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 role on this NCCAT screening proposal will be to provide necessary resources and mentorship at Emory to support Ms. Sarah Strassler in her sample (Trm10 and tRNA) purification and cryo-EM specimen preparation for the proposed research activities at NYSBC. Sarah and my other current trainees are primarily involved in projects investigating: ***i)*** ribosomal RNA, transfer RNA (Sarah's specific project) and protein methyltransferase enzymes related to bacterial resistance to antibiotics or virulence and/ or human health (supported by NIH/NIAID R01 AI088025 and NIH/NIGMS R01 GM130135); ***ii)*** bacterial efflux systems in studies that developed from our on-going work in the area of bacterial antibiotic resistance mechanisms and, ***iii)*** non-coding RNA structure and activity against proteins of the human innate immune response (supported by NIH/NIAID AI144067). Each of these projects involves team members using complementary biochemical, biophysical, and structural approaches (typically single-particle cryoEM) providing a supportive environment for Sarah to complete her proposed studies of Trm10/TRMT10A-tRNA complexes. These efforts will also be supported by Emory's EM core (of which I am one of three Scientific Co-directors) as well as colleagues among Emory's major users of this facility.

Selected current research support (as noted above):

1. NIH/NIAID, R01 AI088025, *RNA modification and antibiotic resistance,* MPI–Conn*,Dunham (5/1/2010–4/30/2025).
2. NIH/NIGMS, R01 GM130135, *Mechanisms and biological functions of SPOUT methyltransferases*, MPI-Jackman*, Conn (09/14/2018–07/31/2023). [Renewal application is pending Council review, 36 Impact, 22nd percentile]
3. NIH/NIAID, R01 AI144067, *dsRNA regulation of the cytosolic innate immune system*, PI–Conn, CoI–Lowen, (3/12/2019–2/28/2023). [Renewal application is pending Council review, 20 Impact, 8th percentile]

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020–present	Co-scientific Director, Robert P. Apkarian Integrated Electron Microscopy Core, Emory University
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.

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.

Other Experience, Service and Professional Memberships

2022-present Associate Editor, npj Antimicrobials and Resistance.
2019 NIH/NIAID ZAI1 LR-M (M1), Special Emphasis Panel (RFA-AI-18-025, Elucidating the Functional Roles of Non-Coding RNAs in Viral Infectious Diseases (R21).
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 (five times total, most recently Nov 2020).
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: *Nature Methods, Nature Protocols, 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-Journal of the 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).

C. Contributions to Science

Complete List of Published Work in My NCBI (75 total publications): My Bibliography-Conn, G.L.
http://www.ncbi.nlm.nih.gov/sites/myncbi/graeme.conn.1/bibliography/40548662/public/?sort=date&direction=descending

Trainees (my lab) are highlighted in the references: **postdoctoral researchers** (bolded and underlined;) and *graduate students* (italicized and underlined; also: †rotation student only, #undergraduate); additionally, trainees from minorities underrepresented in the sciences are noted¶.

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 retained potent activity, leading to a reevaluation of their potential utility in the clinic in the face of increasing resistance to many first line drugs. Clinical aminoglycoside resistance typically arises through the action of aminoglycoside modifying enzymes, whereas drug-producing bacteria also use 16S rRNA methyltransferase enzymes to modify the ribosomal drug binding site, either at the N7 position of G1405 (m^7G1405) or the N1 position of A1408 (m^1A1408). Now, a serious threat to the future clinical usefulness of aminoglycosides has arisen from the acquisition and spread among human bacterial pathogens of these rRNA methyltransferases. rRNA modification confers exceptionally high-level resistance and, combined, these

modifications are capable of blocking the effects of *all* clinically useful aminoglycosides including the latest generation of drugs. Determining the structures of these enzymes and defining the features which govern their interactions with cosubstrate S-adenosyl-L-methionine (SAM) and 30S substrate, have been a major contribution from my lab. Our early work defined critical features for SAM binding and 30S recognition by Sgm (a drug producer m^7G1405 enzyme) and, subsequently, revealed the first structures of m^1A1408 enzymes with cosubstrate from both aminoglycoside-producing and human pathogenic bacteria. Next, in collaboration with Christine Dunham's group (Emory, Chemistry) we presented a breakthrough in the field with the determination of a first structure of a resistance methyltransferase (NpmA) bound to its 30S substrate (**ref. a**). This work was followed by a detailed mechanistic study to dissect the molecular basis of 30S recognition by NpmA (**ref. b**). 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 include complementary studies of members of the clinically more prevalent m^7G1405 family (e.g. **ref. c**) as well as the dual (30S and 50S subunit) methyltransferase TlyA from *Mycobacterium tuberculosis* (e.g. **ref. d**), which is associated with mycobacterial resistance to the tuberactinomycin drugs capreomycin and viomycin. Our long-term goal is to exploit the understanding we develop of these methyltransferase enzymes and their target recognition mechanisms to facilitate development of specific inhibitors of these resistance determinants.

1a. **Dunkle, J.A**., *Vinal, K*., **Desai, P.M**., Zelinskaya, N., *Savic, M.*, **West, D.M.¶**, *Conn, G.L. and *Dunham, C.M. (2014). Molecular recognition and modification of the 30S ribosome by the aminoglycoside-resistance methyltransferase NpmA. *Proc. Natl. Acad. Sci. U.S.A.* **111**(17), 6275-6280. (*Co-corresponding author) [PMCID: PMC4035980]

1b. *Vinal, K.* and Conn, G.L. (2017). Molecular mechanism of substrate recognition and modification by a pathogen-derived aminoglycoside-resistance 16S rRNA methyltransferase. *Antimicrob. Agents Chemother.* **61**(5), pii: e00077-17. [PMCID: PMC5404524]

1c. **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]

1d. *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]

2. Structure, activity, and substrate selection by the *P. aeruginosa* RND pump MexXY-OprM. My lab's 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 (see **Contribution 1** above). 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. in strains isolated from individuals with cystic fibrosis). 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" (**ref. a**). That is, substrates (aminoglycosides) must bind sufficiently tightly to be taken up by MexY but not too tightly, so as to impede movement through the transporter to the 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. Future studies will experimentally test these ideas and define the structure and activity of the *P. aeruginosa* MexXY-OprM efflux system using biochemical, computational, microbiological, structural biology approaches with the long-term goal of developing novel efflux inhibitors to counter the antibiotic resistance the systems provide to pathogenic bacteria.

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]

3. EftM trimethylation of EF-Tu and its impact on *P. aeruginosa* physiology and pathology. In collaboration with Dr. Joanna Goldberg (Emory, Pediatrics) we showed that the activity of the *P. aeruginosa* methyltransferase EftM is regulated via a novel mechanism: direct thermoregulation of the protein's structure itself. EftM trimethylates lysine 5 (K5) of the translation factor EF-Tu in a manner we hypothesize mimics the phosphorylcholine modification which is important for host-cell adhesion and virulence of other opportunistic pathogens. Our first collaborative study was important because it provided a mechanistic basis for how *P. aeruginosa* may control expression of this modification (**ref. a**). Subsequent work revealed an additional contribution to regulation of EftM expression at the transcriptional level and also that EF-Tu modification K5 has no impact on its canonical role in translation, suggesting its purpose is potentially exclusive to the surface-localized role in host cell adhesion (**ref. b**). Finally, we recently completed a study revealing details of the molecular mechanism of EF-Tu recognition by EftM which appears to exploit strategies common to both Class I Rossmann fold and the eukaryotic SET domain methyltransferases (**ref. c**). This work sets the scene for future studies on EftM structure/ enzymatic mechanism (in particular the similarity of its active site to those of SET methyltransferases) that will provide a framework for future development of specific inhibitors of EF-Tu K5 trimethylation to impact *P. aeruginosa* virulence.

3a. Owings, J.P.*, *Kuiper, E.G.**, Prezioso, S.M., Meisner, J., Varga, J.J., Zelinskaya, N., Dammer, E.B., Duong, D.M., Seyfried, N.T., Albertí, S., Conn, G.L. and Goldberg, J.B. (2016). *Pseudomonas aeruginosa* EftM is a thermoregulated methyltransferase. *J. Biol. Chem.* **291**(7), 3280-90 (*Co-first authors) [PMCID: PMC4751374]

3b. Prezioso, S.M., Duong, D.M., *Kuiper, E.G.*, Deng, Q., Dammer, E.B., Seyfried, N.T., Albertí, S., Conn, G.L. and Goldberg, J.B. (2019). Methylation of elongation factor-Tu by the dual thermoregulated methyltransferase EftM does not impact its canonical function in translation. *Scientific Reports*, **9**(1):3553. [PMCID: PMC6401129]

3c. *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)

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 defined the nc886 RNA structure and revealed that only one conformer of nc886 can adopt a (currently undefined) 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 only known 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 (**ref. a**). 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 (**ref. b**). Our most 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. **ref. c**,**d**) and we are currently working to fully define the "rules" that govern potent OAS1 activation by dsRNA 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.**, *Tanguary, 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; **ref. a**). The OAS family of proteins is responsible for sensing foreign (e.g. viral) double-stranded (ds)RNA and promoting 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. In our on-going work, we will next define the molecular basis for OAS1 GoF, including the mechanism of allosteric communication between the GoF sites and residues surrounding the active site. Defining these mechanisms also promises to significantly deepen our understanding of how OAS1 is regulated, both by dsRNA activators and by protein residue networks that limit aberrant activation in the absence of dsRNA. Recently, through our global IEI surveillance network we also identified a first heterozygous missense mutation in *OASL* leading to OASL gain-of-function activity and dysregulation of innate immune signaling. As noted in the NIAID Notice of Special Interest (NOSI) "*Investigations on Inborn Errors of Immunity/Primary Immunodeficiencies*" (NOT-AI-21-032), efforts to understand genetic defects leading to *even exceptionally rare IEIs* offer unparalleled potential for novel insights into the fundamental molecular and cellular mechanisms of immune system function. Our goal will therefore be to establish a foundation from which to exploit the OASL variant and its resultant IEI to define currently unappreciated interplay between innate immune sensing of DNA and RNA viruses via the OASL/ RIG-I and cGAS/ STING pathways.

5a. 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 Immunology*, **6**.PMCID: PMC8392508.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Sarah E. Strassler

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

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 Florida, Gainesville, FL	B. S.	08/2010	05/2014	Chemistry
Emory University, Atlanta, GA	PhD	08/2018	In Progress	Biochemistry

A. Personal Statement

The goal of this proposal is to get accepted into the Grid Preparation and Screening (GPS) program at NCCAT. The GPS program would allow me to determine the correct concentration, grid type, and freezing conditions to prepare grids of my Trm10-tRNA sample for cryo-EM analysis. This will enhance my technical training and allow me to expand on my project characterizing the mechanism of substrate recognition for an important RNA modifying enzyme.

Considering the large gaps in our understanding of Trm10's mechanism of substrate recognition, this research project will provide the exciting opportunity to uncover a new mechanism for protein-RNA recognition which may be employed by other tRNA-modifying enzymes. I will share our findings with other researchers at conferences and will be able to educate others on a seemingly distinct mechanism of substrate recognition. I will also be able to use the training to educate other members of our lab on how to optimize sample and grid preparation.

The abundance of RNA biologists and structural biologists in my department will help to guide me as I work to understand the Trm10-tRNA complex. The Apkarian Integrated Electron Microscopy Core (IEMC) at Emory University will also be available to assist me as a process my dataset and ultimately solve the structure of the Trm10-tRNA complex.

B. Positions and Honors

Positions

ACTIVITY	START DATE	END DATE	FIELD	INSTITUTION/COMPANY
Undergraduate Researcher	05/2012	12/2012	Physical Chemistry	University of Florida Department of Chemistry
Peer Mentor	08/2013	12/2013	General Chemistry	University of Florida Department of Chemistry
Clinical Research Assistant	01/2014	08/2014	Pulmonary	University of Florida Department of Medicine
Organic Chemistry Tutor	01/2014	08/2014	Organic Chemistry	University of Florida Teaching Center
Secondary Education Volunteer	05/2015	08/2017	Science Education	Peace Corps Ghana

High School Programs Manager	08/2017	02/2018	Girls Education	Girls Education Initiative of Ghana
Volunteer Scientist	02/2019	Ongoing	General Science	Atlanta Science Festival
Foundations Teaching Assistant	08/2019	12/2019	Biochemistry	BCDB Graduate Program
Tutor	09/2019	Ongoing	General Science, Pedagogy	Emory-Tibet Science Initiative
Tutor	01/2020	05/2021	BCDB	BCBD Graduate Program
Digital Content Contributor	05/2020	Ongoing	General Science	Science ATL
Student Representative	07/2020	07/2022	Biochemistry	BCDB Executive Committee
Lab Instructor	08/2022	12/2022	General Biology	BIO141L
Booth Organizer	01/2023	03/2023	Structural Biology	Atlanta Science Festival

Academic and Professional Honors

2010-2013 **Dean's List,** *University of Florida* (all 8 semesters): Must be enrolled full-time and earn a grade point average of at least 3.75 per semester.

2014 **Magna Cum Laude**, *University of Florida*: Must graduate with a grade point average of 3.50-3.79.

2017 **Outstanding Foreign Volunteer Award,** *Ministry of Education, Ghana:* Recognized for contributions to Menji Agricultural Senior High School in the field of education.

2019-2020 **National Institute of Health Training Grant (T32),** *Emory University:* Training program in Biochemistry, Cell, and Developmental Biology (5T32GM008367-30)

2020-2023 **Graduate Research Fellowship,** *National Science Foundation*: External funding to support graduate research project.

2020-2023 **Scholarship,** *ARCS Atlanta Chapter*

2021 **Science Communication Fellowship,** *Science ATL*

2022 **Poster Award,** *27th Annual Meeting of the RNA Society*

2022 **Program Scholar of the Year,** *Biochemistry, Cell, and Developmental Biology Graduate Program*

C. Contributions to Science

1. Undergraduate Research

During my time at the University of Florida, I worked under Dr. David Wei to synthesize efficient photocatalysts that act as reducing agents when irradiated with visible light. The photocatalysts were nanorods composed of titanium dioxide doped with gold particles. I used the photocatalysts to lead a comparative study to understand the efficiency of the photocatalysts under sunlight as opposed to the visible light filter and artificial lights utilized by the lab. The experience provided a collaborative environment where I learned how to develop a new protocol and troubleshoot experiments alongside graduate students and other undergrads.

2. Graduate Research Rotations

As a graduate student, I have already had the opportunity to further scientific research through my work during laboratory rotations and as a researcher in Dr. Graeme Conn's lab. During my 8-week rotation in Dr. Conn's lab, I created an expression construct to express and purify the protein RmtB. RmtB is a 16S rRNA methyltransferase that causes aminoglycoside resistance in human pathogenic bacteria. After optimizing expression and purification protocols, I crystallized the RmtB protein during my rotation to allow the lab to test potential RmtB inhibitors which would serve as "resistance breakers". My work during this rotation led to my co-authorship on a paper that details the mechanism of substrate recognition by a closely related rRNA methyltransferase RmtC. The methods outlined in this paper that were used to successfully characterize a mechanism of substrate

recognition closely align with the goals of my research project outlined in this proposal and provide further evidence that I am well suited to investigate the mechanism of substrate recognition by Trm10.

For my second rotation, I worked in the lab of Dr. Stefan Sarafianos. There, I crystallized the HIV capsid protein (CA) in order to test its binding with analogs of PF-74, a known CA-targeting compound which interferes with reverse transcription. I worked with senior scientist Dr. Karen Kirby to solve the crystal structure of CA bound to an inhibitor exhibiting increased antiviral potency. During my third rotation, I gained experience working in a cell biology lab working under Dr. Michael Koval. I spent the rotation optimizing a "rip-off" protocol to separate the apical portion of colon epithelial cells from the basolateral portion to allow the lab to characterize differences in protein expression between the two parts of the cell under varying conditions.

a. Nosrati, M., Dey, D., Mehrani, A., **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.

3. Graduate Thesis Project

Mutations in the *TRMT10A* gene have been linked to neurological disorders such as microcephaly and intellectual disability, as well as defects in glucose metabolism. However, despite the clear biomedical importance of TRMT10A and the tRNA methylation it incorporates, there is still a large gap in our understanding of how this enzyme accurately recognizes its specific substrates to generate the pool of correctly modified tRNAs that is essential for normal brain cell function. The goal of my graduate project is to characterize the mechanism of substrate recognition by the tRNA methyltransferase Trm10. These studies will advance our overall knowledge of how tRNA modifying enzymes are able to discriminate between structurally similar tRNA species for accurate substrate recognition. Specifically, I anticipate that my research will uncover a new mechanism for protein-RNA recognition that relies on the ability of Trm10 to induce specific structural changes only in substrate tRNAs. These studies align well with our lab's overarching goal to define mechanisms of substrate recognition for a broad range of RNA methyltransferases and could reshape our fundamental understanding of RNA modifications and the regulation of their incorporation.

Commented [CG1]: I think you should add some of your detail relating this to brain cells and mental health so it's consistent with the other sections.

a. **Strassler S. E.**‡; Bowles I.‡; Dey D.; Jackman J. E.; Conn G. L. Tied up in knots: Untangling substrate recognition by the SPOUT methyltransferases. *J. Biol. Chem*. 2022.
b. **Strassler, S.E**., Conn, G.L. Substrate Recognition by the tRNA Methyltransferase Trm10. 19th Annual DSAC Student Research Symposium. Atlanta, GA. April 2022. [Oral Presentation]
c. **Strassler, S.E**., Conn, G.L. Substrate Recognition by the tRNA Methyltransferase Trm10. RNA 2022-27th Annual Meeting of the RNA Society. Boulder, CO. June 2022. [Poster Presentation]
d. **Strassler S. E.**; Bowles I. E.; Krishnamohan A.; Kim H.; Kuiper E. G.; Comstock L. R.; Jackman J. E.; Conn G. L. tRNA m1G9 modification depends on substrate-specific RNA conformational changes induced by the methyltransferase Trm10. *J. Biol. Chem*. *(In Revision)*

4. Teaching and Mentoring Students

I consider teaching and mentoring students to be my biggest contribution to science thus far. As a Peace Corps Volunteer, I taught a total of 250 students during my two years as a high school science teacher. I used demonstrations and practical experiments to engage students and help them to develop critical thinking skills. My goal was to make science more accessible and fuel their passion for investigating the world around them. I have continued using these skills to teach and mentor students at Emory University as a teaching assistant for an introductory graduate course and working one-on-one with a high school student. In the latter case, I taught my high school student mentee various biochemical techniques that she will be able to use as we work together to develop a project during school vacations. I will continue working with her, undergraduates, and rotation students in the lab to train them on various biochemical techniques and mentor them as they navigate the path to becoming a scientist.

D. Additional Information: Research Support and/or Scholastic Performance

UNIVERSITY OF FLORIDA (Undergraduate)
GPA: 3.71

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
2010	General Chemistry	A	2010	Calculus I	A
2010	General Chemistry Lab	A	2010	Intermediate Spanish II	A-
2011	General Chemistry II	A	2010	What is the Good Life	A
2011	Accelerated General Chemistry II Lab	A-	2011	Calculus II	A
2011	Biology I	A	2011	Discovering the Universe	B+
2011	Biology I Lab	A	2011	Principles of Sociology	A-
2011	Enhanced Organic Chemistry I	A	2011	Language and Culture	A-
2011	Biology II	B	2011	Calculus III	A
2012	Biology II Lab	A	2012	Social Problems	A-
2012	Enhanced Organic Chemistry II	A	2012	Marriage and Family	A
2012	Organic Chemistry Lab	A-	2012	Human Sexuality & Culture	A
2012	Organic/Biochemistry II	A-	2012	Language & Human Perspective	A
2012	Genetics	A	2013	Plants, Plagues, & People	A
2012	Undergrad Research	A	2013	Italian Regional Cuisine	B
2012	Analytical Chemistry	B-	2013	Beginning Italian I	A
2012	Analytical Chemistry Lab	A-	2013	Survey of American Literature	A-
2012	Plants, Gardening, & You	A	2014	Argument and Persuasion	A
2012	Physics with Calculus I	A-			
2012	Physics I Lab	A-			
2013	Instrumental Analysis	A			
2013	Instrumental Analysis Lab	A-			
2013	Physics with Calculus II	A-			
2013	Physics II Lab	A-			
2013	Inorganic Chemistry	B+			
2013	Physical Chemistry I	B			
2013	Biophysical Chemistry Lab	A			
2014	Physical Chemistry II	B			
2014	Microbiology	B			

EMORY UNIVERSITY (Graduate)
GPA: 3.96

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
2018	Laboratory Rotations	A	2018	Jones Program in Ethics	S
2018	Foundations in BCDB I	A			
2018	Introductory Graduate Seminar	A-			
2018	Laboratory Rotations	A			
2019	Foundations in BCDB II	A-			
2019	Introductory Graduate Seminar	A			
2019	Laboratory Rotations	A			
2019	Hypothesis and Science Writing	A			
2019	Teaching Assistantship	A			
2019-2020	Advanced Graduate Research	A			
2019-2023	Advanced Graduate Seminar	A			
2020	Statistical Design and Analysis of Experiments	A-			
2020-2023	Dissertation Research	A			