

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188		
The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 13-07-2010		2. REPORT TYPE Final Report		3. DATES COVERED (From - To) 10-May-2006 - 9-May-2008	
4. TITLE AND SUBTITLE Selection of tmRNA inhibitors as antibacterial and plasmid elimination agents.			5a. CONTRACT NUMBER W911NF-06-1-0144		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER 106013		
6. AUTHORS Kenneth Keiler, Stephen Benkovic			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES Pennsylvania State University Office of Sponsored Programs The Pennsylvania State University University Park, PA 16802 -			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211			10. SPONSOR/MONITOR'S ACRONYM(S) ARO		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) 49434-CH-TRA.1		
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT The goal of this project is to develop lead compounds for antibiotic development targeted to the tmRNA pathway. These antibiotics are expected to have broad host specificity and will be assayed against pathogens of military interest, including Bacillus anthracis. Specifically, this project aims to: 1) develop screening methods for identification of tmRNA pathway inhibitors; 2) identify and optimize inhibitors from libraries of cyclic peptides; 3) test the bactericidal and plasmid elimination efficacy of lead compounds against B. anthracis and other pathogens;					
15. SUBJECT TERMS antibiotic, cyclic peptide, high-throughput screen					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Kenneth Keiler
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 814-863-0787

Report Title

Selection of tmRNA inhibitors as antibacterial and plasmid elimination agents.

ABSTRACT

The goal of this project is to develop lead compounds for antibiotic development targeted to the tmRNA pathway. These antibiotics are expected to have broad host specificity and will be assayed against pathogens of military interest, including *Bacillus anthracis*. Specifically, this project aims to: 1) develop screening methods for identification of tmRNA pathway inhibitors; 2) identify and optimize inhibitors from libraries of cyclic peptides; 3) test the bactericidal and plasmid elimination efficacy of lead compounds against *B. anthracis* and other pathogens; and 4) use high-throughput screening methods to identify small molecule inhibitors of the tmRNA pathway. Methods for identifying inhibitors of the tmRNA pathway have been developed and cyclic peptide inhibitors of 4 different steps in the tmRNA pathway have been selected and optimized. These inhibitors have antibiotic activity against *B. anthracis*, and are particularly potent when used in combination with the linear antibacterial peptide LL-37. Screening methods were adapted and optimized for high-throughput technology, and, through a collaboration with the Genomics Institute of the Novartis Research Foundation, 52 small molecule inhibitors were identified from a library of over 650,000 compounds. These small molecule inhibitors are potent antibiotics, with MIC values <0.2 mg/l.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Cheng L, Naumann TA, Horswill AR, Hong S-J, Venters BJ, Tomsho JW, Benkovic SJ, and Keiler KC (2007). "Discovery of antibacterial cyclic peptides that inhibit the ClpXP protease." *Protein Sci* 16: 1536-1542.

Number of Papers published in peer-reviewed journals: 1.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

K. C. Keiler (7/9/2006) “Sculpting the proteome with trans-translation” Gordon Research Conference on Microbial Stress Response, West Hadley, MA

Cheng, L. and K. C. Keiler (8/8/2007). “tmRNA in the genetic regulatory network.” Molecular Genetics of Bacteria and Phages, Madison, WI.

“Spatial and temporal control of tmRNA through development” Molecular Genetics of Bacteria and Phages, Madison, WI August 10, 2007

"tmRNA in the genetic regulatory network. Molecular Genetics of Bacteria and Phages, Madison, WI August 12, 2007

“Peptide signals for protein destruction and localization” Pennsylvania State College of Medicine, Hershey, PA October 15, 2007

“Peptide signals for protein destruction and localization” University of Illinois, Urbana, IL November 8, 2007

"Spatial and temporal control of tmRNA through development" Pennsylvania State University, University Park, PA December 2, 2007

“Peptide signals for protein destruction and localization” University of Alabama, Birmingham, AL January 22, 2008

"Temporal and spatial control of tmRNA and trans-translation" Ohio State University, Columbus, OH April 2, 2008

"Temporal and spatial regulation of trans-translation" Gordon Research Conference on Microbial Stress, South Hadley, MA July 9, 2008

"Random paths to many specific locations" Massachusetts Institute of Technology, Cambridge, MA July 12, 2008

"Jumping Ribosomes, Discontinuous Translation and Novel Antibiotics" University of Massachusetts Medical School, Worcester, MA 1/21/2009.

"Peptide signals for protein localization and destruction" University of California, Santa Barbara 4/9/2009.

Number of Presentations: 13.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

Number of Manuscripts: 0.00

Patents Submitted

U. S. Patent Application Serial No. 60/811,967 (pending) “Antibacterial and Plasmid Elimination Agents.” K. C. Keiler (50%) and S. J.

Benkovic (50%) PSU 2006-3177.

U. S. Patent Application Serial No. 60/914,129 (provisional) “Reporter Molecules.” K. C. Keiler (100%) PSU 2007-3320.

Patents Awarded

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Lin Cheng	1.00
Jay Russell	0.13
Xin Zhou	1.00
Nitya Ramadoss	1.00
FTE Equivalent:	3.13
Total Number:	4

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Songon An	1.00
Daniel Haeusser	1.00
Todd Naumann	1.00
FTE Equivalent:	3.00
Total Number:	3

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Kenneth Keiler	0.18	No
Stephen Benkovic		Yes
FTE Equivalent:	0.18	
Total Number:	2	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Sharon Li	
Elizabeth Bailey	
Nur Zafirah Zaidan	
Timothy Rohrbach	
Timothy Rogers	
FTE Equivalent:	
Total Number:	5

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	4.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	4.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	4.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):.....	3.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	3.00

Names of Personnel receiving masters degrees

<u>NAME</u>
Xin Zhou
Total Number: 1

Names of personnel receiving PHDs

<u>NAME</u>
Lin Cheng
Jay Russell
Total Number: 2

Names of other research staff

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	
Teresa Killick	0.66	No
Elisabeth Mahen	0.66	No
FTE Equivalent:	1.32	
Total Number:	2	

Sub Contractors (DD882)

Inventions (DD882)

List of Illustrations and Tables

Table 1: MIC values for cyclic peptide inhibitors against pathogenic bacteria.	2
Table 2: Small molecule inhibitors of <i>trans</i> -translation.	3

Figure 1: Co-antibiotic activity of IXP1.	5
---	---

Statement of the Problem Studied

The goal of this project is to develop lead compounds for antibiotic development targeted to the tmRNA pathway. These antibiotics are expected to have broad host specificity and will be assayed against pathogens of military interest, including *Bacillus anthracis*. Specifically, this project aims to: 1) develop screening methods for identification of tmRNA pathway inhibitors; 2) identify and optimize inhibitors from libraries of cyclic peptides; 3) test the bactericidal and plasmid elimination efficacy of lead compounds against *B. anthracis* and other pathogens; and 4) use high-throughput screening methods to identify small molecule inhibitors of the tmRNA pathway. Methods for identifying inhibitors of the tmRNA pathway have been developed and cyclic peptide inhibitors of 4 different steps in the tmRNA pathway have been selected and optimized. These inhibitors have antibiotic activity against *B. anthracis*, and are particularly potent when used in combination with the linear antibacterial peptide LL-37. Screening methods were adapted and optimized for high-throughput technology, and, through a collaboration with the Genomics Institute of the Novartis Research Foundation, 52 small molecule inhibitors were identified from a library of over 650,000 compounds. These small molecule inhibitors are potent antibiotics, with MIC values <0.2 mg/l.

Summary of the Most Important Results

Reporter strains for identification of inhibitors. Reporter strains were developed for degradation of tmRNA-tagged proteins by producing a variant of the fluorescent protein GFP with the tmRNA-encoded peptide at the C terminus (GFP-AANDENYALAA). Reagents were also engineered to identify inhibitors of the tmRNA pathway that act prior to proteolysis. Eight different sequences with the potential to target proteins to the tmRNA pathway were cloned at the 3' end of three different fluorescent proteins, GFP, YFP, and mCherry. The potential reporters were produced using three different promoters in wild-type *E. coli* and cells deleted for tmRNA. The best reporter was GFP with two tandem targeting sequences, a *trpA* transcriptional terminator followed by two proline codons. This reporter was adapted for high-throughput screening by replacing the gene encoding GFP with one encoding luciferase. This set of reporters allowed us to identify and characterize inhibitors of the tmRNA pathway.

Cyclic peptide inhibitors of proteolysis. Using the GFP-AANDENYALAA reporter, cyclic peptides that inhibit degradation of tagged proteins were isolated from two SICLOPPS libraries. Six peptides were synthesized and assayed in an in vitro reconstituted system to determine the efficiency of inhibition and the precise step in the molecular mechanism of proteolysis that was inhibited. The peptides acted at three distinct steps in the proteolytic pathway: binding of the substrate to the ClpXP protease, binding of the proteolytic adaptor SspB to the ClpX subunit, and translocation of the bound substrate into the ClpXP active site. Three of the isolated inhibitors had bactericidal activity when added to cultures of the model species *C. crescentus*, with the

most efficient inhibitor having a minimum bactericidal concentration (MBC) of 40 μ M. Details of these results were published in the journal *Protein Science* (Cheng et al., 2007). These inhibitors demonstrate that 1) inhibitors of multiple steps in the tmRNA pathway can be isolated using the technologies developed here; 2) inhibitors of the tmRNA pathway are bactericidal; 3) cyclic peptides that inhibit the tmRNA pathway have bactericidal activity, despite anticipated problems with transport across the bacterial membrane. More details antibiotic assays using these peptides are described below.

Cyclic peptide inhibitors of tagging. The Gfp-trpAt reporter was used to screen the SICLOPPS SGWX5 library for inhibitors of the tmRNA pathway, and 10 cyclic peptide inhibitors were identified. In principle, these inhibitors would include those that block proteolysis as well as tmRNA tagging. Therefore, the proteolysis inhibitors were eliminated by screening the peptides using the GFP-AANDENYALAA reporter. Three of the cyclic peptide inhibitors do not block proteolysis, and therefore are inhibitors of tmRNA tagging.

Antibiotic activity of cyclic peptide inhibitors. Four cyclic peptides that block different steps of the tmRNA pathway, XB, IXP1, ITM1, and ITM2, were tested for antibiotic activity against *Bacillus anthracis* using broth dilution assays. The MIC values were 400-1000 μ M. These cyclic peptides were tested for antibiotic activity against a panel of pathogenic bacteria (Table 1). MIC values <125 μ M were obtained for *M. tuberculosis*, *A. baumannii*, *B. pertussis*, *B. cepacia*, *S. marcescens*, *S. pneumoniae*, and *C. freundii*. These species are from a wide phylogenetic distribution of bacteria, and indicate that the isolated inhibitors have a broad specificity.

Table 1: MIC values for cyclic peptide inhibitors against pathogenic bacteria.

species	disease	MIC (μ g/mL)			
		cXB	cIXP1	cITM1	cITM2
<i>B. anthracis</i>	anthrax	250	250	250	250
<i>S. aureus</i>	skin infections	250	250	250	250
<i>M. tuberculosis</i>	tuberculosis	125 - 62.5	125 - 62.5	125 - 62.5	125 - 62.5
<i>A. baumannii</i>	wound infections	125 - 62.5	125 - 62.5	125 - 62.5	125 - 62.5
<i>S. marcescens</i>	wound infections	125 - 62.5	125 - 62.5	125 - 62.5	125 - 62.5
<i>S. pneumoniae</i>	bacterial meningitis	62.5	62.5	62.5	62.5
<i>P. aeruginosa</i>	respiratory infections	125	125	125	125
<i>H. influenza</i>	pneumonia	125	125	125	125
<i>B. cepacia</i>	pneumonia	125 - 62.5	125 - 62.5	125 - 62.5	125 - 62.5
<i>C. freundii</i>	bacteremia	62.5	62.5	62.5	62.5
<i>B. pertussis</i>	whooping cough	125 - 62.5	125 - 62.5	125 - 62.5	125 - 62.5
<i>E. faecium</i>	intestinal infections	>250	>250	>250	>250
<i>C. difficile</i>	colitis	>250	>250	>250	>250
<i>L. monocytogenes</i>	listeriosis	>250	>250	>250	>250
<i>Y. pestis</i>	plague	>250	>250	>250	>250
<i>F. tularensis</i>	tularemia	>250	>250	>250	>250
<i>K. pneumoniae</i>	nosocomial infection	>250	>250	>250	>250
<i>M. catarrhalis</i>	respiratory infection	>250	>250	>250	>250
<i>P. vulgaris</i>	wound infections	>250	>250	250	>250

Small molecule inhibitors of the tmRNA pathway. Based on the success of our screen for cyclic peptide inhibitors of the tmRNA pathway and on the ability of these inhibitors to kill pathogenic bacteria, we collaborated with the Genomics Institute of the Novartis Research Foundation to screen for small molecule inhibitors. To enable high-throughput screening, the tmRNA pathway reporters were adapted by replacing the *gfp* gene with *luc*, encoding firefly luciferase, and the assays were optimized for use in 1536-well plate format. The Luc-trpAt reporter produced a 20-fold dynamic range of luciferase activity, and was used by GNF to screen a library of 663,000 small molecule compounds. All the compounds in this library were predicted to have good pharmacological properties based on Lipinski parameters and other criteria used by GNF and Novartis. Initial hits from this screen were confirmed in duplicate in a dose-response format. 52 compounds produced >2-fold increase in luciferase activity at a concentration of 10 mM, and have been obtained from the supplier indicated in Table 2.

To confirm the hits from high-throughput screening, compounds were added to exponential phase cultures of *E. coli* producing GFP-trpAt and cells were observed by fluorescence microscopy to ensure that *trans*-translation was inhibited. All 52 compounds resulted in fluorescence from cells producing GFP-trpAt, indicating that the compounds inhibit *trans*-translation and do not specifically interfere with the luciferase reporter. To distinguish between inhibitors of tagging and inhibitors of proteolysis, compounds were screened using cells producing the GFP-tag reporter. 27 compounds resulted in fluorescence with GFP-tag, indicating that they are inhibitors of proteolysis (Table 2). 18 compounds resulted in no fluorescence with GFP-tag, indicating that they do not inhibit proteolysis and suggesting that they inhibit tagging. 7 compounds produced inconsistent results and need to be characterized further before an assignment can be made. These data strongly suggest that the set of 52 compounds contains a significant number of inhibitors of both tagging and proteolysis.

Table 2: Small molecule inhibitors of *trans*-translation.

SID	vendor	step inhibited ¹	MIC μ g/ml (μ M) ²	
			<i>S. flexneri</i>	<i>B. anthracis</i>
SID10959901	Life Chemicals	tagging	4.0 (12.5)	7.9 (25)
SID11111812	Chembridge	proteolysis	1.9 (6.25)	15.0 (50)
SID10366423	Life Chemicals		0.6 (1.56)	0.6 (1.56)
SID4926485	Chemdiv	tagging	0.8 (1.56)	12.5 (25)
SID4108743	Chemdiv	proteolysis		4.1 (12.5)
SID11111073	Chembridge	proteolysis		
SID10964929	Life Chemicals	tagging	12.0 (6.25)	0.5 (1.56)
SID5847032	Chemdiv	proteolysis		9.1 (25)
SID10973176	Life Chemicals	proteolysis	17.3 (50)	8.6 (25)
SID10959899	Life Chemicals	proteolysis		
SID11117914	Chembridge		44.3 (100)	11.1 (25)
SID4942270	Chembridge	proteolysis		
SID11110358	Chembridge	proteolysis	23.0 (100)	
SID4132366	Chemdiv	tagging		
SID11110927	Chembridge	tagging		
SID10964938	Life Chemicals	tagging	35.1 (100)	8.8 (25)
SID10961740	Life Chemicals		2.0 (6.25)	0.5 (1.56)
SID11022545	Asinex	proteolysis		35.6 (100)
SID10965015	Life Chemicals			
SID11111947	Chembridge	proteolysis		56.6 (200)
SID1097233	Chemdiv	tagging	50.0 (200)	49.9 (200)
SID4740973	Chemdiv	proteolysis		
SID4244454	Chemdiv			
SID10362497	Life Chemicals	tagging	9.0 (25)	0.6 (1.56)

SID11042239	Asinex	proteolysis		7.8 (25)
SID4741953	Chemdiv	proteolysis		
SID5942530	Chemdiv	proteolysis	11.0 (25)	0.7 (1.56)
SID10366424	Life Chemicals	tagging	1.2 (3.125)	0.3 (0.78)
SID10362524	Life Chemicals	tagging	17.3 (50)	8.7 (25)
SID922315	Asinex	tagging	14.4 (50)	7.2 (25)
SID5200115	Maybridge	tagging		
SID10961694	Life Chemicals	tagging	16.6 (50)	8.3 (25)
SID10961698	Life Chemicals	proteolysis	2.1 (6.25)	2.1 (6.25)
SID11025924	Asinex	proteolysis	57.5 (200)	7.2 (25)
SID10959935	Life Chemicals	proteolysis		8.3 (25)
SID10961733	Life Chemicals	proteolysis	1.8 (6.25)	1.8 (6.25)
SID11021538	Asinex	tagging	0.8 (3.125)	6.6 (25)
SID4856315	Chemdiv	proteolysis	27.7 (100)	
SID10961697	Life Chemicals		2.1 (6.25)	1.0 (3.125)
SID11030386	Asinex	proteolysis		
SID5427002	Asinex	proteolysis		8.3 (25)
SID5942533	Chemdiv	proteolysis	93.3 (200)	11.7 (25)
SID10961710	Life Chemicals	proteolysis	14.2 (50)	14.2 (50)
SID10994442	Chembridge	tagging		
SID4900455	Asinex	tagging		
SID10965069	Life Chemicals	tagging	32.0 (100)	
SID11103495	Chembridge	proteolysis		62.5 (200)
SID1115396	Chemdiv	tagging		5.0 (25)
SID11113620	Chembridge	proteolysis		
SID3324811	Life Chemicals	proteolysis	14.5 (50)	7.3 (25)
SID10959899	Life Chemicals	proteolysis		57.8 (200)

¹Reaction that is affected based on characterization with GFP-tag and GFP-trpAt reporters. Blanks indicate no determination has been made yet.

²Preliminary MIC values from broth microdilution assays. Values in parentheses are for the same assays, but in units of μ M. Blanks indicate no inhibition has been observed. Most numbers are derived from a single experiment.

To determine if *trans*-translation inhibitors have any antibiotic activity, we performed an initial MIC determination using *S. flexneri* 2a 2457T and *B. anthracis* Sterne. Both strains were grown to exponential phase in LB medium at 37°C and diluted to 5×10^5 cfu/ml (the concentration was estimated by optical density at 600 nm and confirmed by plating on LB). 50 ml culture was incubated with 2-fold serial dilutions of each compound, starting at a concentration of 200 mM, in microtiter trays. After incubation at 37°C for 20 h, the trays were visually inspected and the lowest concentration of compound that resulted in a well with no turbidity was scored as the MIC. 27 compounds inhibited growth of *S. flexneri* at <200 mM (Table 2). 7 compounds have MIC values <2 mg/ml, the MIC reported for nalidixic acid, an antibiotic used to treat *S. flexneri* infections. 34 compounds inhibited growth of *B. anthracis*, and although none of these had an MIC as low as ciprofloxacin, 6 have MIC values <1 mg/ml. These results indicate that some of the *trans*-translation inhibitors have antibiotic activity at concentrations that are favorable for drug development.

Co-antibiotic activity of protease inhibitors. The MIC values obtained with tmRNA inhibitors demonstrate that they can inhibit the growth of bacteria in culture, but several lines of evidence suggest that they might be even more effective during pathogenesis. tmRNA and ClpXP are essential in some species, but in many others they are required for virulence but not viability. In addition, inhibitors directed against other proteases decrease the ability of *B. anthracis* and multiple-resistant strains of *S. aureus* (MRSA) to infect and persist in the host. To determine if the cyclic peptide inhibitors of tmRNA and

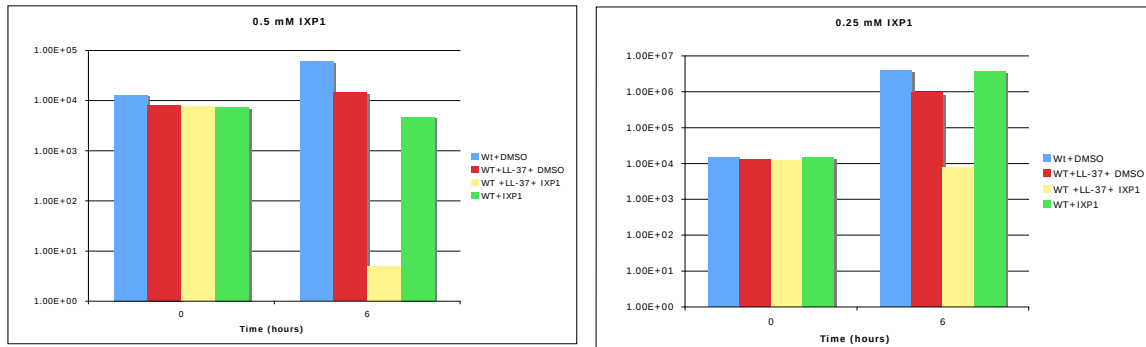


Fig. 1: Co-antibiotic activity of IXP1. *B. anthracis* was grown in the presence of sub-lethal concentrations of IXP1 or the human antimicrobial peptide LL-37, or both

ClpXP affect infection and clearance of *B. anthracis* and MRSA, we initiated a collaboration with Dr. Victor Nizet at the University of California, San Diego. Dr. Nizet is an expert on virulence of *B. anthracis* and MRSA. First, effective use of our ClpXP inhibitor IXP1 as a co-antibiotic against *B. anthracis* was demonstrated. The innate immune system attacks bacteria using antimicrobial peptides, but *B. anthracis* and *S. aureus* mount a defense against these antimicrobial peptides using extracellular peptidases. Production of active peptidases requires ClpXP, so inhibition of ClpXP by IXP1 was expected to make the cells more susceptible to killing by the innate immune system. In collaboration with the laboratory of Victor Nizet at UCSD, we have shown that IXP1 does, in fact, increase the effectiveness of the human antimicrobial peptide LL-37 (Fig. 1). These results indicate that IXP1 may be highly effective in vivo, because it will both inhibit growth of *B. anthracis* and promote killing by the innate immune system. Similar results were obtained with the small molecule inhibitor of proteolysis, SID11042239. Trials for clearance of *B. anthracis* and *S. aureus* are underway using neutrophil and murine model systems.