BIOGRAPHICAL SKETCH

NAME: **Taylor Blackburn**

eRA COMMONS USER NAME (credential, e.g., agency login): TAYLORBLACKBURN

POSITION TITLE: Graduate Student

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Georgia Institute of Technology, Atlanta, GA	B.S.	12/2020	Biochemistry
Emory University, Atlanta, GA	Ph.D.	08/2026 (expected)	Chemistry

A. Personal Statement

My role in this NCCAT GUP3 proposal is to optimize sample and grid preparation that will enable the determination of a high-resolution structure of the HigB toxin – HigA antitoxin complex bound to its DNA repressive element. I am a Chemistry graduate student at Emory University in the Dunham laboratory and my PhD thesis focuses on understanding the activation and regulation of bacterial toxin-antitoxin systems.

The goal of this application is to obtain support and training at the New York Structural Biology Center to advance my project towards a Krios-ready sample. Access to the resources provided through the NCCAT GUP3 will aid my training in cryogenic electron microscopy and eventual transition to being an independent researcher. My educational, research, and personal experiences have led to these career ambitions. I aim to continue investigating biological questions at the molecular level throughout my career, and I am motivated to use the resources that a research-intensive institution offers to ensure that students from underrepresented backgrounds of all kinds are supported in the spaces where major biomedical breakthroughs happen. The GUP3 program would be an important milestone in my journey towards these goals and would help to support foundational research of antimicrobial resistance mechanisms.

I did not know any scientists or people with a Ph.D. when growing up in a rural town in southeast Georgia. I always excelled in school, but never found myself very intellectually stimulated given the resources at my disposal. This made it difficult for me to find my academic passions, until finally in my senior year of high school when I was able to enroll in the only AP Chemistry course to be offered at my high school. I enjoyed the class so much that I decided to study biochemistry in college because I wanted to learn how to apply chemical knowledge to biological systems. At the time, the only public school in Georgia offering a biochemistry degree was the Georgia Institute of Technology (Georgia Tech). I became the first student from my county to be accepted to Georgia Tech.

At Georgia Tech, I joined Dr. Amit Reddi's lab, and with the guidance of my graduate student mentor, I developed and implemented a research plan to build a FRET-based sensor system to examine mechanisms of heme insertion into hemoglobin (Hb). My strategy to fluorescently tag the alpha and beta chains of Hb to measure resonance energy transfer upon Hb maturation, which requires heme, allowed me to develop a strong background in molecular biology laboratory techniques and learn to work independently. This work showed me how fundamental research could contribute to biomedical breakthroughs because studying mechanisms of heme is essential to understanding how to develop therapeutics for diseases such as sickle-cell anemia. Although my undergraduate research was disrupted by the COVID-19 pandemic, I was still able to realize my aspirations to pursue a Ph.D.

To supplement my research skills before transitioning to graduate school, I applied to be a research technician and began working full-time in the lab of Dr. Pamela Peralta-Yahaya to further develop my skills as a scientist. The project I worked on consisted of engineering G protein-coupled receptor (GPCR) based chemical sensors for highly specific detection of chemical environments – specifically, cancer tumor environments. The skillset I had developed under the guidance of my undergraduate research mentor allowed me to excel and work

independently within only a few weeks of being a technician where I applied synthetic biology techniques. After just five months of working, I also trained a post-baccalaureate researcher in the lab. This experience again demonstrated to me how academic research can benefit mankind and gave me a deeper understanding of the importance of mentorship at research-intensive institutions. During this time, I received and accepted an offer to attend Emory University to pursue my Ph.D. in chemistry.

After my graduate school laboratory rotations, I joined a lab for my dissertation research, but the P.I. abruptly left the school and I was tasked with finding a new home for my thesis research. I joined Dr. Christine Dunham's (faculty nominator) lab and began to study mechanisms of bacterial toxin-antitoxin (TA) systems and how these genetic modules contribute to antibiotic resistance and persistence. While I had not done protein biochemistry or structural biology prior to starting this project, my strong background in molecular and synthetic biology techniques have aptly prepared me to pursue this venture. Dr. Dunham is an accomplished structural biologist and biochemist, and her lab provides a supportive environment to conduct my thesis research and numerous opportunities to learn about antimicrobial resistance. Bacterial persistence has been identified as a precursor and promoter of antimicrobial resistance, and my thesis project focuses on characterizing the mechanisms of TA system that lead to bacterial persistence and antibiotic tolerance. By biochemically and structurally characterizing TA system mechanisms, the long-term goal of this work is to identify novel antimicrobial targets that precede and circumvent the development of resistance. Further, the scope of this project will allow me to learn a variety of techniques, share our findings in papers and at conferences, and incorporate the mentorship of an undergraduate student.

With the support of my research lab, mentor, and NCCAT, I will be able to continue building a solid foundation for my scientific career goals.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

July 2022 – Present	Graduate Research Assistant, Dept. of Chemistry, Emory University, Atlanta, GA (Dunham Laboratory)
Jan 2022 – July 2022	Graduate Research Assistant, Dept. of Chemistry, Emory University, Atlanta, GA (Heemstra Laboratory)
Jan 2021 – Aug 2021	Research Technician, Dept. of Chemistry and Biochemistry, Georgia Tech, Atlanta, GA (Peralta-Yahya Laboratory)
Aug 2019 – Dec 2020	Undergraduate Research Assistant, Dept. of Chemistry and Biochemistry, Georgia Tech, Atlanta, GA (Reddi Laboratory)

Honors

2023 – 2025	Antimicrobial Resistance and Therapeutic Discovery Training Program T32
March 2023	NSF GRFP Honorable Mention
Aug 2021	Quayle New Student Award, Emory University
Spring 2020	President's Undergraduate Research Award, Georgia Tech
Fall 2020	President's Undergraduate Research Award, Georgia Tech
Fall 2020	Faculty Honors, Georgia Tech
Spring 2019	Dean's List, Georgia Tech
2018	Dean's List, Georgia Tech
Spring 2017	Dean's List, Georgia Tech
2016 – 2020	Zelle Miller Scholarship, Georgia Tech

C. Contributions to Science

1)Mechanisms of bacterial persistence

My graduate research in the laboratory of Dr. Christine Dunham focuses on biochemically and structurally characterizing the function of the PasTI TA system in uropathogenic *E. coli* (UPEC). This TA system is believed

to promote bacterial persistence and contribute to recurrent urinary tract infections. Persistence arises in a small subset of bacterial populations that are genetically identical to the antibiotic-susceptible parent population. These subsets of cells, called persisters, survive antibiotic exposure by exerting an environmentally influenced phenotypic change. Persisters slow or even halt their cellular growth, thereby evading bactericidal drugs. Once the environmental stressor is removed, persister cells repopulate and their progeny exhibit a similar ratio of susceptible to persistent cells as the parent population. Further treatment is likely to repeat this heterogeneous survival response leading to recurrent infections. TA systems are two-component genetic modules consisting of a toxin protein that inhibits cellular growth and a cognate antitoxin (protein or RNA) that suppresses this toxicity.

In pursuit of biochemically characterizing the PasTI mechanism of action, I have shown that the N-terminus of the toxin PasT is capable of binding to 70S bacterial ribosomes via fluorescence polarization assays. This is consistent with previously published data showing that the N-terminus is vital for the toxic function of PasT (Norton, 2012) and data showing that an orthologous protein is capable of inhibiting protein synthesis (Zhang, 2011). Further, in collaboration with the Mulvey lab at the University of Utah, we have demonstrated via cellular growth curves that UPEC PasT can be changed to a nontoxic version by mutation of residues in the N-terminal domain. Mutating the 6th and 11th residues results in growth comparable to strains complemented with empty vector. Additionally, the reverse outcome is seen when a nontoxic version of PasT found in Salmonella typhimurium is mutated at the same residues.

To structurally characterize PasTI, I subcloned constructs of the PasTI proteins in expression vectors to use for purification of the proteins. These constructs consist of proteins with N- or C-terminal cleavable 6x-His tags, as previous work has shown that modification of either terminus can lead to altered function (Norton, 2012). I have already purified PasT and *E. coli* ribosomes. My next steps are to incubate the proteins with bacterial ribosomes to prepare cryo-grids for solving the structure of PasTI bound to the ribosome via cryogenic electron microscopy (cryo-EM).

i)*Oral Qualifying Exam Presentation:* Persistent Resistance: Examining the Macromolecular Role of PasTI in Bacterial Persistence. (Passed: 03-01-2023)

ii)*Third Year Milestone:* 3 pre-proposals were submitted – “Glyoxal-Caged DNA Nanotubes for Temporally Controlled Drug Release”, “Characterizing tRNA Repair Enzymes in *Mtb*”, and “Exploring the Regulation of Ribosome Rescue”. (Passed 03-05-2024)

iii)*Fourth Year Milestone:* Proposal entitled “Characterizing a tRNA Repair Enzyme in *Mycobacterium tuberculosis*”. (Passed 12-10-2024)

2)GPCR-based chemical sensor system

I worked as a research technician for a project focused on developing new G-protein coupled receptor (GPCR) based multi-sensor systems. Considering that nearly 35% of FDA approved drugs target a GPCR, engineering sensors to detect their activity is important towards pharmaceutical development. I designed, built, and tested dozens of dual-luciferase luminescent sensors in *E. coli* and *S. cerevisiae*. I built these constructs in high-copy number plasmids and used a commercially available luminescence assay to test the sensors’ output upon incubation with their respective GPCRs chemical targets. Results indicated that the use of a dual nano- and firefly-luciferase reporter system showed measurable luminescence upon chemical activation of GPCRs. This work will contribute to the publishing of a future research article.

i)*Mentorship/training of post-baccalaureate researcher:* I trained a post-baccalaureate researcher in molecular biology techniques and data analysis for 4 months before my departure to begin graduate school.

3)Mechanisms of heme acquisition by hemoglobin

As an undergraduate researcher, I developed and implemented a strategy to fluorescently tag the alpha and beta chains of Hb to measure resonance energy transfer upon hemoglobin maturation. Despite the tremendous importance of Hb as an oxygen carrier for much of animal life, it is not known how heme is inserted into Hb. To answer this fundamental biological question, I devised a strategy to make a fluorescent Hb reporter that would fluoresce upon heme binding. Such a reporter, in combination with genetic screens, could reveal proteins and pathways responsible for the proper hemylation of Hb. While this work was performed under the guidance of my graduate student mentor and principal investigator, I was given independence on this project from conception to implementation. To develop a detailed experimental procedure, I conducted thorough literature research with the NCBI database. The goal became to fluorescently tag the alpha and beta chains of Hb with two different

fluorescent proteins, eGFP and mKate2 (respectively), to measure resonance energy transfer upon Hb maturation, which requires heme. Towards this end, I designed and cloned labeled Hb constructs into expression plasmids to test the sensors in yeast, a simple model organism in which human globins have previously been expressed and heme levels can be finely controlled. Due to the pandemic, my research stalled, but I nonetheless successfully expressed the control and test constructs in yeast, and I was able to conduct fluorescence measurements to determine the degree to which these sensors have heme dependent responsiveness. My research strategy for this project won the Georgia Tech President's Undergraduate Research Award two separate times.

i)*Final Undergraduate Project Report:* How does hemoglobin acquire heme? The development of new fluorescent biosensors to probe the mechanisms underlying the insertion of heme into hemoglobin.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	Emory University (GRADUATE) GPA 3.83	
2021	Directed Study (Laboratory Rotations)	S
2021	Biomolecular Chemistry	A
2021	Physical Biochemistry	A
2021	Jones Program in Ethics: Core Class	S
2021	Jones Program in Ethics: Dangers of Invisible Privilege	S
2021	Jones Program in Ethics: When the Law is Not Enough	S
2021	Jones Program in Ethics: Turbulent Futures Social Change	S
2021	Graduate School TATTO Workshop	S
2021	Teaching Assistantship	S
2022	Advanced Biophysical Chemistry	B+
2022	Thesis Research	S
2022	Special Topics: Systems Chemistry	A
2022	Jones Program in Ethics: Your Health is Your Wealth	S
2022	Teaching Assistantship	S
2022	Directed Study	S
2022	Thesis Research	S
2022	Values in Science	S
2022	Becoming a Successful Scientist	S
2023	Thesis Research	S
2023	Research & Evaluation in Chemistry	S
2023	Hypothesis Design & Writing	A-
2023	Thesis Research	S
2023	Thesis Research	S
2024	Third Year Milestone	S
2024	Thesis Research	S
2024	Principles of Anti-Infectives	A
2024	Thesis Research	S
	Georgia Tech (UNDERGRADUATE) GPA 3.52	
2020	Science and Technology in Latin America	A
2020	Research Assistantship	V
2020	Biochemistry Lab II	A
2020	Bioethics	A
2020	Medical Microbiology	A
2020	Environmental Issues in Latin America	A
2020	Research Assistantship	V

2020	Biochemistry Lab I	A
2020	Organic Chemistry Lab	A
2020	Microbiology	A
2019	Cancer Biology/Technology	A
2019	Analytical Chemistry	A
2019	Research Assistantship	V
2019	Business Spanish I	A
2019	Science of Physical Activity & Health	A
2019	Intro to Media Computation	A
2019	Intro Physics II	C
2019	Quantitative Analysis	A
2019	Biochemistry II	B
2019	Biophysical Chemistry	A
2019	Bilingualism in the Spanish World	A
2018	Intro to Organismal Biology	B
2018	Physical Chemistry I	B
2018	Biochemistry I	B
2018	Conversational Spanish I	A
2018	Biological Principles	A
2018	Organic Chemistry II	B
2018	Synthesis Lab I	B
2018	Undergraduate Research	A
2018	Intermediate Spanish II	A
2017	Residence Life Seminar	S
2017	Organic Chemistry I	C
2017	Intro to Computing	W
2017	Undergraduate Research	A
2017	Intermediate Spanish I	A
2017	Chemical Principles II	B
2017	Special Topics: Your Idea Your Invention	A
2017	Intro to Linear Algebra	B
2017	General Psychology	A
2016	Honors Biological Principles	W
2016	Chemical Principles I	C
2016	GT Freshman Seminar	A
2016	Integral Calculus	C
2016	Government of the U.S.	A

Courses at Emory University are graded from an A+ – F scale, with non-letter graded courses offered for Satisfactory/Unsatisfactory (S/U) credit. IP stands for incomplete while passing. Any course taken for S/U credit does not have a letter grade option but requires a B or above to pass.

Courses at Georgia Tech are graded on a 4.00 grading scale (A = 4.00; B = 3.00; C = 2.00; D = 1.00; F = 0.00), and where W = Withdrew from course, S = Satisfactory completion, V = Audit.

BIOGRAPHICAL SKETCH

NAME: **Christine M. Dunham, Ph.D.**

eRA COMMONS USER NAME (credential, e.g., agency login): cmdunham

POSITION TITLE: Professor of Chemistry

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Barnard College, Columbia University, New York, NY	B.A.	09/1993	05/1997	Biochemistry
University of California, Santa Cruz, CA	Ph.D.	09/1997	06/2003	Structural Biology
MRC Laboratory of Molecular Biology, Cambridge, UK	Postdoc	01/2004	04/2008	Structural Biology

A. Personal Statement

My role in this NCCAT GUP3 proposal is to identify conditions that will enable us to determine a high-resolution structure of the HigB toxin - HigA antitoxin complex bound to its DNA repressive element.

The research conducted in my laboratory focuses on the regulation and dysregulation of bacterial gene expression, building from my training in this field as a graduate student in the laboratory of Dr. William G. Scott (at the University of California, Santa Cruz) and then as an American Cancer Society Postdoctoral Fellow in the laboratory of Dr. Venki Ramakrishnan at the MRC Laboratory of Molecular Biology in Cambridge, England. In my independent research group at Emory University, my lab studies the structure, function, and regulation of the bacterial ribosome. Over the course of fifteen years, I have expanded my research interests to include projects centered on the molecular basis for how stress regulates translation and the roles of toxin-antitoxin pairs in inhibiting protein synthesis to alter bacterial physiology using molecular biology, biochemistry, X-ray crystallography and single-particle cryo-electron microscopy (cryo-EM). The awards noted below currently fund these active research projects in my lab.

Research support

NIH/NIGMS R35GM156629 Dunham (PI) 06/01/2025 – 05/31/2030
Ribosome regulation, inhibition, and quality control mechanisms

NIH/NIAID R01 AI088025; Conn*, Dunham (MPI), *No Cost Extension (pending)* 06/01/2020–05/30/2025
RNA modification and antibiotic resistance.

NIH/NIGMS R01 GM121650; Dunham, Keiler* (MPI) 08/24/2022–08/23/2026
Ribosome rescue.

NIH/NIAID R01 AI185192, Conn, Dunham*, Keiler (MPIs) 06/07/2024–06/06/2029
Regulation and mechanism of RND-mediated antibiotic efflux in Pseudomonas.

NIH/NIAID R01 AI158706-01A1; Baugh, Keiler (MPI), Dunham (co-I) 11/01/2021–10/31/2026
Targeting trans-*translation to kill* M. tuberculosis *non-replicating persister cells.*

Pew Innovation Fund Investigators, Barna, Dunham (MPIs) 12/01/2024 – 11/31/2027
A small molecule screen to alleviate lethal disease

Burroughs Wellcome Fund, Dunham (PI) 07/01/2016 – 06/30/2026
Investigator in the Pathogenesis of Infectious Diseases 1015487
Characterization of Pathways involved in Bacterial Persistence and Antibiotic Resistance

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023–present Professor, Dept of Chemistry, Emory University, Atlanta, Georgia.

2021–2023 Professor, Dept of Biochemistry, Emory University School of Medicine, Atlanta, Georgia.
2017–2021 Associate Prof, Dept of Biochemistry, Emory University School of Medicine, Atlanta, Georgia.
2008–2016 Assistant Prof, Dept of Biochemistry, Emory University School of Medicine, Atlanta, Georgia.
2004–2008 American Cancer Society Postdoctoral Fellow, MRC Laboratory of Molecular Biology, Cambridge, UK. Advisor: Group Leader Venki Ramakrishnan.
2004 Medical Research Council Career Development Fellow, MRC Laboratory of Molecular Biology, Cambridge, UK. Advisor: Group Leader Venki Ramakrishnan.
1996 NSF Summer Undergraduate Research Fellow, University of Texas Medical Branch at Galveston. Advisor: Professor Bennett Van Houten.
1994–1995 NSF Summer Undergraduate Research Fellow, Albany Medical College, Albany, New York. Advisor: Professor Peter Weber.

Other Experience, Service and Professional Memberships

2023–present Editorial Board Member, *Nucleic Acids Research*
2023–present Chair, Awards Committee, American Society of Biochemistry and Molecular Biology (ASBMB)
2022–present Chair, Awards Committee, RNA Society
2020–present Publications Committee, American Society of Biochemistry and Molecular Biology (ASBMB)
2018–present Editorial Board Member, *Journal of Biological Chemistry*
2018–present Editorial Board Member, *Molecular Microbiology*
2018–2023 NIH Permanent Study Section Member, Molecular Genetics A (MGA)
2016 Faculty mentor, GRC Microbial Stress Responses, Mt Holyoke, MA.
2015 2016 Conference Organizing committee, ASBMB, San Diego, CA.
2013 Pew Charitable Trusts 2014 Conference organization committee, Chile.
2012 Session chair, “Supramolecular Assemblies”, American Crystallographic Association conference, Honolulu, HI.
2011 Conference organizing committee, Suddath symposium on the Ribosome, Institute for Bioengineering & Bioscience, Georgia Tech, Atlanta, GA.
2008–present Manuscript reviewer: *Nature, Science, PNAS, Cell, Molecular Cell, Nucleic Acids Research*, *Structure*, *J. Biol. Chem., Biochemistry*, *Biophysical Journal, Molecular Microbiology, Nature Structure & Molecular Biology*, *Journal of Bacteriology*, *Journal of American Chemistry Society*, *RNA*, *PLoS Genetics, Scientific Reports, Nature Chemical Biology, PLoS ONE.*
2008–present Temporary grant reviewer/study section: NIH K99 Pathways to Independence Awards study section, Macromolecular Structure and Function C (MSFC) grant study section, ZRG1 Biological Chemistry and Macromolecular Physics; American Heart Association, Basic Cell Protein and Crystallography grant study section; NSF, Division of Molecular and Cellular Biosciences, CAREER, Graduate Student Research Fellowship study sections; American Cancer Society, RNA Mechanisms of Cancer grant study section.
2001–present RNA Society (since 2005), American Crystallographic Association (since 2001), Biochemical Society UK (2004-2007), American Society for Microbiology (ASM; since 2008), and The American Society for Biochemistry and Molecular Biology (ASBMB; since 2011).

Awards/Honors

2022 American Society of Biochemistry and Molecular Biology (ASBMB) fellow
2022 Kavli Fellow, National Academy of Sciences
2021 Emory School of Medicine Innovation for Impact Award
2021–2022 Chair, Molecular Genetics A (MGA) Study Section
2018–2022 NIH Permanent Study Section Member, Molecular Genetics A (MGA)
2018 Cozzarelli Prize, National Academy of Sciences, Best Biological Sciences paper
2018 American Society of Biochemistry and Molecular Biology (ASBMB) Young Investigator
2017 American Crystallographic Association Etter Early Career Awardee
2016–2021 Burroughs Wellcome Investigator in the Pathogenesis of Infectious Diseases
2011–2015 Pew Scholar in the Biomedical Sciences
2010–2015 NSF Early Career Development (CAREER) Award
2003 Best Poster Prize, Gordon Research Conference on Nucleic Acids (Ph.D.)
1999–2003 NSF-GAANN Graduate Research Fellowship

C. Contributions to Science

Complete list of publications in My NCBI:
https://www.ncbi.nlm.nih.gov/myncbi/1hI1rm7vAMckt/bibliography/public/

1. *Antibiotic Resistance Mechanisms*. Modifications to ribosomal RNA (rRNA) and proteins can fine tune protein synthesis or, in other cases, offer a route to antimicrobial resistance in pathogenic bacteria. In the latter case, in collaboration with the Conn lab (MPI of this proposal), we determined the molecular basis for recognition of a complex RNA tertiary structure within the context of the intact 30S subunit by a pathogen-associated aminoglycoside-resistance rRNA methyltransferase (**a**). These studies were the first of a modification enzyme bound to a ribosome and helped rationalize why an intact 30S subunit was required for recognition by this family of antibiotic-resistance enzymes. We further characterize interactions of a different enzyme family and showed the diverse macromolecular recognition by divergent family members (**b**). Modification by *Mycobacterium tuberculosis* ribosomal RNA methyltransferase TlyA requires rRNA modification and we studied the molecular basis for this recognition (**c**) and the role that rRNA modifications play in preordering the capreomycin drug binding site (**d**).

a. Dunkle JA, Vinnal K, Desai PM, Zelinskaya N, Savic M, West DM, Conn GL[#], Dunham CM[#] (2014) Molecular recognition and modification of the 30S ribosome by the aminoglycoside-resistance methyltransferase NpmA. *Proc. Natl Acad. Sci. USA* 111(17):6275-80. PMCID: PMC4035980. [#]Co-corresponding authors.
b. Srinivas P, Nosrati M, Zelinskaya N, Dey D, Comstock LR, Dunham CM[#], Conn GL[#] (2023) 30S subunit recognition and G1405 modification by the aminoglycoside-resistance 16S ribosomal RNA methyltransferase RmtC. *Proc. Natl Acad. Sci. USA.* 120(25):e2304128120 PMID: 37307464. [#]Co-corresponding authors. BioRxiv: doi: 10.1101/2023.03.13.532395.
c. Laughlin ZT, Dey D, Zelinskaya N, Witek MA, Srinivas P, Nguyen HA, Kuiper EG, Comstock LR, Dunham CM, Conn GL (2022) 50S subunit recognition and modification by the *Mycobacterium tuberculosis* ribosomal RNA methyltransferase TlyA. *Proc. Natl Acad. Sci. USA* 119(14):e2120352119 PMCID:PMC9168844. BioRxiv: doi: 10.1101/2021.11.11.467980.
d. Nandi, S., Dey, D., Srinivas, P., Dunham, C.M. and Conn, G.L. (2024). Distant ribose 2'-O-methylation of 23S rRNA Helix 69 pre-orders the capreomycin drug binding pocket at the ribosome subunit interface. *bioRxiv*. doi: 10.1101/2024.11.05.619916. (in revision at *Nucleic Acids Res*.)

2. *Novel inhibitors of bacterial* trans*-translation.* Bacterial ribosomes commonly encounter defective mRNAs that lack stop codons and require rescue to avoid cell death due to accumulation (>5-10%) of non-functional “stalled” complexes. Ribosome are rescued using *trans*-translation, a conserved mechanism mediated by the tmRNA-SmpB complex. *trans*-Translation is essential and bacteria specific, and therefore represents an excellent novel antimicrobial target. The Keiler laboratory (MPI of this proposal) has identified new antimicrobials that specifically inhibit *trans*-translation components at different stages of their interactions with the ribosome, but with no effect on normal translation. This specificity may also allow for fewer opportunities for these molecules to inhibit eukaryotic translation, resulting in low or no toxicity. In collaboration with the Keiler lab, we determined the molecular basis of action of one identified compound, MBX-4132, that inhibits ribosome rescue. Specifically, we solved a cryo-EM structure of the drug bound to a stalled ribosome, revealing that MBX-4132 binds near the peptidyl transferase center (PTC) of the ribosome adjacent to other PTC-binding antibiotics but adopts a distinct mechanism given its unique mechanism of action (**a**). Further, we wrote a review on recent structural insights into *trans*-translation (**b**). Additional molecules were identified that inhibit *trans*-translation but do not bind the ribosome but instead target translation factors. Compound KKL-55, for example, binds to EF-Tu, the translation factor that brings both tRNAs and tmRNA to the ribosome (**c**). We determined the structure of KKL-55 bound to EF-Tu and identified a novel binding site distinct from where other antibiotics bind suggesting a novel mechanism of action. Binding of KKL-55 prevents EF-Tu from binding to tmRNA but not tRNA providing a molecular basis for its specific action against *trans*-translation.

a. Aron ZD*, Mehrani A*, Hoffer ED*, Connolly KL, Torhan MC, Alumasa JN, Srinivas P, Cabrera M, Hosangadi D, Barbor JS, Cardinale S, Kwasny S, Butler M, Opperman T, Bowlin T, Jerse A, Stagg SM, Dunham CM[#], Keiler KC[#] (2021) Ribosome rescue inhibitors clear *Neisseria gonorrhoeae in vivo* using a new mechanism. *Nature Commun.* 12(1):1799. PMCID: PMC7979765. BioRxiv: https://doi.org/10.1101/2020.06.04.132530. [#]Co-corresponding authors.
b. Srinivas P, Keiler KC[#], Dunham CM[#] (2022) Druggable differences: Targeting mechanistic differences between *trans*-translation and translation for selection antibiotic action. *BioEssays* 44(8):e2200046, PMCID:PMC9308750. [#]Co-corresponding authors.
c. Marathe N*, Nguyen HA*, Alumasa JN, Kuzmishin Nagy AB, Vazquez M, Dunham CM[#], Keiler KC[#]

(2023) Antibiotic that inhibits *trans*-translation blocks binding of EF-Tu to tmRNA but not to tRNA. *mBio* e0146123. doi: 10.1128/mbio.01461-23. bioRxiv: doi: 10.1101/2023.06.09.544387. [#]Co-corresponding authors.

3. *Dysregulation of ribosomal function.* Biological fitness is critically dependent upon the accurate flow of genetic information. Although proofreading mechanisms exist, errors still occur during protein synthesis and this breakdown in translational fidelity is detrimental to cells. Our goal is to determine how protein synthesis is influenced by RNA modifications and specific tRNA-mRNA pairings that lead to defects in protein synthesis. We first focused on understanding how tRNA modifications controls the three-nucleotide mRNA frame using factors identified in genetic suppressor studies or through naturally occurring defects and/or mutations. We solved determined how different mRNA frameshift-prone tRNAs interact with the 70S ribosome providing an alternative model for how tRNAs facilitate a change in the mRNA reading frame. We discovered that tRNA modifications help to maintain the mRNA frame and how their absence results in the ribosome losing its grip on the tRNA (**a**,**b**). Related to frameshift errors are miscoding errors and more specifically, why specific mRNA-tRNA pairs are more prone to miscoding despite many structures solved that show very few differences between the decoding of cognate compared to mismatched mRNA-tRNA pairs. We took a different approach and studied the biochemically well-characterized $tRNA^{Ala}$. We found that the ribosome identifies correct from incorrect mRNA-tRNA pairings by directly interacting with the anticodon stem of correct pairs (**c**). These studies provide insight, for the first time, into how tRNA stability and recognition by the ribosome can lead to accurate decoding. Another important question in gene expression is how ribosome quality control mechanisms are activated when incorrect mRNA-tRNA pairing occurs (**d**). Some pairings are commonly incorporated by the ribosome that cause a loss of ribosome fidelity at the adjacent decoding center. We determined that single mismatch mispairings that have bypassed ribosome fidelity mechanisms, disrupt the mRNA path in the decoding center leading to a loss of fidelity. This ensures that incorrect tRNAs and release factors can bind to the ribosome and halt translation.

a. Hong S*, Sunita S*, Dunkle JA, Maehigashi T, Dunham CM (2018) Mechanism of +1 tRNA-mediated frameshifting. *Proc. Natl Acad. Sci. USA* 115(44):11226-31. PMCID: PMC6217423. *These authors contributed equally.
   Commentary by JF Atkins. Culmination of a half-century quest reveals insight into mutant tRNA-mediated frameshifting after tRNA departure from the decoding site. *Proc. Natl Acad. Sci. USA* 115(44):11221-23. PMCID: PMC6217412
b. Hoffer ED, Hong S, Sunita S, Whitford P, Gonzalez RL Jr, Dunham CM (2020) Structural insights into mRNA reading frame regulation by tRNA modification and slippery codon-anticodon pairing. *eLife*. 9:e51898. PMCID: PMC7577736. BioRxiv: https://doi.org/10.1101/2020.09.01.277525.
c. Nguyen HA, Sunita S, Dunham CM (2020) Disruption of evolutionarily conserved tRNA elements impairs accurate decoding. *Proc. Natl Acad. Sci. USA* 117(28):16333–38. PMCID: PMC7368331.
d. Nguyen HA, Hoffer ED, Maehigashi T, Fagan CE, Dunham CM (2023) Structural basis for reduced ribosomal A-site fidelity in response to P-site codon-anticodon mismatches. *J. Biol. Chem.,* 299(4):104608. PMCID: PMC10140155. BioRxiv: https://doi.org/10.1101/2023.01.28.526049.

4. *Role of bacterial toxin-antitoxin modules.* Bacteria adapt to changing environmental conditions by altering their gene expression to facilitate survival. My laboratory has investigated the roles that toxin-antitoxin pairs play in this transition. Most toxins inhibit protein synthesis, and my laboratory has been focused on the largest class of translational inhibitors, ribosome-dependent toxins. These toxins recognize and cleave mRNA bound to the ribosome. We identified the *E. coli* YafQ toxin features required for ribosome binding and catalysis of mRNA cleavage that distinguishes these specialized RNases from general microbial RNases (**a**). In contrast to the prevailing view that bacterial toxins are global translational inhibitors, we demonstrated that the ribosome-dependent HigB toxin only cleaves specific mRNA transcripts which suggests a more specialized role in the regulation of protein synthesis (**b**). To study a bacterial toxin specifically activated during thermal stress, we focused on the *E. coli* YoeB toxin that uniquely adopts a dimeric oligomeric state. Using biochemistry and structural biology approaches, we determined that its dimeric role is not required for activity but rather, simply is needed to withstand elevated temperatures (**c**). Another interest we have is in the transcriptional regulation of toxin-antitoxins that appears to be responsive to changing levels of toxins. We studied the molecular interactions of the HigBHigA toxin-antitoxin complex with its DNA operator to define the mechanistic basis of repression (**d**).

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b. Schureck MA, Dunkle JA, Maehigashi T, Miles SJ, Dunham CM (2015) Defining the mRNA recognition signature of a bacterial protein toxin. *Proc. Natl Acad. Sci. USA* 112(45):13862-7. PMCID: PMC4653167.
c. Pavelich IJ*, Maehigashi T*, Ruangprasert A, Hoffer ED, Miles SJ, Dunham CM. (2019) Monomeric YoeB toxin retains RNase activity but adopts an obligate dimeric form for thermal stability. *Nucleic Acids Res.* 47(19):10400-13. PMCID: PMC6821326. *These authors contributed equally.
d. Pavelich IJ, Schureck MA, Wang D, Hoffer ED, Boamah M, Onouha N, Miles SJ, Okafor D, Dunham CM (2023) Molecular mechanism regulating transcriptional control of the *hig* toxin-antitoxin locus of antibiotic-resistance plasmid Rts1 from *Proteus vulgaris*. bioRxiv: https://doi.org/10.1101/2021.03.04.434028. In press, *Nucleic Acids Research*

5. *Pioneering structural studies of ribosome function.* Protein synthesis is carried out by the ribosome and is one of the most conserved biological processes. As a postdoctoral fellow in Venki Ramakrishnan's lab, I solved the first high-resolution structure of the entire bacterial ribosome (**a**). This work continues to impact the field and has been cited >1000 times. Although this methodology helped push the field forward, the most significant biological achievement has been the mechanistic insights such structures have revealed, including how translation factors facilitate termination and recycling (**b**), how GTPase elongation factors modulate activity (**c**), and how bacterial toxins target the ribosome during the stringent response (**d**).

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