The second compound of interest was added to all wells of the first row. A 2-fold serial dilution was performed in the same manner as the first compound with 100 µL discarded from the last column resulting in a fixed volume in all wells of 100 µL. 5 µL of the overnight culture was diluted in 5 mL of LB, and 10 µL of the cell dilution was added to all wells except for negative control wells in the last column. The second to last column did not have any added compounds as a positive control for cell growth. The plate was sealed with an EXCEL Scientific, Inc., ThermalSeal and incubated for 18-24 hours at 37℃. The synergy of the two compounds was determined by the concentration of each compound where the dilution changed from bacterial growth to no growth.

2.4.6 – Biofilm Assay

The *E. coli* strain of interest was inoculated in 3 mL of LB and incubated at 30℃ or 37℃ for 16-18 hours in a roller drum. The absorbance (600 nm) was measured, and the cultures were diluted 10-fold in LB. 50 µL of cells were pipetted into a 96-well plate. LB was added to one column as a negative control for contamination. The plate was sealed and wrapped in a wet paper towel and plastic wrap prevent evaporation. The plate was incubated at 30℃ for 24 or 48 hours, and the absorbance (600 nm) was measured on a SpectraMax i3 Microplate Reader after incubation. Planktonic cells were removed by inverting the plate and tapping until all liquid was removed, and the plate was washed 2 times with 100 µL of distilled water pipetted into each well. To stain the cells in the biofilm, 100 µL of 0.1% crystal violet was added to each well for 10 minutes. The crystal violet was removed by inverted the plate and tapping to remove all crystal

violet solution, and the plate was washed 3 times with 100 μL distilled water in each well. Another method of washing the cells was used by submerging the plate into distilled water until each well was filled. The water was removed by inverting the plate and tapping until all liquid was removed. Both methods of washing the cells yielded the same results, so no additional data is included for comparison of the two methods. The plate was placed in the incubator for 20 minutes at 37℃ to dry. 60 μL per well of 20% acetone/80% ethanol was used to solubilize the cells. The absorbance (570 nm) was measured on a SpectraMax i3 Microplate Reader to determine the number of cells in the biofilm. The biofilm formation was determined by calculating OD_{570}/OD_{600}.

Chapter 3

Results

Part I – σ^E/Hfq Pathway Small Molecule Inhibition Assay

The σ^E/Hfq pathway inhibition luminescence assay was optimized previously, so no further testing was needed to confirm the efficacy of the assay. 13,000 compounds from Calibr were tested in this assay. Each compound was placed in one well of the Lumitrac 96-well plate by the manufacturer.

The strains used for this assay contain a mutation (*prlF1*) that encodes for a suppressor protein that allows for the cell to survive in the absence of σ^E (Leiser et al., 2012). The screening strain contains *rpoE* on a plasmid in which the activity is controlled by an isopropyl-β-D-1-thiogalactopyranoside (IPTG) inducible promoter. The *rybB* gene encoding *RybB*, a sRNA, is also present on this plasmid and is transcriptionally dependent on σ^E. The control strain contains the pTrc99a plasmid that acts as an empty vector plasmid control. The empty vector plasmid lacks the *rpoE* and *rybB* genes to mimic inhibition of σ^E (El-Mowafi et al., 2015). In addition, both the screening and control strain contain *ompC'-luc* gene on a plasmid that functions to result in the measurable luminescent readout for the assay. The transcription of this gene is induced by the addition of anhydrotetracycline (AHT). Upon transcription, the *ompC* part of the mRNA acts as the target of the sRNA, *RybB*, and can be paired under the control of Hfq to block translation of this *ompC'-luc* mRNA leading to degradation of the mRNA. Blocked translation of this *ompC'*-

luc mRNA prevents the production of luciferase. Luciferase oxidizes its substrate, luciferin, resulting in the release of light as luminescence. This luminescence is the measurable quantity of this assay.

Without inhibition of either σ^E or Hfq and inducing both σ^E expression and *ompC'-luc* transcription with the addition of IPTG and AHT, *RybB* will be transcribed and bound to the *ompC'-luc* mRNA under the control of Hfq. This promotes the degradation of the *ompC'-luc* mRNA thus leading to low luminescence of the cells. If inhibition was present, then either *rybB* transcription by σ^E could be inhibited or *RybB* pairing with *ompC'-luc* by Hfq. This would allow for the transcription and translation of the *ompC'-luc* gene leading to cells having a high luminescence (Figure 3).

To quantify the inhibition of the pathway by these compounds, the luminescence of the oxidized luciferin substrate of each well containing a compound was measured and compared to that of the control strain. DMSO was added to the control strain due to the use of DMSO to create solutions of the chemical library compounds. The DMSO did not have an effect on cell growth. In order to determine hits of this assay, the mean of the luminescence measurements was calculated for all of the compounds tested on a given plate along with the standard deviation. A hit was determined as any compound in which the luminescence was above two standard deviations above the mean. Stronger hits were determined to be any compound with a luminescence that was closer to that of the control strain.

From this assay 23 hits were determined to be potential inhibitors and retested with this assay. Of these hits, 16 had an IC_{50} value greater than 100 μM and 2 did not reach an upper baseline. These 18 hits were not pursued. The 5 remaining hits were shown to have an IC_{50} and

thus were tested further. One of these hits was KKL-17131 (Figure 6). This compound showed fluorescence above the two standard deviations, which tagged it as a possible inhibitor.

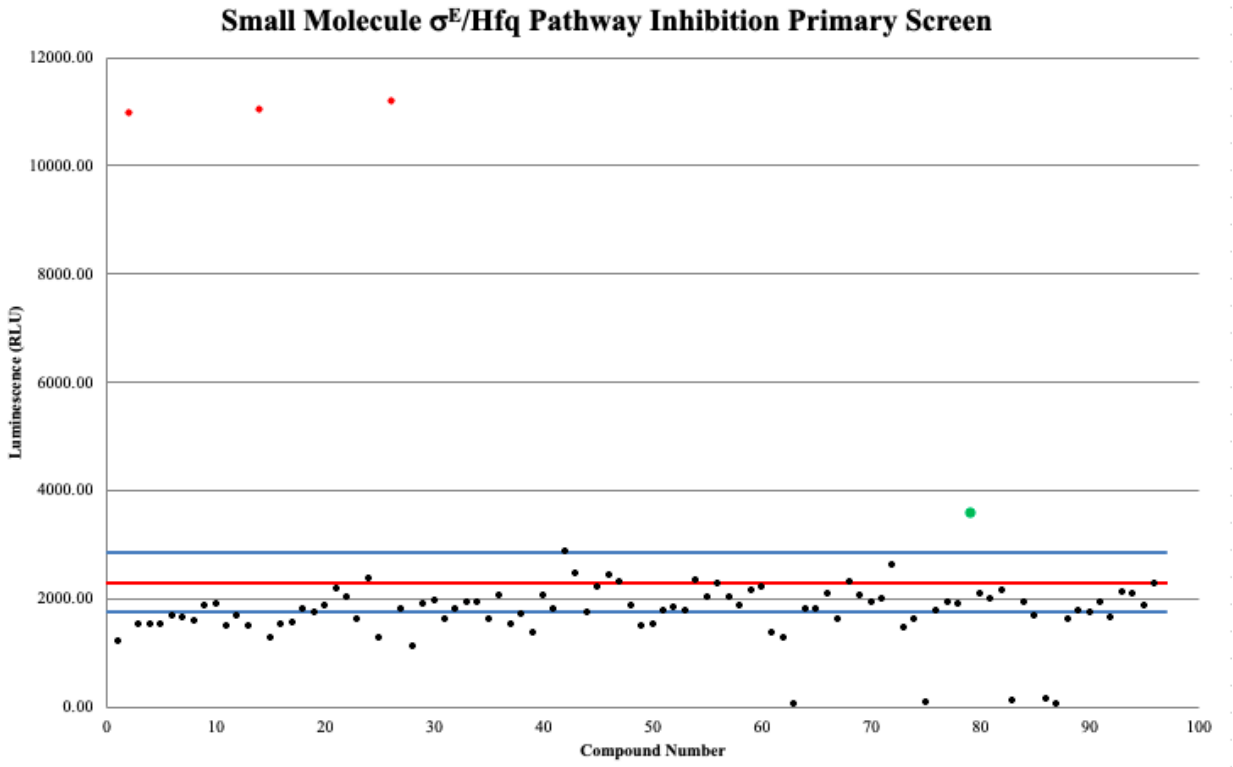

Figure 6. Graph of plate of compounds from Calibr tested in the small molecule σ^E/Hfq inhibition assay. The positive control wells for inhibition with only DMSO added to the control strain are shown in red. The hit, KKL-17131, is depicted in green. The red line represents the mean luminescence of the compounds tested, while the blue lines are indicative of +/- two standard deviations from the mean.

To confirm KKL-17131 as an inhibitor of the σ^E/Hfq pathway, the compound activity was measured again with varying concentrations of KKL-17131. These concentrations were determined by the manufacturer and sent in a similar fashion to the original library of compounds. If a dose response is observed, it would further support the ability of the compound to inhibit the pathway. During the rescreen of this compound, the percent inhibition increased as concentration of KKL-17131 was also increased (Figure 7). The IC_{50} was determined to be 14 μM, while the

IC_{90} was unable to be determined from the concentrations tested (Figure 7). The control strain with DMSO added was used as the upper baseline used to calculate the IC_{50} for this assay.

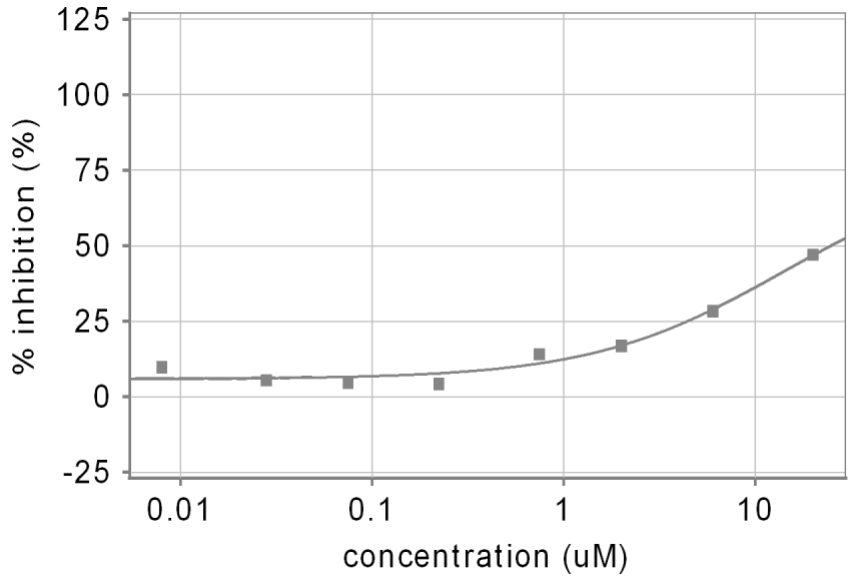

Figure 7. Confirmation of KKL-17131 inhibition of the σ^E/Hfq pathway through repeat of σ^E/Hfq luminescence assay with increasing concentrations of KKL-17131. Luminescence was measured and converted to percent inhibition of the σ^E/Hfq pathway through comparison with 100% inhibition of the pathway of the screening strain without the addition of any compounds.

Another compound determined as an inhibitor of this pathway is KKL-2170. This compound was found in a screening project performed by GlaxoSmithKline (GSK) as a collaboration between the Ades lab and GSK. A high-throughput screening of 2.3 million compounds was performed by GSK. From this project, KKL-2170 was one of the hits and characterized as a σ^E inhibitor through secondary assays (unpublished data). These secondary assays include the secondary σ^E-specific screening assay along with an *in vitro* transcription assay. This screening and characterization occurred prior to the screen of the Calibr chemical library.

Part II – Small Molecule Inhibitor Characterization

3.1 – σ^E-Specific Secondary Assay Optimization

The σ^E-specific secondary assay allows for determination and characterization of the hits from the primary screen as specific inhibitors of σ^E and not Hfq. Before this assay could be utilized to test the specificity of inhibition by KKL-17131, the strains used in the screen were optimized. In order to optimize these strains, a series of strains with yellow, cyan, and green fluorescent proteins with different σ^E promoters were tested (Figure 8). The growth and fluorescence was measured for each strain over the course of four hours after the addition of IPTG to induce σ^E expression. A fold difference of at least 4 was expected to be reached between the fluorescence of the screening and control strain. This would lead to a more accurate visualization of the level of inhibition by compounds tested with this assay. The largest fold difference was observed between SAE051 and SAE052, which contain a GFP reporter protein (Figure 8). These strains were selected for use in this assay.

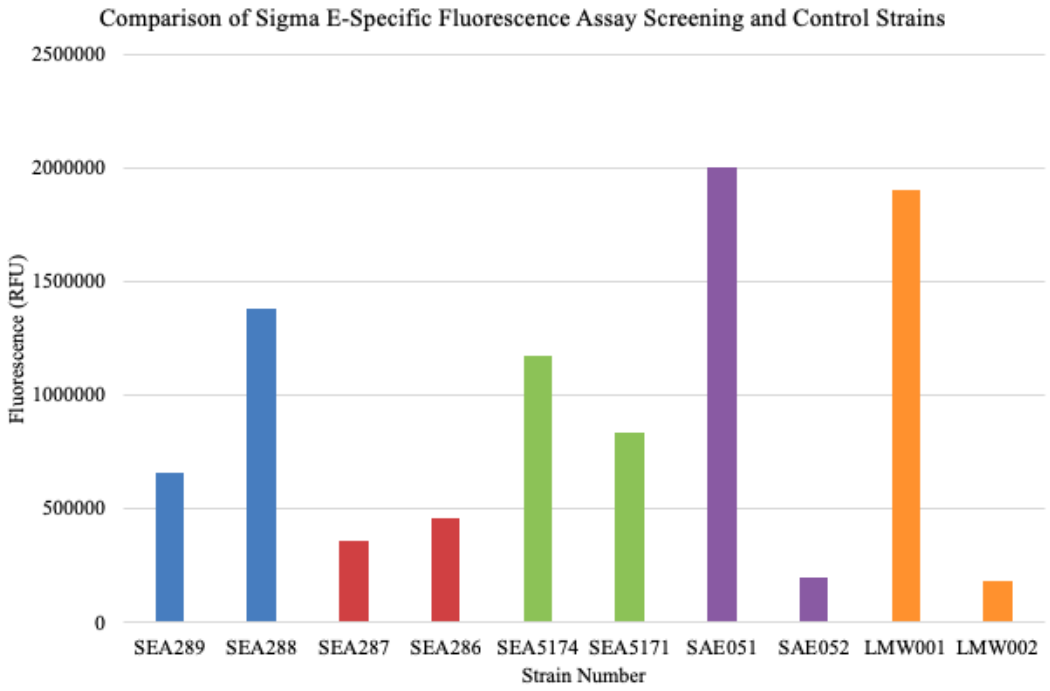

Figure 8. Comparison of different strains with a σ^E-dependent fluorescence reporter. Strains contained either yfp, cfp, or gfp reporters. Pairs of strains (screening and control, respectively) are indicated by the same color. Strain genotypes are indicated in Table 1. SAE051 and SAE052 and LMW001 and LMW002 were used in further assay optimization and testing due to approximate 4-fold difference between the screening and control strains.

Two sets of strains were used for this assay. One set did not have the *tolC* deletion (SAE051 and SAE052), and one did (LMW001 and LMW002) in order to minimize compound efflux from the cells. The screening strains have the *ydcQ* gene deleted. The absence of YdcQ prevents lethality of cells with a *rpoE* deletion, but the mechanism by which it does this has not been determined (Button et al., 2007). Both the screening and control strains contain the p*SRE* plasmid which encodes for green fluorescent protein (GFP) and contains a σ^E-dependent promoter directing *gfp* transcription. Thus, the production of GFP is dependent on σ^E activity. The screening strain has the p*LC245* plasmid transformed into it. This plasmid encodes for the *rpoE* gene and expression is IPTG-induced. When IPTG is added to the cells, there is an overexpression of *rpoE*.

The control strain contains the Trcc99a plasmid, which acts as an empty vector plasmid. This plasmid does not contain the *rpoE* gene inducible by IPTG, so it mimics σ^E inhibition in a cell.

In the absence of an inhibitor and with IPTG induction, σ^E can transcribe the *gfp* gene which will then create a functional GFP. The fluorescence of this protein can be measured. In the presence of a σ^E inhibitor, σ^E would be unable to transcribe the *gfp* gene. Thus GFP is not produced leading to lowered fluorescence of the cells (Figure 4). To confirm that IPTG was able to induce σ^E expression in the screening strain but not in the control strain, both of these strains were grown in the presence in absence of IPTG. Fluorescence and absorbance were measured for these strains over 4 hours to follow the protocol of the assay. The addition of IPTG was confirmed to induce σ^E expression in the screening strain (SAE051). There was baseline fluorescence observed in the absence of IPTG for the screening strain comparable to the control strains (Figure 9). The control strain (SAE052) also did not display any change in fluorescence in the presence or absence of IPTG (Figure 9).

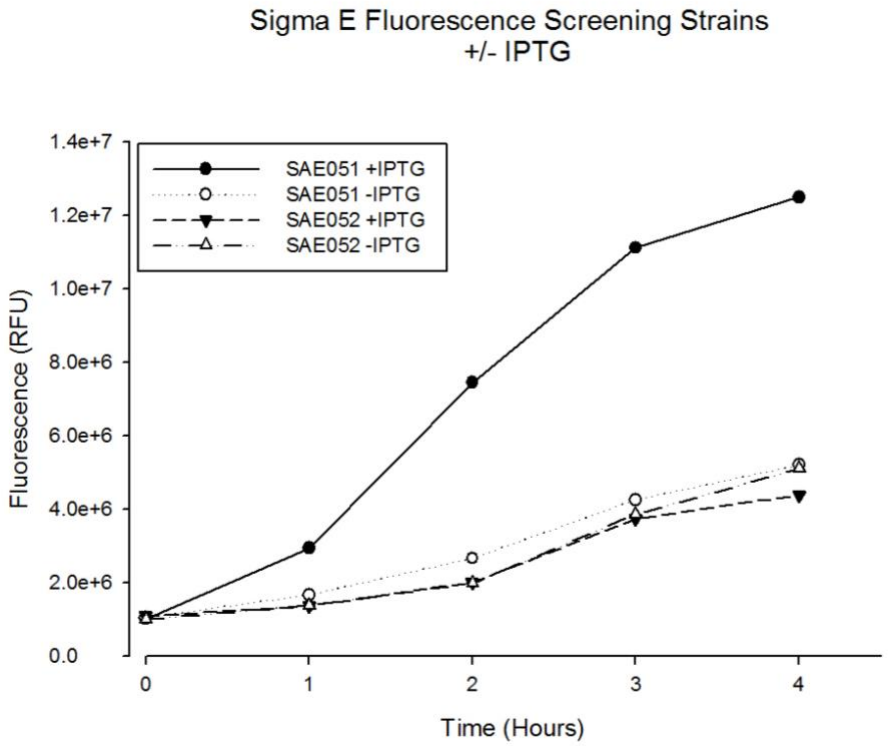

Figure 9. Validation of IPTG-induced σ^E activity in the screening strain (SAE051) for the σ^E-specific inhibition assay. IPTG addition lead to increased σ^E activity which increased *gfp* transcription. The increased *gfp* transcription increased the production of GFP leading to greater fluorescence of the screening strain, but not the control strain (SAE052). In the absence of IPTG, σ^E activity was reduced in the screening strain leading to lower fluorescence comparable to the control strain suggesting no σ^E expression.

The initial strains tested were a wild type background that did not contain a *tolC* deletion. With the *gfp* under the control of a σ^E promoter and the *ydcQ* deletion, there was 4-fold difference in the fluorescence at hour 4 (Figure 10). In addition, this same assay was repeated with strains now containing the *tolC* deletion. This would allow for a lower concentration of the test compounds to be utilized when performing this assay. With the *tolC* deletion, there was also a 4-fold difference in fluorescence at hour 4 (Figure 10). The presence of the 4-fold difference in

fluorescence in both strains provided the necessary difference for further testing and characterization of the small molecule inhibitor, KKL-17131.

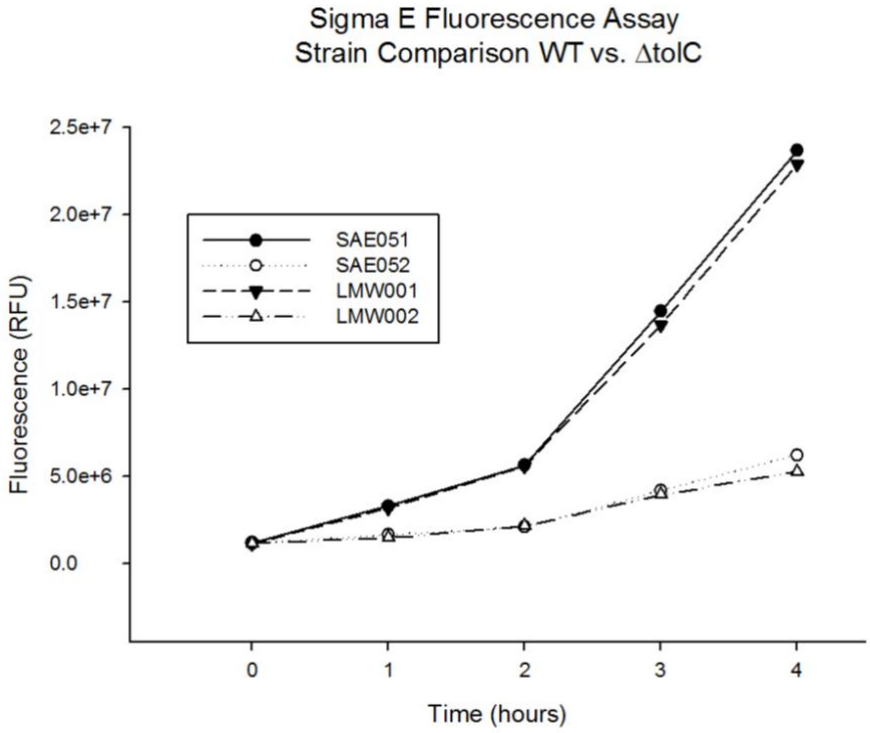

Figure 10. Comparison of the original strains (SAE051 and SAE052) to the screening strains with the Δ*tolC* background (LMW001 and LMW002) in the σ^E-inhibition fluorescence assay. Both the screening strain and the control strain for each background demonstrated comparable fluorescence.

3.2 – Assessment of σ^E/Hfq Pathway Inhibitors with σ^E Secondary Screening Assay

After optimization of the σ^E secondary screening assay, KKL-17131 was tested with this assay to determine if specific inhibition of σ^E could be observed. This compound was initially tested at 100 μM in the strains with the Δ*tolC* background, and some inhibition was observed due to lowered fluorescence in comparison to the screening strain. DMSO was added to both the

screening strain and the control strain to due to the use of DMSO to solubilize KKL-17131. This controls for any results due to an effect of DMSO on the cells. No difference was observed in the presence or absence of DMSO to the screening and control strains. Concentrations of KKL-17131 were tested above and below the MIC previously determined, in order to test for a dose-dependent response of σ^E inhibition. Different concentrations were tested until both an upper and lower baseline was reached. As concentration was increased for KKL-17131, the amount of fluorescence decreased (Figure 11). The IC_{50} of KKL-17131 for inhibition of σ^E was calculated as 90.1 μM.

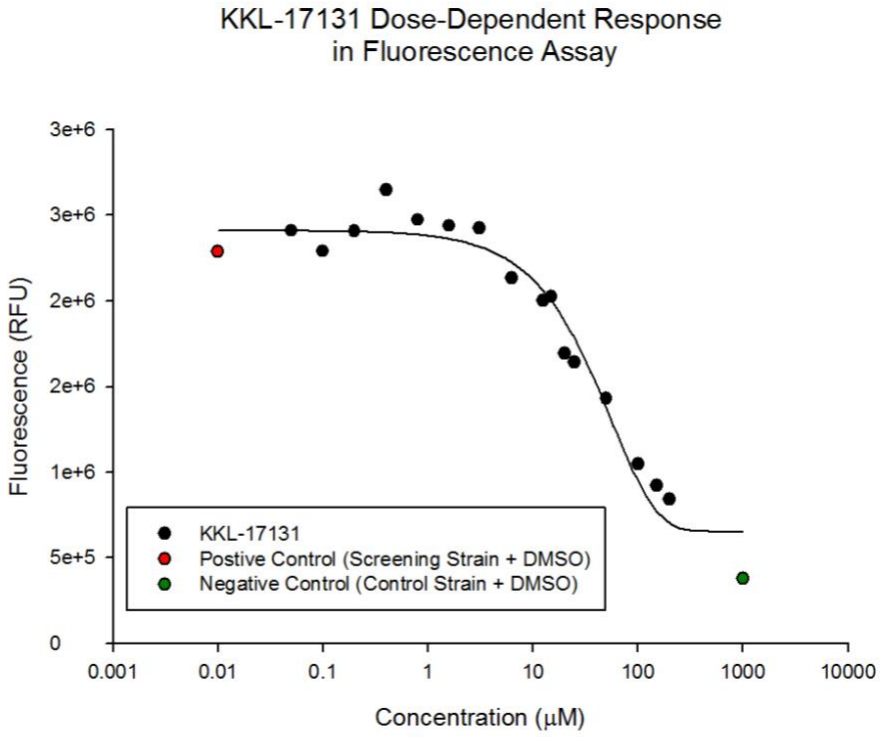

Figure 11. Dose Dependent Response of KKL-17131 in the σ^E-specific secondary screening assay. The positive control is depicted in red, which was the screening strain with DMSO added. The negative control is depicted in green and consists of the control strain with DMSO added. As concentration of KKL-17131 increases, the level of fluorescence decreases.

3.3 – Assessment of σ^E/Hfq Pathway Inhibitors in Growth Inhibition

To further characterize KKL-17131 and KKL-2170 as inhibitors, a series of growth inhibition assays were performed. Some of these inhibition assays included Minimum Inhibitory Concentration (MIC) assays, Minimum Bactericidal Concentration (MBC) assays, and MIC Checkerboard assays. The MIC assay test for the minimum concentration at which a compound can inhibit visible cell growth, while the MBC assay is the minimum concentration at which cells are killed. The MIC Checkerboard assay tests for synergy between two compounds for potential dual drug treatment. Hfq is not essential to cell survival, so it would be expected that any compound acting as a Hfq-specific inhibitor would not be able to actively inhibit growth (Niba et al., 2007). For σ^E-specific inhibitors, growth inhibition would be expected due to the necessity of σ^E in *E. coli* for cell survival (Kazmierczak et al., 2005).

KKL-2170 was previously determined to have an MIC during prior experimentation with this compound, supporting its activity as a σ^E inhibitor (unpublished data). For the MIC and MBC testing with KKL-2170 and KKL-17131, these compounds were tested in Δ*tolC E. coli* strains. This deletion reduces the efflux of the drug from the cell due to the deletion in the TolC efflux pump. This reduces the concentration of compound needed for an effect to be seen. When performing these inhibition assays, Ciprofloxacin was used as a positive control due to its known MIC in *E. coli* (Piddock et al., 1990)(Liu et al., 2011) (Table 2).

The MIC was determined for all three compounds: Ciprofloxacin, KKL-2170, and KKL-17131 as 11.3 nM, 89.5 μM, and 267 μM, respectively (Table 2). For KKL-17131, there was no obtainable MBC. For Ciprofloxacin and KKL-2170, the MBC values were 22.6 nM and 179 μM respectively (Table 2). The IC_{50} of both KKL-2170 and KKL-17131 was determined by measuring the optical density of each well from the plate in which the MIC assay performed. The IC_{50} is determined to be the concentration at which 50% of growth is inhibited, and the IC_{90} is the concentration in which 90% of growth is inhibited. The data was normalized by identifying the optical density for the cells when no compound was added as 100% growth. The optical density for each concentration was divided by this 100% growth optical density to determine percent growth at any given concentration. For KKL-2170, the IC_{50} was determined to be 52.1 μM and the IC_{90} is 96.3 μM (Table 2) (Figure 12). For KKL-17131, the IC_{50} and the IC_{90} were calculated as 258 μM and 413 μM respectively (Table 2) (Figure 12).

Table 2. Small Molecule Inhibitors Growth Inhibition in Δ*tolC E. coli* strain

Compound	MIC	MBC	IC_{50}	IC_{90}
Ciprofloxacin	11.3 nM	22.6 nM	Not tested	Not tested
KKL-2170	89.5 μM	179 μM	52.1 μM	96.3 μM
KKL-17131	267 μM	No MBC observed	258 μM	413 μM

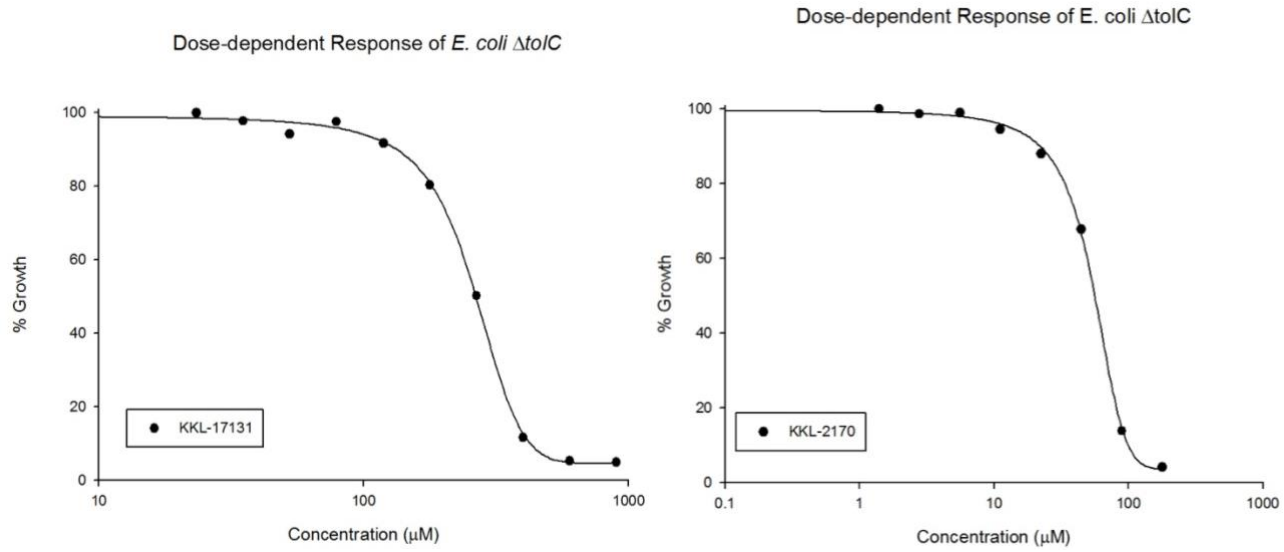

Figure 12. IC_{50} graph of the MIC assay preformed with KKL-17131 (left panel) and KKL-2170 (right panel), respectively, in Δ*tolC E. coli* strain. 100% growth was determined as optical density for no compound added to the cell. Percent growth was calculated for each well.

Further characterization of KKL-2170 was performed to test the synergy of this compound with ciprofloxacin, a known inhibitor of *E. coli*. To determine synergy, a serial dilution of both compounds was performed as a MIC Checkerboard. When synergy is present between two compounds, there is a reduction in the concentration necessary for both compounds to inhibit growth. Essentially the MIC would lower for both compounds in combination treatment as opposed to the use of a single compound. With the combination of cipro and KKL-2170, there was no synergy observed between the two compounds. The MIC did not change for either compound (Table 3).

Table 3. MIC Checkerboard Data with Ciprofloxacin and KKL-2170 in Δ*tolC E. coli* strain

Compound	MIC
Ciprofloxacin (alone)	11.3 nM
Ciprofloxacin with KKL-2170	11.3 nM
KKL-2170 (alone)	89.5 μM
KKL-2170 with Ciprofloxacin	89.5 μM

3.4 – Biofilm Assay Development

For future characterization of hits of the σ^E/Hfq pathway primary screening, a biofilm assay was developed. Due to the importance of Hfq in biofilm formation, inhibitors of Hfq may be identified through the use of this assay. The role of Hfq in biofilm was confirmed through the comparison of a Δ*hfq* strain to a wild type strain. The biofilm formation was greatly reduced in the Hfq deletion strain by approximately 10-fold (Figure 13). In addition, the role of TolC in biofilm formation was examined due to the ability to use a lower concentration of a compound when testing in a Δ*tolC* strain. Based on the results of the assay, the deletion of *tolC* did not have an effect on biofilm formation and was comparable to the wild type strain (Figure 13). This would allow for the interchangeable use of the Δ*tolC* strain and the WT strain for testing potential inhibitors of Hfq.

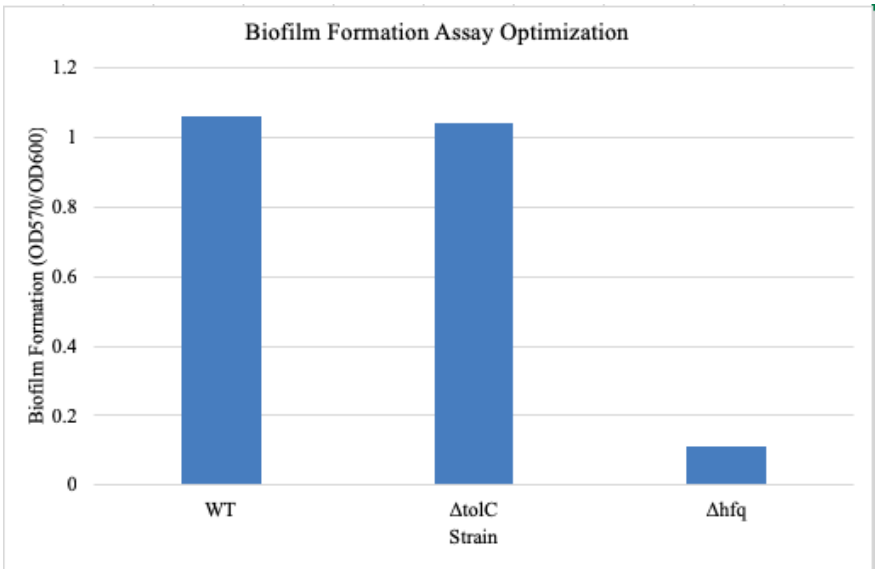

Figure 13. Biofilm formation assay optimization with WT, Δ*tolC* and Δ*hfq* strains of *E. coli*. Biofilm formation of WT and Δ*tolC* strains are comparable. The biofilm formation of the Δ*hfq* strain is reduced greatly compared to the WT and Δ*tolC* strains.

Chapter 4

Discussion

The work here shows that KKL-17131 displays potential as a σ^E inhibitor. Using the primary σ^E-Hfq pathway screening assay, KKL-17131 was determined to be an inhibitor of this pathway. Through the use of the σ^E-specific secondary assay, a dose-dependent response was observed for KKL-17131 (Figure 11). As concentration was decreased for the compound in the σ^E-specific secondary screening assay, a decrease in the level of inhibition of σ^E was also observed. The upper baseline of no inhibition, the positive control, was able to be reached at low concentrations of KKL-17131. The lower baseline was not reached, which is expected due to the concentration of KKL-17131 necessary to reach a lower level would lead to growth inhibition and cell death. When the concentration is high enough to result in growth inhibition and cell death, an accurate measurement of σ^E inhibition is unable to be obtained. The IC_{50} of KKL-17131 in the secondary σ^E assay was calculated as 90.1 μM.

Additionally, the characterization of this compound through growth-inhibition assays provided further support for σ^E-specific inhibition. KKL-171331 was shown to inhibit growth through the presence of a MIC at 267 μM (Table 2). However, an MBC was unable to be determined for this compound. An MIC would not be expected for an inhibitor of Hfq because it is not essential for viability of *E. coli* (Niba et al., 2007).

Through comparison of the IC_{50} values determined for KKL-17131 in the primary, secondary, and growth inhibition assays, the concentrations are different for each assay. In the primary screen, the IC_{50} of 14 μM was significantly lower than the other assays. This low concentration may be due to the sensitivity of the primary screening assay. The IC_{50} from the

secondary σ^E-specific assay is 90.1 μM, which is also lower than the IC_{50} calculated from the growth inhibition assay. A possible explanation for the lower concentration is due the secondary assay measuring inhibition of σ^E. The concentration necessary for sufficient inhibition of σ^E leading to 50% growth inhibition may be higher than what is necessary for 50% inhibition of only σ^E activity. Comparatively, the IC_{50} calculated from the optical density of the growth inhibition assay was only slightly lower than the recorded MIC. The MIC is a measurement based on visual inspection of growth, which may lead to the discrepancy. The IC_{90} calculated from the optical density of the MIC assay was 413 μM, which is higher than 267 μM MIC. This also may be a result of the reliance on visual inspection for growth. It is expected that the IC_{90} concentration is lower than the MIC. Although there are discrepancies in these concentration, there is still evidence from theses assays that KKL-17131 inhibits σ^E.

Given these results there is evidence to support that KKL-17131 may inhibit σ^E; however, more data is necessary to fully classify this compound as a σ^E-specific inhibitor. In order to better classify this compound as a σ^E inhibitor, there multiple different methods in which to test this compound. One method would involve testing KKL-17131 alongside potential Hfq inhibitors identified from the primary screen due to the initial classification of KKL-17131 in a σ^E/Hfq inhibition assay. These compounds could be tested both in the biofilm assay described previously or the Hfq secondary screening assay once it is optimized (El-Mowafi et al., 2014). I would expect that KKL-17131 would not show any inhibition of Hfq in this assay or reduction in biofilm formation at concentrations below the MIC if it is a σ^E inhibitor.

With indication of no direct inhibition of Hfq by KKL-17131, future experimentation with an *in vitro* assay for σ^E inhibition would lead to further validation as a σ^E inhibitor. For an *in vitro*

assay specific to σ^E inhibition, there are a two different functions of σ^E that may be studied. One experiment would look to study the ability of KKL-17131 to prevent binding of σ^E to core RNA polymerase (El-Mowafi et al., 2015). Another experiment could determine if KKL-17131 blocks a part of transcription by the σ^E-RNA polymerase complex (El-Mowafi et al., 2015). Not only would these *in vitro* assays allow for better conformation of KKL-17131 as a σ^E inhibitor, but they could also lead to a better understanding of the mechanism of action of the compound.

In addition to the *in vitro* assays, this compound can be tested in assays that induce cell envelope stress. One such way to induce cell envelope stress involves plating *E. coli* cells on agar plates containing ethylenediaminetetraacetic acid (EDTA) and sodium dodecyl sulfate (SDS). The presence of a chelator and detergent, respectively, would induce cell envelope stress. Due to the role of σ^E in maintaining cell envelope integrity, inhibition of σ^E would be harmful to cells undergoing envelope stress (Nicoloff et al., 2017). A reduction of growth or even cell death should be observed in cells undergoing envelope stress if KKL-17131 inhibits σ^E.

Along with these experiments to characterize KKL-17131, chemical modification is another possible route to improve the efficacy of the compound in inhibition of its target. This also allows for a better understanding of the important components of the molecule. Chemical modification can lead to stronger association to the binding site on the target or better cell uptake (Fernandes et al., 2017). This has been a technique use to modify many different classes of antibiotics, such as macrolides (Fernandes et al., 2017). With further experimentation, KKL-17131 can be better characterized as a σ^E-specific inhibitor and developed to become a more efficacious drug.

Although there is promise for KKL-17131 as a σ^E-specific inhibitor as described before, there are limitations from these results. Through the characterization of KKL-17131 with the growth inhibition assays and the σ^E inhibition fluorescence assay, the strains utilized contained a *tolC* deletion in the genome. This deletion knocks out the TolC efflux pump, a common mechanism for drug efflux from a bacterial cell (Whitneyl, 1971; Nishino and Yamaguchi, 2001). Due to the reduction of drug efflux, a lower concentration of a given compound is necessary to see an effect on the cell. However, this gene is present in wild type cells and thus in clinically important microbes. So with the hope of antibiotic development, KKL-17131 will need to be effective in a wild type background in addition to the Δ*tolC* background. The high concentration of KKL-17131 needed to inhibit growth does not provide evidence for good efficacy of this compound as an antibiotic. Low concentrations for growth inhibition or cell death are often necessary for drug development of a compound.

KKL-2170 also shows promise as an effective inhibitor of σ^E based on the results of the growth inhibition assays tested with this compound. This compound was previously characterized as an inhibitor of σ^E (unpublished data). The low concentrations of KKL-2170 necessary to inhibit growth would allow for this compound to act as a better antibiotic than KKL-17131, which showed much higher concentrations necessary to inhibit growth. This compound also had a low concentration in which it was able to kill the cells, the MBC. Although this compound was unable to show synergy with Ciprofloxacin, there is still the possibility that KKL-2170 may have synergy with other antibiotics. Further testing of this compound with other common antibiotics may lead to the discovery of synergy and a future combination therapy. In addition, chemical modification of KKL-2170, as discussed previously with KKL-17131, would allow for the creation of analogues

that may have higher σ^E inhibition activity. It would also lead to a better understanding of the essential functional groups on the molecule necessary for inhibition.

KKL-17131 and KKL-2170 are promising compounds in a novel class of antibiotics targeting σ^E in *E. coli*. With the increase of antibiotic resistance microbes, novel antibiotics are necessary to help prevent the spread of this global issue (Ventola, 2015). Future characterization and development of KKL-17131 and KKL-2170 can lead to the development of novel antibiotics to combat this issue.

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ACADEMIC VITA
JESSICA REYER

EDUCATION

Pennsylvania State University, Schreyer Honors College — May 2019
Bachelor of Science in Microbiology
Minor in Biochemistry and Molecular Biology

RESEARCH EXPERIENCE

Undergraduate Researcher — 2016 to Present
Ades Lab
Honors Thesis: "Identification of Inhibitors of the SigmaE/Hfq Pathway in *E. coli*"

Reading Hospital Student Summer Internship — 2018
Mentor: Anthony Donato, M.D. — Summer
Reading, PA
Project: "The Influence of Hypochloremia on 30-day Readmission Rates in Patients with Acute Heart Failure Exacerbation"

Undergraduate Research Exhibition — April 2017, 2018
Poster Presentation
University Park, PA
"Identification of Synergy of Sigma E Inhibitor E6 with Common Antibiotics in *E. coli*"
"Identification of Inhibitors of the Sigma E/Hfq Pathway in *E. coli*"

ACTIVITIES

Atlas For The Kids — August 2015 to Present
University Park, PA
Special Interest Penn State Dance Marathon Organization

- Special Events Executive Chair during the 2018-19 THON Fundraising Year
- Special Events Administration Captain and Senior Appreciation Captain during the 2017-18 THON Fundraising Year
- Fun & Games Captain for THON 2017
- Participated in a diverse range of fundraising events to raise money for the Four Diamonds Fund

HONORS AND AWARDS

Academic Excellence Award — 2015 to Present

Undergraduate Research Funding 2017 to Present