BIOGRAPHICAL SKETCH

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NAME: Conn, Graeme Leslie

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

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Edinburgh, UK	B.Sc.	05/1993	Chemistry
University of Edinburgh, UK	Ph.D.	10/1996	Chemistry
Johns Hopkins University	Postdoc	12/1999	Structural Biology

A. Personal Statement

My roles on this NCCAT BAG application (which requests high-resolution data collection on FEI Titan Krios 300 keV microscopes) will be to serve as **Co-Principal Investigator** (with Dr. Dunham) and as the **BAG Spokesperson**. Additionally, I will be responsible for overall direction of studies at NCCAT, in my lab and in coordination with collaborators that are specifically related to **Sub-projects 1 and 2.** These studies center on 30S subunit recognition and modification by acquired antibiotic resistance or intrinsic 16S rRNA methyltransferases, and development and characterization of novel aminoglycoside antibiotics that remain active in the presence of resistance rRNA modification, respectively.

My lab has an established track-record of using a broad array of approaches including structural biology (now primarily single-particle cryoEM), biochemistry, molecular biology microbiology, and computational methods to dissect fundamental biological mechanisms related to bacterial antibiotic resistance, and RNA-mediated regulation of innate immune proteins (see **section C**). Over the last several years, we have transitioned to using single-particle cryoEM in for almost all projects in which we are pursuing high-resolution structural insights. As such, this NCCAT BAG proposal will be essential for successful completion of our NIH-funded studies of the aminoglycoside-resistance 16S rRNA methyltransferases and will allow us to pursue important new directions in this work on intrinsic rRNA methylation and its impact antibiotic resistance (or susceptibility) as proposed in the currently pending renewal application (see below). Specifically, our goals in the studies related to structural targets in this BAG applications are to define the molecular mechanisms of ribosomal subunit substrate recognition by both intrinsic and antibiotic resistance-conferring rRNA methyltransferase enzymes and to understand how rRNA methylation impacts the activity of ribosome targeting antibiotics such as aminoglycosides. These efforts will also be supported by Emory's EM core (of which I was until recently one of three Scientific Co-directors) as well as colleagues among Emory's major users of this facility. Determining the structures proposed in this BAG application will represent a major advance in our understanding of how these RNA modification enzymes carry out their function in relation to antibiotic resistance and, more generally, about how rRNA modification enzymes recognize and site-specifically modify the bacterial ribosome.

Ongoing and recently completed projects that I would like to highlight include:

1. NIH/NIAID, R01 AI088025, *RNA modification and antibiotic resistance,* MPI–Conn*,Dunham (5/1/2010–4/30/2026; NCE–renewal application for 2026-2030 is pending)
2. NIH/NIAID, R01 AI185192, *Regulation and mechanism of RND-mediated antibiotic efflux in Pseudomonas,* MPI–Conn, Dunham*, Keiler (06/2024–05/2029).
3. NIH/NIGMS, R01 GM130135, NIH/NIGMS, *Mechanisms and biological functions of SPOUT methyltransferases,* MPI–Jackman*, Conn (07/01/2022–07/31/2026).
4. NIH/NIAID, R01 AI144067, *dsRNA regulation of the cytosolic innate immune system*, PI–Conn (3/12/2019–4/30/2028).

5. NIH/NIAID, T32 AI106699, *Antimicrobial Resistance and Therapeutic Discovery Training Program,* MPI–Shafer*, Conn (06/1/2014–05/31/2025). [Renewal application received **16 overall impact** and is pending Council Review (MPI-Conn*/Goldberg.]

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022–present	Associate Editor, *npj Antimicrobials and Resistance*
2020–2024	Co-scientific Director, Robert P. Apkarian Integrated Electron Microscopy Core, Emory University
2022–present	Co-Director, Antimicrobial Resistance and Therapeutic Discovery Training Program (ARTDTP) and MPI of the NIAID T32 supporting ARTDTP.
2019–present	Professor, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.
2008–2019	Associate Professor, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.
2007–2008	Senior Lecturer (equivalent to Associate Professor, *with tenure*), Faculty of Life Sciences, University of Manchester, UK.
2000–2007	Lecturer (equivalent to Assistant Professor, *tenure track*), Dept. Biomolecular Sciences, UMIST (2000-2004)/ Faculty of Life Sciences, University of Manchester, UK (2004-2007).
2000–2004	Wellcome Trust Independent Research Career Development Fellow, Dept. Biomolecular Sciences, UMIST, UK.
1999–2000	Wellcome Trust Postdoctoral Fellow, Dept. of Biomolecular Sciences, UMIST, UK.
1996–1999	Wellcome Trust Postdoctoral Fellow, Johns Hopkins University, Baltimore, MD.

Selected Other Experience, Service and Professional Memberships

2023	*Ad hoc* member (subject expert), Board of Scientific Counselors (BSC), review of NIEHS Epigenetics and Stem Cell Laboratory (ESCBL), December 2023.
2019–present	NIH SEPs: **2019**, NIAID ZAI1 LR-M (M1), RFA-AI-18-025, Elucidating the Functional Roles of Non-Coding RNAs in Viral Infectious Diseases (R21); **2020**, ZRG1 BCMB-G (02)– Member Conflict-Chemistry and Biological Chemistry; **2021**, ZRG1 AIDC-V (02)–Conflict: Topics in infectious diseases vaccines, therapeutics, and vector biology.
2017-2019	Guest Editor, special topic “Bacterial Mechanisms of Antibiotic Resistance: A Structural Perspective”, *Frontiers in Molecular Biosciences*.
2015-present	NIH/ CSR F13, Fellowships: Infectious Diseases and Microbiology (six times total, most recently July 2023)
2014-present	Frontiers in Molecular Biosciences, Reviewing Editor (Structural Biology)
2013-2014	Chair, Proteins and Crystallography Committee 2, American Heart Association.
2012	Co-chair, Proteins and Crystallography Committee 2, American Heart Association.
2012	‘Recombinant and *in vitro* RNA synthesis: Methods and Protocols’ (Editor), Methods in Molecular Biology series (Walker, J.M., series Editor), Humana Press.
2001-present	*Ad hoc* grant reviewer/ study section: The Wellcome Trust, BBSRC (UK), American Heart Association, American Cancer Society, NIH/CSR (IMST-G 30 (S10 Shared Equipment review), ZRG1 BCMB-R 02 M, ZDC1 SRB-K17 (R03 review) and DP5 Director’s Early Independence Award), American Heart Association and the Canadian Council for the Arts (Killam Research Fellowship).
2001-present	Manuscript reviewer: *Nat. Meth., Nat. Prot., Na. Chem. Biol., PNAS, J. Mol. Biol.*, *Nucleic Acids Res.*, *J. Biol. Chem., BioTechniques*, *Biochemistry*, *Cell. Mol. Life Sci., Chem. Senses*, *Biol. Cell., J. Biotechnology, Current Biology, Molecular Microbiology, FEMS Letters and PLoS-ONE, ChemBioChem., RNA (*RNA Society*), Frontiers Microbiology, mBio..*
2000-present	Biochemical Society UK (2003-2007), Association for Chemoreception Sciences (2003-2011), American Society for Microbiology (ASM; since 2008), American Association for the Advancement of Science (AAAS; since 2009), American Crystallographic Society (ACA; since 2010), The American Society for Biochemistry and Molecular Biology (ASBMB; since 2011), and the RNA Society (since 2017).

Awards/Honors

2019	Researcher Appreciation Day recognition, Emory University School of Medicine.
2018	Hidden Gem award, Emory University School of Medicine.
2000–2004	Wellcome Trust Independent Research Career Development Fellowship.

1996–2000 Wellcome Trust International Traveling Prize Fellowship (Postdoctoral).
1993–1996 Royal Society of Edinburgh Caledonian Trust Scholarship (PhD).
1990–1994 1st, 3rd, and 4th Year Undergraduate Class Prize (top ranked student), Department of Chemistry, University of Edinburgh, UK.
1990–1993 Faculty of Science and Engineering Bursary, University of Edinburgh, UK.

C. Contributions to Science

Complete List of Published Work in My NCBI (85 total citations):
https://www.ncbi.nlm.nih.gov/myncbi/graeme.conn.1/bibliography/public/

1. Bacterial ribosomal RNA modification and antibiotic resistance. Aminoglycoside antibiotics typically act by binding and inducing specific conformational changes in the ribosome "decoding center" that result in aberrant protein synthesis. Aminoglycosides have potent activity, leading to a reevaluation of their potential utility in the clinic in the face of increasing resistance to many other (first line) drugs. Clinical aminoglycoside resistance commonly arises through the action of aminoglycoside modifying enzymes, but an additional serious threat to the future clinical usefulness of aminoglycosides has arisen from the acquisition and spread among human bacterial pathogens of 16S rRNA methyltransferase enzymes that modify the ribosomal drug binding site, either at the N7 position of G1405 (m^7G1405) or the N1 position of A1408 (m^1A1408). These rRNA modifications confer exceptionally high-level resistance and combined, these modifications can block the effects of *all* clinically useful aminoglycosides including the latest generation of drugs such as plazomicin. Determining the structures of these enzymes and defining the features which govern their interactions with cosubstrate S-adenosyl-L-methionine (SAM) and the 30S substrate, have been a major contribution from my lab. In 2014, in collaboration with Christine Dunham's group we presented a major breakthrough in the field with the determination of a first structure of a resistance methyltransferase (NpmA) bound to its 30S substrate. This work was followed by a detailed mechanistic study to dissect the molecular basis of 30S recognition by NpmA and together these publications revealed the basis for the requirement of mature 30S as substrate, and the molecular details underpinning specific target recognition, including flipping of the target A1408 base into the NpmA active site. Our on-going studies in collaboration with the Dunham group and Lindsay Comstock (Wake Forest University) include efforts to obtain: *1)* structure-function insights for other members of the m^1A1408 enzyme family which, unexpectedly, exhibit distinct details in their molecular mechanisms of 30S recognition and modification and *2)* complementary studies of members of the clinically more prevalent m^7G1405 family (e.g. **1a**,**1b**) and another antibiotic resistance associated enzyme that modifies an adjacent site (C1409, *Mycobacterium tuberculosis* TlyA; **1c,d**). Our long-term goal is to exploit the understanding we develop of the enzymes and their target recognition mechanisms to facilitate development of specific inhibitors of these resistance determinants.

1a. Nosrati, M., Dey, D., Mehrani, A., D. Strassler, S.E., Zelinskaya, N., Hoffer, E.D., Stagg, S.M., Dunham, C.M. and **Conn, G.L.** (2019). Functionally critical residues in the aminoglycoside resistance-associated methyltransferase RmtC play distinct roles in 30S substrate recognition. *J. Biol. Chem. 294, 17642-17653.* [PMCID-PMC6873201]

1b. Srinivas, P., Nosrati, M., Zelinskaya, N., Dey, D., Comstock, L.R., Dunham, C.M.* and **Conn, G.L.*** (2023). 30S subunit recognition and G1405 modification by the aminoglycoside-resistance 16S ribosomal RNA methyltransferase RmtC, *Proc. Natl. Acad. Sci. USA,* **120**(25):e2304128120. *Co-corresponding authors. [PMCID: PMC10288597] [*CryoEM structure determined using data collected at the PNCC national center*]

1c. Laughlin, Z.T., Nandi, S., Dey, D., Zelinskaya, N., Witek, M.A., Srinivas, P., Nguyen, H.A., Kuiper, E.G., Comstock, L.R., Dunham, C.M. and **Conn, G.L.** (2022) 50S subunit recognition and modification by the Mycobacterium tuberculosis ribosomal RNA methyltransferase TlyA. *Proc. Natl. Acad. Sci. U. S. A.*, **119**, e2120352119. [PMCID: PMC9168844] [*CryoEM structure determined using data collected at NCCAT*]

1d. 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. (Also in revision at *Nucleic Acids Res*.) [*CryoEM structures determined using data collected at NCCAT*]

2. Structure, activity, and substrate selection by the *P. aeruginosa* RND pump MexXY-OprM. Our work on the resistance-nodulation-cell division (RND) family efflux pumps represents a relatively new direction in our fundamental studies of bacterial antibiotic resistance. These studies originated from on-going *in silico* analyses of aminoglycoside antibiotic dynamics and interactions with *methylated* 30S ribosome subunits to understand how some drugs of this class appear to be able to "evade" the effects of RNA modification by the

aminoglycoside-resistance 16S rRNA methyltransferases. Our current focus is on *P. aeruginosa* MexXY-OprM which is unique in both its dependence on aminoglycosides for regulation of its expression and its ability to preferentially efflux these antibiotics leading to clinical resistance (e.g., strains isolated from cystic fibrosis patients). Using phylogenetic analyses, computational molecular modeling of the MexXY-OprM complex, and *in silico* ligand docking in the MexY distal binding pocket (DBP), we proposed a novel hypothesis: that the physicochemical properties of the MexY DBP have evolved to prefer aminoglycosides over β-lactams via a "Goldilocks" binding affinity (**2a**). That is, substrates must bind tightly enough to be taken up by MexY but not too tightly, so as to impede movement through the transporter to the periplasm adaptor protein MexX. Supporting this novel concept, β-lactams are universally predicted to bind more tightly to MexY, and the converse is also true: aminoglycosides were predicted, without exception, to bind more tightly to MexB, of the homologous RND pump MexAB-OprM which exports β-lactams but not aminoglycosides. More recently, building on these initial studies of MexY we used computational (docking, molecular dynamics) and microbiology approaches to identify analogs of the natural compound berberine that can serve as starting point for development of novel probes or efflux pump inhibitors of MexXY-OprM (**2b**). In our on-going studies using interdisciplinary approaches including microbiology, biochemistry, structural biology and computation (e.g. MD simulations), we are defining the substrate preferences of MexXY-OprM (**2c**), substrate entry points and translocation pathway(s), and the molecular basis of substrate selectivity among different RND systems in *P. aeruginosa*. Finally, we recently produced a comprehensive review of the RND efflux pump family structure, mechanism, and role in clinical antimicrobial resistance (**2d**).

2a. Dey, D., Kavanaugh, L.G. and **Conn, G.L.** (2020). Antibiotic substrate selectivity of *Pseudomonas aeruginosa* MexY and MexB efflux systems is determined by a Goldilocks affinity. *Antimicrob. Agents Chemother.* **64**(8), e00496-20. [PMCID: PMC7526836]

2b. Kavanaugh, L.G., Mahoney, A.R., Dey, D., Wuest, W.M.* and **Conn G.L.*** (2023). Di-berberine conjugates as chemical probes of Pseudomonas aeruginosa MexXY-OprM efflux function and inhibition. *npj Antimicrobials and Resistance* 1:12. [PMC10327050; also posted on *bioRxiv*]. *Co-corresponding authors

2c. Kavanaugh, L.G, Hariharan, S.M. and **Conn, GL***. (2025). Determination of *Pseudomonas aeruginosa* MexXY-OprM substrate profile in a major efflux knockout system reveals distinct antibiotic substrate classes. *Microbiol Spectr*. Feb 6;:e0290324. doi: 10.1128/spectrum.02903-24'. [PMCID in process; also posted on *bioRxiv*]

2d. Kavanaugh, L.G.†, Dey, D.†, Shafer, W.M.* and **Conn, GL***. (2024). Structural and functional diversity of Resistance-Nodulation-Division (RND) efflux pump transporters with implications for antimicrobial resistance. *Microbiol. Mol. Biol. Rev.* **88**(3):e0008923. doi: 10.1128/mmbr.00089-23. †Co-first authors, *Co-corresponding authors. [PMCID: PMC11426026]

3. Mechanisms and biological functions of SPOUT methyltransferases. My group's studies of RNA methyltransferases began with the thiostrepton antibiotic resistance methyltransferase, TsnR, a member of the SpoU/TrmD (SPOUT) enzyme family. Our studies of TsnR revealed the first structure of the enzyme (**3a**) and the role of binding-induced rRNA conformational changes and catalytic residues in substrate recognition and site-specific 2-O-ribose modification (e.g. **3b**). More recently, our focus in the area of SPOUT methyltransferases has been on the tRNA m^1G9/m^1A9 modifying enzyme Trm10, in collaboration with Dr. Jane Jackman's group at OSU. Here, our goal has been to define how Trm10 selects correct substrates from the pool of available tRNAs in the absence of any common sequence or prior modifications to distinguish them. We recently described the use of SHAPE RNA probing to define both inherent tRNA flexibility and Trm10-binding induced conformational changes as key elements of correct substrate recognition (**3c**). Additionally, we are in the final stages of determining a first structure of a stand-alone Trm10 enzyme bound to a substrate tRNA using cryo-electron microscopy (cryo-EM). Finally, through our collaboration with Dr. Jackman and her team, we recently published a comprehensive review on current knowledge and the major open questions on substrate recognition by SPOUT methyltransferases, including Trm10 and TsnR (**3d**).

3a. Dunstan, M.S., Hang, P.C., Zelinskaya, N.V., Honek, J.F., and **Conn, G.L.** (2009). Structure of the thiostrepton resistance methyltransferase:S-adenosyl-L-methionine complex and its interaction with ribosomal RNA. *J Biol. Chem.* **284**(25):17013-17020. [PMCID: PMC2719339]

3b. Kuiper, E.G. and **Conn, G.L.** (2014). Binding induced RNA conformational changes control substrate recognition and catalysis by the thiostrepton resistance methyltransferase (Tsr). *J. Biol. Chem.* **289**(38):26189-26200. [PMCID: PMC4176221]

3c. Strassler, S.E., Bowles, I.E., Clio Hancock, Krishnamohan, A., Kim, H., Edgington C.B., Kuiper, E.G., Comstock, L.R., Jackman, J.E.* and **Conn, G.L.*** (2023). tRNA m^1G9 modification depends on substrate-

specific RNA conformational changes induced by the methyltransferase Trm10, *J. Biol. Chem*., **299**(12):105443 [PMCID: PMC10704376]

3d. Strassler, S.E., Bowles, I.E., Dey, D., Jackman, J.E. and **Conn, G.L** (2022). Tied up in knots: Untangling substrate recognition by the SPOUT methyltransferases. *J. Biol. Chem.* **298**(10):102393. [PMCID: PMC9508554]

4. Non-coding RNA structure and regulation of proteins of the human innate immune system. My lab has long-standing interests in understanding the structure and activity of viral and cellular non-coding RNAs, and their mechanisms of regulation of the host cell innate immune system double-stranded (ds)RNA-sensors, dsRNA-activated protein kinase (PKR) and oligoadenylate synthetase 1 (OAS1). Our early work defined the stabilities and roles of the conserved domains within the adenovirus non-coding RNA, VA RNA_I, and more recently centered on nc886, a *cellular* ncRNA proposed to be an endogenous regulator of PKR. These studies have begun defining the nc886 RNA structure and revealed that only one conformer of nc886 can adopt a (currently unknown) tertiary structure that confers both potent inhibition of PKR and activation of a second dsRNA-sensing innate immune protein, OAS1, which has become the main current focus of our work in this area. In response to dsRNA, 2',5'-oligoadenylate synthetase (OAS) proteins produce 2',5'-linked oligoadenylate second messengers for which the major target is the latent ribonuclease, RNase L. Activation of the OAS/RNase L pathway triggers a program of cellular and viral RNA degradation designed to halt protein synthesis in the infected cell. First, we reported the discovery of a novel *single-stranded* RNA motif (termed 3'-ssPy, or "*three prime spy*") that strongly potentiates OAS1 activation by a short model dsRNA duplex as well as structured viral and cellular non-coding RNAs (**4a**). Subsequently, we identified the role of a novel tertiary structure within nc886 in potent the activation of the OAS/RNase L pathway both *in vitro* and in the context of cellular innate immune signaling (**4b**). Our more recent studies have revealed that even for "simple" model dsRNAs there is a potentially complex interplay of RNA features that controls whether OAS1 becomes activated or not (e.g. **4c**,**4d**) and we are currently working to fully define the "rules" that govern potent OAS1 activation by natural dsRNAs of cellular or viral origin, and the impacts of motifs like 3'-ssPy.

4a. Vachon, V.K., Calderon, B.M. and **Conn, G.L.** (2015). A novel RNA molecular signature for activation of 2'-5' oligoadenylate synthetase-1. *Nucleic Acids Res.* **43**(1), 544-552. [PMCID: PMC4288181]

4b. Calderon, B.M. and **Conn, G.L.** (2018). A human cellular noncoding RNA activates the antiviral protein 2'–5'-oligoadenylate synthetase 1. *J. Biol. Chem.* ***293****, 16115-16124.* [PMCID: PMC6187638]
This article was an Editors' Pick for the October 12th, 2018 issue of *JBC*, see:
Dinman, J.D. (2018). Shapeshifting RNAs guide innate immunity. *J. Biol. Chem.* ***293****, 16125-12126.*

4c. Schwartz, S.L., Park, E.N., Vachon, V.K., Danzy, S., Lowen, A.C. and **Conn, G.L.** (2020). Human OAS1 activation is highly dependent on both RNA sequence and context of activating RNA motifs. *Nucleic Acids Res*. **48**(13), 7520–7531. [PMCID PMC7367156]

4d. Schwartz, S.L., Dey, D., Tanquary, J., Bair, C.R., Lowen, A.C. and **Conn, G.L.** (2022) Role of helical structure and dynamics in oligoadenylate synthetase 1 (OAS1) mismatch tolerance and activation by short dsRNAs. *Proc. Natl. Acad. Sci. U. S. A.*, **119**. [PMCID: PMC8784149].

5. Molecular bases of OAS IEIs. As part of a multinational interdisciplinary team, we recently reported the identification and mechanistic basis of a new IEI resulting from four distinct heterozygous mutations in the human *OAS1* gene which encodes the enzyme oligoadenylate synthetase 1 (OAS1; **5a**). The OAS family of proteins is responsible for sensing foreign (e.g. viral) double-stranded (ds)RNA and effecting an interferon-induced innate immune response. In humans, three catalytically active OAS proteins (OAS1-3) accomplish this by dsRNA-activated synthesis of 2',5'-linked oligoadenylate (2,5-A) second messengers which then activate the latent ribonuclease (RNase L). Our studies showed that the four OAS1 variants possess low-level gain-of-function (GoF) activity, i.e., 2,5-A synthesis in the *absence of dsRNA activation* and thus result in a new IEI we termed OAS1-associated polymorphic auto-inflammatory immunodeficiency disorder (OPAID). Using computational modeling and molecular dynamics (MD) approaches, my group that showed each OAS1 GoF amino acid substitution results in similar changes in protein dynamics surrounding the active site, despite being distributed across one half of the protein. This initial study opens the exciting new opportunity to exploit these gain-of-function variants to substantially deepen our understanding of OAS1 regulation, including the mechanism of allosteric communication between the GoF sites and residues surrounding the active site, and how these protein residue networks limit aberrant activation in the absence of dsRNA.

5a. Magg, T., Okano, T., Koenig, L.M., Boehmer, D.F.R., Schwartz, S.L., … **Conn, G.L**., Sullivan, K.E., Klein, C., Morio, T. and Hauck, F. (2021) Heterozygous OAS1 gain-of-function variants cause an autoinflammatory immunodeficiency. *Science Immunology*, **6**. [PMCID: PMC8392508].

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 on this NCCAT BAG application (which requests FEI Titan Krios 300 keV microscope access for high-resolution data collection) will be to serve as **Co-Principal Investigator** (with Dr. Conn). Additionally, I will be responsible for overall direction of studies at NCCAT, in my lab and in coordination with our collaborators, within **Sub-projects 2 and 3.** These studies will specifically focus on: **Sub-project 2–**novel trans-translation-targeting molecules as potential new antimicrobials and stop-codon read-through drugs; and, **Sub-project 3–** the impact of mRNA chemical modifications on translation.

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 (cryoEM). The awards noted below currently fund these active research projects in my lab.

Research support

NIH/NIGMS R01 GM093278; Dunham (PI), *No Cost Extension* 09/01/2019–08/31/2025
Molecular basis of ribosomal frameshifting.

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.*

NIH/NIGMS R01 GM12359, Yap (PI), Dunham (co-I) 04/01/2022–03/31/2026
Regulation and function of bacterial hibernating 100S ribosome.

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**).

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. 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.

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**).

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

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NAME: Dey, Debayan

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

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Presidency College, University of Calcutta, India	B.Sc	07/2007	Physics
University of Calcutta, Dept. of Biophysics, India	M.Sc	07/2009	Biophysics
Indian Institute of Science, Bangalore, India	Ph.D	07/2016	Structural Biology
Poornaprajna Institute of Scientific Research, India	Postdoc	06/2017	Structural Biology
Emory University, Atlanta GA, USA	Postdoc	05/2022	Structural Biology

A. Personal Statement

My role on this NCCAT BAG application will be as a **primary user**, performing task including specimen preparation and shipment to NCCAT, monitoring of data collection, and data processing and structure determination. I have over 16 years of experience in structural biology, molecular modeling, chemoinformatics, structural bioinformatics, computer-aided drug discovery (CADD), phylogenetics, and protein biochemistry. My research journey began with my Master's project in structural bioinformatics and machine learning, where I developed models for predicting secondary structural elements using neural network architecture and supervised learning. I pursued my Ph.D. at the Indian Institute of Science, Bangalore, where I focused on the structural biology of bacterial nucleoid-associated proteins. During this time, I identified first-in-class inhibitors of the regulatory protein HU in Mycobacterium tuberculosis through computational methods, which were experimentally validated. Additionally, I explored the structure and evolutionary dynamics of the SPOUT family rRNA methyltransferases in bacteria, which play a significant role in bacterial antibiotic resistance.

In my postdoctoral work, partially supported by a Cystic Fibrosis Foundation (CFF) postdoctoral fellowship at Emory University, I applied computational chemistry, bioinformatics, and structural biology to investigate the fundamental mechanisms of antibiotic resistance, bacterial virulence, and immunity. I have also contributed to the understanding of the structural evolution and substrate recognition mechanisms of various RNA methyltransferases (see citations **1-3**, below).

Relevant Citations:

1. Nosrati, M., **Dey, D.**, Mehrani, A., D. Strassler, S.E., Zelinskaya, N., Hoffer, E.D., Stagg, S.M., Dunham, C.M. and Conn, G.L. (2019). Functionally critical residues in the aminoglycoside resistance-associated methyltransferase RmtC play distinct roles in 30S substrate recognition. *J. Biol. Chem.* **294***, 17642-17653.* [PMCID-PMC6873201]
2. Srinivas, P., Nosrati, M., Zelinskaya, N., **Dey, D.,** Comstock, L. R., Dunham, C. M., & Conn, G. L. (2023). 30S subunit recognition and G1405 modification by the aminoglycoside-resistance 16S ribosomal RNA methyltransferase RmtC. *Proc. Natl. Acad. Sci. U. S. A.*, **120**(25), e2304128120. [PMCID: PMC10288597]
3. Laughlin, Z.T., Nandi, S*., **Dey, D.*,** Zelinskaya, N., Witek, M.A., Srinivas, P., Nguyen, H.A., Kuiper, E.G., Comstock, L.R., Dunham, C.M. and Conn, G.L. (2022) 50S subunit recognition and modification by the Mycobacterium tuberculosis ribosomal RNA methyltransferase TlyA. *Proc. Natl. Acad. Sci. U. S. A.*, **119**, e2120352119. *equal contribution as second author. [PMCID: PMC9168844]

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2024-current Assistant Professor, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.
2022-2024 Instructor, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.
2017-2022 Postdoctoral Fellow, Department of Biochemistry, Emory University School of Medicine, USA.
2017 Postdoctoral Fellow, Indian Institute of Science, Bangalore, India.

Other Experience, Service and Professional Memberships

2022-2024 Member of Emory University senate Committee for Open Expression (CFOE)
2022-2023 National Summer Undergraduate Research Program (NSURP) mentor
2021 Hub organizer for Americas, Society for Molecular Biology and Evolution (SMBE) 2021, Virtual, July 2021.
2021-present Frontiers in Bioinformatics, Reviewing Editor (Drug Discovery)
2017-present American Society for Microbiology (ASM; since 2020), American Association for the Advancement of Science (AAAS; since 2019), The American Society for Biochemistry and Molecular Biology (ASBMB; since 2020), and Society of Molecular Biology and Evolution (SMBE; since 2020), The Surveillance and Epidemiology of Drug-resistant Infections Consortium (SEDRIC; since 2022)
2017-present Manuscript reviewer: *Nature Communications*, *Journal of Molecular Graphics and Modeling*, *Medicine*, *Frontiers in Microbiology*, *Molecular Microbiology, Computational Biology and Chemistry*, *Translational Medicine Communications*, *MDPI Applied Sciences*, *MDPI Sci*, *MDPI Crystals*, *MDPI Sustainability*, *MDPI Pharmaceutics*, *MDPI Mathematics*, *MDPI International Journal of Environmental Research and Public Health*, *Frontiers Cell and Developmental Biology*.

Awards/Honors

2022 American Society of Microbiology, Future Leaders Mentoring Fellowship.
2020 ASBMB Travel award.
2019 Cystic Fibrosis Scholars Program at CF@tlanta
2018 Cystic Fibrosis Foundation Postdoctoral fellowship (DEY18F0)
2017 DST-SERB National Post-Doctoral fellowship by Science & Engineering Research Board (SERB) of Department of Science and Technology, India.
2016 Department of Biotechnology Research Associateship by Department of Biotechnology, India.
2014 ICTP travel award by International Center for Theoretical Physics, Trieste Italy.
2012 Okinawa Institute of Science and Technology (OIST) Travel award.
2011 Research Fellowship in Sciences for meritorious students BSR scheme, awarded by the University Grants Commission, India.
2010 First prize for the Best Entrepreneurial Project Nationwide, at BEST-India 2010.
2009 Silver medalist at M.Sc. in Bioinformatics and Biophysics, Calcutta University,India.

C. Contributions to Science

Complete List of Published Work in My NCBI (19 total publications):
https://www.ncbi.nlm.nih.gov/myncbi/1b3Ui55CoCVks/bibliography/public/

1. Antibiotic resistance mediated by RNA methyltransferases and resistance evasion by aminoglycosides. My research is focused on unraveling the molecular mechanisms of antibiotic resistance mediated by RNA methyltransferases and developing inhibitors to counteract them. Additionally, my focus extends to understanding how various aminoglycosides manage to evade resistance mediated by these methylations which could enable rational drug redesign. Such understanding would be crucial for designing combinations of newer-generation aminoglycosides and resistance-breaking drugs. Aminoglycosides, as broad-spectrum antibiotics, exert their action by binding to the ribosomal decoding center and inhibiting protein synthesis. Resistance to aminoglycosides poses a significant threat to their current clinical utility, and it can arise from aminoglycoside-modifying enzymes (AMEs), 16S rRNA methyltransferases, and aminoglycoside-selective efflux pumps. Among the mechanisms of resistance, 16S rRNA methyltransferases play a pivotal role by modifying the ribosomal drug binding site—either at the N7 position of G1405 (m^7G1405) or the N1 position of A1408 (m^1A1408). The m^7G1405 methyltransferase family is particularly clinically relevant, conferring pan-aminoglycoside resistance for a specific class of aminoglycosides. I have contributed to the functional and structural understanding of one such m^7G1405 methyltransferase, RmtC (**ref. 1a,b**). In ongoing work, computational techniques have been employed to discover 'first-in-class' lead molecules targeting these

methyltransferases. These molecules, under development by the Wuest lab at Emory, aim to overcome the aminoglycoside resistance conferred by these enzymes. Furthermore, my contributions extend to the structural and evolutionary understanding of the dual-substrate methyltransferase TlyA from *Mycobacterium tuberculosis* (**ref. 1c,d**). This enzyme is associated with mycobacterial resistance to tuberactinomycin drugs like capreomycin and viomycin. Ongoing efforts are directed toward understanding methyltransferase recognition by the bacterial ribosome, the role of loop dynamics in these methyltransferases, and gaining a broader understanding of protein-ribosome interaction and allosteric transitions. Additional endeavors involve deciphering aminoglycoside dynamics and determining factors influencing resistance evasion by different drug scaffolds.

1a. Nosrati, M., **Dey, D.**, Mehrani, A., D. Strassler, S.E., Zelinskaya, N., Hoffer, E.D., Stagg, S.M., Dunham, C.M. and Conn, G.L. (2019). Functionally critical residues in the aminoglycoside resistance-associated methyltransferase RmtC play distinct roles in 30S substrate recognition. *J. Biol. Chem.* **294***, 17642-17653.* [PMCID-PMC6873201]

1b. Srinivas, P., Nosrati, M., Zelinskaya, N., **Dey, D.,** Comstock, L. R., Dunham, C. M., & Conn, G. L. (2023). 30S subunit recognition and G1405 modification by the aminoglycoside-resistance 16S ribosomal RNA methyltransferase RmtC. *Proc. Natl. Acad. Sci. U. S. A.*, **120**(25), e2304128120. [PMCID: PMC10288597]

1c. Laughlin, Z.T., Nandi, S*., **Dey, D.*,** Zelinskaya, N., Witek, M.A., Srinivas, P., Nguyen, H.A., Kuiper, E.G., Comstock, L.R., Dunham, C.M. and Conn, G.L. (2022) 50S subunit recognition and modification by the Mycobacterium tuberculosis ribosomal RNA methyltransferase TlyA. *Proc. Natl. Acad. Sci. U. S. A.*, **119**, e2120352119. *equal contribution as second author. [PMCID: PMC9168844]

1d. 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. (Also in revision at *Nucleic Acids Res*.)

2. Antibiotic resistance mediated by efflux pumps in bacteria. In *Pseudomonas aeruginosa*, the MexXY-OprM efflux pump drives clinical aminoglycoside resistance, notably in cystic fibrosis patients. Belonging to the RND superfamily, these pumps feature an inner membrane transporter, a periplasmic adaptor, and outer membrane component, forming a continuous channel for antibiotic export. Through molecular modeling, phylogenetic analysis, and docking studies, I proposed a 'Goldilocks affinity' hypothesis, i.e., a binding strength sufficiently robust to engage the transporter but not excessively tight to hinder substrate movement (**ref. 2a**). Further, I used computational techniques to identify di-berberine conjugate scaffolds as promising EPI leads against MexXY-OprM; MIC assays revealed improved efficacy of aminoglycosides when used in conjunction with them, marking them as lead candidates for combating MexXY-OprM-mediated resistance (**ref. 2b**). This discovery holds clinical significance for addressing antibiotic resistance. The broader goal involves employing various computational methods, including classical MD simulations, mixed solvent MD simulations, ensemble docking, and steered MD simulations, to comprehensively understand efflux pump functionality. Ongoing efforts include developing frameworks integrating structural, evolutionary, and network-based metrics, to understand the mechanisms underlying substrate preference, translocation dynamics, and allosteric pathways of the efflux pump essential for designing inhibitors against these pumps, thereby preserving antibiotic efficacy. Recently, I also contributed as co-first author on a comprehensive review of the RND efflux pump family structure, mechanism, and role in clinical antimicrobial resistance (**ref. 2c**).

2a. **Dey, D.,** Kavanaugh, L. G., & Conn, G. L. (2020). Antibiotic substrate selectivity of Pseudomonas aeruginosa MexY and MexB efflux systems is determined by a Goldilocks affinity. *Antimicrob. Agents Chemother.*, **64**(8), e00496-20. [PMCID: PMC7526836]

2b. Kavanaugh, L.G., Mahoney, A.R., **Dey, D.,** Wuest, W.M. (2023). Conn, G.L**.** Di-berberine conjugates as chemical probes of Pseudomonas aeruginosa MexXY-OprM efflux function and inhibition. *npj Antimicrob. Resist.* **1**:12. [PMC10327050; also posted on *bioRxiv*].

2c. Kavanaugh, L.G.†, **Dey, D.†,** Shafer, W.M.* and Conn, GL*. (2024). Structural and functional diversity of Resistance-Nodulation-Division (RND) efflux pump transporters with implications for antimicrobial resistance. *Microbiol. Mol. Biol. Rev*. **88**(3):e0008923. doi: 10.1128/mmbr.00089-23. †Co-first authors, *Co-corresponding authors. [PMCID: PMC11426026]

3. Dynamics and allostery of macromolecules involved in innate immunity. The dynamics of macromolecules play a crucial role in molecular recognition, activity and allostery. I have used computational techniques to study the molecular evolution and dynamics of oligoadenylate synthetase 1 (OAS1), a dsRNA

sensor which play an important role in sensing cytosolic double-stranded RNA (dsRNA) as part of the host cell innate immune system. With Dr. Conn's lab and as a part of a multinational team, I studied the changes in dynamics of gain-of-function (GoF) mutations using computational modeling and molecular dynamics (MD) approaches. We showed each OAS1 GoF affects the protein dynamics surrounding the enzyme active site, although the sites of these mutations are distributed across different regions of the protein (**ref. 3a**). My ongoing work in this area focusses on applying enhanced sampling molecular dynamics methods to understand the mechanism of allosteric communication between the GoF sites and residues surrounding the active site. In this work, I have also used computer aided drug design to discover first-in-class inhibitors for OAS1 and our ongoing efforts are focused on lead optimization leading to pre-clinical studies. In other published work, I have applied MD approaches to understanding a complex interplay of RNA features leads to more potent OAS1 activation (**ref. 3b**). Using classical molecular dynamics simulations of dsRNA and analysis of the conformational parameters, we are exploring the rules that govern potent OAS1 activation by dsRNA.

3a. Magg, T., Okano, T., Koenig, L.M., Boehmer, D.F.R., Schwartz, S.L., Inoue, K., Heimall, J., Licciardi, F., Ley-Zaporozhan, J., Ferdman, R.M., Caballero-Oteyza, A., Park, E.N., Calderon, B.M., **Dey, D.,** Kanegane, H., Cho, K., Montin, D., Reiter, K., Griese, M., Albert, M.H., Rohlfs, M., Gray, P., Walz, C., Conn, G.L., Sullivan, K.E., Klein, C., Morio, T. and Hauck, F. (2021) Heterozygous OAS1 gain-of-function variants cause an autoinflammatory immunodeficiency. *Science Immunol.*, **6**. [PMCID: PMC8392508].

3b. Schwartz, S.L., **Dey, D.,** Tanquary, J., Bair, C.R., Lowen, A.C. and Conn, G.L. (2022) Role of helical structure and dynamics in oligoadenylate synthetase 1 (OAS1) mismatch tolerance and activation by short dsRNAs. *Proc. Natl. Acad. Sci. U. S. A.*, **119**, [PMCID: PMC8784149].

4. Host-pathogen interactions and regulation of virulence *Pseudomonas aeruginosa*. As an opportunistic pathogen, *P. aeruginosa*, engages in complex interactions with its host, often evading host responses. The pathogen's ability to evade host defenses and establish persistent infections underscores its adaptability. Understanding the intricacies of this host-pathogen interplay is crucial for developing effective strategies to manage Pseudomonas infections. In a discovery by the Conn and Goldberg labs at Emory University, a novel methyltransferase named EftM was identified. EftM trimethylates the translation factor EF-Tu at Lys5, binds to Platelet-Activating Factor Receptor (PAFR), mimicking a phosphorylcholine modification crucial for host-cell adhesion and virulence. Enhanced adhesion of *P. aeruginosa* to host epithelial cells is facilitated by surface-exposed translation elongation factor EF-Tu with Lys5 trimethylation. I delved into the molecular mechanisms underlying EF-Tu recognition by EftM, revealing distinct structural features shared with both Class I Rossmann fold and eukaryotic SET domain methyltransferases (**ref. 4a**). This discovery expands our understanding of the intricate host-pathogen interactions. The future goal of this project aims to deepen our understanding of the role of EF-Tu in recognition and interaction with other modification machines in bacteria and host receptors. By elucidating these processes, I aspire to develop targeted interventions against EF-Tu, using computer aided drug discovery and structural biology. In another project, I used computational and phylogenetic techniques, to model MucP and AlgT to gain insights into clinical mutations and indels in protein structure and function (**ref. 4b**). *P. aeruginosa* isolates from chronic lung infections produce alginate, resulting in a mucoid phenotype that is challenging to treat with antibiotics. The sigma factor AlgT and metalloprotease MucP play a crucial role in regulating alginate biosynthesis, forming a multiprotein complex. Leveraging computational, biochemical, and structural approaches, my broader goal is to understand the *Pseudomonas*-host factors responses, focusing on therapeutic outcomes.

4a. Kuiper, E.G.*, **Dey, D.*,** LaMore, P.A., Owings, J. P., Prezioso, S. M., Goldberg, J. B., and Conn, G.L. (2019). Substrate recognition by the Pseudomonas aeruginosa EF-Tu methyltransferase EftM. *J. Biol. Chem.*, **294***,* 20109-20121. [PMCID: PMC6937583] (*Co-first authors)

4b. Cross, A. R., Raghuram, V., Wang, Z., **Dey, D.**, & Goldberg, J. B. (2020). Overproduction of the AlgT sigma factor is lethal to mucoid *Pseudomonas aeruginosa*. *J. Bacteriol.*, **202**(20), e00445-20. [PMCID: PMC7515251]

5. Global regulation of bacterial DNA transaction processes by HU and IHF. In bacteria, nucleoid-associated proteins (NAPs) represent a crucial group of global regulators that govern genome compaction, chromosomal architecture, and various DNA transactions, including replication, transcription, recombination, and repair. HU stands out as a key NAP in eubacteria, proving essential in Gram-positive bacteria and actinobacterial pathogens like Mycobacterium tuberculosis. Despite decades of biochemical and genomic studies on HU and IHF-like proteins, questions regarding their DNA binding specificity and their differential

ability to bend DNA, thereby affecting the binding site length, have persisted. My studies have provided a rational explanation, grounded in phylogenetic and structural analyses, for the sequence and structural determinants influencing the DNA binding specificity and bending of nucleoid-associated proteins HU and IHF (**ref. 5a**). Furthermore, my research has proposed that the molecular mechanisms underpinning specificity or multi-specificity depend on a co-factor. In subsequent studies, I delved into the role of crucial residues in HU family proteins to unravel their evolutionary history, leveraging these insights for inhibitor design targeting *M. tuberculosis* HU (MtbHU; **ref. 5b**). Using computational techniques, including virtual screening, precision docking, cheminformatics, and molecular dynamics simulations, I designed stilbene derivatives that inhibit HU–DNA binding, disrupt nucleoid architecture, and reduce tuberculosis growth. Furthermore, my research has utilized computational drug discovery, using suramin as a lead, to identify novel scaffolds for targeting HU (**ref. 5c**). MtbHU features a C-terminal (CTR) low-complexity disordered region, enhancing its DNA compaction capabilities and displaying striking similarities with histone tails. Bioinformatics analysis of this low-complexity region unveiled different nucleic acid binding proteins associated with this motif (**ref. 5d**). To gain a deeper understanding of the structure and dynamics of this intrinsically disordered region, I conducted replica exchange molecular dynamics (REMD) simulations, complemented by circular dichroism (CD) spectroscopy and nuclear magnetic resonance (NMR) studies by other team members. These investigations highlighted the role of proline and lysine residues in amplifying conformational heterogeneity in these CTRs, offering a first glimpse into conformation selection and binding to DNA by lysine and proline-rich "tails."

5a. **Dey, D.**, Nagaraja, V. and Ramakumar, S., 2017. Structural and evolutionary analyses reveal determinants of DNA binding specificities of nucleoid-associated proteins HU and IHF. *Mol Phylogenet Evol.* 107:356-366.

5b. Bhowmick, T., Ghosh, S., Dixit, K., Ganesan, V., Ramagopal, U.A., **Dey, D.,** Sarma, S.P., Ramakumar, S. and Nagaraja, V., 2014. Targeting Mycobacterium tuberculosis nucleoid-associated protein HU with structure-based inhibitors. *Nature Communications*, 5/ 10.1038/ncomms5124.

5c. **Dey, D**., Ramakumar, S., & Conn, G. L. (2021). Targeted redesign of suramin analogs for novel antimicrobial lead development. *J. Chem. Inform. Model.*, 61(9), 4442-4454.

5d. Khare, H., **Dey, D.,** Madhu, C., Govindaraju, T., Ramakumar S. Conformational heterogeneity in tails of DNA-binding proteins is augmented by proline containing repeats. *Mol Biosyst.* 2017 Nov 21;13(12):2531-2544.

BIOGRAPHICAL SKETCH

NAME: Suparno Nandi

eRA COMMONS USER NAME (credential, e.g., agency login): SNANDI21

POSITION TITLE: Postdoctoral Fellow

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)*	Completion Date MM/YYYY	FIELD OF STUDY
St. Xavier's College, Kolkata, India	MSC (integrated)	06/2013	Biotechnology
The University of Iowa, Iowa City, Iowa, USA	PHD	12/2020	Chemistry
Emory University, Atlanta, USA	Postdoctoral	In Progress	Biochemistry

A. Personal Statement

I am a **primary user** of this NCCAT BAG application, and I will be involved in preparing samples and shipping them to NCCAT, remotely collaborating with the cryo-electron microscopist at the facility and monitoring data collection, followed by data processing and structure determination at Emory. As a postdoctoral fellow, I have 4 years of experience in cryo-EM, and during this time, I have determined eight cryo-EM structures of *M. smegmatis* (*Msm*) 70S ribosomes, complexes of Trm10-tRNA and RsmF-*E. coli* (*Eco*) 30S ribosome, from data collected at NCCAT/PNCC with sizes ranging from 65 kDa to 2.7 MDa. In the process, I have acquired proficiency in negative staining, sample preparation in a variety of grids by plunge freezing technique, clipping grids, and handling microscopes such as Talos L120C and Talos Arctica. Additionally, I am skilled in the analysis and interpretation of cryo-EM data using RELION/CryoSPARC, PHENIX/CCP-EM based-refinement, and model building using COOT/Chimera/ChimeraX. My understanding of cryo-EM technology has allowed me to determine the structure and decipher the mechanism of different methyltransferases such as TlyA, RsmF, and Trm10 in complex with ribosomes and tRNA, which are involved in the modification of the macromolecules (see citations 1-2 below).

1. **Nandi, S.**, Dey, D., Srinivas, P., Dunham, C. M., Conn, G. L. Distant Ribose 2'-O-Methylation of 23S rRNA Helix 69 Pre-Orders the Capreomycin Drug Binding Pocket at the Ribosome Subunit Interface. *bioRxiv* 2024.11.05.619916
2. Laughlin, Z.T., **Nandi, S*.**, Dey, D.*, Zelinskaya, N., Witek, M.A., Srinivas, P., Nguyen, H.A., Kuiper, E.G., Comstock, L.R., Dunham, C.M. and Conn, G.L. (2022) 50S subunit recognition and modification by the Mycobacterium tuberculosis ribosomal RNA methyltransferase TlyA. Proc. Natl. Acad. Sci. U. S. A., 119, e2120352119. *equal contribution as second author.

B. Positions, Scientific Appointments, and Honors

Positions and scientific appointments

2021-present Postdoctoral fellow, Department of Biochemistry, Emory University School of Medicine, USA.
2024-present Reviewer, Nature Chemical Biology, Biochemistry, PLOS One, PeerJ
2021 Judge, 19th Annual GDBBS Research Symposium, Emory University, Atlanta, USA
2015-2020 Graduate Research Assistant, Department of Chemistry, University of Iowa, Iowa City, IA, USA
2015-2020 Graduate Teaching Assistant (Fall '20, '18, '16, '15: General Chemistry I (CHEM 1070); Spring '19, '17, '16, Summer '16: Principles of Chemistry I (CHEM 1110) Department of Chemistry, University of Iowa, Iowa City, IA, USA

2013-2014 Project fellow, CSIR-Indian Institute of Chemical Biology, Kolkata, India
2013 M.Sc. dissertation, Indian Institute of Science, Education, and Research (IISER)-Kolkata, India
2012 Summer trainee, IISER-Kolkata, India

Honors

2018 Named a champion of student success at the University of Iowa for creating a positive impact on new students
2017 Director's award for the best poster at the Center for Biocatalysis and Bioprocessing (CBB) Conference, USA
2015 Travel award from the University of Iowa for an outstanding undergraduate record, and past achievements
2014 Selected for a project fellowship by the CSIR, Ministry of Science & Technology, Govt. of India
2012 Selected for a summer research fellowship by the IISER-Kolkata, Ministry of Human Resource and Development, Govt. of India

C. Contributions to Science

1. Mechanistic studies of methyltransferases involved in antibiotic resistance and tRNA recognition

My postdoctoral research is directed towards understanding the mechanism of action of different methyltransferases such as TlyA, Trm10, and RsmF. My studies with TlyA have been instrumental in determining the molecular surface employed by the enzyme to interact with the Msm ribosome. Specifically, the structure of TlyA solved in complex with Msm 50S ribosome using data collected at NCCAT has uncovered a hitherto unknown mechanism of base flipping used by the enzyme to modify its target nucleotide. Additionally, from the data collected at NCCAT of methylated and unmethylated Msm 70S ribosomes, I, with a team of collaborators, have deciphered the effect of TlyA methylation in capreomycin binding and uncovered the existence of an allosteric signaling mechanism induced by methylation in the H69. As part of another project, I, along with another graduate student and other team members, have discovered the mechanism of selective methylation of G9 nucleotide in tRNA by yeast Trm10 enzyme by solving three cryo-EM structures of the enzyme-tRNA complex from the data obtained at NCCAT. Finally, I and other team members have obtained preliminary cryo-EM maps and structures of another methyltransferase, RsmF, in complex with Eco 30S ribosomal subunit in a variety of conformations that shed light on the mechanism of recognition and modification of 30S by the enzyme.

a. **Nandi, S.**, Dey, D., Srinivas, P., Dunham, C. M., Conn, G. L. Distant Ribose 2'-O-Methylation of 23S rRNA Helix 69 Pre-Orders the Capreomycin Drug Binding Pocket at the Ribosome Subunit Interface (2024). *bioRxiv* 2024.11.05.619916
b. Laughlin, Z.T., **Nandi, S*.**, Dey, D.*, Zelinskaya, N., Witek, M.A., Srinivas, P., Nguyen, H.A., Kuiper, E.G., Comstock, L.R., Dunham, C.M. and Conn, G.L. (2022) 50S subunit recognition and modification by the Mycobacterium tuberculosis ribosomal RNA methyltransferase TlyA. Proc. Natl. Acad. Sci. U. S. A., 119, e2120352119. *equal contribution as second author.
c. Strassler, S.E.*, **Nandi, S.*,** Comstock L. R., Jackman, J. E., Conn, G. L. Cryo-EM structure of the tRNA methyltransferase Trm10 bound to substrate tRNA, poster presentation, 75th Southeastern Regional Meeting of the American Chemical Society (SERMACS), Atlanta, GA (2024). *equal contribution as first author
d. **Nandi, S.**, Dey, D., Srinivas, P., Dunham, C. M., Conn, G. L. Distant Ribose 2'-O-Methylation of 23S rRNA Helix 69 Pre-Orders the Capreomycin Drug Binding Pocket at the Ribosome Subunit Interface., Poster presentation, Gordon Research Conference on Nucleic Acids, Newry, ME (2023).

2. Allosteric Regulation of Pyruvate Kinase Muscle Isoform 2 (PKM2)

My graduate research was to determine the allosteric regulation mechanism of PKM2 enzyme by posttranslational modifications and small molecules. My biochemical and biophysical studies of PKM2 with asparagine, aspartic acid, valine, serine, and cysteine, along with six crystal structures of the enzyme with the small molecules, showed that the non-polar side chains of valine and cysteine inhibit PKM2 whereas, the polar side chains of asparagine, aspartic acid, and serine activate PKM2 by altering the oligomeric state of the enzyme. Site-directed mutagenesis, coupled with activity assays, gel filtration studies, and a crystal structure of a PKM2 variant, showed that a β strand between the active site and the AA binding pocket was responsible for bidirectional signaling between the sites. Also, our crystallographic studies of four PTM mimetics of PKM2 showed that phosphorylation and acetylation of the enzyme cause minor structural changes in the binding pocket

of fructose 1, 6 bisphosphate (FBP), which is an allosteric activator of PKM2. This leads to a decrease in the FBP binding affinity of the enzyme, which causes a shift in the oligomeric state of PKM2 from a tetramer to a dimer. Overall, our explanation of the allosteric regulation mechanism of PKM2 will aid in the development of small molecule agonists and antagonists against PKM2, which can influence the proliferation of tumors.

a. **Nandi, S.**, Dey, M. Biochemical and structural insights into how amino acids regulate pyruvate kinase muscle isoform 2 (2020). *Journal of Biological Chemistry*, 295, 16, 5390 – 5403 (PMID: 32144209).
b. **Nandi, S.***, Razzaghi, M*., Srivastava, D., Dey, M. Structural basis for allosteric regulation of pyruvate kinase M2 by phosphorylation and acetylation (2020). *Journal of Biological Chemistry*, 295, 51, 17425 – 17440 (PMID: 33453989). *equal contribution as first author
c. **Nandi, S.**, Dey, M. Identification of residues involved in allosteric signal transmission from amino acid binding site of pyruvate kinase muscle isoform 2 (2023). PLoS ONE 2023 Mar 10;18(3):e0282508 (PMID: 36897854).
d. **Nandi, S.**, Dey, M. Biochemical and Structural Investigation of the Dynamic Regulation Mechanism of Pyruvate Kinase Muscle Isoform 2 using Amino Acids (2019) *Acta Cryst.* A75, a406.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/suparno.nandi.1/bibliography/public/

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: Mohamed Issam Barmada

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

POSITION TITLE: Graduate Student

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 Pennsylvania, Philadelphia, PA	B.A.	08/2017	05/2021	Biochemistry
University of Pennsylvania, Philadelphia, PA	M.S.	08/2019	05/2021	Chemistry
Emory University, Atlanta, GA	Ph.D.	08/2022	In Progress	Biochemistry and Structural Biology

A. Personal Statement

I am currently a 3rd year student in Emory's Biochemistry, Cell and Developmental Biology (BCDB) graduate program and I am completing my thesis research in Dr. Graeme Conn's lab in the Department of Biochemistry. My role on this NCCAT BAG will be as a **primary user**, performing task including specimen preparation and shipment to NCCAT, monitoring of data collection, and data processing and structure determination. My thesis project centers on defining the mechanism of 30S subunit recognition by two intrinsic *E. coli* 16S rRNA methyltransferases (RsmH and RsmI) as well as the impact of the loss of the methylations they incorporate on ribosome function and sensitivity to antibiotics. I am currently in the final stages of completing studies and preparing a manuscript that will describe the high-resolution structure of the 30S-RsmI complex which was determined using data from NCCAT (during our previous BAG award period).

B. Positions, Scientific Appointments and Honors

Positions and Employment

Aug 2023 - Dec 2023 Foundations in Biochemistry, Cell, and Developmental Biology (BCDB) Teaching Assistant, BCDB Graduate Program, Emory University (Course Directors: Homa Ghalei, Ph.D., and Lefteris Michailidis, Ph.D.).

Mar 2023 - present Graduate Student, Department of Biochemistry, Emory University School of Medicine (Mentor: Graeme Conn, Ph.D.).

2020 - 2021 Organic Chemistry Lab Teaching Assistant, Department of Chemistry, University of Philadelphia (Instructor: Jennifer Rutherford, Ph.D.).

2020 – 2021 Master's Student, University of Pennsylvania's Children's Hospital of Philadelphia (Mentors: Marni Falk, M.D., and Eiko Nakamura-Ogiso, Ph.D.).

2019 - 2020 Tutor, University of Pennsylvania Tutoring Center.

2018 - 2019 Undergraduate Researcher, Department of Biology, University of Pennsylvania (Mentor: Michael Lampson, Ph.D.).

2017 - 2018 Lab Assistant, Department of Biology, University of Pennsylvania (PI: Lori Splinder, Ph.D.).

Scientific Appointments and Other Experiences

May 2024 – present Member of the Emory RNA Salon.

Fall 2023 - present Reviewer, Journal of Emerging Investigators.

Fall 2023 - present	Scientist Mentor, Letters to a Pre-Scientist.
2023	Volunteer, Atlanta Science Festival.
2022 - 2023	Graduate Student Mentor, Emory Residence Hall Association Meet and Mentor Initiative.
2020 - 2021	Tutor, CovEducation Online Mentorship and Tutorship Program.
2019 - 2020	Clinical Volunteer, Corporal Michael J. Crescenz, VA Medical Center.
2019 - 2020	Mentor, University of Pennsylvania's English Language Program.

Honors and Awards

03/2025	RNA Society Research Presentation Fellowship
07/2024 - present	Ruth L. Kirschstein National Research Service Award 1F31AI186518-01.
2023	Margaret and Thomas Lew First Year Award, BCDB Program, Emory University (awarded to the top performing 1st year BCDB student).
2023	BCDB T32 Training Grant Appointment, BCDB Graduate Program, Emory University.
2022 - 2027	Laney Graduate School Fellowship, Emory University.
2021	Graduated with *Summa cum laude* honors, University of Pennsylvania.
2021	Award for exceptional teaching service from the Department of Chemistry, University of Pennsylvania.
2018	Ernest M. Brown, Jr. College Alumni Society Undergraduate Research Grant, University of Pennsylvania (1000$ award given to students with exceptional research proposals)
2018	Penn Undergraduate Research Mentorship Program Award, University of Pennsylvania (ten-week funded summer research program for students).
2017 – 2019	Dean's List, University of Pennsylvania (awarded to students with a GPA of 3.7 or higher).

C. Contributions to Science

1. Ph.D. Thesis Research in the Conn Lab (Emory University)

I joined Dr. Graeme Conn's Lab in March 2023, where I have been working on my thesis project examining the role of ribosomal RNA methyltransferases RsmH and RsmI, and the modifications they incorporate in the ribosome decoding center, in the response of bacteria to antibiotics. My thesis work will determine the molecular basis of how RsmH and RsmI recognize and site-specifically modify their unique target sites on the same 16S rRNA nucleotide (C1402), the full extent of how they influence antibiotic susceptibility, and their capacity to influence antibiotic resistance via nearby acquired modifications found in some pathogenic bacteria. For my preliminary work on my thesis project, I presented a poster at the 21st GDBBS Symposium at Emory. Additionally, to begin my training in high-resolution single-particle electron microscopy as early as possible, I took over a related project from a former graduate student in which I aim to obtain the first high-resolution structure of the thiostrepton-resistance methyltransferase (TsnR) bound to the 70S ribosome. This work is still on-going. More recently, I have used a high-resolution data set collected at NCCAT to determine a 2.4Å structure of the dimeric RsmI enzyme bound to its 30S subunit substrate. I have also completed comprehensive complementary analyses of RsmI enzyme variants to further test insights from my structure and I am currently preparing a first author manuscript describing this work.

a) **Barmada MI,** Zelinskaya N, Conn GL. Intrinsic Ribosomal Decoding Center Methylation and the Bacterial Antibiotic Response. [Poster Presentation]. 21st Annual GDBBS Research Symposium, Emory University, Atlanta, GA, 2024.
b) **Barmada, M.I.** and Conn, G.L. (2024). Evading Resistance at the Double. *Nat. Chem Biol.* **20**(12):1555-1556. doi: 10.1038/s41589-024-01772-5
c) **Barmada, M.I.**, Zelinskaya, N., Conn, G.L. "Intrinsic Ribosomal Decoding Center Methylation and the Bacterial Antibiotic Response", research talk, Emory GA RNA Salon Meeting, Sep 2024.
d) **Barmada, M.I.**, Zelinskaya, N., McGinity, E., Conn, G.L. **"**Mechanism of Substrate Recognition and Modification by Ribosomal Methyltransferase RsmI," poster presentation, 2024 Southeastern Regional Meeting of the American Chemical Society (SERMACS), Atlanta, GA.

2. Ph.D. Rotation Research

At Emory, I had the chance to conduct rotations with three labs, so I decided to use this opportunity to explore wide fields of science that interested me. In my first seven-week rotation in Dr. Shashank Shekhar's lab, I studied actin dynamics using a microfluidics-based total internal reflection fluorescence microscopy technique. I

examined how the toxin VopF elongates actin at the minus end of an actin filament in the presence of the actin-binding partner profilin, which ordinarily forces actin to elongate from the plus end. I discovered that the presence of profilin does not impact the rate of minus end elongation of actin filaments by the toxin.

In my second rotation in Dr. Graeme Conn's lab, I worked alongside postdoc Dr. Suparno Nandi to investigate how the mycobacterial 2'-O-methyltransferase TlyA mediates the addition of methyl groups to its two ribosomal RNA (rRNA) nucleotide substrates. I examined the role of the four amino acid linker region that connects the rRNA binding N-terminal domain (NTD) and the catalytic methyltransferase C-terminal domain (CTD) of TlyA. Using a radioactive *in vitro* methylation assay, I compared the activity levels of cleaved TlyA domains with and without the linker region and discovered that the linker region is needed for the cleaved TlyA domains to exhibit residual methyltransferase activity. For my work on this project, I presented a poster at the 13th Annual Southeast Enzyme Conference at Georgia State (**ref. a**). Dr. Nandi's on-going work on the long-distance structural impact of TlyA's modification in Helix 69 (of the large subunit, 23S rRNA) on the capreomycin binding site within the 16S rRNA of the small subunit also provided inspiration and precedence for my hypothesis regarding the impact of loss of C1402 modifications on h44 structure.

In my third rotation in Dr. Renhao Li's lab, I studied the binding properties of the pro-clotting nanobody 6C11 using ELISA and biolayer interferometry approaches. The Li Lab had characterized two nanobodies 6D12 and 6C11 which promote Von Willebrand Factor (VWF) induced clotting. I discovered that 6C11 has comparable abilities to 6D12 to promote clotting at nM concentrations of nanobody, but 6D12 vastly eclipses 6C11 in performance when the concentration of nanobody is in the µM range.

a) **Nandi S, Barmada MI, Laughlin ZT, Conn GL.** Deciphering the Mechanism of TlyA-Mediated Ribosomal RNA Methylation: Insights into Ribosome Subunit Recognition & Interdomain Signaling. [Poster Presentation]. 13th Annual Southeast Enzyme Conference, Georgia State University, 2023

3. Master's Research in the Falk Lab:

As a junior in college, I decided to submatriculate and graduate with both a master's degree in chemistry in addition to a bachelor's degree in biochemistry, which lead me to joining the Falk Lab. There, I conducted a master's thesis project focused on establishing a polyacrylamide-based staining method to detect a variant of glycoproteins, N-linked glycoproteins, within mitochondria. The Falk Lab specializes in mitochondrial diseases, and one specific area of interest within the lab was establishing the existence of N-linked glycoproteins in mitochondria. Specifically, we hypothesized that the glycosylation status of mitochondrial proteins might regulate their functions and activities based on glucose availability to adjust cellular metabolism. Additionally, we had some preliminary evidence that MSR2, a mitochondrial magnesium transporter protein in mammals, could be an N-linked glycoprotein. However, the glycoprotein visualization method we were using to obtain this evidence often produced ambiguous results. I was therefore tasked with establishing alternative and more reliable methods to detect N-linked glycoproteins selectively and sensitively in mitochondria. After extensively studying the existing literature on methods to visualize and detect N-linked glycoproteins, as well as on the history of attempts to uncover N-linked glycoproteins within mitochondria, I identified five promising glycoprotein detection techniques that had never been tried, to my knowledge, on mitochondrial protein extracts. The detection methods consisted of colorimetric or fluorescent tags conjugated with hydrazides that selectively reacted with the sugar components of glycoproteins via periodic acid-Schiff base chemistry to exclusively stain glycoproteins on polyacrylamide gels. I conducted sensitivity and selectivity tests for the five glycoprotein stains using SDS PAGE assays, where I identified that the fluorescent stain Emerald 488 was best suited to my purposes. Then, I used Emerald 488 staining in combination with the protein separation techniques SDS PAGE and Blue Native PAGE - 2D SDS PAGE to detect three potential glycoproteins within mouse mitochondria, which I confirmed preliminarily to be N-linked using N-linked glycoprotein digestive enzymes. Not only did my work provide the Lab with a method that could definitively identify whether MSR2 was an N-linked glycoprotein, but it also revealed the existence of other potential mitochondrial N-linked glycoproteins that the Lab could explore next. Because of the pandemic, the opportunities I had to share my work were limited, but I was able to write a master's thesis (**ref. a**), giving me extensive scientific manuscript writing experience.

a) **Barmada, MI.** Optimizing Glycoprotein Stains for Detection of N-linked Glycoproteins in Mitochondria. Master's thesis. University of Pennsylvania, 2021.

4. Undergraduate Research in the Lampson Lab:

In the Lampson Lab, I worked with a postdoctoral researcher (Dr. Arunika Das) to study centromeric protein A (CENPA) using mice as a model organism. CENPA, a histone H3 variant, is an incredibly stable protein that is passed down epigenetically, and Dr. Das's research involved testing how well CENPA is inherited from parents

to offspring when such stability is compromised via mutations. One reason for the stability of CENPA is the presence of a hydrophobic core in its sequence that interacts with histone H4, so we aimed to test the effects of disrupting this interaction. We substituted an alanine residue in CENPA's hydrophobic core with hydrophilic serine in mice embryos using CRISPR/Cas9 and tested the subsequent stability of CENPA in the germline of these embryos when they developed. My roles in the project included genotyping, mouse husbandry, collecting mouse germline cells, imaging germline cells by immunofluorescence, and data quantification. I was also heavily involved with checking the efficiency and viability of the CRISPR guide RNAs designed to create the mutation, for which I presented a poster (**ref. a**) about during the University of Pennsylvania Center for Undergraduate Research and Fellowships 2018 Fall Research Expo.

a) **Barmada MI, Das A, Ma J, Lampson MA.** Testing The Internal Stability of CENPA in Mice. [Poster Presentation]. University of Pennsylvania Center for Undergraduate Research and Fellowships Fall Research Expo, 2018.

5. Teaching and Mentorship Contributions

Apart from research, I have heavily immersed myself in teaching and mentorship experiences. During my undergraduate career, I was both a chemistry tutor and an organic chemistry lab teaching assistant. As a teaching assistant, I held weekly lab sessions where I taught my students how to conduct lab techniques, interpret data, and write reports. This teaching experience improved my time management skills, as I had to prepare lessons and grade reports before next week's session, and it advanced my ability to teach science in a comprehensive manner to my peers. I also served as a mentor for the University of Pennsylvania's English Language Program, where I helped a foreign student apply to colleges in the US and get acclimated to the social environment here. Additionally, I mentored and tutored a high school student disproportionately impacted by the Covid-19 pandemic as part of my role with the organization CovEducation. As a Ph.D. student, I became a mentor as part of the Emory Residence Hall Association Meet and Mentor Initiative, where I assisted a college junior in applying to graduate school. Moreover, I joined the Letters to a Pre-Scientist Program as a mentor, where I have been exchanging letters about science with a middle school student. I also am currently a teaching assistant for the BCDB Foundations graduate course, in which I am responsible for leading weekly classes where I recap the course's weekly modules to first year graduate students. Lastly, I am always on the lookout for new opportunities to engage with future generations of scientists. For instance, I have recently become a reviewer for the Journal of Emerging Investigators, which is an open access journal that publishes original science research by middle and high school students.

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: Jacob M. Mattingly

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

POSITION TITLE: Postdoctoral Fellow

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)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Kentucky, Lexington, KY	B.S.	05/2016	Chemistry, Philosophy
Emory University, Atlanta, GA	Ph.D.	11/2024	Biochemistry, Cell, and Developmental Biology
Emory University, Atlanta, GA	Postdoc	Present	Biochemistry

A. Personal Statement

As a member of the Dunham Lab, my role in this proposal is to use electron cryomicroscopy to determine structures of ribosome complexes of particular interest for studies of translational fidelity and antibiotic resistance. These studies will generate important findings related to the fundamental biological processes underlying bacterial protein synthesis and can support the development and optimization of antibiotics to treat bacterial infections.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2024 - Present Postdoctoral Research Fellow, Emory University, Atlanta, GA
2019 - 2020 Graduate Teaching Assistant, Emory University, Atlanta, GA
2018 - 2024 Graduate Student, Emory University, Atlanta, GA
2016 - 2018 Research Technician, University of Chicago, Chicago, IL
2014 - 2016 Undergraduate Research Assistant, University of Kentucky, Lexington, KY
2014 - 2014 Summer Research Intern, University of Louisville, Louisville, KY
2013 - 2013 Summer Research Intern, University of Louisville, Louisville, KY

Honors

2019 - 2020 Training Grant Appointment (T32 GM008367-30), Emory University Biochemistry, Cell, and Developmental Biology Graduate Program
2019 National Science Foundation Graduate Research Fellowship Program Honorable Mention, National Science Foundation
2019 Margaret and Thomas Lew First Year Achievement Award in Biomedical Sciences (competitive award provided to one student annually in Emory University's Biochemistry, Cell, and Developmental Biology PhD program based on performance in first-year classes and on the first-year qualifying exam), Emory University Biochemistry, Cell, and Developmental Biology Graduate Program
2018 - 2023 Laney Graduate School Fellowship (competitive supplementary graduate fellowship), Emory University Laney Graduate School

2016	Magna Cum Laude, University of Kentucky
2016	Hammond Undergraduate Service Award (awarded to one student annually for departmental and community service), University of Kentucky Department of Chemistry
2014 - 2016	Gaines Fellowship for the Humanities (cometitive, two-year humanities research program emphasizing rhetorical skills; fellows produce a thesis project during their senior years of undergraduate study), University of Kentucky Gaines Center for the Humanities
2013 - 2014	Chellgren Fellowship for Undergraduate Excellence (competitive, one-year undergraduate fellowship emphasizing research, professional skill development, rhetorical training, and preparation for postgraduate studies), University of Kentucky Chellgren Center for Undergraduate Excellence
2012 - 2015	Dean's List, University of Kentucky

C. Contributions to Science

1. Macrolide-induced frameshifting. Macrolides are an important class of translation-targeting antibiotics targeting the large subunit of the bacterial ribosome. Bacteria have evolved means to resist their activity, including through methylation of their 23S rRNA at the macrolide binding site near the ribosome's peptidyl transferase center. Activation of these 23S rRNA methylation systems often occurs through a translation attenuation-mediated mechanism, whereby the activity of macrolide antibiotics causes ribosomes to stall while translating a small leader peptide whose coding sequence is located upstream of the coding sequence for a resistance methyltransferase on the same mRNA transcript. Ribosome stalling induces a change in the mRNA secondary structure, liberating the ribosome binding site for the sequence encoding the resistance methyltransferase. Translation attenuation of the leader mRNA sequence *ermCL* is the canonical activation mechanism for the macrolide resistance gene *ermC*. Ketolides are a subclass of macrolide antibiotics which lack a sugar modification at position C3 on their characteristic macrolactone ring. Because this sugar modification is necessary for ribosome stalling during translation of *ermCL*, ketolides would not canonically be expected to activate translation of the *ermC* mRNA sequence. However, ketolides do activate *ermC* translation, instead proceeding through a mechanism involving a macrolide-dependent shift in the mRNA reading frame. The ability of macrolides to cause frameshifts during translation is striking due to the distance between their binding site and the mRNA path of the ribosome and is not well understood. I plan to use electron cryomicroscopy studies of macrolide-bound ribosomes translating frameshift-prone mRNA sequences across the translation cycle to uncover the molecular mechanism of macrolide-induced frameshifting, including the signal transmitted from the macrolide binding site to the mRNA path of the ribosome.

2. Impact of ribosome-tRNA interactions on the fidelity of translation initiation. Translation initiation is a highly regulated, multi-step process whose accuracy and efficiency is necessary for protein synthesis. In bacteria, the initiation factors IF1, IF2, and IF3 drive selection of the initiator tRNA, $tRNA^{fMet}$, at the canonical mRNA start codons AUG, GUG, and UUG. Along with the fMet moiety at the tRNA acceptor end, the ribosome uses a series of three consecutive G-C base pairs in the tRNA anticodon stem to select $tRNA^{fMet}$ from the pool of cellular tRNAs. When the middle G-C base pair is flipped to C-G (a $tRNA^{fMet}$ variant referred to as $tRNA^{fMet}$ M1), the ribosome loses its ability to discriminate against the noncanonical start codon CUG (versus the canonical start codons AUG, GUG, and UUG), suggesting that there may be poorly understood crosstalk between the strength of tRNA-ribosome and tRNA-mRNA interactions during translation initiation. Additionally, this loss in initiation fidelity is suppressed by IF2, suggesting that IF2 serves a poorly understood quality control role during initiation. Using cryo-EM to determine structures of *E. coli* ribosomes containing the M1 variant of $tRNA^{fMet}$ bound to various NUG start codons in the presence or absence of IF2, we determined that the M1 base pair flipping mutation disrupts interactions between the 16S rRNA in the ribosomal P site and the $tRNA^{fMet}$ minor groove, leading to the observed dysregulation of start codon selection during initiation. Additionally, IF2 partially restores the strength of these interactions, explaining its ability to suppress translational infidelity during initiation.

Mattingly JM, Nguyen HA, Roy B, Fredrick K, Dunham CM. Structural analysis of noncanonical translation initiation complexes. *Journal of Biological Chemistry* (2024) 300(10). DOI: 10.1016/j.jbc.2024.107743

3. Antibiotic Resistance Evasion. Aminoglycoside antibiotics are a crucial class of antimicrobial drugs which target the fidelity of bacterial protein synthesis. An increasingly common mode of resistance to aminoglycoside antibiotics is methylation of 16S ribosomal RNA (rRNA) nucleotides G1405 or A1408 to yield m^7G1405 or m^1A1408, respectively. While methylation of G1405 yields pan-resistance to the clinically important 4,6-disubstituted deoxystreptamine (4,6-DOS) subclass of aminoglycosides (including drugs like gentamicin and kanamycin), 4,6-DOS aminoglycosides display a spectrum of activity against bacteria expressing m^1A1408, with some 4,6-DOS aminoglycosides maintaining their activity and others having their activity nearly completely abolished. With A1408 methylation-mediated resistance on the rise, it would be of benefit for rational optimization of aminoglycoside drug structures to understand the chemical features which give rise to the ability of some aminoglycoside drugs to evade resistance; however, these features have not previously been determined. Using a combined molecular dynamics and electron cryomicroscopy (cryo-EM) approach, we have determined chemical features of 4,6-DOS aminoglycosides (including ring substituents at specific positions in the aminoglycoside scaffold) which enhance aminoglycoside activity in the presence of m^1A1408. These findings can aid in the design of aminoglycosides whose activity is maintained in the presence of resistance-associated rRNA methylation.

Dey D*, **Mattingly JM***, Zelinskaya N, Dunham CM, Conn GL. Basis for selective drug evasion of an aminoglycoside-resistance ribosomal RNA modification. (2024). Under review.

4. Ribonucleases Regulating Translation. Ribosome hibernation is a crucial cellular process enabling cells to conserve energy and resources during periods of stress or nutrient starvation. In bacteria, hibernation promoting factor (Hpf) drives hibernation by preventing mRNA binding to ribosomes and, in many species, by inducing dimerization of 70S ribosomes to form a 100S hibernating ribosome complex. The *hpf* mRNA transcript encoding Hpf protein was recently discovered to be regulated by YhaM, a purported 3'-to-5' exoribonuclease present in Gram-positive bacteria, where it trims the 3' end. However, much is unknown about YhaM, with no empirically determined structure and no clear consensus on how it selects mRNA substrates. Using electron cryomicroscopy, we have determined the structure of YhaM to an overall resolution of 3.3 Å. YhaM uses an oligonucleotide-oligosaccharide binding (OB) fold domain to bind its RNA substrates and uses a histidine-aspartate-rich (HD) phosphorohydrolase domain to iteratively remove nucleotides from the 3' end.

Tanquary JR*, **Mattingly JM***, Lipońska A*, Yap MN, Dunham CM. Exploring the structure of Staphylococcus aureus exoribonuclease YhaM: Implications for RNA Processing and Virulence. (2025). In preparation.

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: Usoro, Edu Nkeneke

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

POSITION TITLE: Graduate Student

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
Michigan State University, East Lansing, MI		08/2016		Biochemistry
University of Michigan, Ann Arbor, MI	B.S.	09/2017	05/2020	Biomolecular Science
Emory University, Atlanta, GA	Ph.D.	08/2021	In Progress	Molecular and Systems Pharmacology

A. Personal Statement

I am currently a 4th year student in Emory's Molecular and Systems Pharmacology (MSP) graduate program and I am completing my thesis research in Dr. Christine Dunham's lab in the Department of Chemistry. As a **primary user** on this NCCAT BAG application, I will perform specimen preparation and shipment to NCCAT, monitoring of data collection, and data processing and structure determination. By supporting my research to investigate the mechanisms of ribosome rescue using *trans*-translation inhibitors, access to this facility will enhance my technical and professional skills.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

May 2021 – Present Graduate Student Researcher, Department of Biochemistry, Emory University, Atlanta, GA
Aug 2020 – Jul 2021 Lead Molecular Technologist II, Tempus Labs, Inc., Chicago, IL
May 2020 – Aug 2020 Extraction Technologist, Tempus Labs, Inc., Chicago, IL
Sep 2017 – May 2020 Laboratory Assistant, Department of Internal Medicine, University of Michigan, Ann Arbor, MI
May 2017 – Aug 2017 Research Intern, National Science Foundation Research Experience for Undergraduates, Clemson University, Clemson, SC

Teaching Experience

Aug 2022 – Dec 2022 Graduate Teaching Assistant for Introduction to Pharmacology, Molecular and Systems Pharmacology Program, Emory University, Atlanta, GA

Honors

2021 – Present Initiative to Maximize Student Development Fellowship (IMSD, NIH 5R25GM125598-04), Emory University
2021 – Present Centennial Scholars Fellowship, Emory University
2018 University Honors, University of Michigan
2017 Dean's list, Michigan State University
2016 Dean's list, Michigan State University

C. Contributions to Science

1. Bacterial ribosome rescue as a novel antimicrobial pathway, May 2022-Present

My dissertation research is dedicated to unraveling the molecular mechanism of newly identified antibiotics against *Mycobacterium tuberculosis* (Mtb). This compound is broad spectrum against diverse pathogenic bacteria and is exciting because it is potent against latent or persistent Mtb. This new antibiotic targets *trans*-translation, a specialized quality control pathway only present in bacteria. My research aims to elucidate the molecular basis of inhibition of *trans*-translation in Mtb. Additionally, this research will also provide significant insights into the role of *trans*-translation in bacterial survival and persistence. Unraveling the mechanism of action of this new antibiotic is promising in the development of targeted therapies against Mtb. Moreover, by delving into this mechanism, my research will advance our understanding of fundamental bacterial processes such as *trans*-translation and its effect on bacterial physiology.

a) Poster: **E. Usoro**, P. Srinivas, A. Varshney, K.C. Keiler, C.M. Dunham. *Inhibition of trans-translation as a novel antibiotic strategy to combat* M. tuberculosis. Division Student Advisory Council's 2024 Graduate Division of Biological and Biomedical Sciences Symposium, Atlanta, GA, 2024
b) Poster: **E. Usoro**, P. Srinivas, A. Varshney, K.C. Keiler, C.M. Dunham. *Inhibition of trans-translation as a novel antibiotic strategy to combat* M. tuberculosis. National Organization for the Professional Advancement of Black Chemists and Chemical Engineers Annual Conference, Orlando, FL, 2024
c) Poster: **E. Usoro**, P. Srinivas, A. Varshney, K.C. Keiler, C.M. Dunham. *Inhibition of trans-translation as a novel antibiotic strategy to combat* M. tuberculosis. The 75th Southeastern Regional Meeting of the American Chemical Society, Atlanta, GA, 2024

2. A New Role for bL27 in *trans*-Translation

This research, conducted in collaboration with the Keiler laboratory, investigates the role of ribosomal protein bL27 in the *trans*-translation process, which is vital for bacterial ribosome rescue and pathogenic virulence. We examine how bL27 undergoes significant conformational changes upon binding with *trans*-translation inhibitors and analyze the effects of specific mutations on its sensitivity to inhibitors KKL-35 and MBX-4132. The study uses both *in vivo* and *in vitro* assays to show that while bL27 is not essential for *trans*-translation, its movement and positioning affect protein tagging and inhibition. This work enhances our understanding of *trans*-translation inhibitors and their interactions with ribosomal components.

a) M. Cabrera, **E. Usoro**, P. Srinivas, C.M. Dunham, K.C. Keiler. bL27 in Trans-Translation: A New Role in the Peptidyl Transferase Center. *In preparation.*

3. Roles of DNA nucleases in maintenance of genome stability

I worked with Prof. JoAnn Sekiguchi in the Department of Internal Medicine at the University of Michigan as an undergraduate researcher. My project aimed to investigate how the absence of the nuclease ARTEMIS, involved in DNA repair and V(D)J (variable, diversity, and joining) recombination, alters DNA-damage responses. particularly, we focused on understanding the mechanism by which ARTEMIS functions in repairing DNA damage induced by the alkylating agent busulfan. These studies laid the groundwork for future research directed toward developing potential therapies for Severe Combined Immunodeficiency (SCID), a disease resulting from ARTEMIS mutations. Using diverse biochemical and genetic approaches, including tissue culture, Bicinchoninic acid (BCA) assays, and Western blot analyses, I found that longer busulfan exposure in ARTEMIS-deficient cells led to increased DNA damage compared to wildtype cells.

a) Poster: **E. Usoro** and J. Sekiguchi. *Roles of DNA nucleases in maintenance of genome stability*. Undergraduate Research Opportunity Program's Annual Research Symposium, Ann Arbor, MI, 2018

4. Mutagenesis of the gene *DMC1* in *Saccharomyces cerevisiae*

I was selected to participate in the highly competitive National Science Foundation (NSF) Research Experience for Undergraduates at Clemson University, where I worked under the guidance of Prof. Michael Sehorn in the Department of Genetics and Biochemistry. The focus of my project was to determine if mutagenesis of the DMC1 protein that is involved in homologous recombination (HR) affects the genetic makeup during meiosis and to explore whether such effects are beneficially utilized to treat genetic disorders. In my research, my goal was to identify which DMC1 protein region resulted in defective HR using molecular biology and biochemical approaches. Although mutagenesis was successful, I was not able to detect protein expression, suggesting the mutations may have resulted in protein instability and degradation, preventing further analysis.

a) Poster: **E. Usoro**, S. Goodson, M. Sehorn. *Mutagenesis of the Gene DMC1 in Saccharomyces cerevisiae*. 5th Annual Summer Undergraduate Research Poster Symposium, Clemson, SC, 2017