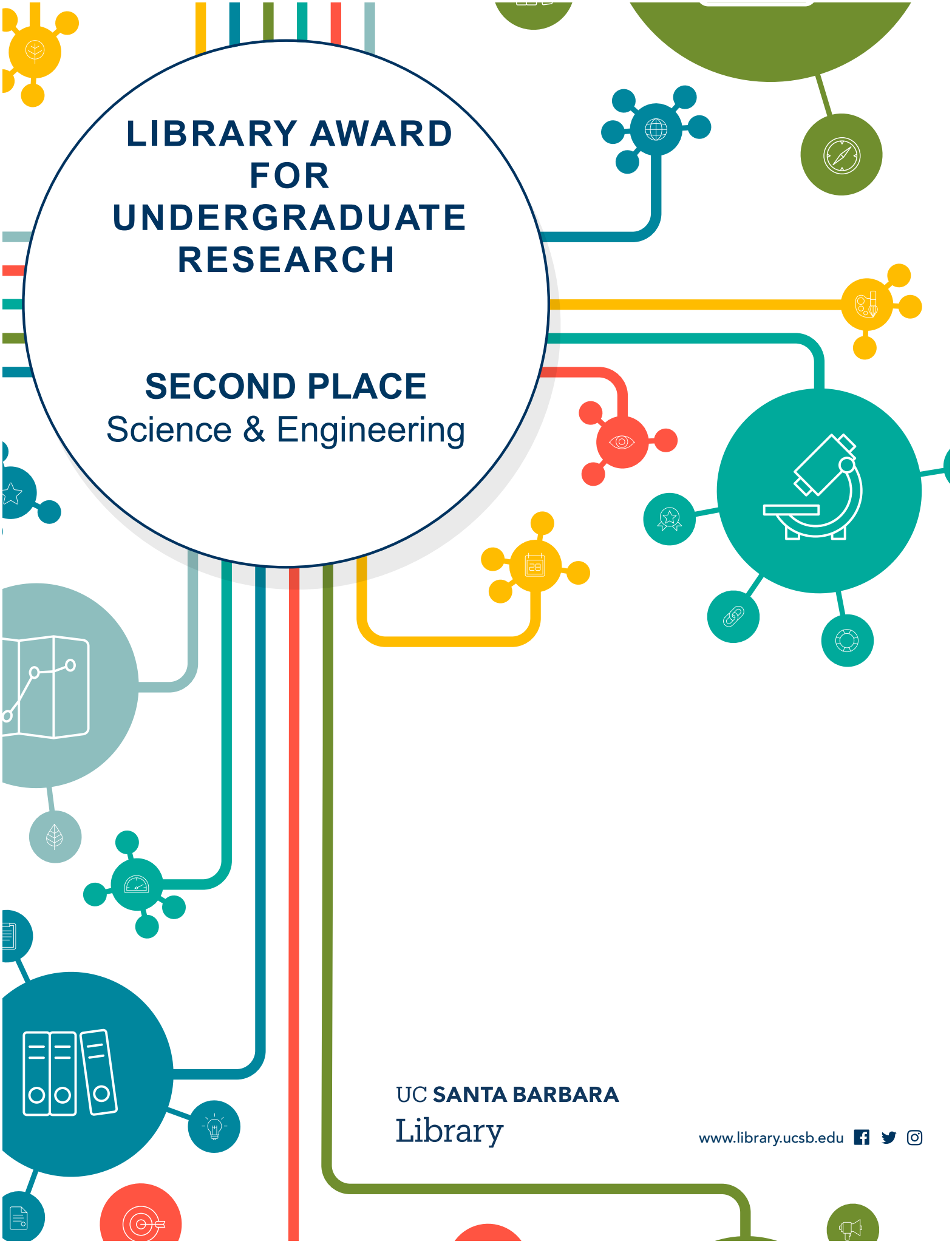
LIBRARY AWARD FOR UNDERGRADUATE RESEARCH

SECOND PLACE
Science & Engineering



UC SANTA BARBARA
Library

www.library.ucsb.edu

Jeffrey Rong

Reflective Essay

The lab I work in studies a process that some bacterial strains exhibit called contact-dependent inhibition, or CDI. CDI is a mechanism which some species of bacteria use to inhibit the growth of competing strains by binding to specific target receptors through the binding domains of extracellular CDI proteins. This binding triggers the release of C-terminal toxins into the target cells, inhibiting their growth.

In my research project, I have been working with a specific CDI mechanism found in *E. coli* EC93, called CdiA, in which I am assessing its binding ability to three conserved extracellular loops of BamA: Loop 4, Loop 6, and Loop 7. BamA is targeted because it is a conserved and essential outer membrane β-barrel protein found in gram-negative bacteria. Given how it is necessary for the viability of gram-negative bacteria, it is a reliable target for CDI. If CdiA is proven to be able to bind properly to the three extracellular loops of BamA through its binding domain, then the CdiA protein, especially its binding domain, can be used in phage therapy applications by fusing it to the hypervariable regions of the tail fibers of T2 phage. These chimera phages could potentially target pathogenic gram-negative bacteria by landing on BamA receptors. Since BamA is essential for viability, it would have a hard time acquiring sufficient resistance mutations.

This project has been going on for many months, as my lab is collecting the data that is necessary to further confirm the hypothesis that CdiA binds at a high affinity to the three indicated extracellular loops of BamA. Therefore, when I got started in the project, I was already given the help and resources I need to test the binding of CdiA to BamA.

During my time in undergraduate research, I had and have been presenting my research through poster presentations at colloquia and symposia, as stipulated by a research program I

am in called UC LEADS. This means that I must collect the data from my experiments and make sense of them by providing background information on CDI in general and providing a question that my lab is trying to answer. Fortunately, I was able to learn what to put on scientific posters and papers through the online library resources, which contain citation styles and guides on writing scientific papers and posters.

Given how I had to make posters for undergraduate research colloquia and symposia, I knew that I must obtain a list of references that support the details of my experiment and provide a background on why I am conducting this research project. I discovered some my cited sources from the UCSB library research databases, where I searched up published research papers that contained details related to m project, such as details on the BamA receptor, the CdiA protein, and further details on CDI in general. I also discovered some of my sources from my faculty mentor and the other members in my lab, as my lab have been working with different classes of CDI mechanisms, especially CdiA, for many years. Therefore, I was able to obtain as much information as possible to back up information on my posters and to help me understand what I am trying to discover, what I am working with, and what direction should I proceed towards.

As I spend time doing research as an undergraduate student, I gradually learn the process of discovering knowledge through scientific experiments and data collection. In addition, I've learned that going through the research process require the collection of previous research publications in order to provide background information that would help provide a guide for experiments and lay out a general direction for research projects. Using my lab's publications and the library resources, such as the research databases, help me understand why I am performing a certain experiment, which helps me brainstorm ideas for future project proposals. Combining knowledge from performing experiments, from reading and understanding referenced scientific publications, and from library resources also helps me properly

communicate my research contributions through scientific posters. I still have a lot to learn in my undergraduate research career and I will continue to learn through my lab, the library, and other resources.

Deducing the Binding Specificity and Affinity of CdiA to the BamA Receptor Protein

Jeffrey Rong, Natasha I. Edman, Cyril X. George, David A. Low
University of California, Santa Barbara
Department of Molecular, Cellular, and Devlopmental Biology

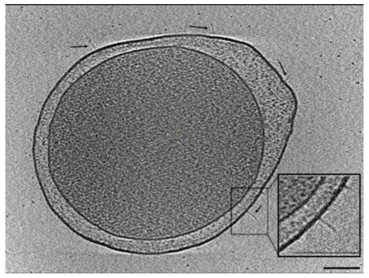
Abstract

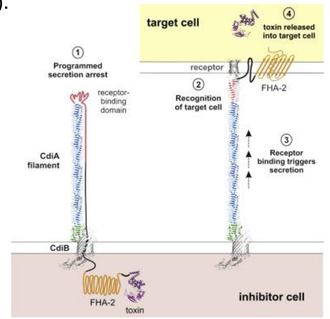
Contact-dependent inhibition (CDI) is a series of mechanisms that some bacteria use to inhibit competing bacterial strains. This allows them to better compete for resources. Many Cdi mechanisms have been discovered, such as CdiA in *E. coli* EC93. CdiA is used in this experiment to target BamA, an essential and highly conserved β-barrel protein found in gram-negative bacteria. Cdi's ability to inhibit target bacterial cells was tested through a growth competition assay between an inhibitor EC93 strain, DL4608, with a target strain with wild-type BamA, DL7282, and a target with a BamA$^{\Delta F675}$ mutation, DL8830. Through a baseline growth competition assay, there was inhibition of DL7282, but not for DL8830, since it may have mutated BamA receptors, confirming that CdiA binds to BamA and allows the inhibitor strain to inhibit the growth of the target strain. To further confirm CdiA binding to BamA, a fusion protein, MBP-CdiA, a competitive inhibitor to bacterial CdiA, was introduced. The protein slightly narrowed inhibition during competition assays, further confirming that CdiA binds effectively to wild-type BamA. The overall goal is to determine CdiA's minimum binding specificity and affinity to to BamA. Through these series of experiments, the data from this experiment will be compared to the binding ability of the J protein of Stxϕ24 phage, which binds to similar extracellular loops of BamA as CdiA. If proven to be a reliable ligand, CdiA will be fused to the tail fibers to T2 phage to possibly target the BamA receptors of pathogenic bacteria.

Introduction

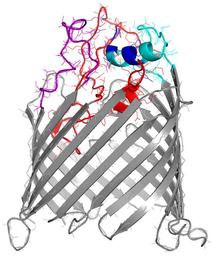
Contact-dependent inhibition (CDI) is a mechanism which some species of bacteria release toxins to other target bacterial species, inhibiting their growth through binding domains that recognize specific receptors of target cells (Ruhe et al. 2017). This binding allows toxins to be inserted into the target cells, inhibiting their growth (Ruhe et al. 2013).

Evidence of CDI has been discovered in a strain of *E. coli* called EC93, which uses a Cdi mechanism called CdiA. CdiA contains a C-terminal toxin that is injected into a target bacterial cell (Aoki et. Al 2010). When it recognizes and binds to a receptor through its receptor binding domain, the C-terminal toxin is ejected from the periplasm through the FHA-2 domain and then transferred to the target cell's periplasm (Ruhe et al. 2018).





- CdiA targets BamA, an essential outer membrane β-barrel protein
  - CdiA binds to the following extracellular loops: Loop 4, Loop 6, and Loop 7 (Aoki et al. 2008).
  - Mutations in either loops prevent CdiA from binding (Edman 2015)

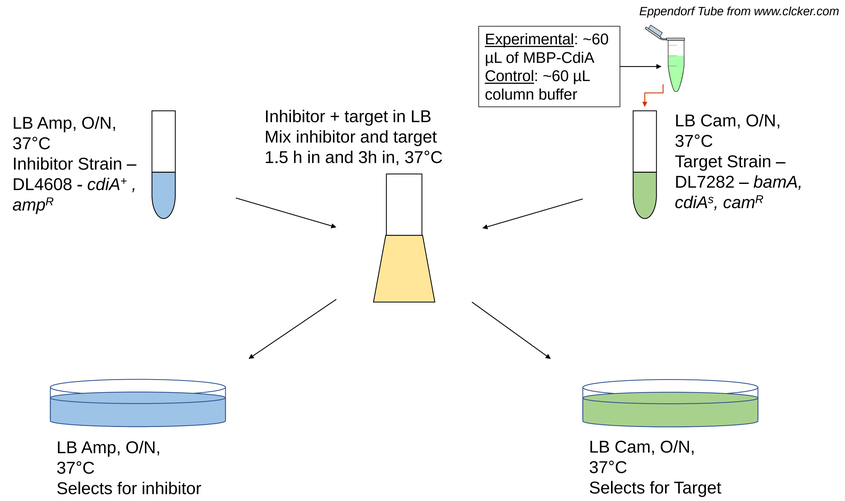


This computer-generated image of BamA via PyMol displays the three extracellular loops in which CdiA binds to: Loop 4 (cyan), Loop 6 (red), Loop 7 (purple), and mutated residues (blue).

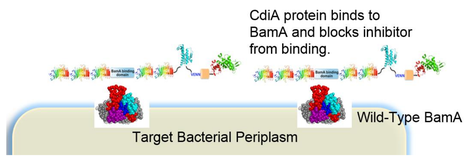
- BamA is targeted because it is a conserved and essential protein (Noinaj et al. 2013) [Malinverni & Silhavy 2011].
  - This makes it a viable target for CDI, allowing for more practical applications.

- Experiment: Test the ability of CdiA to target and inhibit BamA^{+} *E. coli* strains through growth competition assays between an inhibitor strain and two target strains.
  - Add fusion protein, MBP-CdiA, which will inhibit competition via competitive inhibition
  - If protein inhibited the CDI effect of the inhibitor, then it shows that CdiA binds effectively to BamA, even when it is not part of the bacterium.

Growth Competition Assay



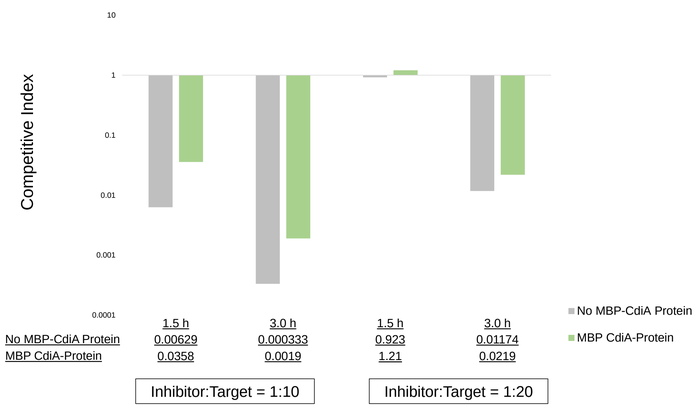
- **Rationale**:



$$Competitive\ Index = \frac{Target: Inhibitor\ at\ n\ Hours}{Targets: Inhibitor\ at\ Initial\ Time\ of\ Mixture}$$

Results

Competition Between Inhibitor and Target



	1.5 h	3.0 h	1.5 h	3.0 h
No MBP-CdiA Protein	0.00629	0.000333	0.923	0.01174
MBP CdiA-Protein	0.0358	0.0019	1.21	0.0219

- Theory: Judging by how the MBP-CdiA slightly blocks inhibition, it seems that CdiA does bind to BamA, even if it's not part of a bacterium.
- Note: More work needs to be done to verify the binding of the MBP-CdiA to BamA.

- Competition Between Inhibitor and BamA$^{\Delta F675}$ Target:
  - This was performed as a negative control.
  - Mutant Target: DL8830 *bamA$^{\Delta F675}$, cdiA^{R}, kan^{R}*
    - ΔF675: UV mutation on one of the phe residues on loop 6 of BamA (Edman 2015)
  - **CI = 2.457**
    - There is no inhibition of mutant target.
    - This shows how mutations in the loops of BamA affect CdiA binding.

Future Directions

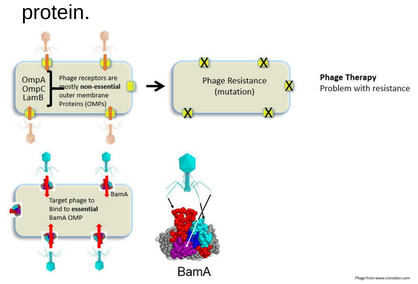
- The data from this assay will be used as a comparison to the binding of BamA by the J protein of Stxϕ24 phage, which is located on the phage's tail fiber (Fogg et al. 2007)
  - The J protein of Stxϕ24 phage recognizes similar protein markers on the extracellular loops of BamA, just like CdiA^{EC93}.
    - ∴ The mechanisms of the J protein can be used in phage therapy, providing antimicrobial applications (Chanisvili 2012).

Table 1. Effect of *bamA* mutations on CDI resistance and Stx phage resistance. *bamA^{EC}* = *E. coli bamA*; *bamA^{ECL}* = *Enterobacter cloacae bamA*; *bamA$^{ECL+EC\text{-}loop6}$* = *bamA^{ECL}* with loop 6 of *E. coli* BamA replacing loop 6 of *E. cloacae*.

***bamA* mutation**	**Loop affected**	**CDI^{EC93} phenotype**	**Stx$^{\Phi 24}$ phenotype**
bamA^{EC}	None	S	S
*bamA^{EC}*_V543N/W546C	4	R	R
*bamA^{EC}*_DR547/Y548H	4	R	R
*bamA^{EC}*_DA672/N681	6	R	R
*bamA^{EC}*_DF675	6	R	R
*bamA^{EC}*_Dloop4	6	R	R
*bamA^{EC}*_Dloop6	6	R	R
*bamA^{EC}*_Dloop7	7	R	R
bamA^{ECL}	None	R	R
bamA^{ECL}+EC-loop6	6	R	R
bamA^{ECL}+ EC-loop4/6	4,6	R	R
bamA^{ECL}+ EC-loop6/7	6,7	S	S

Phage from www.cronodon.com

  - Problem: Stxϕ24 phage is lysogenic (Fogg et al. 2007).
- To get around this, T2 phage with CdiA^{EC93} fused to the four hypervariable gp38 regions of its tail fibers will be used (Montag et al. 1987).
  - These lytic chimera phages can potentially bind to the extracellular loops (specifically Loop 4 and Loop 6) of Bam A through the fused CdiA protein.



- Potential Problem: The target bacteria can become resistant to the phage.
- Potential Solutions:
  - Find other extracellular regions of BamA that the phage can bind to.
  - Have the phage bind to Loops 4 and 6 and have another chimera phage or CDI protein potentially bind to other hypervariable loops of BamA.
    - Rationale: Double mutations are rare.

References

- Ruhe ZC, Subramanian P, Song K, Nguyen JY, Stevens TA, Low DA, Jensen GJ, Hayes CS. Programmed Secretion Arrest and Receptor-Triggered Toxin Export during Antibacterial Contact-Dependent Growth Inhibition. Cell. 2018; 175(4): 921-933. doi: 10.1016/j.cell.2018.10.033
- Edman NI. 2015. The Contact-Dependent Growth Inhibition Pathways of *Burkholderia pseudomallei* 1026b and *Escherichia coli* EC93 [Thesis] Department of Molecular, Cellular, and Developmental Biology: University of California, Santa Barbara. Chapter 2, Identification of BamA residues critical for interaction with CdiA^{EC93}; p. 30-55.
- Ruhe ZC, Low DA, Hayes CS. Bacterial Contact Dependent Growth Inhibition. Trends in Microbiology. 2013;21(5):230–237.
- Aoki SK, Pamma R, Hernday AD, Bickham JE, Braaten BA, Low DA. Contact-dependent inhibition of growth in Escherichia coli. Science. 2005;309(5738):1245-1248. doi: 10.1126/science.1115109.PubMed PMID: 16109881.
- Aoki SK, Diner EJ, de Roodenbeke CT, Burgess BR, Poole SJ, Braaten BA, Jones AM, Webb JS, Hayes CS, Cotter PA, Low DA. A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. Nature. 2010;468(7322):439-442. doi: 10.1038/nature09490.
- Aoki SK, Malinverni JC, Jacoby K, Thomas B, Pamma R, Trinh BN, Remers S, Webb J, Braaten BA, Silhavy TJ, Low DA. Contact-dependent growth inhibition requires the essential outer membrane protein BamA (YaeT) as the receptor and the inner membrane transport protein AcrB. Mol Microbiol. 2008;70(2):323-340. doi: 10.1111/j.1365-2958.2008.06404.x.
- Ruhe ZC, Nguyen JY, Xiong J, Koskiniemi S, Beck CM, Perkins BR, Low DA, Hayes CS. CdiA Effectors Use Modular Receptor-Binding Domains To Recognize Target Bacteria. MBio. 2017;8(2).doi: 10.1128/mBio.00290-17. PubMed PMID: 28351921; PMCID: PMC5371414.
- Noinaj N, Kuszak AJ, Gumbart JC, Lukacik P, Chang H, Easley NC, Lithgow T, Buchanan SK. Structural insight into the biogenesis of β-barrel membrane proteins. Nature. 2013;501(7467):385–390.
- Malinverni JC, Silhavy TJ. Assembly of Outer Membrane beta-Barrel Proteins: the Bam Complex. EcoSal Plus. 2011;4(2). doi: 10.1128/ecosalp
- Fogg PC, Gossage SM, Smith DL, Saunders JR, McCarthy AJ, Allison HE. Identification of multiple integration sites for Stx-phage Phi24B in the Escherichia coli genome, description of a novel integrase and evidence for a functional anti-repressor. Microbiology. 2007;153(Pt 12):4098-4110.doi: 10.1099/mic.0.2007/011205-0
- Chanishvili N. Phage therapy--history from Twort and d'Herelle through Soviet experience tocurrent approaches. Adv Virus Res. 2012;83:3-40. doi: 10.1016/B978-0-12-394438-2.00001-3.
- Montag D, Riede I, Eschbach M-L, Degen M, Henning U. Receptor-recognizing proteins of T-even type bacteriophages: Constant and hypervariable regions and an unusual case of evolution. Journal of Molecular Biology. 1987;196(1):165–174. doi: 10.1016/0022-2836(87)90519-5

Acknowledgements

- This overall project is made possible by the help from the NIH, the NSF, and the Gates Foundation.
- My work is made possible by Dr. Cyril George, who trained me and assisted me in this project, and Dr. David Low, who mentored me in the lab and provided me with the resources and support for developing my poster and my aptitude as a student researcher.
- I would like to give acknowledgements to the UC LEADS program for mentoring me and funding me for the summer and throughout the academic year.

Bibliography

Citation Format: CSE (Council of Science Editors)

RuheZC, Subramanian P, Song K, Nguyen JY, Stevens TA, Low DA, Jensen GJ, Hayes CS. Programmed Secretion Arrest and Receptor-Triggered Toxin Export during Antibacterial Contact-Dependent Growth Inhibition. Cell. 2018; 175(4): 921-933. doi: 10.1016/j.cell.2018.10.033

Edman NI. 2015. The Contact-Dependent Growth Inhibition Pathways of Burkholderia pseudomallei 1026b and Escherichia coliEC93 [Thesis] Department of Molecular, Cellular, and Developmental Biology: University of California, Santa Barbara. Chapter 2, Identification of BamA residues critical for interaction with CdiAEC93; p. 30-55.

Ruhe ZC, Low DA, Hayes CS. Bacterial Contact Dependent Growth Inhibition. Trends in Microbiology. 2013;21(5):230–237.

Aoki SK, Pamma R, Hernday AD, Bickham JE, Braaten BA, Low DA. Contact-dependent inhibition of growth in Escherichia coli. Science. 2005;309(5738):1245-1248. doi: 10.1126/science.1115109.PubMed PMID: 16109881.

Aoki SK, Diner EJ, de Roodenbeke CT, Burgess BR, Poole SJ, Braaten BA, Jones AM, Webb JS, Hayes CS, Cotter PA, Low DA. A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. Nature. 2010;468(7322):439-442. doi: 10.1038/nature09490.

Aoki SK, Malinverni JC, Jacoby K, Thomas B, Pamma R, Trinh BN, Remers S, Webb J, Braaten BA, Silhavy TJ, Low DA. Contact-dependent growth inhibition requires the essential outer membrane protein BamA (YaeT) as the receptor and the inner membrane transport protein AcrB. Mol Microbiol. 2008;70(2):323-340. doi: 10.1111/j.1365-2958.2008.06404.x.

Ruhe ZC, Nguyen JY, Xiong J, Koskiniemi S, Beck CM, Perkins BR, Low DA, Hayes CS. CdiA Effectors Use Modular Receptor-Binding Domains To Recognize Target Bacteria. MBio. 2017;8(2).doi: 10.1128/mBio.00290-17. PubMed PMID: 28351921; PMCID: PMC5371414.

Noinaj N, Kuszak AJ, Gumbar tJC, Lukacik P, Chang H, Easley NC, Lithgow T, Buchanan SK. Structural insight into the biogenesis of β-barrel membrane proteins. Nature. 2013;501(7467):385–390.

Malinverni JC, Silhavy TJ. Assembly of Outer Membrane beta-Barrel Proteins: the Bam Complex. EcoSal Plus. 2011;4(2). doi: 10.1128/ecosalp

Fogg PC, Gossage SM, Smith DL, Saunders JR, McCarthy AJ, Allison HE. Identification of multiple integration sites for Stx-phage Phi24B in the Escherichia coli genome, description of a novel integrase and evidence for a functional anti-repressor. Microbiology. 2007;153(Pt 12):4098-4110.doi: 10.1099/mic.0.2007/011205-0

Chanishvili N. Phage therapy--history from Twort and d'Herelle through Soviet experience tocurrent approaches. Adv Virus Res. 2012;83:3-40. doi: 10.1016/B978-0-12-394438-2.00001-3.

Montag D, RiedeI, Eschbach M-L, Degen M, Henning U. Receptor-recognizing proteins of T-even type bacteriophages: