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 supervise Tiffany Trieu, a graduate student in my lab. Tiffany proposes to optimize grid preparation and ice thickness of her DcpG sample to ultimately solve a high resolution structure.

The Dunham lab research centers on understanding how stress alters translation, the mechanism of ribosome dysregulation, and the roles of toxin-antitoxin pairs in inhibiting protein synthesis to alter bacterial physiology. Starting 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 and how bacterial protein toxins regulate translation. Over the course of fourteen years, I have expanded my research interests to 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).

Select current research support

NIH/NIGMS R01 GM093278; Dunham (PI), No Cost Extension 09/01/2019–08/31/2023
Molecular basis of ribosomal frameshifting.

NIH/NIAID R01 AI088025; Conn, Dunham (MPI) 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 AI158706-01A1; Baugh, Keiler (MPI) 11/01/2021–10/31/2026
Targeting trans*-translation to kill* M. tuberculosis *non-replicating persister cells*

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023– 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.

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

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).

C. Contributions to Science

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

1. *Ribosomal mRNA frameshifting.* mRNA frameshifting controls gene expression in that the noncanonical reading of the genetic code facilitates expression of different protein products. Frameshift-prone tRNAs and mRNAs that contain complex RNA tertiary structures are two major causes for the change in the mRNA reading frame. To study this, we focused on factors identified in genetic suppressor studies or naturally occurring defects and/or mutations. As a postdoctoral fellow, I solved the first structure of a frameshift suppressor tRNA bound to the 30S decoding center (PMCID: PMC1869038). These studies provided an alternative model for how tRNAs facilitate a change in the reading frame. In my own lab, we have extended these initial observations by solving a number of different frameshift-prone tRNAs bound to the 70S ribosome that have defined how additional tRNA nucleotides and modifications in the anticodon loop regulate the mRNA reading frame (**a,b**). Further, we discovered how tRNA modifications maintain the mRNA frame and how dysregulation results in the ribosome losing its grip on the mRNA (**c,d**).

a. Maehigashi T*, Dunkle JA*, Miles SJ, Dunham CM (2014) Structural insights into +1 frameshifting promoted by expanded or modification-deficient anticodon stem-loops. *Proc Natl Acad Sci* 111(35):12740-5. PMCID: PMC4156745. *These authors contributed equally.
b. Hong S*, Sunita S*, Dunkle JA, Maehigashi T, Dunham CM (2018) Mechanism of +1 tRNA-mediated frameshifting. *Proc Natl Acad Sci* 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* 115(44):11221-23. PMCID: PMC6217412
c. Nguyen HA, Hoffer ED, Dunham CM (2019) Importance of tRNA anticodon loop modification and a conserved, noncanonical anticodon stem pairing in $tRNA^{Pro}$-CGG for decoding. *Journal of Biological Chemistry* 294(14):5281-91. PMCID: PMC6462517. *Selected as the Editor's Pick, an honor bestowed on the top 2% of JBC papers.*
d. 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.

2. *Molecular basis of translational dysregulation.* Biological fitness is critically dependent upon the accurate flow of genetic information. Although proofreading mechanisms exist, errors still occur and this breakdown in translational fidelity is detrimental to cells. Miscoding or misreading of the genetic code can occur with high frequency with specific mRNA-tRNA pairs and the basis of this dysregulation is not currently understood at the molecular level. We first studied this question using 16S rRNA *ribosome ambiguity mutants* (*ram*), a known hyperaccurate phenotype, as a model system. We identified global changes of these *ram* ribosomes that were allosterically communicated to the decoding center providing a molecular basis of this hyperaccurate phenotype (**a**). The molecular basis for why specific mRNA-tRNA pairs are more prone to miscoding has also been an elusive question despite many structures solved that show very little differences between the decoding of cognate as compared to near-cognate mRNA-tRNA pairs. We took a different approach and studied the biochemically well-studied $tRNA^{Ala}$. We found that the ribosome identifies correct from in correct mRNA-tRNA pairing by directly interacting with the anticodon stem of correct pairs (**b**). These studies provide insight, for the first time, into how tRNA stability and recognition by the ribosome can lead to accurate decoding. **(c)** Near-cognate mRNA-tRNA pairs that lead to activation of the post-peptidyl quality control pathway result in a loss of ribosome fidelity at the decoding center. We determined that near-cognate 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. (**d**) Defects in the rescuing of bacterial ribosomes that have stalled due to issues with mRNAs (5-10% of all cellular mRNAs) lead to cell death. Therefore, the ability to inhibit rescue was identified as a possible new antimicrobial target and compounds that specifically inhibit ribosome have been identified. In collaboration with the Keiler lab, we determined the molecular basis of action of one identified compound that inhibits rescue and specifically, we solved cryo-EM structures of the drug bound to a stalled ribosome.

a. Fagan CE, Dunkle JA, Maehigashi T, Dang MN, Deveraj A, Miles SJ, Qin D, Fredrick K, Dunham CM (2013) Reorganization of an intersubunit bridge induced by disparate 16S ribosomal ambiguity mutations mimics an EF-Tu-bound state. *Proc Natl Acad Sci* 110(24):9716-21. PMCID: PMC3683721.
    Commentary by PB Moore. Ribosomal ambiguity made less ambiguous. *Proc Natl Acad Sci* 110(24):9627-8. PMCID PMC3683732.

b. Nguyen HA, Sunita S, Dunham CM (2020) Disruption of evolutionarily conserved tRNA elements impairs accurate decoding. *Proc Natl Acad Sci* 117(28):16333–38. PMCID: PMC7368331.
c. 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. *Journal of Biological Chemistry,* 299(4):104608. PMCID: PMC10140155.
d. 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 Communications.* 12(1):1799. PMCID: PMC7979765. [#]Co-corresponding authors.

3. *Role of Modifications in Protein Synthesis*. Modifications to ribosomal RNA and proteins can tune protein synthesis or in other cases, modifications by pathogens are an antimicrobial strategy to gain resistance. In the latter case in collaboration with the Conn lab, we determined the molecular basis for recognition of a complex RNA tertiary structure within the context of the intact 30S subunit by a pathogen-derived 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 enzymes. We further characterize interactions of a different enzyme family and showed the diverse macromolecular recognition by divergent family members (**b,c**). In collaboration with the Ibba lab, we identified the molecular basis for how oxidative stress in Salmonella enterica serovar Typhimurium causes a tRNA synthetase to become more accurate to combat changing levels of amino acids (**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* 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. *Proceedings of the National Academy of Sciences.* 120(25):e2304128120 PMID: 37307464. [#]Co-corresponding authors.
c. Nosrati M, Dey D, Mehrani A, Strassler SE, Zelinskaya N, Hoffer ED, Stagg SM, Dunham CM, Conn GL (2019) Functionally critical residues in the aminoglycoside resistance-associated methyltransferase RmtC play distinct roles in 30S substrate recognition. *Journal of Biological Chemistry* 294(46):17642-53. PMCID: PMC6873201
d. Srinivas P, Steiner RE, Pavelich IJ, Guerrero-Ferreira R, Juneja P, Ibba M, Dunham CM (2021) Oxidation alters the architecture of the phenylalanyl-tRNA synthetase editing domain to confer hyperaccuracy. *Nucleic Acids Research* 49(20):11800-11809.

4. *Role of bacterial toxin-antitoxin modules.* Bacteria quickly 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 mRNA catalysis 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 understanding the proteolysis of antitoxins or antidote proteins that occurs during stress to release its cognate toxin. We identified the importance of the C-terminus of the *E. coli* DinJ antitoxin required for its recognition by the Lon protease (**d**).

a. Maehigashi T*, Ruangprasert A*, Miles SJ, Dunham CM (2015) Molecular basis of ribosome regulation and mRNA hydrolysis by the *E. coli* YafQ toxin. *Nucleic Acids Res* 43(16):8002-12. PMCID: PMC4652777. *These authors contributed equally.
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* 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 Research* 47(19):10400-13. PMCID: PMC6821326. *These authors contributed equally.
d. Ruangprasert A, Maehigashi T, Miles SJ, Dunham CM (2017) Importance of the *E. coli* DinJ antitoxin carboxy terminus for toxin suppression and regulated proteolysis. *Mol Micro* 104(1):65-77.

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 >950 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**).

a. Selmer M*, Dunham CM*, Murphy IV FV, Weixlbaumer A, Petry S, Kelley AC, Weir J, Ramakrishnan V (2006) Structure of the 70S Ribosome Complexed with mRNA and tRNA. *Science* 313(5795):1935-42. *These authors contributed equally.
b. Weixlbaumer A, Petry S*, Dunham CM*, Selmer M*, Kelley AC, Ramakrishnan V (2007) Crystal structure of the ribosome recycling factor bound to the ribosome. *Nat Struct Mol Biol* 14(8):733-7. *These authors contributed equally.
c. Gao Y-G, Selmer M, Dunham CM, Weixlbaumer A, Kelley AC, Ramakrishnan V (2009) The Structure of the Ribosome with Elongation Factor G Trapped in the Posttranslocational State. *Science* 326(5953):694-99. PMCID: PMC3763468.
d. Neubauer C*, Gao, Y-G*, Andersen KR*, Dunham CM, Kelley AC, Hentschel J, Gerdes K, Ramakrishnan V, Brodersen DE (2009) The structural basis for mRNA recognition and cleavage by the ribosome-dependent endonuclease RelE. *Cell* 139(6):1084-1095. PMCID: PMC2807027. *These authors contributed equally.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Trieu, Tiffany

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

POSITION TITLE: Graduate Student Research Assistant

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Central Florida	BS	08/2014	05/2018	Chemistry – Biochemistry Track
Emory University	PHD	08/2018	08/2024 (expected)	Chemistry

A. Personal Statement

My research career began during my undergraduate years, where I worked in the lab of Dr. Karin Chumbimuni-Torres to develop an electrochemical sensor specific to the Zika and Dengue viruses. My research contributed to a publication and was presented at Pittcon in 2018. While I enjoyed applied research in electrochemistry, I decided to branch out to fundamental research at Emory University. I took a leave of absence during the 2020 pandemic and decided to switch labs once I was readmitted. Currently, I am working with Dr. Christine Dunham on investigating the sequence specificity of type II ribosome-dependent toxins. I am also working on solving the structure of a diguanylate cyclase phosphodiesterase with sensor globin from *Paenibacillus dendritiformis* in collaboration with the Weinert lab at Penn State University. In the Dunham laboratory, I have been trained in various biochemical (i.e. stopped flow spectroscopy, denaturing gels) and structural biology (i.e. X-ray crystallography, cryo-EM) techniques.

1. Mills DM, Foguel MV, Martin CP, **Trieu TT**, Kamar O, Calvo-Marzal P, Kolpashchikov DM, Chumbimuni-Torres KY. Rapid detection of different DNA analytes using a single electrochemical sensor. Sensors and Actuators B: Chemical. 2019 August.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2018 – Present Graduate Student Research Assistant, Emory University
2016 – 2018 Undergraduate Student Research Assistant, University of Central Florida

Honors

2023 Poster Presenter, Southeast Enzyme Conference
2022 Poster Presenter, GA RNA Salon
2019 Outstanding Teaching Assistant Award, Emory University
2018 Poster Presenter, Pittcon
2017 Office of Undergraduate Research (OUR) Grant
2014 – 2018 Scholarship, Pegasus Scholarship
2014 – 2018 Scholarship, Florida Bright Futures Scholarship Program

C. Contributions to Science

1. **Undergraduate Career:** I researched the development of an electrochemical sensor that was specific to the sequences found in the Zika and Dengue viruses. What made this electrochemical sensor unique was that it was reusable and can be applied to test for different viruses. I mainly worked with optimizing the sensor and calibrating its output signal.
    1. **Trieu TT**, Mills DM, Martin CP, Kamar O, Calvo-Marzal P, Parks GD, Kolpashchikov DM, Chumbimuni-Torres KY. A Highly Selective Electrochemical Biosensor for the Detection of Zika Virus. Pittcon; 2018 February; Orlando, FL.
    2. Mills DM, Foguel MV, Martin CP, **Trieu TT**, Kamar O, Calvo-Marzal P, Kolpashchikov DM, Chumbimuni-Torres KY. Rapid detection of different DNA analytes using a single electrochemical sensor. Sensors and Actuators B: Chemical. 2019 August.
2. **Graduate Career:** My current research is on understanding how ribosome-dependent toxins specify their target sequences. Toxin-antitoxin systems are ubiquitous in bacteria and are linked to stress response and phage defense. In addition, I study globin-coupled sensors in collaboration with the Weinert lab. I utilize techniques in structural biology and biochemistry to investigate the questions for my projects.
    1. **Trieu TT**, Pavelich IJ, Dunham CM. Investigating the Specificity of the Endoribonuclease RelE. GA RNA Salon; 2022 December; Atlanta, GA.
    2. **Trieu TT,** Gonzalez SM, Pavelich IJ, Dunham CM. Mechanism of Toxin-mediated mRNA Cleavage. Southeast Enzyme Conference; 2023 April; Atlanta, GA.