PI: **Schwartz, Samantha Lynne**	Title: Regulation of 2'-5'-oligoadenylate synthetase 1 (OAS1) by dsRNA	
Received: 12/08/2016	FOA: PA16-309	Council: 05/2017
Competition ID: FORMS-D	FOA Title: RUTH L. KIRSCHSTEIN NATIONAL RESEARCH SERVICE AWARD (NRSA) INDIVIDUAL PREDOCTORAL FELLOWSHIP (PARENT F31)	
1 F31 AI133950-01	Dual:	Accession Number: 4001165
IPF: 2384501	Organization: EMORY UNIVERSITY	
Former Number:	Department: GRS: GDBBS BCDB	
IRG/SRG: ZRG1 F13-C (20)L	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A)	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Samantha Schwartz	Emory University	PD/PI
Graeme Conn	Emory University	Other (Specify)-Sponsor
Anice Lowen	Emory University	Other (Specify)-Co-sponsor

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED 2016-12-08

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: Emory University
Department: Office of Sponsored Programs
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Holly Middle Name: Last Name*: Sommers Suffix:
Position/Title: Director, Pre-award Grants Adm
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍Women Owned ❍Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍Resubmission
❍ Renewal ❍Continuation ❍Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Regulation of 2'-5'-oligoadenylate synthetase 1 (OAS1) by dsRNA

12. PROPOSED PROJECT
Start Date* 07/01/2017
Ending Date* 06/30/2020

13. CONGRESSIONAL DISTRICTS OF APPLICANT
GA-005

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Samantha Middle Name: Last Name*: Schwartz Suffix:
Position/Title: Research Fellow/Trainee
Organization Name*: Emory University
Department: [redacted]
Division: [redacted]
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City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* [redacted]
b. Total Non-Federal Funds* [redacted]
c. Total Federal & Non-Federal Funds* [redacted]
d. Estimated Program Income* [redacted]

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Maggie Middle Name: Last Name*: Hassan Suffix:
Position/Title*: Associate Director
Organization Name*: Emory University
Department: Office of Sponsored Programs
Division: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative* [redacted]

Date Signed* [redacted]

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_Schwartz1032089591.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Emory University
Duns Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: GA-005

Additional Location(s) File Name:

Funding Opportunity Number: PA-16-309. Received Date: 2016-12-08T16:40:52.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other ProjectInformation

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: 1 _ 2 _ 3 _ 4 _ 5 _ 6 If

NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as ahistoric place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_Summary_Schwartz1032089604.pdf
8. Project Narrative*	Project_Narrative_Schwartz1032089601.pdf
9. Bibliography & References Cited	References_Schwartz1032089677.pdf
10. Facilities & Other Resources	Facilities_and_Other_Resources_Schwartz1032089597.pdf
11. Equipment	Key_Equipment_Schwartz1032089598.pdf

Funding Opportunity Number: PA-16-309. Received Date: 2016-12-08T16:40:52.000-05:00

PROJECT SUMMARY

The innate immune system is a broad set of critical intracellular and extracellular processes that limit viral infectivity. In order to provide its essential first line of defenses against pathogens, the innate immune system must be able to accurately distinguish “self” from foreign molecules. Misregulation of the innate immune system can cause increased persistence and susceptibility to viral infection and human diseases, such as interferonopathies. The 2’-5’-oligoadenylate synthetase (OAS) family of enzymes are important innate immune sensors of cytosolic double-stranded RNA (dsRNA). Attesting to the importance of the OAS/RNase L pathway, viruses have developed ways to evade OAS. Previous structural studies have revealed that dsRNA binding allosterically induces structural changes in OAS1 that reorganize the catalytic site to drive synthesis of 2’-5’-oligoadenylates from ATP. These 2’-5’-oligoadenylate secondary messengers activate a single known target, the latent ribonuclease (RNase L). Active RNase L in turn degrades viral and cellular RNA to halt viral replication. Although X-ray crystal structures have given some insight into how OAS1 is activated by dsRNA, we still understand very little about how specific features of the dsRNA contribute to the level of OAS1 activation. To address which specific features of dsRNA are required for potent OAS1 activation, we designed dsRNA hairpin variants, based on the RNA duplex used in the structural studies. Remarkably, while a single point mutation on one strand resulted in *complete loss* of OAS1 activity, the equivalent mutation on the opposite strand led to *increased* OAS1 activity. Despite these stark differences in ability to activate OAS1, both variants appear to bind OAS1 with similar affinity. Given these preliminary findings, **I hypothesize that dsRNAs may contain competing OAS1 binding sites with remarkably different capacities to activate the protein in a context dependent manner.** However, the molecular signatures defining these sites as activating and non-activating are unknown. The goal of this project is to determine how specific sequences in dsRNA, and their context, control regulation of OAS1 in the following two Specific Aims. **Aim 1.** To use complementary assays of OAS1 activity *in vitro* and in human cells to determine the features of dsRNA that lead to potent activation of OAS1. **Aim 2.** To use biochemical, biophysical, and structural approaches to define the molecular mechanism(s) by which the dsRNA hairpin variants differ in their effects upon OAS1 activation. These experiments will reveal new insights into the regulation of OAS1 by dsRNA. In doing so, I will enhance our understanding of host-pathogen interactions, such as how viruses might circumvent the OAS1/RNase L pathway by masking activating motifs to evade detection. My results will furthermore provide new insights into cellular translational control in the context of infection and potentially strengthen the foundations necessary to design effective treatments for viral infection.

PROJECT NARRATIVE

The innate immune system is our cell's front line defense against infecting pathogens. This project will investigate how one important RNA-sensing component of the innate immune system is regulated by specific molecular signatures within double-stranded RNA molecules. Such studies are essential to understand how the innate immune system is controlled, how its effects can be circumvented by infecting viruses, and as a potential platform to design effective antiviral treatments.

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FACILITIES AND OTHER RESOURCES

INTELLECTUAL ENVIRONMENT:

I am well positioned to take advantage of the many outstanding intellectual resources Emory has to offer. In the Conn and Lowen labs, I will work directly alongside graduate students from several programs (including BCDB, Immunology and Molecular Pathogenesis (IMP), and Microbiology and Molecular Genetics (MMG)) as well as postdocs and other lab personnel with diverse backgrounds. Lab meetings, BCDB program-sponsored events, research group meetings (e.g. Structural Biology and Emory RNA Club), and other Emory or local symposia will afford me many opportunities to seek important critical feedback on my work and enrich my intellectual and professional development.

PHYSICAL RESOURCES:

As described below, my Sponsor's and Co-sponsor's labs have the physical resources necessary for me to complete my proposed research (please also see *Equipment* for description of Conn and Lowen lab equipment, as well as Departmental shared equipment and Emory core facilities). Additionally, both labs have sufficient funding to provide me with any additional reagents/small equipment I will need.

Laboratory:

Dr. Conn's research group occupies ~1250 sq. ft. of laboratory space on the fourth floor of the Rollins Research Center on the Emory University School of Medicine Clifton Road campus. I have designated bench space, complete with ample storage, and my own set of four pipetman and small equipment (microfuge, vortex, etc.). The laboratory is divided into four main rooms for organizational and containment purposes: the larger main lab space (where each lab member has their own bench), and three small labs for microbiology work (bacterial culture for protein expression, etc.), a hot lab for all radioisotope work, and crystallography area, including a stereo microscope and storage space for crystallization screens (three dedicated crystallization incubators). The laboratory is equipped with standard wet benches, certified fume hood, sinks, utilities, deionized water, etc. for experimental biochemistry, biophysics, and molecular and structural biology research. The Conn laboratory is **approved for Biosafety Level 2 (BSL-2)** work and has currently approved biosafety and radioisotope protocols. I also have completed all of the required safety training established by the Environmental Health and Safety Office, including research laboratory safety, biosafety, and radiation safety. Adjacent shared laboratory space is also available in common laboratories, computer rooms, darkrooms, temperature-controlled environments (-20, 4, 30 and 37 ˚C), libraries, and meeting areas. The Conn laboratory has (or has immediate access to) all of the essential equipment required for the proposed work (see *Equipment* for further details).

Dr. Lowen's research group occupies 850 sq. ft. of recently renovated laboratory space on the third floor of the Rollins Research Center. The laboratory is equipped with standard wet benches, a certified fume hood, sink, utilities, deionized water, etc. and includes an adjoining room dedicated for cell culture. Additionally, Dr. Lowen has 50% use of a 100 sq. ft. cold room and 10% use of a freezer room. All equipment required is housed within the laboratory. The Lowen laboratory is **approved for Biosafety Level 2 (BSL-2)** work with current biosafety approvals.

Clinical:
N/A

Animal:
N/A

Computer:
I have an Apple MacBook Pro loaded with the Microsoft Office suite, GraphPad Prism, Adobe Illustrator and Photoshop, Endnote, and Findings electronic notebook software. I have wireless Internet access throughout the lab and office spaces, as well as access to a departmental server with a backup system. The laboratory and office are each supplied with networked computers and printers. In addition to my personal computer, a shared facility on the ground floor of the Rollins Research Center building adjacent to the X-ray laboratory

has an extensive molecular graphics area in which the Conn Laboratory has dedicated space for their existing computer workstations (2 x Apple Mac OSX) for data processing, model building, and refinement.

Office:
I have access to ~200 sq. ft. of shared office space for Conn lab postdocs, students, and staff. Drs. Conn and Lowen each have ~200 sq. ft. of office space with enough room to meet with students and staff located adjacent to their respective labs.

INSTITUTIONAL SUPPORT:

X-ray Crystallographic Facilities. The Department of Biochemistry maintains an ~150 sq. ft. state-of-the-art automated crystallization and 'in-house' X-ray data collection facility to support the research programs of four structural biology groups (see *Equipment* for details).

***Synchrotron (Advanced Photon Source, APS)*:** Emory is a member of SER-CAT (Southeast Regional Collaborative Access Team) at the Advanced Photon Source (APS), providing 12 days ID (undulator insertion device) beamtime and 12 days of BM (bending magnet) beamtime per year to the Biochemistry X-ray crystallography groups. Further details are provided in the *Equipment* section.

Emory Core Facilities. This proposal will use facilities within several Emory centers and core facilities: ***(1)*** The *Emory Chemical Biology Discovery Center* (http://www.pharm.emory.edu/ECBDC/capabilities.html) houses 'hands-on' equipment for quantifying molecular interactions using label-free methods, including a FortéBIO OctetRED^{384} bio-layer interferometry instrument (see attached letters). The ECBDC is located on the fourth floor of the Whitehead Research building, which is connected directly by internal bridge walkway on each floor to the Rollins Research Center (<1 minute walk from our lab). ***(2)*** The *Emory Integrated Genomics Core* (EIGC, http://cores.emory.edu/eigc/) houses instrumentation we will use for analysis of extracted ribosomal RNA to assess RNase L activity in transfected and/ or virus infected cells. The EIGC is located in a Clinical Laboratory Improvement Amendments (CLIA) certified (CLIA ID:11D1086150) laboratory located on the seventh floor of the Woodruff Memorial Research Building on the Clifton Road campus (about a 5 minute walk from our lab in the Rollins Research Center), with 2400 sq. ft. of dedicated wet-lab space. The EIGC's laboratory areas include dedicated pre- and post-PCR spaces. ***(3)*** *HDX-MS* is available on campus through a new core facility established by Dr. Renhao Li in the Department of Pediatrics at Emory (see attached letter and *Equipment* for further details on the facility). The facility is housed in ~250 sq. ft. of newly renovated space adjacent to Dr. Li's lab in the Emory Childrens' Center on the Clifton Road campus (about a 10 minute walk from our lab in the Rollins Research Center). ***(4)*** the *Emory Comprehensive Glycomics Core* (ECGC; http://www.cores.emory.edu/ecgc/) houses additional 'hand-on' instrumentation for quantifying molecular interactions using label-free methods (BiaCore X100 surface plasmon resonance and a Malvern/ MicroCal Auto-iTC$_{200}$ isothermal titration calorimetry instrument) in a 250 sq. ft. laboratory on the fourth floor of the Rollins Research Center.

EQUIPMENT

As described in the sections below, collectively the Sponsor and Co-sponsor laboratories, the Departments of Biochemistry and Microbiology & Immunology as well as the Emory core facilities can provide me with access to all of the equipment I will need to complete my proposed research.

Conn Laboratory: All necessary equipment for RNA *in vitro* transcription and protein synthesis, purification, analysis, and crystallization is already available in the Conn laboratory on the fourth floor of the Rollins Research Center. We have Infors Multitron dual and Ecotron orbital shaker incubators (both with cooling module for low temperature expression) for bacterial culture for protein expression; a Beckman Allegra 25R table top centrifuge with swinging bucket and fixed angle rotors; two ÄKTA Purifier10 (FPLC) systems each housed in a dedicated 4 ˚C chromatography refrigerator and equipped with column selection kit for protein and protein-RNA complex purification; a BioTek Synergy 4 multimode (absorbance, luminescence, fluorescence) plate reader on which we have optimized our 96-well plate chromogenic assay of OAS activation; a Cary400 UV/Vis spectrophotomer with 6x6 cell multichanger (used for RNA UV thermal melting analysis); a Nanodrop 2000 UV/Vis spectrophotomer; three incubators for macromolecular crystallization and Leica optical microscope with digital camera for viewing and recording crystallization experiments; electrophoresis equipment for horizontal agarose, vertical mini/ preparative PAGE (the latter custom designed for large scale purification of *in vitro* transcribed RNA), three BioRad Sequi-Gen GT sequencing gel systems, and power supplies; all other essential small equipment, *e.g.* pH meter, balances, PCR thermocycler, microcentrifuges; and, low temperature storage units, *e.g.* chiller cabinets and refrigerator (4 ˚C) and freezers (-20 and -80 ˚C).

Lowen Laboratory: Dr. Lowen's laboratory is located on the third floor of the Rollins Research Center and is fully equipped for molecular and cellular biology experiments. Major equipment includes two laminar flow hoods, CO_2 incubators, an inverted phase contract microscope, microcentrifuges, a table-top centrifuge, several electrophoresis systems, water baths, a bacterial culture shaker/incubator, liquid nitrogen tank, and a -80 ˚C freezer. Additionally, a BioRad ChemiDoc imager, two DNA thermal cyclers, two BioRad real-time PCR instruments with 384-well capability, and a new droplet digital PCR instrument (BioRad QX200) are located in Dr. Lowen's laboratory. BioRad GenePulser Xcell and Lonza Nucleofector devices for electroporation of cells are available in an adjacent lab. A Zeiss fluorescent microscope with digital recording system is available in the department, and the lab has full access to a Becton Dickenson FACS Calibur flow cytometer that is located down the hall. An ultracentrifuge and darkroom facilities are located within 100 feet of thelab.

Departmental Shared Equipment: To support X-ray crystallographic studies, the Biochemistry Department provides a state-of-the-art facility for automated crystallization and 'in-house' X-ray data collection to support the research programs of three structural biology groups (led by Drs. Conn, Eric Ortlund, and Christine Dunham). This equipment is housed in dedicated laboratory space within the Rollins Research Center and includes a Phoenix nanoliter dispensing crystallization robot (Art Robbins Instruments), a Formulator liquid handling robot (Formulatrix) for crystal optimization, and a Rigaku MicroMax007HF generator with an ACTOR automated crystal mounting robot, Varimax-HF optics, a Saturn 944+ CCD detector, and Oxford Cryosystem X-Stream system. Adjacent to these facilities is an extensive molecular graphics area with dedicated space for computers for X-ray data processing, model building, and refinement. Emory is also a member of **SER-CAT** (Southeast Regional Collaborative Access Team) at the Advanced Photon Source (APS), providing 12 days ID (undulator insertion device) beamtime and 12 days of BM (bending magnet) beamtime per year to the Biochemistry X-ray crystallography groups.

For recording and quantitation of OAS assays using ^{32}P or fluorescently labeled RNAs, we have access to two imaging systems: a GE **Typhoon FLA9000** (for ^{32}P only) in the Department of Biochemistry and a GE Typhoon Trio variable mode imager system (^{32}P and multi-color fluorescence) located in the Department of Microbiology and Immunology on the third floor of the Rollins Research Center building (adjacent to Dr. Lowen's laboratory).

Additional shared laboratory areas immediately adjacent to the Conn Lab house equipment available as needed for this project, including preparative and ultracentrifuges, autoclaves, DNA/protein gel-imaging system, scintillation counter, and X-ray film developer. Another shared gel imaging system, scintillation counter, and X-ray developer are available as in the Department, adjacent to the X-ray crystallography suite on the ground floor of the Rollins Research Center building.

Emory Core Facilities:

The following core facilities house equipment that I will use during the course of my project:

***Emory Chemical Biology Discovery Center (ECBDC)*:** For analysis of OAS1 interaction with dsRNAs, we have access to a high-throughput bio-layer interferometry (BLI) instrument, the **FortéBIO OctetRED^{384}**. The ECBDC is located in the adjacent building to the Rollins Research Center (<1 minute walk via an internal connecting bridge). Further info: http://www.pharm.emory.edu/ECBDC/.

***Emory HDX-MS Core*:** This newly established **HDX-MS facility** is located in the Department of Pediatrics in the Emory Childrens' Center (~5 minute walk from the Rollins Research Center) on the Emory Clifton Road Campus. The instrument consists of an ACQUITY UPLC System with HDX Manager and automatic sampler paired with a Waters Q-TOF Premier mass spectrometer capable of a maximum resolution of 1/17,500 Da. Masslynx 4.1, ProteinLynx Global SERVER™ (PLGS 3.0), and Dynamix 3.2 software packages control the instrument and HDX-MS analysis. The instrument collects an H/D exchange data profile after each sample injection, with as many as 50 sequential injections setup in one experiment using the autosampler program.

Emory Integrated Genomics Core (EIGC): For analysis of RNase L activation in cell assays the **Agilent 2100 Bioanalyzer** on a chip platform is available through the EIGC. This system allows for rapid quantification of nucleic samples (here, ribosomal RNA using the RNA nano chip) and information about the size distribution of the fragments. Further info: *http://cores.emory.edu/eigc/.*

***Emory Comprehensive Glycomics Core (ECGC)*:** The ECGC houses additional equipment potentially relevant to this proposal in a dedicated laboratory in the same hall as the Conn lab on the fourth floor of the Rollins Research Center building. Specifically, for measurement of molecular interactions, we have available a shared a GE Biacore X-100 surface plasmon resonance (SPR) instrument and also an automated small cell isothermal titration calorimeter (Malvern/ MicroCal Auto-iTC$_{200}$). These instruments are maintained under service agreements by the ECGC to ensure instrument availability.
Further info: *http://www.cores.emory.edu/ecgc/*.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Samantha Middle Name Last Name*: Schwartz Suffix:

Position/Title*: Research Fellow/Trainee
Organization Name*: Emory University
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Fax Number:

E-Mail*:

Credential, e.g., agency login:

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: NIH_Biosketch_Schwartz_12_20161032089585.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-16-309 . Received Date: 2016-12-08T16:40:52.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: Graeme Middle Name Leslie Last Name*: Conn Suffix:

Position/Title*: Asc Professor
Organization Name*: Emory University
Department: Biochemistry
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Fax Number:

E-Mail*:

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Sponsor

Degree Type: PHD, Chemistry Degree Year: 1996

Attach Biographical Sketch*: File Name: NIH_Biosketch_Conn_12_20161032089586.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Anice Middle Name Carmen Last Name*: Lowen Suffix:

Position/Title*: Assistant Professor
Organization Name*: Emory University
Department: Microbiology & Immunology
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Fax Number:

E-Mail*:

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Co-sponsor

Degree Type: PhD Degree Year: 2005

Attach Biographical Sketch*: File Name: NIH_Biosketch_Lowen_12_5_161032089587.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

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: Schwartz, Samantha Lynne

eRA COMMONS USER NAME: [redacted]

POSITION TITLE: Graduate Student

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Armstrong State University, Savannah, GA	B.S.	08/2007	05/2012	Biology
Emory University, Atlanta, GA	Ph.D.	08/2015	In progress	Biochemistry, Structural Biology

A. Personal Statement

The goal of this proposal is to obtain extramural funding to support my Ph.D. thesis research. This independent funding will enhance my technical and professional training experience through my project investigating the molecular mechanisms of RNA-mediated regulation of innate immune system proteins. I hope that securing this fellowship award will be an important step on the path to a career as an independent researcher.

Being a first-generation college student was not without its challenges. At the beginning of my sophomore year I received my first and only 'F' grade in organic chemistry. This result initially shook my confidence but also made me realize that I had a lot of growing to do. Recognizing that this might not be my last setback, I used this renewed motivation to work harder and to not be intimidated by obstacles. Determined to show (mainly to myself) that I could overcome such obstacles, I later repeated organic chemistry and earned the 'A' I knew I was always capable of achieving. Although my confidence in my own ability and potential has grown considerably since those early days in college, the determination to overcome such setbacks and to continue to learn and improve as I work towards my career goals has stuck with me. My persistent-nature has been an asset and I think a valuable quality to have in the field of scientificresearch.

Later in my undergraduate career, I pursued a research opportunity with one of my professors, Dr. Jennifer Brofft, investigating microbes that cause sea turtle eggs' failed development. After undergrad, I knew I wanted to pursue graduate school, but I wanted to make a more informed decision prior to making a commitment. To gain this additional experience, I became a research technician at Emory University where I worked on the double-stranded RNA (dsRNA)-activated protein kinase (PKR). My thesis project will not only allow me to gain new technical skills (as well as perfect existing ones), but to also use this opportunity to develop as an independent scientist. I plan to seek opportunities that will aid in my professional development, such as attending conferences, giving poster presentations and talks, as well as writing primary literature to improve the way I communicate science. It is important to me to have both the technical and professional skills necessary to be successful as I will require these skill sets to secure a position as a postdoctoral fellow and ultimately lead an independent research team.

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	END DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Undergraduate Researcher	01/2011	05/2012	Microbiology	Armstrong State University	Jennifer Brofft, Ph.D.

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	END DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Tutor, Writing Center	08/2011	05/2012	Communication, Writing	Armstrong State University	Deborah Reese, Ph.D.
Research Specialist	06/2012	08/2015	Biochemistry	Emory University	Graeme Conn, Ph.D.

Academic and Professional Honors

2007-2012 **HOPE Scholarship,** *Armstrong State University:* Merit-based award available to Georgia residents who have demonstrated academic achievement. Must graduate from high school with a minimum 3.0 grade point average and maintain a minimum 3.0 cumulative post-secondary grade point average to remain eligible.

2009-2012 **Dean's List,** *Armstrong State University:* Must be enrolled full-time and earn a grade point average of at least 3.6 per semester.

2010-2012 **Tri-Beta National Biological Honor Society,** *Armstrong State University:* Must maintain a grade point average of at least 3.0 in all biology major courses.

2011-2012 **National Science Foundation STEM Talent Expansion Program,** *Armstrong State University:* Bridging the Gap: Using Research and Learning Communities to Increase STEM Majors (0856593).

2011-2012 **Biology Faculty Recognition Award,** *Armstrong State University:* Faculty nominate and award a student who demonstrates outstanding leadership abilities, excels in undergraduate research, and achieves other notable academic distinctions.

2012 **Phi Kappa Phi Honor Society,** *Armstrong State University:* By invitation only. Must be a senior in the top 10 percent of their class.

2012 ***Magna Cum Laude*,** *Armstrong State University:* Must graduate with a grade point average of 3.50-3.79.

2016-present **National Institutes of Health Training Grant (T32),** *Emory University:* Training Program in Biochemistry, Cell, and Developmental Biology (5T32GM008367-27).

C. Contributions to Science

1. Regulation of 2'-5'-oligoadenylate synthetase 1 (OAS1) by double-stranded RNA.

My thesis project in Dr. Graeme Conn's lab involves elucidating the mechanisms underlying OAS1 regulation by RNA. This work will provide novel insights into cellular translational control as well as the foundations necessary to design effective treatments for viral infection. I am currently working to develop bio-layer interferometry and cell-based assays as outlined in this proposal. My preliminary data will also be presented at the 14th Annual DSAC Student Research Symposium (ref. **a**) sponsored by the Laney Graduate School of Emory University.

a) **Schwartz, S.L.**, Conn, G.L. Differential regulation of 2'-5'-oligoadenylate synthetase 1 (OAS1) by small double-stranded RNAs. 14th Annual DSAC Student Research Symposium. Atlanta, GA. 19 January 2017. [Poster]

2. Droplet digital PCR: A novel method for detection of influenza virus defective interfering particles.

During my ten-week rotation in Dr. Anice Lowen's lab, I developed a method based on reverse transcription droplet digital PCR. To apply droplet digital PCR technology for the measurement of defective interfering (DI) genomes, I designed primers targeting: **i)** nucleotides 50–150 of each gene segment, a region typically present in both DI and standard segments, and **ii)** an internal site lacking in all DI segments described to date. The ratio of internal to terminal copies/µl for a given segment was used to indicate what proportion of the total copies was full-length. All segments were analyzed for three stocks of influenza A/Panama/2007/99 (H3N2)

virus of differing passage histories. Evidence of DI particles was seen in each stock, but their abundance differed substantially and as expected based on passage history. I performed and analyzed the results of all experiments, designed figures, and wrote the manuscript (ref. **a** and **b**) with insight from Dr. Lowen.

a) **Schwartz, S.L.**, Lowen, A.C. (2016). Droplet digital PCR: A novel method for detection of influenza virus defective interfering particles. *J. Virol. Meth*. **237**, 159-165 (PMCID: PMC5056858).
b) **Schwartz, S.L.**, Lowen, A.C. Droplet digital PCR assay for quantification of defective interfering influenza A viruses. 9th Annual NIAID Centers of Excellence for Influenza Research and Surveillance Network Meeting. Memphis, TN. 26-29 June 2016. [Poster]

3. Developed and applied a high throughput assay to measure PKR activity.
During my time as a research specialist in Dr. Graeme Conn's lab, I developed a high-throughput radiometric assay used to measure PKR activity. The activity assays allowed the discovery of novel findings: the interdomain linker appeared to be unexpectedly unnecessary for regulation of PKR (activation or inhibition), domain swapping showed that both the double-stranded RNA-binding domain (dsRBD) and kinase domain are important for inhibition, and the general scheme of inhibition seemed to be the same for different inhibitory RNAs. The PKR activity assay became the key feature of two of my publications (ref. **a** and **b**) and has become the standard assay still used by others in the lab today. I was also invited to my undergraduate institute, Armstrong State University, to give a lecture (ref. **c**) on these published works (ref. **a** and **b**) with the goal of sharing my experiences and inspiring undergraduates with the knowledge I have garnered through my time both as a research specialist working at the bench full-time and as a current graduate student.

a) Wilson J.L.*, Vachon V.K.*, Sunita S., **Schwartz S.L.**, Conn, G.L. (2014). Dissection of the adenoviral VA RNA_I central domain structure reveals minimal requirements for RNA-mediated inhibition of PKR. *J. Biol. Chem*. **289**(33), 23233-23245 (PCMID: PMC4132820). [**Co-first author*]
b) Sunita, S.*, **Schwartz, S.L.***, and Conn, G.L. (2015). The regulatory and kinase domains but not the interdomain linker determine human double-stranded RNA-activated kinase (PKR) sensitivity to inhibition by viral non-coding RNAs. *J. Biol. Chem*. **290**(47), 28156-28165 (PCMID: PMC4653674). [**Co-first author*]
c) **Schwartz, S.L.** (2016). Human double-stranded RNA-activated kinase (PKR) inhibition by viral RNA requires both structured domains. Armstrong State University, Savannah. 3 November 2016. Lecture. <*https://www.armstrong.edu/academic-departments/biology-spring-seminar-series*>.

PUBLICATIONS
https://www.ncbi.nlm.nih.gov/sites/myncbi/1XQwdr7tzrPQ1/bibliography/51319590/public

D. Scholastic Performance

ARMSTRONG STATE UNIVERSITY (UNDERGRADUATE)
GPA: 3.60

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
2007	Principles of Chemistry I	A	2007	Composition I	A
2008	Principles of Chemistry II	C	2007	Civilization I	B
2008	Principles of Biology I	A	2007	Pre-calculus Mathematics	B
2008	Principles of Biology II	A	2008	Composition II	A
2008	Organic Chemistry I	F	2008	Calculus I	B
2008	Organic Chemistry I Lab	A	2008	Ethics/Morals in Science	A
2009	Microbiology	B	2008	Beginning Weight Training	A
2009	Zoology	A	2008	Introduction to Psychology	A
2009	Introductory Physics I	A	2009	Elementary Statistics	A
2009	Introductory Physics I Lab	A	2009	Aerobic Dance	A
2009	Introductory Physics II	A	2009	Introductory Sociology	A

YEAR	SCIENCE COURSE TITLE	GRADE
2009	Introductory Physics II Lab	A
2009	Principles of Modern Biology	B
2010	Cell Biology	A
2010	Histological Technique	A
2010	Survey of Kingdoms Plantae and Fungi	C
2010	Mammalian Physiology	B
2010	Virology	B
2011	Natural History of Vertebrate Animals	A
2011	Applied Microbiology	A
2011	Organic Chemistry I	A
2011	Tropical Field Biology	A
2011	General Ecology	B
2011	Senior Seminar (General Biology)	S
2011	Organic Chemistry II	B
2011	Organic Chemistry II Lab	B
2012	Genetics	A
2012	Cell and Molecular Biology Lab	A
2012	Biochemistry I	B

YEAR	OTHER COURSE TITLE	GRADE
2009	Elementary French I	A
2009	Elementary French II	A
2009	Political History of America and Georgia	B
2009	Yoga for Beginners	A
2010	Literature and Humanities	A
2010	Cultural Geography	B
2010	Shakespeare	A
2011	Popular Literature	A
2012	Art History I	A

EMORY UNIVERSITY (GRADUATE)
GPA: 3.90

YEAR	SCIENCE COURSE TITLE	GRADE
2015	Foundations in BCDB I	A
2015	Introductory Graduate Seminar	A-
2015	Laboratory Rotations	A
2016	Foundations in BCDB II	A-
2016	Introductory Graduate Seminar	A
2016	Laboratory Rotations	A
2016	Advanced Graduate Research	A
2016	Hypothesis Design and Scientific Writing	A
2016	Advanced Graduate Seminar	S
2016	Advanced Graduate Research	A

YEAR	OTHER COURSE TITLE	GRADE
2015	Jones Program in Ethics	S
2016	Jones Program in Ethics: Workshop	S
2016	Graduate School Teaching Assistant Workshop	S

For letter grades: A = 90-100%, A- = 90-92%, B = 80-89%, C = 70-79% (grades with +/- distinction only apply to Emory University). Attendance and/or participation were used to grade Satisfactory (S) or Unsatisfactory (U) courses.

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

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: Conn, Graeme Leslie

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

POSITION TITLE: Associate 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	Structural Biology
Johns Hopkins University	Postdoc	12/1999	Structural Biology

A. Personal Statement

My role as the Sponsor of this Ruth L. Kirschstein National Research Service Award (NRSA) F31 Pre-doctoral fellowship application by Ms. Samantha (Sam) Schwartz will be to provide a research environment dedicated to Sam's mentorship, training, and scientific development. As her graduate thesis research advisor, I am committed to overseeing Sam's technical training and development of laboratory and analytical skills through the execution of the project she has devised. Further, supported by the outstanding resources and facilities of the Biochemistry, Cell and Developmental Biology (BCDB) graduate program, the Laney Graduate School, and Emory University, I am also committed to Sam's broader professional development to support her goal of a future independent research career in the area of innate immunity and molecular virology.

I have been actively involved in mentoring and training graduate students and other junior trainees throughout my independent research career over the last ~17 years (at two different institutes, the University of Manchester, United Kingdom, 2000-2008, and Emory University, 2008-present). Since I arrived at Emory in 2008, I have been an active training faculty member of two graduate programs, BCDB (Sam's program) and the Microbiology and Molecular Genetics (MMG) program. I have twice served on the BCDB Executive Committee (EC; 2009-2012 and 2014-present) and currently serve as the Program's Director of Graduate Studies. I also serve on the MMG EC through my role on the Program's student recruitment committee, and on the EC of a cross-program NIH T32 *Antimicrobial Resistance and Therapeutic Discovery Program (ARTDTP)* (PI: William Shafer, PhD). I also contribute extensively to graduate education in these programs, including the BCDB Year 1 *BCDB-501 Foundations in BCDB I* (a 2-week minicourse on macromolecular structure), BCDB/ MMG Year 2 *IBS-522r Hypothesis Design and Scientific Writing*, and ARTDTP *IBS-568 Principles of Anti-Infectives*. The development, design and delivery of the BCDB IBS-522r course was the subject of an article I recently coauthored (see **ref. a**, below). Finally, in addition to being the direct advisor and mentor in my lab to 5 Emory graduate students (2 graduated and 3 current, including Sam), I have served on the thesis committees of 17 Emory graduate students (9 present). In total, 8 PhD students have successfully graduated from my lab (6 Manchester and 2 Emory) and these former pre-doctoral trainees have gone on to the successful positions in academia and industry they desired. My current trainees are involved in projects investigating ribosomal RNA methyltransferase enzymes that confer bacterial resistance to antibiotics, or viral/ cellular non-coding RNA structure and activity against proteins of the human host cell antiviral response. Sam's project represents an important new contribution in the latter area.

Sam joined my lab as a BCDB graduate student in Summer 2016 and has developed a new project focused on understanding the regulation of activation of the innate immune system double-stranded RNA (dsRNA) sensor oligoadenylate synthetase 1 (OAS1). As Sam describes elsewhere in her application, her project is based on an initial observation by a former graduate student (Dr. Ginny Vachon) that minor changes to a model dsRNA containing two OAS1 activation consensus sequences had dramatically different impacts on the RNA's ability to activate OAS1. From that starting point, with my help Sam has developed a plan to define the molecular basis for this unexpected phenomenon and assess its impact on the OAS/RNase L pathway in cells. Sam's project will deliver broad experimental training that exploits both the strengths of my lab in biochemistry, structural and other biophysical methods, molecular biology and microbiology, as well as other

resources and expertise across campus (such as the HDX-MS core). Additionally, Sam will have Co-sponsor Dr. Anice Lowen as a co-mentor and advisor. Dr. Lowen is an expert in the cell-based assays and models of viral infection that will extend and enhance the impact of Sam's *in vitro* analyses of OAS1 regulation by RNA. Dr. Lowen is a colleague at Emory in the Department of Microbiology and Immunology, one floor down from us in the same building. Sam and Dr. Lowen have already established an excellent rapport through the BCDB program's Year 1 rotations and Sam's project will further build on established interactions between our groups [redacted]

In summary, I have a demonstrated record of successful mentorship and engagement in graduate training in my lab and programs at Emory. Together with Dr. Lowen, I am committed to providing Sam with a research environment that offers outstanding breadth of technical training, professional and transferable skills development, mentorship and the career guidance needed to promote her development as an independent researcher of outstanding promise.

a. Kahn, R.A., Conn, G.L., Pavlath, G.K. and Corbett, A.H. (2016). Use of a grant writing class in training Ph.D. students. *Traffic*. **17**(7), 803-814.

B. Positions and Honors

Positions and Employment

1996–1999 Wellcome Trust Postdoctoral Fellow, Johns Hopkins University, Baltimore, MD.
1999–2000 Wellcome Trust Postdoctoral Fellow, Dept. of Biomolecular Sciences, UMIST, UK.
2000–2004 Wellcome Trust Independent Research Career Development Fellow, Department of Biomolecular Sciences, UMIST, UK.
2000–2004 Lecturer (equivalent to Assistant Professor, *tenure track*), Department of Biomolecular Sciences, UMIST, UK.
2004–2007 Lecturer (equivalent to Assistant Professor, *with tenure*). Faculty of Life Sciences, University of Manchester, UK.
2007–2008 Senior Lecturer (equivalent to Associate Professor), Faculty of Life Sciences, University of Manchester, UK.
2008– Associate Professor, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.

Other Experience, Service and Professional Memberships

2000-present Biochemical Society UK (2003-2007), Association for Chemoreception Sciences (since 2003), American Society for Microbiology (ASM; since 2008), American Association for the Advancement of Science (AAAS; since 2009), American Crystallographic Society (ACA; since 2010) and The American Society for Biochemistry and Molecular Biology (ASBMB; since 2011).
2001-present *Ad hoc* grant reviewer/ study section: The Wellcome Trust, BBSRC (UK), 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.*
2012 'Recombinant and *in vitro* RNA synthesis: Methods and Protocols' (Editor), Methods in Molecular Biology series (Walker, J.M., series Editor), Humana Press.
2012 Co-chair, Proteins and Crystallography Committee 2, American Heart Association.
2013-2014 Chair, Proteins and Crystallography Committee 2, American Heart Association.
2014-present Frontiers in Molecular Biosciences, Reviewing Editor (Structural Biology)
2015 NIH/CSR ZRG1 F13-C 20 L, Fellowships: Infectious Diseases and Microbiology (twice).
2016 NIH/CSR ZRG1 F13-C 20 L, Fellowships: Infectious Diseases and Microbiology (twice).

Awards/Honors

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

2000–2004 Wellcome Trust Independent Research Career Development Fellowship.

C. Contribution to Science

Complete List of Published Work in My NCBI (61 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 (in my lab only) are highlighted as follows in the references in the following sections: **undergraduate†/ graduate students** (bolded and underlined) and *postdoctoral researchers* (italicized and underlined); additionally, students from underrepresented minorities are noted¶.

1. Non-coding RNA structure and regulation of the innate immune protein PKR. My lab has long-standing interests in the structure and activity of viral non-coding RNAs, such as Adenovirus VA RNA_I – an essential, pro-viral RNA best known for inhibition of the double-stranded (ds)RNA-activated protein kinase (PKR). Our early work defined the stabilities and roles of the conserved domains within VA RNA_I (e.g. **refs a,b**), including the remarkable finding that the entire Terminal Stem could be deleted without loss of activity (whereas smaller deletions were detrimental). Our finding complemented the discovery that VA RNA_I is similarly processed in the cell by Dicer, offering the prospect (still to be fully explored) that Adenovirus may exploit Dicer activity to tune the activity of VA RNA_I appropriately to the stage of viral replication. More recent work has defined the minimal requirements for PKR inhibition by VA RNA_I, offering a satisfying explanation for why VA RNAs from different serotypes are equally effective despite their wide variation in sequence and length (**ref. c**). Finally, we recently demonstrated that both the N-terminal dsRNA binding domain and the C-terminal kinase domain of human PKR, but not the interdomain linker, contain important determinants for inhibition by human viral non-coding RNAs (**ref. d**).

a. **Wahid, A.M.**, **Coventry, V.K**. and Conn, G.L. (2008). Systematic deletion of the adenovirus-associated RNA_I terminal stem reveals a surprisingly active RNA inhibitor of double-stranded RNA-activated protein kinase. *J. Biol. Chem.* **283**(25), 17485–17493. [PMCID: PMC2427366]

b. **Wahid, A.M.**, **Coventry, V.K.** and Conn, G.L. (2009). The PKR-binding domain of adenovirus VA RNA_I exists as a mixture of two functionally non-equivalent structures. *Nucleic Acids Res.* **37**(17), 5830-5837. [PMCID: PMC2761268]

c. Wilson*, J.L, **Vachon*, V.K**., *Sunita, S.*, Schwartz, S.L. and **Conn, G.L.** (2014). Dissection of the adenoviral VA RNA_I Central Domain structure reveals minimal requirements for RNA-mediated inhibition of PKR. *J. Biol. Chem*. **289**(33), 23233-23245. (*Co-first author) [PMCID: PMC4132820]

d. *Sunita S.*, Schwartz SL, Conn GL. (2015) The Regulatory and kiinase domains but not the Interdomain linker determine human double-stranded RNA-activated kinase (PKR) sensitivity to inhibition by viral non-coding RNAs. *J. Biol. Chem*. **290**(47):28156-28165. (*Co-first author) [PMCID: PMC4653674]

2. Molecular mechanisms of RNA-mediated regulation of OAS1. 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. 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**). Our current studies have revealed that even for "simple" model dsRNAs there is a potentially complex interplay of RNA features that controls whether OAS1 becomes activated. We next aim to extend these initial finding to define the "rules" that govern potent OAS1 activation by dsRNA and the impacts of motifs like 3'-ssPy or a novel tertiary structure within the cellular non-coding RNA 886 (nc886) in non the activation of the OAS/RNase L pathway both *in vitro* and in the context of cellular infection. *Sam's project will be a critical component of this long-term goal*. Finally, in unpublished work, we have recently cloned and expressed human OAS3 in *E. coli* and will extend our studies to this additional component of the OAS/ RNase L pathway. For example, we will test whether similar rules about OAS-activating RNA features apply to this RNA or if OAS3 has evolved as a more general sensor longer dsRNAs.

a. **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]

3. Molecular basis for aminoglycoside-resistance arising from 16S rRNA methylation. 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, while drug-producing bacteria invariably 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 potential 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 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 expanded in a later study which experimentally determined the methylation target for a representative group of putative m^7G1405 enzymes. Subsequently, we determined the first structures of m^1A1408 enzymes with cosubstrate, KamB from the aminoglycoside-producer *S. tenebrarius*, and NpmA which was identified as the source of treatment failure in a clinical infection (**ref. a**). More recently, 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 (**ref. b**). This structure 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 efforts to obtain complementary structure-function insights for members of the m^7G1405 family, and investigating the molecular mechanisms of action of enzymes from both families (e.g. see **ref. c,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.

a. *Macmaster, R.,* Zelinskaya, N., *Savic, M.*, **Rankin, C.R.** and Conn, G.L. (2010). Structural insights into the function of aminoglycoside-resistance A1408 16S rRNA methyltransferases from antibiotic-producing and human pathogenic bacteria. *Nucleic Acids Res.* **38**(21), 7791-7799. [PMCID: PMC2995053]

b. *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]

c. *Savic, M., Sunita, S.*, Zelinskaya, N., *Desai, P.M.*, *Macmaster, R.*, **Vinal, K.** and Conn, G.L. (2015). 30S subunit-dependent activation of the *S. cellulosum* So ce56 aminoglycoside-resistance 16S rRNA methyltransferase Kmr. *Antimicrob. Agents Chemother.* **59**(5), 2807-2816. [PMCID:PMC4394793]

d. *Witek, M.A.* and Conn, G.L. (2016). Functional dichotomy in the 16S rRNA (m^1A1408) methyltransferase family and control of catalytic activity via a novel tryptophan mediated loop reorganization. *Nucleic Acids Res.* **44**(1), 342-353 [PMCID: PMC4705659]

4. Mentorship of Trainees. As described in my Personal Statement (see section A, above), I have a strong track record of mentorship of junior trainees in my lab. My former lab members have gone on to successful graduate/ medical school training, postdoctoral positions, and science-related careers in academia and industry. To date, my lab has graduated 8 PhD students, and contributed to the training of three current graduate students, 8 postdoctoral scientists, 12 graduate rotation students (at Emory only, 2008-present), and >30 undergraduates or high school students during summer and other research experiences. The publications below are the most recent from my current or past trainees that are not included in the specific Contribution sections above.

a. **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]

b. Myers, C.L., **Kuiper, E.G.**, Grant, P.C., **Hernandez, J.**¶†, Conn, G.L. and Honek, J.F. (2015). Co-substrate binding and enzyme catalysis by the thiostrepton resistance RNA methyltransferase (Tsr) from *Streptomyces cyaneus, FEBS Lett.* **589**(21), 3263-3270. [PMCID: PMC4661090]

c. **Vachon, V.K.** and Conn, G.L. (2016). Adenovirus VA RNA: An essential pro-viral non-coding RNA. *Virus Res.* **212**, 39-52.

d. 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]

D. Research Support

Ongoing Research Support

R01 AI088025 (Conn, PI) 05/01/2010 – 04/30/2020
NIH/NIAID
RNA modification and antibiotic resistance.
This project will investigate 16S ribosomal RNA methyltransferase enzymes that confer resistance to aminoglycoside antibiotics. The goals are to determine methyltransferase and methyltransferase-30S substrate complex X-ray crystal structures and to define the molecular mechanisms which underpin target (30S ribosome) recognition by these resistance enzymes. **Role: PI.**

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

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: Lowen, Anice C

eRA COMMONS USER NAME: [redacted]

POSITION TITLE: Assistant Professor of Microbiology and Immunology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
University of Alberta, Edmonton, Canada	B.Sc.	2001	Biochemistry
University of Glasgow, Scotland	Ph.D.	2005	Virology
Mount Sinai School of Medicine, New York, NY	Postdoc	2005-2009	Virology

A. Personal Statement

My role in this proposal as Co-sponsor will be to provide Ms. Samantha Schwartz (Sam) with mentorship and technical guidance for her proposed experiments to correlate OAS1 activity *in vitro* with that in human lung carcinoma (A549) cells (**Aim 1.2**). I am an Assistant Professor in Microbiology and Immunology at Emory University, having joined this Department in 2011. Effective mentoring of graduate students is one of my highest priorities in the lab. I have taken on four students for their dissertation work to date; two of these students have obtained their PhDs and both are now employed in related fields. I also serve on the committees of a number of graduate students and participate actively in annual recruiting, teaching and seminar series associated with the Microbiology and Molecular Genetics (MMG) and Immunology and Molecular Pathogenesis (IMP) graduate programs. Along with Sam's mentor and Sponsor on this application, I am currently a member of the MMG Program Executive Committee and Graduate Recruitment Committee. I have studied the biology of influenza viruses for the past 11 years, working in animal models and cell-based culture systems. As a post-doctoral fellow working with Peter Palese, I gained a firm grounding in molecular virology and virus-host interactions, including innate antiviral responses to infection. I have continued this work as an independent researcher, with a current focus on mechanisms of influenza A virus evolution. My lab is therefore well equipped for the experiments Sam describes in her proposal, including cell-based experiments involving delivery of specific RNA molecules to the cytoplasm, assessment of OAS activation and impact on viral infection. By combining biochemical and virological approaches, Sam's project promises to reveal novel insights into the molecular mechanisms by which viral pathogen-associated molecular patterns (PAMPS) are sensed and responded to within infected cells. In carrying out this project, she will furthermore receive valuable training in biochemistry and virology, two highly complementary disciplines. Sam had a very productive rotation in my lab during the first year of her graduate studies and we have an excellent relationship. Based on this foundation, the established ties between my lab and Dr. Conn's, my track-record of mentorship, and relevant research expertise, I am well-placed to provide Sam with technical and professional training that will support her career goal of becoming an independent researcher further exploring the field of innate immunity.

B. Positions and Honors

Positions

2009-2010 Assistant Professor, research track, Department of Microbiology, Mount Sinai School of Medicine

2011-present Assistant Professor, tenure track, Department of Microbiology and Immunology, Emory University School of Medicine

Other experience and professional memberships

2005-present Member, American Society for Virology

2008-present Editorial board, *Journal of Virology*
2011-present Member, American Society for Microbiology
2013-2015 Ad hoc reviewer for Virology B study section
2015-present Editor, *mSphere*

Honors

2002	Wellcome Trust Studentship of the Wellcome Trust
2002-2004	Overseas Research Student Award of the Universities, UK
2003	Joel M. Dalrymple award from the American Society for Virology
2007-2010	Parker B Francis Fellow in Pulmonary Research
2013	Member of "The Emory 1%" awarded in recognition of perfect score on NIH grant application
2013	One of the top 25 peer reviewers for the *Journal of Virology* (see JVI 87:13087)
2015	Ann C. Palmenberg Award for Junior Investigators from the American Society forVirology
2015	One of the top 25 peer reviewers for the *Journal of Virology* (see JVI 89:12233)
2016	Finalist for Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Disease award

C. Contribution to Science

1. A system for the study of influenza virus reassortment. Since starting my own laboratory I have developed novel system that, for the first time, allows influenza virus reassortment to be studied directly. The system relies on a matched pair of parental viruses that differ only by a handful of silent mutations and offers an important advantage over traditional approaches: all 256 progeny genotypes emerging from mixed infection are of equivalent fitness. For this reason, reassortment efficiency can be measured in an unbiased fashion. Already, we have made strides in understanding reassortment at intracellular and organismal levels. We have shown that genome mixing and therefore reassortment are efficient in co-infected cells and that reassortment occurs routinely in an animal host. Our recent data indicate that semi-infectious influenza virus particles, which fail to deliver one or more gene segments to the site of replication, are important determinants of the high efficiency of reassortment. Together, our results suggest that, rather than being an episodic event that occurs when conditions permit (as was thought previously), reassortment is a routine feature of influenza virus infections that contributes to viral evolution on a continual basis.

a. Marshall N, Priyamvada L, Ende Z, Steel J and **Lowen AC**. 2013. Influenza virus reassortment occurs with high frequency in the absence of segment mismatch. PLOS Pathogens. 9(6):e1003421. PMC3681746
b. Tao H, Steel J and **Lowen AC**. 2014. Intra-host dynamics of influenza virus reassortment. Journal of Virology. 88(13):7485-7492. PMC4054463
c. Tao H, Li L, White MC, Steel J and **Lowen AC**. 2015. Influenza A virus co-infection through transmission can support high levels of reassortment. Journal of Virology. 89(16):8453-61. PMC4524221
d. Fonville JM, Marshall NM, Tao H, Steel J and **Lowen AC**. Influenza virus reassortment is enhanced by semi-infectious particles but can be suppressed by defective interfering particles. PLOS Pathogens. 11(10): e1005204. PMCID: PMC4595279

2. Guinea pig model of influenza virus transmission. As a post-doctoral fellow with Dr. Peter Palese, I developed the guinea pig as a model for the study of influenza virus transmission, providing a valuable alternative to the well-established ferret model. The utility of the model is evidenced by the fact that a number of laboratories have adopted its use and generated important new knowledge on influenza virus transmission.

a. **Lowen AC**, Mubareka S, Tumpey TM, García-Sastre A, and Palese P. 2006. The guinea pig as a transmission model for human influenza viruses. Proceedings of the National Academy of Sciences. 103(26):9988-9992. PMC1502566
b. Bouvier N, **Lowen AC**. 2010. Animal Models for Influenza Virus Pathogenesis and Transmission. Viruses. 2(8):1530-1563. PMC3063653
c. **Lowen AC**, Bouvier N, Steel J, 2014. Transmission in the guinea pig model. Current Topics in Microbiology and Immunology. 385: 157-183. PMID: 25001209

3. An explanation for winter-time seasonality of influenza. The guinea pig system allowed my colleagues and I to address a long-standing question in the influenza field - that of the underlying basis for winter-time

seasonality. We found that influenza virus transmission was strongly favored by low temperature and low humidity, suggesting that environmental conditions account for the seasonality of epidemics. This work offered the first experimental evidence supporting a role for climatic factors in determining the seasonality of influenza and has reinvigorated the influenza field with respect to understanding the timing with which epidemics occur and what factors drive that timing. Our article in PLOS Pathogens was featured by several news outlets, including on the front page of the New York Times, has been cited 374 times, and has stimulated a number of epidemiological and modeling-based studies aimed at understanding how humidity and temperature impact influenza at a population level.

a. **Lowen AC**, Mubareka S, Steel J, and Palese P. 2007. Influenza virus transmission is dependent on relative humidity and temperature. PLOS Pathogens. 3(10):1470-1476. PMC2034399
b. **Lowen AC**, Steel J, Mubareka S and Palese P. 2008. High temperature (30°C) blocks aerosol but not contact transmission of influenza virus. Journal of Virology. 82(11):5650-5652. PMC2395183
c. Steel J, Palese P, **Lowen AC**. 2011. Transmission of a 2009 pandemic influenza virus shows a sensitivity to temperature and humidity similar to that of an H3N2 seasonal strain. Journal of Virology. 85(3):1400-1402. PMC3020521
d. **Lowen AC** and Steel J. 2014. Roles of humidity and temperature in shaping influenza seasonality. Journal of Virology. 88(14):7692-7695. PMC4097773

4. Identification of determinants of influenza virus transmission. The efficiency with which influenza viruses transmit from one individual to the next is a key factor in determining the occurrence of influenza pandemics and the severity of influenza epidemics. In total, I have co-authored 19 publications focused on influenza virus transmission, a subject of clear significance to public health. This body of work has contributed significantly to the advancement of the field. While much remains to be learned, a picture of the viral, host and environmental factors required to support transmission among mammals is nowemerging.

a. Steel J, **Lowen AC**, Mubareka S and Palese P. 2009. Transmission of influenza virus in a mammalian host is increased by PB2 amino acids 627K or 627E/701N. PLOS Pathogens. 3(10):1470-1476. PMC2603332
b. Mubareka S, **Lowen AC**, Steel J, Coates AL, García-Sastre A and Palese P. 2009. Transmission of influenza virus via aerosols and fomites in the guinea pig model. Journal of Infectious Diseases**.** 199(6):858-865. PMC4180291
c. **Lowen AC**, Steel J, Mubareka S, Carnero E, García-Sastre A and Palese P. 2009. Blocking inter-host transmission of influenza virus by vaccination in the guinea pig model. Journal of Virology. 83(7):2803-2818. PMC2655561
d. Chutinimitkul S, Herfst S, Steel J, **Lowen AC**, Ye J, van Riel D, Schrauwen EJ, Bestebroer TM, Koel B, Burke DF, Sutherland-Cash KH, Whittleston CS, Russell CA, Wales DJ, Smith DJ, Jonges M, Meijer A, Koopmans M, Rimmelzwaan GF, Kuiken T, Osterhaus AD, García-Sastre A, Perez DR, Fouchier RA. 2010. Virulence-associated substitution D222G in the hemagglutinin of 2009 pandemic influenza A(H1N1) virus affects receptor binding. Journal of Virology. 84(22):11802-13. PMC2977876

5. A robust reverse genetics system for Bunyamwera virus. As a PhD student with Dr. Richard Elliott, I was fortunate to work with the only reverse genetics system available at that time for a virus of the *Bunyaviridae*. In addition to gaining novel insights into the bunyavirus life cycle, my work in the Elliott lab helped to vastly improve the efficiency of virus rescue and the simplicity of theprotocol.

a. **Lowen AC**, Noonan C, McLees A, Elliott RM. Efficient bunyavirus rescue from cloned cDNA. 2004. Virology. 330(2):493-500. PMID: 15567443
b. Kohl A, **Lowen AC**, Léonard VH, Elliott RM. 2006. Genetic elements regulating packaging of the Bunyamwera orthobunyavirus genome. Journal of General Virology. 87(1):177-87. PMID: 16361430
c. **Lowen AC**, Elliott RM. 2005. Mutational analyses of the nonconserved sequences in the Bunyamwera Orthobunyavirus S segment untranslated regions. Journal of Virology.79(20):12861-70.PMC1235861
d. **Lowen AC**, Boyd A, Fazakerley JK, Elliott RM. 2005. Attenuation of bunyavirus replication by rearrangement of viral coding and noncoding sequences. Journal of virology. 79(11):6940-6. PMC1112153

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/anice.lowen.1/bibliography/40895322/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

Title: Reassortment of influenza viruses in a co-infected host
Number: R01 AI099000
Principal investigator: Anice Lowen
Funding Agency: National Institutes of Health
Award period: 9/1/2012 – 8/31/2017
The main goals of this project are to determine the baseline frequency of influenza virus reassortment under unbiased conditions, and test whether a number of factors, such as timing and dose of co-infection, increase or decrease that frequency.

Title: Host adaptation, reassortment and transmission of influenza viruses at the animal-human interface
Number: HHSN272201400004C
Funding Agency: National Institutes of Health
Principal investigator: Walt Orenstein
Role: Co-project leader on Project 2
Award period: 4/1/2014- 3/31/2021
Project 2 within Emory/UGA Center of Excellence for Influenza Research and Surveillance NIH/NIAID contract. The main goals are to test the impact of host adaptation and host cell origin on reassortment outcomes, and to evaluate the potential for transmissible viruses to arise through reassortment.

Title: Identification and Hit-To-Lead Development of Influenza A Virus Inhibitors
Number: R21 AI119196
Funding Agency: National Institutes of Health
Principal investigator: Richard Plemper
Role: sub-contractor
Award period: 7/1/2015 – 6/30/2017
The main goal is to identify several novel small molecule inhibitors of influenza A viruses with potential for clinical development.

Title: Dynamics and evolution of recall immune responses to influenza viruses
Number: U19 AI117891
Funding Agency: National Institutes of Health
Principal investigator: Rustom Antia
Role: Co-investigator
Award period: 06/01/2015 – 05/31/2020
The major aim is to develop a robust computational model, informed by experimental data, of humoral and cell mediated immune responses to influenza virus infection and vaccination.

Title: Impact of selective genome packaging on influenza A virus reassortment
Number: R01 AI125268
Funding Agency: National Institutes of Health
Principle investigator: Anice Lowen
Award Period: 08/04/2016 – 07/31/2020
The main aim of this project is to test the hypothesis that sequence divergence in RNA packaging signals limits reassortment between heterologous strains of influenza A virus.

Title: Host dependence of influenza A virus reassortment
Number: R01 AI127799
Funding Agency: National Institutes of Health
Principle investigator: Anice Lowen
Award Period: 12/05/16-11/30/21
Research funded through this grant will indicate the efficiency of influenza A virus reassortment in a broad set of natural hosts, reveal whether the prevalence of viral genetic exchange varies among host species, and give insight into the features of viral within-host dynamics that allow for co-infection (and therefore reassortment) to occur in vivo.

Completed Research Support

Title: Contribution of virion morphology to influenza viral fitness
Number: HHSN266200700006C
Principal investigator: Orenstein
Funding Agency: National Institutes of Health
Award period: 4/1/2011 – 3/31/2013
Role: Project lead of pilot project
The main goal of this project is to define the relative fitness and transmissibility of matched spherical and filamentous influenza viruses.

PHS Fellowship Supplemental Form

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction

1. Introduction
(RESUBMISSION)

Fellowship Applicant Section

2. Applicant's Background and Goals for Fellowship Training*	Applicant_s_Background_and_Goals_for_Fellowship_Training_Schwartz103208959 3.pdf

Research Training Plan Section

3. Specific Aims*	Specific_Aims_Schwartz1032089674.pdf
4. Research Strategy*	Research_Strategy_Schwartz1032089676.pdf
5. Respective Contributions*	Respective_Contributions_Schwartz1032089594.pdf
6. Selection of Sponsor and Institution*	Selection_of_Sponsor_and_Institute_Schwartz1032089595.pdf
7. Progress Report Publication List (RENEWAL)	
8. Training in the Responsible Conduct of Research*	Responsible_Conduct_of_Research_Schwartz1032089596.pdf

Sponsor(s), Collaborator(s) and Consultant(s) Section

9. Sponsor and Co-Sponsor Statements	Sponsor_Cosponsor_Training_Plan_Schwartz1032089592.pdf
10. Letters of Support from Collaborators, Contributors and Consultants	LoS_Renhao_Li HDX_MS NIH_F31_Dec2016V21032089849.pdf

Institutional Environment and Commitment to Training Section

11. Description of Institutional Environment and Commitment to Training	Institutional_Environment_and_Commitment_to_Training_Schwartz1032089602.pdf

Other Research Training Plan Section

Human Subjects

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the involvement of human subjects, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Human Subjects Involved? ☐ Yes ✔ No

12. Human Subjects Involvement Indefinite?
13. Clinical Trial?
14. Agency-Defined Phase III Clinical Trial?
15. Protection of Human Subjects
16. Data Safety Monitoring Plan
17. Inclusion of Women and Minorities
18. Inclusion of Children

Vertebrate Animals

The following item is taken from the Research & Related Other Project Information form and repeated here for your reference. Any change to this item must be made on the Research & Related Other Project Information form.

Are Vertebrate Animals Used? ☐ Yes ✔ No

19. Vertebrate Animals Use Indefinite?

Funding Opportunity Number: PA-16-309. Received Date: 2016-12-08T16:40:52.000-05:00

PHS Fellowship Supplemental Form

20. Are vertebrate animals euthanized?	
If "Yes" to euthanasia Is method consistent with American Veterinary Medical Association (AVMA) guidelines?	
If "No" to AVMA guidelines, describe method and provide scientific justification	
21. Vertebrate Animals	

Other Research Training Plan Information

22. Select Agent Research	
23. Resource Sharing Plan	Resource_Sharing_Plan_Schwartz1032089603.pdf
24. Authentication of Key Biological and/or Chemical Resources	

PHS Fellowship Supplemental Form

Additional Information Section

25. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ☐ Yes ☑ No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s), using the registry information provided within the agency instructions. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s):

26. Alternate Phone Number: [redacted]

27. Degree Sought During Proposed Award:

Degree:	If "other", please indicate degree type:	Expected Completion Date (month/year):
PHD: Doctor of Philosophy		05/2021

28. Field of Training for Current Proposal*: 100 Biochemistry

29. Current Or Prior Kirschstein-NRSA Support?* ☑ Yes ☐ No

If yes, please identify current and prior Kirschstein-NRSA support below:

Level*	Type*	Start Date (if known)	End Date (if known)	Grant Number (if known)
Predoctoral	Institutional	06/01/2016	05/31/2017	5T32GM008367-27

30. Applications for Concurrent Support?* ☐ Yes ☑ No

If yes, please describe in an attached file:

31. Citizenship*

U.S. Citizen — U.S. Citizen or Non-Citizen National? ☑ Yes ☐ No

Non-U.S. Citizen
- ☐ With a Permanent U.S. Resident Visa
- ☐ With a Temporary U.S. Visa

If you are a non-U.S. citizen with a temporary visa who has applied for permanent resident status and expect to hold a permanent resident visa by the earliest possible start date of the award, please also check here. ☐

Name of Former Institution:*

32. ☐ Change of Sponsoring Institution

Funding Opportunity Number: PA-16-309. Received Date: 2016-12-08T16:40:52.000-05:00

PHS Fellowship Supplemental Form

Budget Section

All Fellowship Applicants:

1. Tuition and Fees*:

☐ None Requested ✔ Funds Requested

Year 1	[redacted]
Year 2	[redacted]
Year 3	[redacted]
Year 4	
Year 5	
Year 6 (when applicable)	
Total Funds Requested:	[redacted]

Senior Fellowship Applicants Only:

	Amount	Academic Period	Number of Months
2. Present Institutional Base Salary:			

3. Stipends/Salary During First Year of Proposed Fellowship:

	Amount	Number of Months
a. Federal Stipend Requested:		

	Amount	Number of Months
b. Supplementation from other sources:		

Type (sabbatical leave, salary, etc.)

Source

Appendix

Tracking Number: GRANT12302947 Funding Opportunity Number: PA-16-309. Received Date: 2016-12-08T16:40:52.000-05:00

APPLICANT'S BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING

A. Doctoral Dissertation and Research Experience

My previous research experiences have all revolved around microbiology, biochemistry, or a combination of the two. I had the opportunity to try two very different research experiences before entering graduate school at Emory University. I began my research career as an undergraduate at Armstrong State University and then worked for three years as a research technician at Emory University. Upon joining Emory University's Biochemistry, Cell, and Developmental Biology (BCDB) Program, I completed three different laboratory rotations prior to joining the Conn lab in May 2016.

As an undergraduate at Armstrong State University, I wholeheartedly applied myself to volunteering in Dr. Jennifer Brofft's lab investigating microbes that penetrate sea turtle eggs and which are implicated with failed turtle embryo development. My research culminated in a poster presentation at two American Society for Microbiology meetings (Gainesville, Florida in 2011 and San Francisco, California in 2012), for which I was awarded travel funds via the National Science Foundation's STEM program. I also gave a formal oral presentation at Armstrong State University's Student Scholars Symposium. Following this experience, I felt sure graduate school was in my future, but I wanted to explore additional areas of research before applying. While this research opportunity at my undergraduate institution was instrumental in my early development as a researcher, other options were limited in scope due to the small size of the institution. To broaden my horizons and develop an understanding of the rigors of a major research institution, I applied for a research specialist (technician) position at Emory University, an institute I had also identified as a potential top choice for graduate school. I took a leap and left for Atlanta for no other reason than to pursue academic research, despite my lack of exposure to graduate-level research and no family role model for higher education.

In Dr. Graeme Conn's lab at Emory University, I developed technical and organizational skills to efficiently carry out full-time research. My work ranged from laboratory prep work to troubleshooting complex assays and processing resultant data. My three years of lab experience as a research specialist provided an important initial exposure to aspects of being a researcher, such as reading of primary research articles, attending research seminars, giving presentations, and contributing to manuscript preparation. From these initial insights, I gained a clear concept of the critical skills I would develop for success in graduate school. My research in the Conn lab focused on elucidating the molecular details of protein kinase R (PKR) regulation by viral non-coding RNAs (VA RNA$_I$ and HIV-1 TAR) as part of the host innate immune response. I gained skills in RNA *in vitro* transcription and purification, and developed a new high-throughput version of the radiometric assay used by the lab to measure PKR activity. Developing this assay in particular permitted me to evolve from becoming frustrated by experimental road blocks to a scientist who is excited and driven by the often subtle complexities that define a highly reproducible, invaluable experimental tool. What initially began as a test of perseverance grew into a love of experimental science. It was in this transformation that I knew I was ready to take the next step: graduate school. Upon joining Emory University's Biochemistry, Cell, and Developmental Biology (BCDB) Program, I completed three laboratory rotations prior to joining my thesis lab.

My first rotation was in the research group of Dr. Christine Dunham. The Dunham lab uses X-ray crystallography to study ribosome structure and translational regulation. Here, my work focused on the toxin MazF-mt6 from *Mycobacterium tuberculosis* that inhibits protein synthesis during stress by cleaving 23S rRNA at Helix 70. The goal of my project was to determine MazF-mt6 residues important for cleavage of Helix 70 RNA. Using X-ray crystal structures of MazF-mt6 and *Bacillus subtilis* MazF-mRNA complex as a guide, I substituted predicted catalytic residues with alanine and tested the variant MazF proteins for ribonuclease activity using an *in vitro* cleavage assay. Dr. Dunham also entrusted me with an X-ray crystallography side project where I attempted to co-crystallize a novel inhibitor compound with the ribosome. I gained experience setting trays, optimizing conditions, and testing crystals remotely at the SER-CAT synchrotron beamline at the Advanced Photon Source.

My next rotation was with Dr. Daniel Reines' research group. The Reines lab uses yeast as a model system to study RNA-binding proteins containing low complexity domains and their propensity to form amyloid filaments. Here, my work focused on the RNA-binding protein, Pcf11, a component of the cleavage and polyadenylation complex that is involved in orchestrating transcription termination in yeast. Computational analysis suggested that Pcf11 possesses a prion-like domain predicted to form amyloid filaments. The Reines lab has shown through biochemical data and electron microscopy imaging that the low complexity domain of Pcf11 alone was able to form amyloid filaments. The main focus of my project was to address whether or not the low complexity domain of Pcf11 could form filaments when linked to structure-containing domains, as it exists naturally in the

My last rotation was with the Lowen lab. Dr. Anice Lowen's group uses molecular and classical virology techniques to study influenza A virus reassortment, the process by which two viruses that co-infect the same cell exchange intact gene segments. Here, my work focused on developing a novel technique using droplet digital PCR (ddPCR) as a method for detecting influenza virus defective interfering (DI) particles within a virus preparation. Three A/Panama/2007/99 (H3N2) virus stocks were used in these experiments that were either enriched in DI particles, purified to remove possible DI's, or unknown. My results revealed that ddPCR can successfully detect DI particles within these virus stocks and confirmed the approach to be more sensitive and precise when compared to traditional quantitative PCR methods. My ten-week rotation proved to be very successful: I collected publishable data, and wrote and published a methods paper. Perhaps most importantly, I forged an excellent mentor-mentee relationship with Dr. Lowen who is now the Co-sponsor on this fellowship application. During my rotation, she also encouraged me to attend the 14th Annual Southeastern Regional Virology Conference at Emory University and that we present my work at the 9th Annual NIAID Centers of Excellence for Influenza Research and Surveillance (CEIRS) Network Meeting at St. Jude Children's Research Hospital.

Narrative of Doctoral Dissertation. Since joining the Conn laboratory, I have focused my studies on the regulation of OAS1 by double-stranded RNA (dsRNA). Data from previous lab members showed that mutations to particular consensus sequence nucleotides cause stark differences in OAS1 activation. However, the molecular basis for these differences was not clear from available structural and functional data. The goal of my dissertation research is to understand how RNA features (sequences, structural motifs, etc.) contribute to OAS1 activation. Preliminary data suggests that the stark differences observed in OAS1 activity by these dsRNA are not due to differences in binding affinity. From these data, I hypothesize that the differences in OAS1 activity likely lie in differences in enzyme kinetics or structural rearrangements and will test these hypotheses as part of my current proposal.

B. Training Goals and Objectives

My first goal for the training period is to continue developing my skills in independent experimental design, execution, and interpretation of results obtained. To date, I have refined our expression and purification scheme for OAS1, resulting in a better yield of more active protein. As presented in the preliminary data in this proposal, I have also begun experiments using bio-layer interferometry (BLI) (using a new biotinylated OAS1 construct I generated) and produced the samples for the hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) peptide mapping study.

The proposed research will aid my scientific career by first allowing me to acquire many new technical skills e.g. in performing and analyzing the results of BLI binding assays, HDX-MS analyses of protein dynamics, X-ray crystallographic structure determination, OAS enzyme assays, cell culture, and viral infection assays. These new skills will complement those from my time as a research specialist (e.g. in RNA synthesis and purification, and protein kinase assays) as well deepen my knowledge of critical practical skills for any biochemist, such as protein expression and purification. I will be supported by my Sponsor and Co-sponsor in many of these approaches but will also be able to call on other researchers and experts to develop new protocols and troubleshoot difficult problems in approaches, including BLI and HDX-MS. I am excited to be contributing to bringing these new approaches into our lab's repertoire. With the help of Drs. Conn and Lowen, I have developed an integrated plan to assess OAS1 regulation by specific RNA features using the above broad range of approaches. In Specific Aim 1, I will measure OAS1 enzyme kinetics and correlate the differences *in vitro* with their impact on the OAS1/RNase L pathway in human cells by measuring ribosomal RNA (rRNA) cleavage and transcript level changes of known RNase L targets, all of which is new to me. In Specific Aim 2, I will measure dsRNA binding kinetics (on/off rates) and OAS1 structural dynamics using BLI and HDX-MS, respectively, both entirely new techniques to me. These experiments will allow us to gain insight into exactly how the RNA can mechanistically alter OAS1 structure to achieve different levels of activation. By building upon my current foundation of knowledge, I am setting myself up for the best possible post-doctoral experiences available.

I will also continue to develop my communication skills. Dr. Conn places a strong emphasis on perfecting presentation and writing skills, which is one of the main reasons I ultimately chose to join his lab for my thesis

research. I practice my seminars in front of Dr. Conn and lab members to improve my style by becoming more clear and polished. The process of developing and writing this fellowship with Dr. Conn and others, and my manuscript with Dr. Lowen from my rotation in her lab, not only has been an amazing experience, but also it revealed I still have much to learn from them in this area. Developing both of these communication skills is an ongoing process as they are essential to my advancement in science. Throughout the training period, I will attend meetings to present my research and network with other scientists. These meetings will not only further my research through the critical feedback I will receive, but will also help me build connections in my chosen field. Additionally, I will take advantage of the many training opportunities provided by the BCDB graduate program (yearly student seminars, journal clubs, etc.) to refine and practice my communication skills. Finally, I will expand my writing skills to include the preparation and submission of my work for publication. My expectation is that, from the project I have developed, I will be able to publish at least two first-author peer-reviewed papers in leading journals in my new field.

My long-term career goal is to become an independent researcher using biochemical and virological approaches to study mechanisms underpinning cellular function and disease related to infection and immunity. Given my early stage in graduate school, I currently aim to keep an open mind about the ultimate setting in which I will build this career (e.g. academia, government lab, or industry). I plan to take advantage of opportunities to learn about all career options, including a career seminar series, “Pathways Beyond the Professoriate,” offered by the Laney Graduate School (LGS), and professionalization workshops offered by my program. One important strength of the BCDB Program is its requirement that students produce an individual development plan (IDP) in their first year and then update this plan on an annual basis, including short/ long term research goals and career plans. This process will help Dr. Conn and I ensure that my technical and other professional training continues to be well suited to my future career. Dr. Conn has been exceptionally supportive of my career goals to date, and I am optimistic that the training and mentorship I will receive from him and Dr. Lowen will allow me to reach my goal. My initial target following successful graduate work will be to secure a post-doctoral training position at a top research university in a lab working in the areas of innate immunity field but using approaches tangential from my current studies. Long term, I want to continue studying innate immunity with the goal that my research will lend insight into both basic cellular mechanisms and disease.

C. Activities Planned Under this Award

I am currently a second year in the BCDB graduate program at Emory University. As such, I have completed the majority of the required BCDB coursework, including the intensive, literature- and discussion-based course Foundations in BCDB I and II, Beginning Seminar, and research rotations in Year 1. I have also completed the Year 2 course “Hypothesis Design and Scientific Writing” in which I developed this proposal (see *Respective Contributions* for details). I also passed my written qualifying exam in April 2016. My remaining coursework and program-related activities include a Year 2 spring course on statistics for biology research and a semester-long teaching assistantship (as part of the formal LGS TATTO program). I am therefore currently focusing the majority of my time between research and coursework. I spent the summer prior to the fall semester of my second year writing up and publishing my methods paper with Dr. Lowen, and I have spent the fall semester obtaining preliminary data presented here and writing this proposal. I have also submitted an abstract for a poster presentation at the 14th Annual DSAC Student Research Symposium at Emory in January 2017. Importantly, both of my remaining Program obligations (biostatistics course and teaching assistantship) will be completed in the Spring 2017 semester before this NRSA F31 fellowship would be awarded. This will allow me to devote my time primarily to research and other related activities, including mentoring undergraduates or other junior lab members, preparing manuscripts, and attending local, national, or international conferences to present my work, as outlined in the Table below.

Grad Year	F31 Year	Research*	Data collection/ analysis	Mentoring	Manuscript/ thesis prep	BCDB Program Activities	Seminars, Conferences, & Symposia
3	1	90	75	5	10	3	7
4	2	90	65	15	10	3	7
5	3	90	55	5	30	3	7

*Research time includes three specific activities: Data collection/ analysis, Mentoring, and Manuscript/ thesis prep. Therefore, the sum of these three categories equal the total percentage indicated for Research.

At Emory, I have the opportunity to attend and participate in many excellent seminars. I attend a weekly Advanced Seminar through the BCDB graduate program. Here, two students give half hour presentations on their research, and I am required to present once a year. I also participate in two monthly special interest seminars. The first is a Joint Structural Biology Groups meeting where one or more lab members from one of the Emory structural biology research groups gives a one-hour presentation on their project(s) and any problems or difficulties encountered using biophysical methods, including X-ray crystallography. This meeting involves four groups from the Department of Biochemistry (Drs. Conn, Dunham, Bo Liang, and Eric Ortlund) and others from across campus (e.g. Drs. Renhao Li and Elizabeth Wright from the Department of Pediatrics). The other special interest seminar series is the 'Emory RNA club,' which brings together labs with varied interests in RNA biology from both the School of Medicine (e.g. Drs. Conn, Dunham, and Daniel Reines from Biochemistry, and Gary Bassell from Cell Biology) and Emory College (e.g. Drs. Khalid Salaita and Anita Corbett from Chemistry and Biology, respectively). At these meetings, two students or postdocs each give a 30 minute presentation on their research, with an emphasis on diverse methods used related to RNA biology (e.g. structural biology, cell biology, fluorescence microscopy, and RNA-seq/ bioinformatic methods). Here, I am exposed to the many techniques used to study RNA outside the mostly *in vitro* structure-function assays I perform. I will continue to participate in local scientific seminar series and plan to attend both national and international conferences. Future conferences I would like to attend include: RNA Society, American Society for Biochemistry and Molecular Biology, Cold Spring Harbor, Keystone, and a Gordon Conference.

I also enjoy volunteering and contributing to the BCDB program and plan to maintain this level of involvement throughout my time at Emory. Currently, I am a "Student Director" for the first year Foundations course, an instructor for the Methods workshop, an editor for The Leading Edge BCDB Newsletter, and an organizer for the program's Professionalization Workshops. I also helped to lead the organization of this year's BCDB retreat (August 2016), and was asked to help organize recruitment for the 2017 admissions cycle. Being a part of these non-research activities will help me gain important transferable skills, like communication, organization, and collaboration that are essential for my professional development and are not achievable by exclusively working at the bench. It is important to me to give back to the program. I find these experiences incredibly valuable, and I want to contribute directly to future BCDB student's personal and professional development.

SPECIFIC AIMS

The innate immune system must accurately distinguish self from foreign molecules to provide a critical first line of defense against pathogens. Double-stranded RNAs (dsRNAs) are produced during the life cycles of many viruses and their detection by cellular sensors signals that infection has occurred and antiviral defenses should be activated. 2'-5'-oligoadenylate synthetase 1 (OAS1) is an important innate immune sensor of cytosolic dsRNA. OAS1 activates the latent ribonuclease (RNase L) via dsRNA-dependent production of 2'-5'-oligoadenylate second messengers. Activated RNase L then targets viral and cellular RNA for degradation and thereby halts viral replication. Attesting to the importance of the OAS1/RNase L pathway, viruses have evolved mechanisms to evade its effects by directly inhibiting OAS1, sequestering dsRNA produced as a result of viral infection, or producing 2'-5'-oligoadenylate analogs. Determining the molecular mechanism of dsRNA-mediated regulation of OAS1 is vital for understanding how the OAS1/RNase L pathway controls viral infection.

Previous structural studies revealed that dsRNA binding allosterically induces conformational changes in OAS1 to form its active site and thus drive polymerization of ATP into 2'-5'-oligoadenylates. These structures have given us some insight into how OAS1 is activated by dsRNA; however, we still understand very little about how *specific features of the dsRNA* contribute to the level of OAS1 activation. The dsRNA used in these structural studies contained two overlapping and antiparallel copies of a known OAS1 activation consensus sequence, one on each strand of the helix. Yet the dsRNA bound OAS1 in a single unique orientation. Our preliminary data indicate that this selectivity in binding orientation also exists in solution and plays a critical role in determining the potency of OAS1 activation. We designed three RNA hairpin constructs based on the model dsRNA used in the structural studies: the wild-type dsRNA hairpin with the two strands linked by a short stable loop, and two variants where a critical residue of one OAS1 consensus sequence was mutated. Remarkably, while mutation of one consensus sequence resulted in *complete loss* of OAS1 ("non-activating"), [redacted] *increased* OAS1 ("hyper-activating"). Despite these differences in ability to activate OAS1, preliminary data from our lab suggested that both variants bind OAS1 with similar affinity.

These findings suggest that the wild-type dsRNA hairpin contains competing OAS1 binding sites with remarkably different capacities to activate the protein. However, the molecular signatures defining these sites as activating and non-activating are unknown. The dsRNA hairpins we have generated are ideal tools for deciphering the structural requirements and molecular mechanisms of OAS1 activation. Thus, in the following two Specific Aims, I will use complementary assays of OAS1 activity *in vitro* and in human cells as well as biochemical, biophysical, and structural approaches to define the differences in protein-RNA interaction that lead to differential activation of OAS1 by these three dsRNA hairpin sequences.

SPECIFIC AIM 1: Define dsRNA features and their context that lead to potent activation of OAS1. *Hypothesis: Extent of OAS1 activation is determined by the context of potentially overlapping RNA features with the ability to activate or inhibit OAS1.* I will examine the role of specific RNA features *in vitro* by using an established assay to measure OAS1 enzyme kinetics in the presence of the wild-type and each variant dsRNA hairpin. I will correlate the differences in ability of each RNA to activate OAS1 *in vitro* with their impact on the OAS1/RNase L pathway by transfecting these dsRNAs into A549 cells and measuring cellular messenger RNA transcript level changes of known RNase L targets and cleavage of ribosomal RNA by RNase L. These experiments will provide important new insight into currently unknown features of dsRNA that result in potent OAS1 activation and the potential for competition between OAS1 binding sites.

SPECIFIC AIM 2: Determine the molecular mechanism(s) by which the "non-activating" and "hyper-activating" dsRNAs differ in their effects upon OAS1 activity. *Hypothesis: "Non-activating" and "hyper-activating" RNAs exert their effects through different impacts on binding kinetics and/or OAS1 structural changes despite both having similar binding affinity.* Biotinylated OAS1 will be immobilized on a streptavidin biosensor and dsRNA binding affinity and kinetics (on/off rates) measured using bio-layer interferometry. Next, I will use hydrogen-deuterium exchange coupled with mass spectrometry to determine the impact of each dsRNA hairpin on OAS1 conformational dynamics, including critical allosteric changes in the enzyme active site. Lastly, I will use x-ray crystallography to solve structures of dsRNA hairpins bound to OAS1. Collectively, these experiments will reveal the molecular mechanism(s) by which specific dsRNA signatures drive different levels of OAS1 activation.

Summary: The goal of this project is to determine how specific features in dsRNA, as well as their context, control regulation of OAS1. Such studies are critical to gain a broader understanding of host-pathogen interactions and the fundamental mechanisms of cellular translational control. For example, this work will give insight into how viruses could mask otherwise activating motifs in order to evade detection by the innate immune system and may provide a foundation for the development of novel antiviral therapeutics.

RESEARCH STRATEGY

A. SIGNIFICANCE

2'-5'-oligoadenylate synthetase 1 (OAS1) is an important component of the innate immune system that provides a critical first line of defense against viral infection. OAS1 is a sensor of cytosolic double-stranded RNA (dsRNA) (1-3), a potent pathogen-associated molecular pattern (PAMP), present in some viral genomes or produced as a consequence of viral gene expression or replication (4). Viral dsRNA-promoted OAS1 activation leads to subsequent activation of the latent endoribonuclease L (RNase L) via dsRNA-dependent production of 2'-5'-oligoadenylate secondary messengers. RNase L activation effectively halts viral replication through the degradation of target viral and cellular RNAs (**Fig. 1A**) (5-8). The goal of this project is to reveal novel insight into the importance of RNA sequence, structure, and context to dsRNA regulation of OAS1 activity. These details will aid in our understanding of host-pathogen interactions and, for example, how viruses might circumvent the OAS1/RNase L pathway.

Viruses have evolved ways of circumventing the innate immune system. As obligate parasites, viruses depend on the host cell to complete their life cycle. In particular, all viruses require the cellular translation machinery for expression of their proteins. Viruses must also avoid detection by innate antiviral factors that can potently restrict viral replication, often by interfering with cellular translation. Attesting to the specific importance of the OAS1/RNase L pathway in this process, many viruses have developed ways to evade detection by directly inhibiting OAS1, sequestering dsRNA produced as a result of viral infection, or producing 2'-5'-analogs (1,9-17). Therefore, *determining the molecular mechanisms of dsRNA-mediated OAS1 regulation is a vital component of understanding how the OAS1/RNase L pathway contributes to the control of viral infection.*

OAS1 shuts down host translation through the OAS/RNase L pathway. OAS1 is an important innate immune sensor that detects cytosolic dsRNA produced as a consequence of viral replication (1-3). Previous structural studies revealed that dsRNA binding allosterically induces the conformational changes necessary in OAS1 to form its active site and thus drive polymerization of ATP into 2'-5'-linked oligoadenylates (18). These 2'-5'-oligoadenylate secondary messengers promote the dimerization and subsequent activation of RNase L (**Fig. 1A**) (7,8). Activated RNase L degrades both viral and cellular RNA to halt viral replication and limit the spread of infection (19-22). RNase L targets specific messenger (m)RNA transcripts encoding proteins known to regulate cell adhesion and proliferation (23) as well as ribosomal (r)RNA to prevent translation and viral spread to neighboring cells (24).

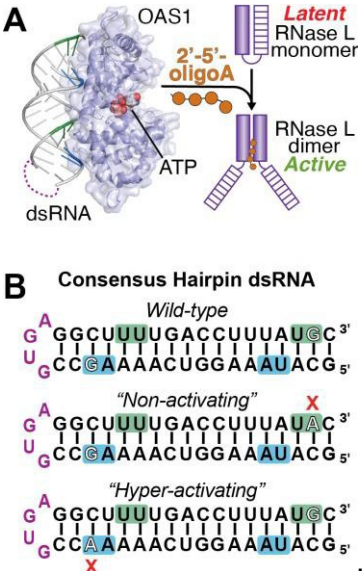

Fig. 1. OAS1/RNase L pathway and dsRNA hairpin construct design. ***A,*** Upon binding dsRNA, OAS1 forms 2'-5'-oligoadenylates, which activate RNase L. The location of the stable RNA tetraloop sequence added to my dsRNA constructs is shown by the *dashed purple line* on the OAS1-dsRNA crystal structure (PDB 4IG8). ***B,*** The wild-type dsRNA hairpin (*top*) with two overlapping and anti-parallel copies of a known OAS1 activation consensus sequence (shaded *blue* and *green*). The tetra-loop added to connect the strands is in *purple*. Single G to A mutations that result in dramatic differences in OAS1 activation are indicated for the "Non-activating" and "Hyper-activating" dsRNA hairpin variants.

OAS1 is activated upon binding viral dsRNA. OAS1 lacks an RNA-binding motif and instead interacts with dsRNA through patches of positive residues on the protein's surface (18). RNA binding to OAS1 requires double stranded regions with a minimum length of 18 base pairs (bp) (25). However, while OAS1 can be activated by dsRNA that meets this 18 bp requirement, activation is strongly potentiated by activating consensus sequences and the 3'-end single-stranded pyrimidine (3'-ssPy) motif that our lab recently identified (26-28). *However, how these molecular signatures in dsRNA affect the level of OAS1 activity, and thus the potency of its antiviral effect, is not well understood.*

The dsRNA used in previous structural studies (18) contained two overlapping and antiparallel copies of a known OAS1 activation consensus sequence WWN_9WG, where W is A or U, and N is any nucleotide (26). Although one consensus sequence was present on each strand of the helix, the dsRNA bound OAS1 in a single, unique orientation (18). Our preliminary data indicate that selectivity in binding orientation also exists in solution and plays a critical role in determining the potency of OAS1 activation. We designed a model dsRNA hairpin construct based on the dsRNA used in these structural studies where the "wild-type" dsRNA strands are linked by a short stable loop (**Fig. 1B**). We chose to use short dsRNA hairpins for the studies proposed here to simplify preparation (by *in vitro* transcription), for ease of transfection, and because due to their small size they should not activate other dsRNA sensors in cells. Two additional hairpin variants were also created where the G residue of one OAS1 consensus sequence was mutated. This G residue (in the top strand, green shaded consensus; **Fig 1B**) was the only base-specific contact between OAS1 and the RNA in the crystal structure (18). Remarkably, our preliminary data showed that

while the mutation in the top strand consensus resulted in a complete loss of OAS1 activity ("Non-activating" variant), the equivalent change on the other stand *increased* OAS1 activity ("Hyper-activating" variant). Despite displaying stark differences in their ability to activate OAS1, both dsRNA hairpin variants retain the ability to bind OAS1 (see *Approach* section for details).

Scientific Premise: Although crystal structures have been solved of OAS1 in complex with dsRNA (18,29), we still do not fully understand the mechanisms OAS1 employs to discriminate between viral and cellular RNAs. Some previous work has been done to tease out the molecular requirements for OAS1 activation (18,25-27), but our lab's recent discovery of the novel [redacted] motif suggests our understanding of dsRNA-mediated OAS1 regulation is still incomplete (28). Based on the preliminary findings presented here, **I hypothesize that the dsRNA hairpin contains competing OAS1 binding sites with remarkably different capacities to activate the protein**. However, the molecular signatures defining these sites as activating and non-activating are not known. I will test my hypothesis through the following aims (summarized in **Fig. 2**). In **Aim 1**, I will use OAS1 kinetics assays and RNase L activation in cells to define the features of dsRNA that lead to potent OAS1 activation using the model dsRNA hairpins. In **Aim 2**, I will determine the molecular mechanism(s) by which the "non-activating" and "hyper-activating" dsRNAs differ in their interaction with OAS1 and effects on OAS1 structural dynamics. Through these complementary specific aims, I will determine how specific features in dsRNA and their context contribute to regulation of OAS1.

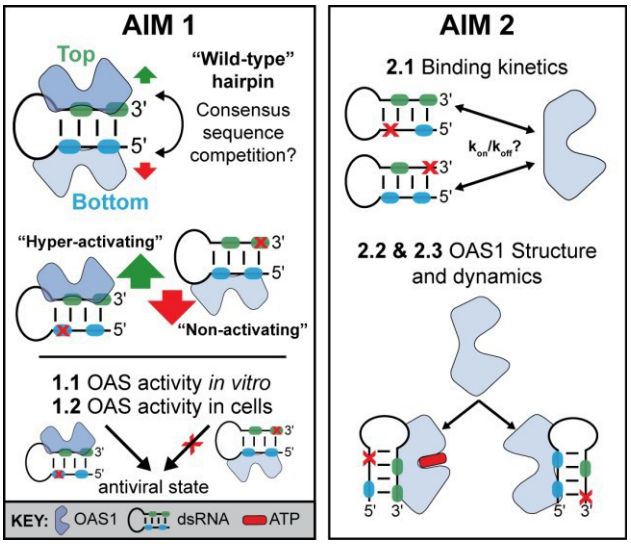

Fig. 2. Summary of Aims. In ***Aim 1***, I will test my model for competition between OAS1 binding sites (*top*) using complementary assays of OAS1 activation *in vitro* and in A549 cells using the "hyper-activating" (*green arrow*) or "non-activating" (*red arrow*) dsRNA variants. In ***Aim 2***, I will examine RNA binding affinity and kinetics (*top*) and structural changes (*bottom*) to define the mechanistic basis to "hyper-activating" or "non-activating" OAS1 binding sites in dsRNA.

B. APPROACH

SPECIFIC AIM 1: Define dsRNA features and their context that lead to potent activation of OAS1.

Rationale: dsRNA binding to OAS1 requires a minimum of 18 bp and activation is potentiated by the presence of recently identified molecular signatures, including [redacted] However, how these molecular signatures act in concert or competition to affect the level of OAS1 activity and thus an innate antiviral response is unknown. Our preliminary data (**Fig. 3**) illustrate that [redacted] in each of the two consensus sequences leads to opposing affects: one no longer activates OAS1 ("non-activating") while the other dramatically increases OAS1 activation ("hyper-activating"). Determining which RNA features create a preferred OAS1 binding site activating or non-activating will give critical new insight into how viruses could mask otherwise activating motifs to evade detection by the innate immune system. *Hypothesis: Extent of OAS1 activation is determined by the context of potentially overlapping RNA features with the ability to activate or inhibit OAS1.*

Overview of Experimental Design: I will examine how specific dsRNA features control the extent of OAS1 activity *in vitro* by using our established chromogenic assay (28) to measure OAS1 enzyme kinetics for the wild-type and each variant dsRNA hairpin (see **Fig. 1B**). I will next compare the differences in the ability of each dsRNA to activate OAS1 *in vitro* with their impact on the OAS1/RNase L pathway in living cells. These experiments will provide important new insight into currently unknown features of dsRNA that result in potent OAS1 activation *in vitro* and in human cells.

Aim 1.1: Define the features of dsRNA that lead to potent activation of OAS1 *in vitro*.

Experimental Approach: I will *in vitro* transcribe the dsRNA hairpins from linearized plasmid DNA templates using T7 RNA polymerase and purify the RNAs by denaturing polyacrylamide gel electrophoresis using established protocols (30-33). Two "scrambled" versions of the dsRNA hairpin will also be transcribed as controls: Scramble 1 will maintain the wild-type activation consensus sequences, but will randomize all other nucleotides and in Scramble 2 all nucleotides will be randomized (while maintaining an 18 bp duplex in both RNAs). Human OAS1 will be expressed in *E. coli* BL21(DE3) and purified using sequential Ni^{2+}-affinity, heparin-affinity, and gel filtration chromatography (28). I will use our lab's established 96-well plate format *in vitro* chromogenic assay (28) to test the effects of mutations in defined RNA signatures in the dsRNA model

hairpin on OAS1 enzyme kinetics (**Fig. 3A**). This assay measures the amount of inorganic pyrophosphate (PP_i) produced as a consequence of 2'-5'-oligoadenylate synthesis as a readout of OAS1 activity. The PP_i is detected by quantifying the change in color (Abs at 580 nm) upon adding an ammonium molybdate reagent, as shown for the initial enzyme progress curves at a single concentration of the wild-type (*purple*), "non-activating" (*blue*), and "hyper-activating" (*green*) dsRNA hairpins (**Fig. 3B**). In this subaim, I will perform assays at a range of RNA concentrations to determine enzyme kinetic parameters (V_{max} and K_{app}) (28) to fully define the capacity of each RNA to activate OAS1 *in vitro*.

I will also perform competition assays with a fixed amount of the "hyper-activating" dsRNA and titrating increasing amounts of the "non-activating" dsRNA. This experiment will directly test the consensus competition model and show that the "non-activating" RNA interacts with OAS1 (as suggested by preliminary binding data, see **Aim 2**). The following controls will be used in all experiments in this subaim: ATP degradation (no OAS1), no RNA (negative), poly(rI:rC) dsRNA (positive), wild-type and both scramble dsRNA hairpins.

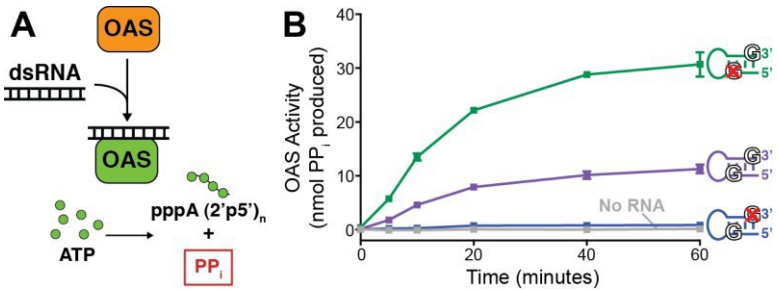

Fig. 3. *In vitro* OAS1 activation assay reveals stark differences between the two consensus sequence variants. *A,* OAS1 activity is measured via PP production during 2'-5'-oligoadenylate synthesis. A color product is produced upon the addition of ammonium molybdate and absorbance is measured at 580 nm using a BioTek Synergy 4 plate reader. ***B,*** OAS1 enzyme progress curves are shown for a single concentration of wild-type (*purple*), "hyper-activating" (*green*), and "non-activating" (*blue*) dsRNAs; a no RNA control (*grey*).

Data Analysis/Rigor: To ensure rigorous and reproducible data are obtained, each experiment will be performed in three technical replicates and use at least two different preps of OAS1. Prep-to-prep variation in OAS activity will be assessed and controlled for by including poly(rI:rC) dsRNA and wild-type dsRNA samples on each plate. After background correction, absorbance measurements are converted to nmol PP_i produced using a standard curve of known PP_i concentrations. Data will be averaged and plotted using standard error of the mean on GraphPad Prism software and curves will be fit using non-linear regression to obtain V_{max} and K_{app} values using the Michaelis-Menten model equation, $Y=(V_{max}X)/(K_{app}+X)$.

Outcomes/Interpretations: This subaim will measure *in vitro* OAS1 enzyme activity to discern potential differences in OAS1 kinetic parameters (V_{max} and K_{app}) that lead to the dramatically different extents of OAS1 activation by the dsRNA hairpins suggested by the preliminary studies using single RNA concentrations (**Fig. 3B**). Our lab's previous analysis of the [redacted] (28) suggest that I may observe an increase in maximal enzyme rate (V_{max}) for the "hyper-activating" and a decrease in this parameter for the "non-activating" dsRNA compared to "wild-type." For each dsRNA, I may also observe a change in the apparent RNA affinity (K_{app}). A decrease in apparent affinity from enzyme kinetic measurements for the "hyper-activating" dsRNA hairpin could, for example, reflect a mechanistic requirement for OAS1 to bind and release the dsRNA between rounds of catalysis. Competition experiments will explore a model that the "wild-type" dsRNA contains competing sites (productive vs non-productive); if the "non-activating" dsRNA can still bind OAS1 then it should be able to compete for binding (and thus regulation of OAS1) in *trans*. The mechanistic basis for the differences in OAS1 activation by different dsRNAs and the potential for competition between activating and inhibitory sequences with be fully explored in **Aim 2**. As these experiments progress, new dsRNA variants could be designed to answer additional questions such as whether consensus sequence position within the duplex influences activation by inserting/ deleting base pairs between the consensus G and the RNA terminus.

Aim 1.2: Determine the ability of dsRNA hairpins to activate the OAS/RNase L pathway in living cells.
Experimental Approach: I will test the effects of the three dsRNA model hairpin variants (**Fig. 1B**) on OAS1 activation by transfecting each dsRNA into human lung carcinoma (A549) cells and measuring OAS/RNase L activity. A549 cells are optimal for these experiments as they express OAS1 without interferon stimulation, thus reducing the complexity of experimental design. I will transfect cells with my three dsRNA hairpins using lipofectamine 2000 (ThermoFisher) accompanied by the following controls: mock (no RNA), poly(rI:rC) dsRNA (positive control), and the scramble dsRNA hairpins (see *Aim 1.1*). Mock transfections will allow me to confirm that the transfection process does not stimulate an innate immune response as well as assess RNA quality during extraction. I will isolate total RNA from A549 cells at specific established time points (e.g. 1 and 3 hours) post-transfection (23,34). RNase L activity will then be determined by: 1) reverse transcription droplet digital PCR (RT ddPCR) with primers for known specific RNase L targets (FAT4, FREM2, PCDHB5, and ZEB1) (23), and 2) assess ribosomal RNA (rRNA) cleavage via agarose gel (23,35-37). Partitioning of cDNAs into droplets

prior to amplification makes ddPCR ideal for detecting small differences between samples or minor species in a population (36). ddPCR also allows for absolute quantification of cDNA copy number without the need for a standard curve. Pre-determining the number of cells in the starting material allows back calculation and precise quantification of the number of mRNA transcripts per cell. rRNA cleavage assays will be used to visualize cleavage patterns characteristic of RNase L activation and is well established in the field (19,20,34,35). These two methods will allow me to measure a gradation of OAS1 activation (e.g. ddPCR is sensitive tool that will allow weak activation to be detected, whereas robust activation can be monitored by rRNAcleavage).

Data Analysis/Rigor: Each experiment will be performed in biological triplicates. Data will be plotted using GraphPad Prism software, replicates will be analyzed using standard deviation, and statistical significance determined using unpaired Student's t-test ($p<0.05$).

Outcomes/Interpretations: I anticipate that the cell data will recapitulate my *in vitro* results on OAS1 activation, e.g. cellular mRNA and rRNA degradation following transfection with the "hyper-activating" and "non-activating" dsRNA hairpins will be significantly increased and decreased, respectively, compared to the "wild-type" RNA. These experiments will reveal the importance of the identified RNA signatures and their context on OAS1 activation in cells and will thus serve as an important validation of the *in vitro* enzymatic studies of this aim and the mechanistic studies in **Aim 2**. It is possible that differences in OAS1 (and thus RNase L) activation in cells might not be as dramatic as shown by the *in vitro* data. However, by testing RNAs with a range of potential capacities to activate OAS1, my experiments may reveal in more detail the nature of the cellular response to low extents of OAS1 activation. For example, does low level activation (which might reflect the early stages of infection) lead to only mRNA degradation, while extended time of high OAS1 activation subsequently results in additional degradation of the cellular translational machinery?

Potential Pitfalls/Alternative Approaches: The *in vitro* chromogenic assay outlined in *Aim 1.1* is for OAS1 enzyme kinetics assays established in our lab (28), and therefore I do not expect technical issues with these experiments. However, an alternative approach also used in our lab would be to analyze 2'-5'-oligoadenylate synthesis by [α^{32}P]-ATP incorporation via denaturing sequencing gels (28). This method, unlike the chromogenic assay, is not high throughput but is useful to directly visualize 2'-5'-oligoadenylate products and measure very weak activation (18,25). In *Aim 1.2*, RT ddPCR is an established method that I have used in the Lowen lab previously (36) therefore I do not expect to run into technical issues adapting the method for the proposed analyses. However, qRT-PCR is an alternative method (23) that can be used if needed and is also well established in the Lowen lab. rRNA cleavage can also be determined using a Bioanalyzer (available in the Emory Integrated Genomics Core) if agarose gels prove to be inconclusive. Although my dsRNA constructs are designed to minimize activation of other innate immune sensor proteins in A549 cells such as dsRNA-activated protein kinase (PKR), if such issues are identified I would use knockout cell lines which have been constructed (38) or can be generated in the Lowen lab.

SPECIFIC AIM 2: Determine the molecular mechanism(s) by which the "non-activating" and "hyper-activating" dsRNAs differ in their effects upon OAS1 activity.

Rationale: Our preliminary data suggest that dsRNAs may contain potentially competing OAS1 binding sites with remarkably different capacities to activate the protein (see **Aim 1** and **Fig. 3**). However, the molecular mechanisms defining these sites as activating and non-activating are unknown. Defining such differences will provide novel insight into the regulation of OAS1 and could reveal both potential new strategies to manipulate OAS1 for the design of effective treatments for viral infection and mechanisms by which viruses could potentially mask otherwise activating dsRNA motifs. *Hypothesis: "Non-activating" and "hyper-activating" RNAs exert their effects through different impacts on binding kinetics and/or OAS1 structural changes despite having similar binding affinity.*

Overview of Experimental Design: I will elucidate the molecular mechanism(s) responsible for the dramatic differences in OAS1 activation by the dsRNA hairpins by measuring binding affinity and kinetics (on/off rates) using bio-layer interferometry (BLI), comparing dsRNA-induced changes in OAS1 structural dynamics using hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS), and determining x-ray crystal structures of the "non-activating" and "hyper-activating" dsRNAs bound to OAS1. These experiments will reveal important new insight into the molecular mechanisms that modulate OAS1 activation.

Aim 2.1: Determine the impact of dsRNA binding affinity and/or kinetics on OAS1 activation.

Experimental Approach: To immobilize OAS1 on a streptavidin biosensor for BLI OAS1-dsRNA interaction studies, I used inverse PCR to add an AviTag sequence to the C-terminus of OAS1 to create an OAS1-AviTag

construct (39,40). AviTag is a 15 amino acid tag (GSGLNDIFEAQKIEWHE) containing a specific recognition sequence for biotinylation by BirA biotin ligase (39). I purified biotinylated recombinant OAS1-AviTag from *E. coli* and showed, using the OAS1 activation assay (see *Aim 1.1*), that the C-terminal AviTag does not alter OAS1 activity (**Fig. 4A,B**). I have also shown that OAS1-AviTag can be successfully immobilized on streptavidin biosensors for RNA binding affinity (K_d) and kinetics (k_{on} and k_{off}) experiments on a state-of-art FortéBio OctetRED384 instrument (**Fig. 4C**) available to us through Emory's Chemical Biology Discovery Center (*see letter from Dr. Du*). Preliminary BLI data shown here (**Fig. 4C**) not only demonstrates my ability to collect data using this method, but also the important finding that the "non-activating" dsRNA hairpin can still bind OAS1. Therefore, the observed non-activation by this hairpin in *Aim 1.1* (see **Fig. 3B**) is not due to lack of OAS1 binding. Experiments will be performed at a fixed density of OAS1-AviTag (e.g. 180s loading, 20 µg/ml) with increasing amounts of dsRNA (e.g. 0.1-100 µM). Each sensor will use a different RNA concentration and on/off rates will be measured by dipping sensors into RNA (*association*) and then into buffer (*dissociation*). Controls will include: OAS1 in buffer only (background) and biocytin (a biotin analog) with and without RNA.

Parameters that may require optimization include buffer conditions, OAS1 and dsRNA concentrations, molecule orientation, and reference sensors. I will test different assay buffers using the OAS1 activation assay (see *Aim 1.1*) buffer as a starting point. Loading density of biotinylated OAS1 and analyte concentration will be optimized to ensure the appropriate signal intensity is achieved. I will also verify that there is no preferred molecule orientation by performing experiments using immobilized biotinylated dsRNA. Although an important control, this set up is not ideal, as every dsRNA construct will need to be synthesized with a biotin modification.

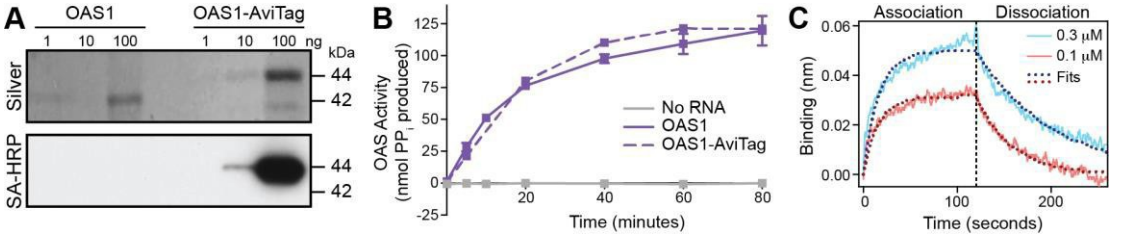

Fig. 4. OAS1-AviTag is active and can be biotinylated for BLI assays. *A,* Serially diluted wild-type OAS1 and OAS1-AviTag were analyzed by SDS-PAGE and visualized using silver stain (*top*) or blotted with streptavidin-horseradish peroxidase (*bottom*). ***B,*** OAS1 activation by the "wild-type" RNA hairpin is shown for wild-type OAS1 (*solid purple*) and OAS1-AviTag (*dashed purple*). OAS1 activity was also measured using a no RNA control (*grey*). **C,** OAS1-AviTag was immobilized on a streptavidin biosensor and the "non-activating" RNA hairpin was added at 0.3 mM (*blue*) and 0.1 mM (*red*). Dotted lines show the curve fits for each concentration. These data show that OAS1-AviTag is specifically biotinylated, retains enzyme activity, and preliminary BLI data demonstrate our ability to measure protein-RNA binding affinity andkinetics.

Data Analysis/Rigor: Data will be subtracted from reference streptavidin sensors coated with biocytin. After background correction, the interference shift (in nanometers) is plotted, fit, and analyzed using the Octet Data Analysis Software. To ensure rigorous and reproducible data, each experiment will be performed in three technical replicates using at least two different preps of OAS1 to address prep-to-prep variability.

Outcomes/Interpretations: If the ability of a dsRNA to activate OAS1 is *dependent* on binding affinity or kinetics I expect to identify significant differences between "non-activating" and "hyper-activating" dsRNAs. For example, I may find that differences in binding affinity whereby higher affinity is correlated with either the more or less activating dsRNA. For the "hyper-activating" dsRNA, higher affinity could result in a great ability to drive OAS1 conformational changes (see *Aim 2.2*), while lower affinity with altered kinetics would be consistent with a mechanistic requirement to bind and release dsRNA between rounds of catalysis. It is thus also possible that the functionally critical difference could be based in alteration of a specific kinetic parameter (k_{on} and/ or k_{off}); a kinetic basis for regulation would be a novel finding. If both dsRNAs have the same (or similar) affinity and kinetics, this would suggest that binding differences are not the basis for their distinct capacities to activate OAS1. In this case, I will focus on the protein dynamics and structural approaches in *Aims 2.2 and2.3*.

Aim 2.2: Define the role of dsRNA-driven structural changes in OAS1 enzyme activation.

Experimental Approach: I performed an OAS1 peptide mapping experiment by in-line pepsin digest and MS on our HDX-MS system (*see letter from Dr. Li*) revealing my ability to achieve 100% OAS1 sequence coverage and high (33-fold) redundancy (**Fig. 5A**). An earlier comparative deuterium exchange experiment showed that OAS1 residues [redacted] dsRNA-induced conformational changes (18), exhibited high deuterium uptake in *apo* OAS1, indicating the dynamic nature of these residues in the absence of RNA (**Fig. 5B**). These data show that HDX/MS will be an ideal tool to monitor differences in the capacity of my dsRNAs to functionally alter the OAS1 structure. I will therefore use HDX-MS to examine differences in OAS1 protein conformational dynamics in the absence or presence of the "wild-type," "non-activating," and "hyper-activating" dsRNA hairpins (see **Fig. 1B**). The automated system adds deuterium-containing solution to the sample (OAS1 alone

or OAS1-dsRNA complex), quenches the reaction at 4-5 individual time points (e.g. 0, 15, 60, 180, 600 seconds), and injects the samples individually onto the in-line pepsin column prior to MS analysis. Differential deuterium uptake in each measured OAS1 peptide (*apo* vs each dsRNA, "hyper-activating" vs "non-activating") will be calculated and mapped on to the OAS1 structure.

Data Analysis/Rigor: Duplicate experiments will be conducted using protein from independent preps, with six separate injections of each sample into the MS to ensure high redundancy. I will analyze the data in the HDX core with Masslynx 4.1, PLGS 3.0, and Dynamix 3.2 software.

Outcomes/Interpretations: If an ability to drive changes in OAS1 conformational dynamics contributes to differences observed in OAS1 activation, I expect that the "hyper-activating" dsRNA will cause changes in [redacted] and potentially other ***activating*** changes elsewhere in OAS1. In contrast, the "non-activating" dsRNA may fail to induce the functionally critical changes in [redacted] or may cause additional ***inhibitory*** changes that contribute to loss of OAS1 activation. The role of newly identified regions in OAS1 that may be allosterically regulated by dsRNA could next be tested by mutagenesis and functional analyses like those of **Aim 1**.

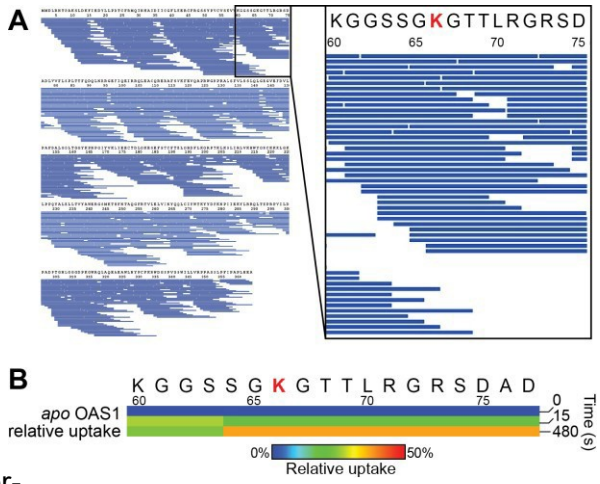

Fig. 5. Preliminary HDX-MS data. ***A,*** OAS1 mapping experiment demonstrating the potential for acquiring high quality HDX-MS data (100% coverage, 33-fold redundancy). Each blue bar represents a unique peptide and the zoomed in region shows the loop [redacted] which undergoes conformational change upon dsRNA binding. ***B,*** Deuterium uptake from a pilot exchange experiment. These data show uptake ***only*** for [redacted] demonstrating that RNA-induced changes can be monitored by this method. [Data in *panel B* collected by G. Conn].

Aim 2.3: Determine structures of 'non-activating' and 'hyper-activating' dsRNA in complex with OAS1.

Experimental Approach: We have obtained initial crystals from 96-well screens for both the "non-activating" and "hyper-activating" dsRNA complexes with OAS1 that diffract to medium resolution (~7 Å) (**Fig. 6**). I will next optimize these crystals using additional screens around the initial conditions, adjusting parameters such as the concentrations of OAS1-dsRNA complex, salt, or precipitant, pH, and temperature. If needed, I will also test detergents and other additives to improve crystal formation. Diffraction data sets will be collected at the SER-CAT beamline at the APS. Once suitable quality data are obtained, I will determine the structure by molecular replacement using the structure of RNA-bound OAS1 (PDB 4IG8) as a starting model. Our lab has all the software needed for structure determination, including *HKL2000* for X-ray data processing (41); *Phenix* for molecular replacement, refinement, and validation (42); and *Coot* for manual model building (43).

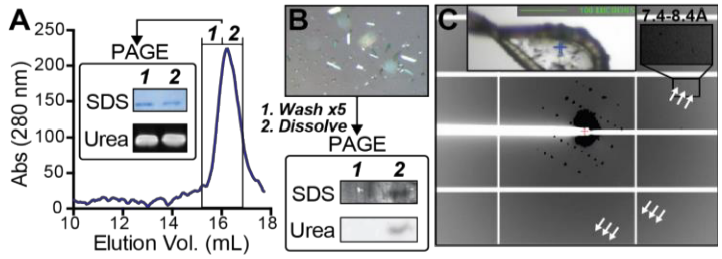

Fig. 6. Crystallization of OAS1:dsRNA complexes. ***A,*** OAS1 and dsRNA elute from a Superdex S200 gel filtration column. ***B,*** Demonstration that crystals contain both RNA and protein. Crystals were (*1*) washed five times with crystallization solution then (*2*) dissolved and analyzed by PAGE. ***C,*** Diffraction from a crystal taken from the drop shown in *panel B* extends to at least 7Å (*white arrows*). These data demonstrate our ability to crystallize OAS1 with a dsRNA hairpin from our library. [Data collected by B. Calderon and G. Conn].

Data Analysis/Rigor: Structures will be validated during refinement with Phenix (42), and I will use the co-Protein Data Bank (PDB) server to ensure our data and final structures are of optimal quality prior to deposition (44).

Outcomes/Interpretations: Structures of the OAS1-dsRNA complexes will reveal the molecular details underpinning the differing ability of each dsRNA to activate OAS1. These structures will provide a detailed platform for interpretation of my findings in other subaims as well as allowing me to develop and test (by mutagenesis and functional assays) new hypotheses about critical OAS1-dsRNA interactions for potent activation or for dsRNA binding in the absence of OAS1activation.

Potential Pitfalls/Alternative Approach: My preliminary data indicate BLI will be applicable to OAS-dsRNA binding affinity/ kinetics but, if needed other approaches are well established in our lab (45-47) and several options are available (e.g surface plasmon resonance or isothermal titration calorimetry in the Emory Comprehensive Glycomics core). I also do not anticipate technical issues with HDX-MS as our preliminary data (**Fig. 5**) clearly support the feasibility of this approach. Finally, X-ray crystallography is inherently full of potential pitfalls but progress in obtaining diffracting crystals of both complexes and our lab's track record in structure determination (48-54) give strong cause for optimism that I will obtain high-resolution structures.

RESPECTIVE CONTRIBUTIONS

The project described in this proposal is founded on an initial observation made by a previous Conn Lab graduate student, Dr. Virginia Vachon. Specifically, she found that single point mutations within each OAS1 activation consensus sequence in the double-stranded RNA duplex used in previous structural studies had starkly different impacts on the ability of the RNA to activate OAS1. After I joined Dr. Conn's lab for my thesis work, we discussed this observation and together outlined a set of experimental approaches that could tease out the molecular basis for how RNAs with similar affinity could have such different capacities to activate OAS1.

Following these discussions in early summer 2016, I began developing a form of this proposal in the IBS 522r Hypothesis Design and Scientific Writing course taken as part of the Biochemistry, Cell, and Developmental Biology (BCDB) Program core curriculum. This course is co-directed by Drs. Conn, Anita Corbett, and Joanna Goldberg. The course includes all second year BCDB and Microbiology and Molecular Genetics (MMG) Program students, as well as other MD/PhD students enrolled in various graduate programs at Emory. This course taught us how to think critically and to communicate our science. As part of the course, I prepared an NRSA-format research proposal in a step-wise manner with weekly assignments to outline, write, and re-write each section of the proposal. As a result, I received feedback on my developing proposal from a large number of individuals. For each week's assignment, Dr. Conn worked closely with me, typically through an initial discussion of ideas and then written comments on a draft version of the section to be submitted. During the entire process, Dr. Conn assisted by critically evaluating my writing and ideas and always followed up with helpful advice and suggestions. I worked very closely with my "class mentor," Dr. Corbett, who would provide written comments on each week's assignment to offer an additional perspective to help guide me as I developed, wrote, and re-wrote the various sections of my fellowship. In several of the classes, I also received feedback from classmates through a presentation of aims and a model figure and other in-class exercises. Once the complete proposal was compiled, I received additional comments from a senior BCDB student (Mr. Ed Quach) before finally "submitting" my proposal for review by two faculty members (all mentors of students in the class are required to review two "grants" of other students in the class). Comments from these anonymous reviews were also provided to me before I prepared the current proposal. Finally, I also received comments from Dr. Conn and my Co-sponsor, Dr. Lowen, on the version of my proposal that I ultimately submitted.

The preliminary data presented in this proposal was collected and analyzed by me unless otherwise specifically noted in the corresponding figure legend.

Moving forward, for the experiments described in this proposal, I will design, conduct, analyze, and interpret the results with assistance from Drs. Conn and Lowen. My sponsors will also assist me with correctly interpreting and presenting the results of my experiments, as well as advising me on experimental design for the next steps through both our regular scheduled meetings and informal discussions. Dr. Conn's office is adjacent to the lab, and he is always available and eager to talk about experiments or data. Though I strive for independence in the execution of my experiments, his expertise and availability has been a tremendous asset to my training thus far. For preparation of manuscripts, I will construct a thorough outline prior to seeking assistance from Drs. Conn and Lowen so that we can further organize my thoughts and findings into a cohesive story. I will then write initial drafts with my sponsors' guidance until I can piece together a well-written first draft independently. I will also seek feedback on my results and manuscripts from my thesis committee, other colleagues (e.g. Dr. Christine Dunham and her group, with whom we have joint lab meetings), and current Conn and Lowen lab members.

SELECTION OF SPONSOR AND INSTITUTE

After making my decision to pursue graduate school, I narrowed my difficult decision down to two excellent graduate programs: University of Texas Southwestern Medical Center's Biological Sciences Umbrella Program and Emory University's Biochemistry, Cell, and Developmental Biology (BCDB) Program. BCDB really stood apart from UTSW and the other programs I had considered because of the attractive training opportunities and the warm, close-knit feel the faculty and students share (and which I had had the opportunity to witness in detail through my role as a research specialist in a lab hosting BCDB students). These opportunities include a first year discussion-based course called "Foundations" that allows for extensive time one-on-one with the faculty and my peers, a first year seminar course that prepares students for public speaking and presentations, a second year scientific writing course where students write their own research proposals using an NRSA-style format, yearly student seminars, workshops to explore potential career opportunities, and a guaranteed stipend throughout training. BCDB also offers 1) a program retreat at the start of each academic year that is full of team-building activities, science, and the opportunity to catch up with BCDB alums to discuss their experiences and career paths, 2) a methods workshop (which I currently organize with two senior students in my program), and 3) a professionalization workshop (I currently organize this also!). As a result, BCDB students have many opportunities to hone their oral and written communication skills, as well as to access substantial education about career development. In addition, the pervasively collaborative and supportive environment of the BCDB program convinced me to attend. Professors and students routinely consult each other for advice or to develop new collaborations. In addition to my experience working at Emory, this strong graduate program environment was obvious to me at my interview because members of the program clearly knew each other and each other's research, despite the breadth of research in the program. I see this type of environment being a particular asset to my career because I find it easy to approach a professor at an event or in their office to talk about an experimental method, my data, or my proposal. Although the BCDB program is relatively small (roughly 40 students and 45 faculty), as one of nine programs in the Emory School of Medicine's Graduate Division of Biological and Biomedical Sciences I am part of an exceptionally broad, dynamic, and exciting research and intellectual environment. As a result, on an institutional level, Emory's graduate support structure and core facilities are outstanding (I am even implementing two of them in this proposal!). Collectively, these many strengths from the individual program level and across the whole of Emory assured me that I would have everything I need to perform cutting-edge research and all of the training opportunities to make me exceptionally competitive for my post-doctoral training and career in research beyond.

As a first-generation college student, I was unsure about the next steps and not yet prepared to commit to graduate school following my graduation from Armstrong State University. At the time, I bounced back and forth between pursuing a Master's or a Ph.D. but could not firmly commit to one field of study over another, having had limited research opportunities to that point. I knew, however, that Emory University was one of my top choices for graduate school, so I thought what better way to figure out my path than to work at a university I was interested in attending. This experience would allow me to work alongside the graduate students and principal investigators of programs I would potentially apply to. I worked for three years as a research technician in Dr. Conn's lab. I had no prior experience working in a biochemistry lab, nor had I ever done bench science full-time, but Dr. Conn took a chance on me. I was able to witness first-hand how invested Dr. Conn was to training, mentoring, and challenging his students to improve. He carefully adapts his mentoring style to each of his students based on their individual needs. He will be hands-off when independence is needed or warranted, but always has an open door if you want to discuss a result or troubleshoot a problem. Dr. Conn is also always very involved in the graduate programs he is a member of, including BCDB of which he is currently the Director of Graduate Studies. He is one of the main instructors for the Hypothesis Design and Scientific Writing course and Foundations, and is a regular participant in student-lead seminars and workshops. Dr. Conn and I worked closely throughout my three years in his lab, from troubleshooting assays to writing a manuscript I coauthored with a postdoc in the lab, and this showed me the qualities I valued in a mentor and advisor. He was always very supportive of my decision to apply to graduate school, a career goal he knew of from the day I interviewed for the position. My experience as a technician was pivotal in my career, because without a "good" experience I am convinced I would have changed career paths. The time I spent in the Conn lab solidified for me that I was meant to pursue a career in research. Although I explored other options (at Dr. Conn's insistence!) based on the qualities outlined above, I chose to stay at Emory to pursue my graduate school career. After three very different rotations (see *Research Experience*), I ultimately chose to return to Dr. Conn's lab. Because of his unprecedented commitment to training and mentoring his students in all aspects of graduate student development, I was sold.

RESPONSIBLE CONDUCT OF RESEARCH

All Emory University graduate students are required to take a six-hour ethics seminar course, called Jones Program in Ethics (JPE 600), at the start of their first year. The ethics course covers general topics applicable to all disciplines and is supported by the Laney Graduate School in collaboration with the Emory Center for Ethics. I am also required to attend at least four additional workshops sponsored by the Laney Graduate School or the Emory Center for Ethics. I attended my first workshop this semester, where I met the Editor-in-Chief of the European Journal of Neuroscience, Dr. Paul Bolam. We discussed the ethical conundrums that plague science publishing, such as the "crisis" in the peer review process, conflicts of interest, and plagiarism. He also gave us an inside look at the publishing timeline and the stages manuscripts must go through from submission to acceptance.

As a graduate student in the Biochemistry, Cell, and Developmental Biology (BCDB) Program, I attend monthly, one-hour ethics workshops (eight per school year) sponsored by the program and currently directed by Drs. Guy Benian (Professor, Pathology) and William Dynan (Professor, Biochemistry). For my first two years in the program, I am required to attend all eight of the monthly ethics workshops. As a third year and beyond, I am required to attend at least one ethics workshop per semester. This requirement therefore meets the National Institutes of Health (NIH) requirement for retraining every four years. Students, typically a junior and senior student, are paired with a faculty advisor to put together each interactive, discussion-based workshop, supported by a Powerpoint presentation on one of the following topics outlined in the table below. Many of the faculty advisors attend multiple sessions, and other BCDB faculty, e.g. Drs. Richard Kahn (Biochemistry) and Michael Koval (Medicine), also attend to contribute to the discussions.

Topic	Year	Faculty Advisor (Department)
Conflict of Interest	2015-16* 2016-17	Paul Doetsch (Biochemistry) Kenneth Moberg (Cell Biology)
Animal Experiments and IACUC	2015-16 2016-17*	David Pallas (Biochemistry) David Katz (Cell Biology)
Human Subjects and Tissues	2015-16 2016-17	Judy Fridovich-Keil (Human Genetics)
Mentor-Mentee Responsibilities and Relationships	2015-16 2016-17	G. Benian (Path.), W. Dynan (Biochem.) Keith Wilkinson (Biochemistry)
Collaborative Research	2015-16	Eric Ortlund (Biochemistry)
Peer Review	2015-16 2016-17	Graeme Conn (Biochemistry) Alexa Mattheyses (Cell Biology)
Data Management and Sharing	2015-16 2016-17	Ichiro Matsumura (Biochemistry) Maureen Powers (Cell Biology)
Research Misconduct and Data Manipulation	2015-16 2016-17	Christine Dunham (Biochemistry) David Pallas (Biochemistry)
Responsible Authorship and Publication	2015-16 2016-17	Judy Fridovich-Keil (Human Genetics) William Kelly (Biology)

*Denotes the two ethics presentations I have given to date.

SPONSOR AND CO-SPONSOR STATEMENT

A. RESEARCH SUPPORT AVAILABLE

Source	Identifier	Title	PI	Dates	Direct $/ yr
Current-Conn (Sponsor)					
NIH/ NIAID	R01 AI088025-07	RNA modification and antibiotic resistance	Conn	6/1/10-5/30/20	$304,000
Pending-Conn (Sponsor)					
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Current-Lowen (Co-sponsor)					
NIH/ NIAID	R01 AI099000	Reassortment of influenza viruses in a co-infected host	Lowen	9/1/12-8/31/17	$250,000
NIH/ NIAID	R01 AI125268	Impact of selective genome packaging on influenza A virus reassortment	Lowen	8/4/16-7/31/20	$250,000
NIH/ NIAID	R01 AI127799	Host dependence of influenza A virus reassortment	Lowen	12/5/16-11/30/21	$493,000
NIH	HHSN272201400004C	Host adaptation, reassortment and transmission of influenza viruses at the animal-human interface	Orenstein (Lowen, Project 2 Co-leader)	4/1/14-3/31/21	$259,462
Pending-Lowen (Co-sponsor)					
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

B. SPONSOR'S/ CO-SPONSOR'S PREVIOUS FELLOWS/ TRAINEES

Conn (Sponsor)

- **Eight (8) previous** and **three (3) current** pre-doctoral trainees, including the applicant Ms. Schwartz.
- **Six (6) previous** postdoctoral trainees.

Five representative previous trainees:

Name	Pre-/ Postdoctoral	Current Position
Miloje Savic	Pre (Manchester)/ Post (Emory)	Senior Advisor on Global Pharmaceutical Policy, Norwegian Institute of Public Health, Norway.
Catherine Templeton	Pre (Manchester, PhD)	Senior Scientific Officer, Inst. for Cancer Research, UK
Ahmed Wahid	Pre (Manchester, PhD)	Associate Prof., Biochemistry, Minia University, Egypt
Emily Kuiper	Pre (Emory BCDB program)	Postdoctoral Fellow, Dana Farber Cancer Institute
Pooja M. Desai	Post (Emory)	Research Scientist, Therapure Biopharma Inc., Canada

Lowen (Co-sponsor)

- **Two (2) previous** and **two (2) current** pre-doctoral trainees.
- **One (1) current** postdoctoral trainee.

Previous trainees:

Name	Pre-/ Postdoctoral	Current Position
Jillian Seladi-Schulman	Pre (Emory MMG program)	Associate Lab Project Set-up Manager, Quintiles, Atlanta
Nicolle Marshall-Baird	Pre (Emory MMG program)	Postdoctoral Fellow, CDC Influenza Division

C. TRAINING PLAN, ENVIRONMENT AND RESEARCH FACILITIES

C1. TRAINING PLAN. Samantha (Sam) Schwartz is a student in the Biochemistry, Cell & Developmental Biology (BCDB) Graduate Program at Emory University. Sam will be co-mentored by Dr. Graeme L. Conn (Associate Prof., Department of Biochemistry) and Dr. Anice Lowen (Assistant Prof., Department of Microbiology & Immunology). Dr. Conn has many years of mentoring experience and a research focus on

understanding the structures, interactions and biological functions of RNA molecules and their protein binding partners. Relevant to Sam's proposal, Dr. Conn's group has a well-established record in the area of viral non-coding RNAs and their interactions with innate immune proteins, PKR and OAS1. Dr. Lowen will additionally bring a distinct perspective to Sam's mentoring and career guidance as well as outstanding expertise in molecular virology and the cell culture systems Sam will use in her proposal.

Sponsor Background. I (Dr. Conn) place a great emphasis on student mentorship and development in my lab, and I have been actively engaged in graduate education since arriving at Emory in 2008 (please see my Biosketch Personal Statement for details). I am currently a member of two Emory Graduate programs, BCDB and Microbiology & Molecular Genetics (MMG), as well as the NIAID-funded T32 "*Antimicrobial Resistance and Therapeutic Discovery Training Program (ART-DTP).*" I currently serve on the Executive Committees of each of these programs, as the BCDB Program Director of Graduate Studies (DGS), and as one of four MMG Program student recruiters (along with Sam's Co-sponsor Dr. Lowen). I am also actively involved in directing and teaching courses for each of these programs. In total, I have mentored 11 PhD students and served on the thesis committees of an additional 17 Emory graduate students. My lab currently consists of three graduate students (including Sam), and one senior (PhD level) Research Specialist Lead (technical staff). I am currently in the process of recruiting a postdoctoral fellow to my lab to work on our antibiotic-resistance rRNA methyltransferase project. In addition to the 8 PhD students who have graduated from my lab, I have mentored and trained 5 former postdoctoral researchers who have gone on to successful positions in academia and industry (e.g. see Table above). Through this experience I have developed, and try to continue to evolve, a broad perspective on training and mentorship. I recognize that each of my lab members may require a style of mentorship specifically tailored to them and I continually assess each individual's strengths, weaknesses, expectations and ambitions.

Co-Sponsor Background. Dr. Lowen is similarly engaged in graduate education having been an active member of the MMG and Immunology and Molecular Pathogenesis (IMP) programs since joining Emory in 2011. Dr. Lowen teaches in "*Principles of Basic Biomedical and Biological Sciences*" and co-directs the "*Virology"* course required for students of both the MMG and IMP program, and which Sam will either audit or take as an elective to strengthen her fundamental virology knowledge. Dr. Lowen also serves on the MMG recruitment committee, regularly attends seminars hosted by both of her programs, and is currently a member of eight thesis committees. The Lowen lab currently comprises two PhD students, one post-doc, two senior technicians (Research Specialist Senior) and a more junior Research Specialist. The lab is expanding due to new funding and Dr. Lowen expects to take on an additional student and post-doc in the next year. Since starting her lab in 2011, Dr. Lowen has graduated two PhD students who are now employed in related fields (see Table above). She strives to provide constructive mentorship and scientific direction while leaving trainees enough independence to develop critical thinking and creativity. Dr. Lowen meets regularly with her trainees to discuss progress and plans, and is available for input as needed.

Overview of Training Objectives. My current trainees are engaged in projects investigating ribosomal RNA methyltransferase enzymes that confer bacterial resistance to antibiotics, or viral/ cellular non-coding RNA structure and activity against proteins of the human host cell antiviral response (PKR and OAS1). Sam's project represents an important new direction in the latter area. My plans with Dr. Lowen to support Sam's technical, professional and other training away from the bench are outlined in the following sections.

Training in Diverse New Experimental Approaches. When Sam began her thesis work my lab last summer she chose to develop a new direction in our studies on OAS1 which had begun a couple of years earlier (see Vachon, V.K. *et al. Nucleic Acids Res.* 2015). Specifically, Sam decided to devise a project to investigate the molecular basis for an unexpected observation, made by former graduate student Dr. Ginny Vachon, that very small changes in a model dsRNA used for prior structural studies of the OAS1-dsRNA complex could either substantially increase OAS1 activation or completely eliminate it. Sam's project will employ diverse approaches that will provide new training in biochemical (OAS1 enzyme kinetics), structural (x-ray crystallography) and other biophysical approaches (BLI and HDX-MS) to tease apart new details of OAS1 regulation by RNA. The use of cell culture approaches in Dr. Lowen's lab will further broaden Sam's technical training while also providing greater biological context to increase the impact of her findings.

Plan for Mentorship, Professional Development, & Opportunities for Scientific Networking. Sam is part of a dynamic, interactive and collaborative group of researchers studying various aspects of RNA biology and translational control using many complementary biochemical, microbiological and biophysical techniques. Sam's primary day-to-day interactions will be with me, Dr. Lowen, and the current members of our research groups. We also collaborate closely with Dr. Christine Dunham's group in Biochemistry, with

whom we have weekly joint lab meetings; Dr. Lowen's group similarly works closely with that of Dr. John Steel in Microbiology & Immunology. In addition to the technical training and individual mentorship Sam will receive in my laboratory and that of her Co-sponsor Dr. Lowen, Sam's project will bring a wealth of opportunities to interact with and learn from a diverse collection of scientists in the Emory University School of Medicine and more broadly across the Emory campus and its healthcare system.

One-on-one meetings: My office is located adjacent to the lab and one door down from our shared student/ postdoc office; as far as possible, my door is "open" for informal discussions. Sam is very proactive in seeking my input (and that of Dr. Lowen) on her experimental plans, the subsequent interpretation of her results, and decisions on the next priorities. We typically meet briefly in my office or in the lab to discuss the latest developments at least 3-4 times a week. Should I feel such interactions are too infrequent, during periods of particular challenge, or when greater input might be needed (e.g. finalizing a manuscript), I typically schedule individual meetings (at least once a week) to give these one-on-one discussions additional structure. In Sam's case, our meetings have largely been informal but through the Fall semester regular scheduled meetings helped us keep on track with my feedback on her weekly assignments for the intensive IBS522r *Scientific Writing and Hypothesis Design* course. Sam was also a willing volunteer to pilot the use of an electronic notebook in my lab, using "Findings" (http://findingsapp.com/), allowing us to share protocols and experimental results via Emory Box (an Emory-wide implementation of the Box.net file sharing and collaboration tool). This approach has allowed me to track progress with experiments, offer immediate input on record keeping, analysis, interpretation and, most importantly, prepare for our regular discussions with greater depth of insight. Dr. Lowen will also continue to be available to address Sam's questions and discuss her project as needed, and will also begin meeting with Sam regularly once she reaches the stage of her project that relies on use of cell culture systems. Though I expect Sam to coordinate these interactions with Dr. Lowen, I plan to participate in any regular scheduledmeetings.

Weekly laboratory meetings: My lab holds weekly joint meetings with Dr. Christine Dunham's group who have related research interests in translation control, including bacterial toxin-antitoxin systems, ribosomal frameshifting, and ribosome quality control. At these meetings, one member of each lab will typically present either their latest results or a relevant paper from the recent literature. We also use these meetings as a forum for students and postdocs to practice formal presentations of a more complete "story", e.g. prior to a conference talk or using the figures from anin prep manuscript to get group feedback. Dr. Lowen (and potentially members of her group) will attend the Conn/ Dunham lab meetings when Sam is presenting her work. Similarly, Dr. Lowen will include Sam on the regular presentation schedule for her group's lab meetings which are held jointly with Dr. Steel's group. Sam will be a regular participant in the Lowen/ Steel meetings and I will also attend when Sam is presenting.

Presentations: I encourage my lab members to present their research early and often in order to hone their presentation skills. Sam has already benefited from the BCDB Program's focus on improving presentation skills through the Year 1 Introductory Seminar class, and is scheduled to present her first BCDB "Advanced Seminar" on Feb. 15th 2017. Ahead of this presentation, I will work closely with Sam, as will all my students and postdocs, to ensure her presentation is clear, polished and successful in making its main points. As noted above, Sam will also be scheduled to present her talk at least a week before in our weekly lab meeting. I also expect that Sam will present her work extensively at local journal clubs, the monthly Joint Structural Biology Groups Meeting and Emory RNA Club, and the annual Emory Graduate Symposium organized by the Graduate Division Advisory Council (DSAC). Sam will present a poster at the next DSAC Symposium in January 2017 and my expectation is that Sam submit an abstract for this event each year and will request a podium presentation in the second and/ or third year of this fellowship. Dr. Lowen and I will also strongly encourage Sam to attend and present her work at local, e.g. the Southeastern Regional Virology Conference (SERVC), and national scientific meetings (see below). Sam attended SERVC held near Emory in April 2016 while rotating in the Lowen lab and this is an excellent forum for students to present their research in a workshopsetting.

Scientific Conferences and Meetings: I will encourage Sam to attend at least one scientific meeting per year to present her results and to develop a network of interactions with senior colleagues and peers at other institutes. Specific conferences for Sam to target will be discussed ~6-9 months prior to planned attendance but over the course of her time in my lab will include both more focused meetings, like the GRC on Nucleic Acids or a Keystone meeting on Innate Immunity, and broader conferences such as the annual meetings of the RNA Society, American Society for Microbiology (ASM) or American Society for Biochemistry and Molecular Biology (Experimental Biology). A first priority for Sam will be attendance at a meeting of broader scope to immerse herself in her new field. As a goal of Sam's technical training is to

learn x-ray crystallography, I will also encourage Sam to attend an intensive training course in macromolecular crystallography and at least one conference related to this technique. Several courses on x-ray data collection and/or structure determination are held annually at US synchrotron sources and elsewhere, and I will encourage Sam to apply once she has fully embarked on that aspect of her project. Upon completing the second aim of her proposal I would encourage Sam to present her structures and complementary binding and protein dynamics (HDX-MS) data at the national meeting of the American Crystallographic Association. In addition to lab funds, the Laney Graduate School provides "Professional Development Support (PDS)" funds (up to [redacted] per student, potentially more on a competitive basis) to support conference attendance for students to present their work (poster or talk is required). Dr. Lowen and I will also guide Sam in the preparation of applications for travel awards and other meeting fellowships to maximize the range of her conference attendance and experiences throughout her graduate career.

Thesis Committee: Sam has assembled a thesis committee with diverse expertise to help guide her research and oversee her progress towards her career goals. In addition to Dr. Lowen and I, the committee comprises three Biochemistry Department faculty, Drs. Christine Dunham, Rick Kahn, and Daniel Reines. As noted above, Dr. Dunham brings relevant expertise in structural biology, protein synthesis and RNA-protein interactions. Dr. Reines was one of Sam's BCDB rotation mentors and brings complementary expertise in RNA biology and protein structure related to transcriptional control. Dr. Kahn is a biochemist and cell biologist with expertise in cell signaling/ regulation by GTP-binding proteins, membrane traffic, cell division, and energy metabolism. Sam's thesis committee will meet for 1-2 hours every six months as dictated by the BCDB program and she will meet individually with members of her committee as needed.

Publications: All students in my laboratory are required to complete an independent project leading to first author publications. When Sam is nearing the point of preparing her first manuscript, I will assist her in developing a framework for the paper as she prepares draft figures (a process that may identify any "gaps" in the data). Next, Sam will write each section in turn to prepare a complete first draft: Methods, Results, Discussion, Introduction and finally the Abstract. In my lab we typically share manuscripts via OneDrive (for sharing and team editing), which allows me to track progress and offer quick comments as each section develops. Once Sam completes a first draft I will provide detailed feedback-usually I do this as handwritten comments on a printed copy. I will then sit down with her and discuss my comments and explain why I made specific suggestions. My experience is that students may go through several drafts before a final product is reached; this can be a time-consuming process (at least for the first manuscript), but one that I believe is critical arming my students with effective skills for scientific writing.

Mentoring as a component of training: Later in her graduate career I plan to provide Sam with several opportunities to mentor undergraduates (we typically host one or two each summer), rotation or other junior graduate students, to gain skills as a mentor. My approach to this, as with previous trainees, will be to have an initial discussion with Sam and her mentee about the overall project goals and the timeframe, the general experimental plan, and the specific details of the first experiments. After this, I will allow Sam the freedom to train and mentor the student directly. I expect that Sam will be the first person her mentee will seek input from, e.g. on experimental details or later for reviews of abstracts, poster drafts, etc. However, I will schedule regular meetings with Sam and her mentee to ensure things are progressing well. At the end of the student's time in the lab, I will discuss the outcomes with Sam and ask her to prepare a reference letter for the student on which I will provide feedback to help her develop this essential skill.

C2. ENVIRONMENT

Biochemistry, Cell & Developmental Biology (BCDB) Program. Sam is a member of the BCDB program and will continue to follow the innovative BCDB curriculum (please see *Institutional Environment and Commitment to Training*). Sam has completed most requirements for Years 1-2 (a Spring semester in biostatistics and teaching assistantship remain). Beyond Year 2, coursework centers mostly on honing presentation and other professional skills, through courses such as BCDB Advanced Seminar and Ethics in Research. Sam will also attend a twice-monthly BCDB journal club, monthly Professionalization Workshops covering topics such as Individual Development Plans (IDP) and time management, and an annual scientific and team building retreat with other students and many of the Program faculty.

Biochemistry Department. The Conn lab is part of the Biochemistry Department in the Emory University School of Medicine so Sam is also affiliated with the Department. Additional groups Sam will have opportunities to interact with (in addition to those already noted above) include those of Drs. Eric Ortlund (protein structure, protein-DNA/RNA interactions, X-ray crystallography), and Bo Liang (viral RNA polymerase structure, high-resolution cryo-EM). The Department hosts a weekly seminar series and students are invited to have lunch with the speakers. I encourage my students to participate in these

meetings and I expect that Sam will have opportunities to meet with a seminar speaker 2-3 times/ semester.

Microbiology & Immunology Department. The Lowen lab is part of the Microbiology & Immunology Department, located one floor below Biochemistry. Virology is a strength at Emory and Sam will have the opportunity to interact with trainees and PIs from the Steel, Speck, Mocarski, Ahmed, Suthar, Hunter and Grakoui laboratories, to name a few. Dr. Lowen is a member of the Emory-UGA Center of Excellence for Influenza Research and Surveillance (CEIRS), which offers a number of opportunities for trainees to present their work, meet virologists, and travel to other institutions to learn pertinenttechniques.

Emory as an Outstanding Training Environment. Emory has an extremely collaborative research environment and many of our current studies rely on the expertise of colleagues. For our students, this collaborative environment creates a unique working environment where they can draw on the expertise of many individuals in the absence of formal training relationships. The interdisciplinary nature of the GDBBS graduate programs also enhances our research environment creating interactions and bonds between College departments (such as Biology and Chemistry) and those in the School of Medicine. Thus, students have the opportunity to broaden their training and learn new approaches from experienced experts.

As noted above, the Graduate Division holds an annual student symposium providing opportunities for poster and oral research presentations. I require that my students submit an abstract each year and Sam will begin participating in January 2017. Various additional seminar series are available that will provide Sam with opportunities to become familiar with diverse techniques, biological systems and biomedical problems. These include: 1) *Emory RNA Club*, a bimonthly seminar series that brings together diverse research groups using biochemical, cellular, genetic, developmental, chemical biological and structural biological techniques that focus on understanding the emerging world of RNA biology. More than 10 research groups at Emory are involved in this series that will provide an excellent forum for Sam to present her research and hone her presentation skills for a diverse audience. 2) *Structural Biology Joint Group Meeting*, a monthly meeting involving groups with expertise in x-ray crystallography (Drs. Conn, Dunham, and Ortlund), cryo-EM and tomography (Drs. Liang and Elizabeth Wright), and other biophysical approaches such as HDX-MS (Dr. Renhao Li). In both cases, students and postdocs give presentations on their work or recent literature in an informal setting where discussion is strongly encouraged.

Relationship of Training to Applicant's Career Goals. Sam's immediate goal after completing her PhD is to secure a postdoctoral position at a top research university to continue on the path to a career as an independent researcher. My goal in training Sam is to provide the laboratory setting, atmosphere and continuous mentorship and feedback that will allow her to develop the technical and professional skills necessary to accomplish this goal. Sam's project will give her experience of experimental design and interpretation, and essential technical skills in protein expression/ purification of RNA, biochemical assays of enzyme activity, cell culture, and biophysical approaches to study protein structure, dynamics and interactions. I am also committed to ensuring Sam is trained as broadly as possible in all other essential professional skills, including scientific writing (e.g. manuscripts, grants), presentations (posters, talks) and in mentorship. Our efforts will be complemented by the comprehensive BCDB programmatic activities and the outstanding intellectual environment at Emory. Taken together, all the requisite resources are in place to promote Sam's development as an independent research leader and an outstanding scientist.

C3. RESEARCH FACILITIES

The Conn and Lowen laboratories, Departments of Biochemistry and Microbiology and Immunology, and Emory core facilities will provide everything Sam needs to complete the experiments described in her proposal (please see *Facilities and Resources* and *Equipment* sections for more detail). Briefly, the Conn lab has dedicated spaces for bacterial culture (for protein expression, etc), radioisotope use, and macromolecular crystallography; a main lab space where each member has their own dedicated bench; and, is well equipped for protein and RNA preparation/purification and *in vitro* biochemical/ structural studies of OAS1 regulation by dsRNA. Of particular relevance for Sam's cell culture experiments, the Lowen lab has three Class II biosafety cabinets, four CO_2 incubators and a light microscope for cell culture work, and is also well equipped with quantitative PCR instrumentation. The Department of Biochemistry has modern x-ray crystallography suite with crystallization robotics and x-ray generator with sample mounting robot, and also provides regular scheduled time at the SER-CAT beamline at the Advanced Photon Source. These outstanding resources and regular availability of synchrotron beamtime at APS will expedite Sam's research program and enhance her training in x-ray crystallography. Finally, Sam's project will make use of equipment in two Emory core facilities (for HDX-MS and BLI analyses); it is important to note that both facilities require thorough user training from the core but will subsequently allow Sam to independently

design, run and analyze the results of her experiments under their expert guidance.

D. NUMBER OF TRAINEES

In addition to Sam, I currently advise two other pre-doctoral trainees and a PhD-level technician. I expect one student to graduate by summer 2017 and my lab is open to a new student for their thesis research. I am also actively recruiting a new postdoctoral fellow. Over my time at Emory, my group has typically had 5-8 members (graduate students, postdocs and technicians) plus 1-2 undergrads or other visiting scientists. Dr. Lowen currently advises two pre-doctoral trainees and one postdoctoral fellow. Her lab has consisted of ~4 members since its inception in 2011, but is currently transitioning to a larger group; Dr. Lowen plans to take one additional student and aims to recruit a postdoctoral fellow or staff scientist within the next year.

E. APPLICANT'S QUALIFICATIONS AND POTENTIAL FOR A RESEARCH CAREER

Sponsor (Conn): Sam is smart, driven, and a careful and already highly competent experimentalist. These qualities, among many others, point to her outstanding potential as a future research leader. Not all these abilities are innate, some were built on hard work over 3 years as a research technician; some of them she now continues to develop in grad school. As Sam describes elsewhere, being a first generation college student and completing her undergrad degree at an institute with limited research opportunities, she took the (very mature) decision to gain further research experience before committing to grad school. As a result, I have in effect recruited Sam to my lab twice, each time with very different goals. When Sam applied for the open Research Specialist position in my lab in 2012, her CV made my short list but I had reservations both about her limited research experience and some "features" of her undergrad transcript. Needless to say, the interview convinced me she was the right choice for my lab. Two main factors contributed to this decision. The first was Sam's clear objective to gain research experience with graduate school the future goal (something I felt my lab and I were well placed to help her achieve). Second, was how Sam dealt with my list of standard "lab tech" questions, gleaned from many co-interviews with experienced HR professionals, as mandated in Manchester for all tech hires. While I had unknowingly always found myself going too deep into specific research experiences or future career goals, these questions were simple, direct, and, to my initial surprise, *highly* discriminating. These included asking about task prioritization or how conflicts should be handled, to the seeming simple question, "*If I asked you to make a 1M solution of NaCl, what would you need to know?*" which completely throws >90% of my interviewees. How about one who got an F grade in Organic Chemistry? As with everything else I threw at her from my repertoire, Sam aced the answers (I suppose I should have known, since she retook the class and got an A!). I was completely convinced and Sam was a stellar technician. What about her potential for grad school and beyond? As Sam began to develop her innate scientific curiosity to complement her excellent experimental hands, I knew it was time she left. I encouraged Sam to explore broadly and she did (choosing an interview at UTSW over waiting on an invitation to the Emory BCDB event the same weekend). Having seen the BCDB Program up close already, she chose to stay at Emory (I didn't blame her). Next, I insisted she explore other labs during her rotations. While she ultimately chose to return to a new project in my lab, she did so with a new perspective on her abilities, interests, and future goals and an outstanding co-mentor in Dr. Lowen! I cannot over state how different I view Sam's future grad experience and training in my lab (and my role in that training) from what she has already experienced and accomplished. What I know is that I am fortunate to have an amazingly talented student in my lab who is destined for great things in her future career in research.

Co-Sponsor (Lowen): During her rotation in my lab, Sam demonstrated excellent technical ability, maturity, organizational skills and an ideal balance of independence and willingness to ask for help. Sam's impressive productivity during her 10-week rotation is evidenced by her publication on the use of ddPCR to detect and quantify defective viral genomes. Sam is the only author on this paper with me because she did all the experiments, analyzed the data and, with my guidance, interpreted the data and wrote the first draft of the paper. She accomplished all of this because she was thoughtful in her experimental design, ensuring that all necessary pilot experiments had been run and all reagents were in good condition before starting a larger series of experiments. Sam had very few, if any, failed experiments. Sam is also an excellent communicator: she reported her results regularly without me having to prompt her, she came to my door or set up meetings as needed to discuss future plans, and she worked very well with other group members. Although in my lab only briefly, Sam became our expert on ddPCR, which was new to us at the time of Sam's rotation. I therefore asked her to write up a protocol and to train others to use the instrumentation and software; she took these tasks on willingly and did a great job. Sam was also a lot of fun to have in the lab because she is very enthusiastic about her research and science in general. She is ambitious, willing to work hard to achieve her goals, and has excellent potential for a successful career in research.

INSTITUTIONAL ENVIRONMENT AND COMMITMENT TO TRAINING

Samantha Schwartz is a 2nd-year student (rising 3rd year by time of potential award of this NRSA F31 fellowship) in the interdepartmental Biochemistry, Cell, and Developmental Biology (BCDB) Graduate Program at Emory University. Sam has completed all required core Program coursework to date and passed her written qualifying exam in April 2016. Remaining Program commitments include a biostatistics course and a semester-long teaching assistantship. Both of these obligations will be completed in the Spring 2017 semester prior to an award of this NRSA F31 fellowship. Sam has assembled her thesis committee, comprising Drs. Conn (Sam's Sponsor on this application), Lowen (Co-sponsor), Christine Dunham, Rick Kahn, and Daniel Reines.

Technical, Intellectual and Other Supporting Resources at Emory University. Sam's research and professional training will benefit from outstanding technical, intellectual, and other resources available directly within the Conn and Lowen laboratories, as well as the Departments of Biochemistry (Conn) and Microbiology & Immunology (Lowen), the Emory School of Medicine and its Core facilities, the BCDB Graduate Program, and the Laney Graduate School (LGS) at Emory University. In terms of equipment and other facilities, both the Sponsor and Co-sponsor labs are well-equipped for the proposed studies. The Conn lab has the equipment and resources needed for modern biochemical and biophysical approaches to study the structures, interactions, and biological functions of biomedically important RNA and protein molecules. Additional shared facilities that Sam will use in her work include the Biochemistry structural biology facility, which contains robotics for automated crystallization screens and an X-ray generator for crystal screening. The Department also supports regular access to the SER-CAT beamlines at APS (see Equipment and Facilities sections for details). The Lowen lab is similarly well-equipped for cell culture and viral infection experiments. Sam will use several of Emory's excellent core facilities, including the Emory Chemical Biology Discovery Core for their OctetRED^{384} and the HDX-MS Core. In both cases, Sam will work directly on protocol development, data collection, and analysis on these state-of-the-art instruments under the guidance of expert core staff.

Sam's research is centered on understanding how a key component of the human innate immune system (OAS1) is regulated by specific features within double-stranded RNA (dsRNA) molecules. Among labs working today on the OAS1/ RNase L pathway, Sam's project is unique with its specific focus on understanding the *RNA features* that potently activate OAS1 or that can lead to sequestration of its activity. Sam will extend the Conn lab's strong record of using RNA biochemistry/structural biology approaches to investigate the regulation of dsRNA sensors of the innate immune system by viral and cellular non-coding RNAs. This project builds directly on that of a former graduate student (Dr. Virginia Vachon) and will complement that of a current BCDB graduate student (Ms. Brenda Calderon) whose project is centered on the cellular structured non-coding RNA nc886. The environments in which Sam will work are also enriched by other PhD and graduate student investigators working on RNA-protein interactions related to bacterial antibiotic resistance (Conn lab) and viral transmission, reassortment, and host immunity (Lowen lab). Outside of the Conn and Lowen labs, Sam will have the opportunity to interact with other faculty members and their teams in both informal and formal settings. For example, Sam's thesis committee has three additional members in addition to Drs. Conn and Lowen who bring diverse expertise, including translational control and ribosome structural biology (Dunham), RNA biology and protein-RNA interactions related to eukaryotic transcription regulation (Reines), and cell and protein biology (Kahn). Other faculty members in Biochemistry with strong research programs in structural biology include Drs. Eric Ortlund (steroid hormone receptors, including protein-RNA interactions, using X-ray crystallography) and Bo Liang (viral RNA-dependent RNA polymerase structures by high-resolution cryo-EM). Sam will participate in the monthly Joint Structural Biology Groups (JSBG) meeting and the "Emory RNA Club," which connects researchers with interests in RNA biology from Biochemistry, Biology, Cell Biology, and other departments across campus. More broadly, through her interactions with Dr. Lowen's lab, Sam will benefit from exposure to the great strengths at Emory in virology and vaccine development.

Sam will have many opportunities to present and receive feedback on her work. She will present research updates at least 3-4 times a year in bi-weekly joint Conn-Dunham and Lowen lab meetings. Sam will meet twice a year with her thesis committee and will present her latest research to faculty and students in the BCDB Advanced Seminar course once per year until graduation. Sam will present her work at the JSBG and RNA Club meetings, as well as at Emory symposia (including the Division Student Advisory Council (DSAC) Annual Research Symposium (sponsored by the LGS)) and other local meetings, such as the Southeastern Regional Virology Conference. The LGS also provides professional development support funds for students to present their work at national and international meetings. Finally, Sam will benefit from a number of innovative approaches the BCDB program has implemented to support student professional development, e.g. by requiring yearly updates to her individual development plan (IDP) and allocating time during thesis committee

meetings to discuss her career goals. Sam also has access to career seminars, such as “Pathways Beyond the Professoriate” and professionalization workshops offered by the BCDB program.

ADDITIONAL EDUCATIONAL INFORMATION

Program Structure. The BCDB Program is one of 9 graduate training programs in the Graduate Division of Biological and Biomedical Sciences (GDBBS), part of the LGS of Emory University. The BCDB Program is highly interdisciplinary with ~45 training faculty representing 13 basic science and clinical departments and offering an incredible breadth of potential training. Faculty research is grouped into four overarching themes: Biochemistry/ Structural Biology, Cancer Biology, Cell Biology, and Developmental Biology. The Program has a history of implementing innovative approaches to graduate education that emphasize oral and written communication skills, quantitative skills, career development, and professionalization, along with cutting edge technical research training. Our graduates are expected to have the technical, analytical, and communication skills necessary to pursue an independent career in academic, industrial, or government research or in careers where their training will facilitate the business, application, or public understanding of biomedicalresearch.

Required Milestones & Timing. ***(a) Coursework***. The BCDB curriculum is intended to provide students with the basic knowledge and skills to pursue doctoral dissertation research and a career in the disciplines represented by the Program. All required coursework is completed by the end of the Spring semester of Year 2, and electives may be taken starting in Year 2 if desired. *The following core courses are required for all BCDB students*: *Year 1*, Foundations in BCDB I and II (BCDB 501/502), Beginning Seminar (BCDB 570r; including attendance at Advanced Seminar), and Jones Program in Ethics (JPE 600); *Year 2*, Statistical Design and Analysis of Experiments (IBS 538), Hypothesis Design and Scientific Writing (IBS 522r), and, Advanced Seminar (BCDB 790r). Additionally, three 10-week research rotations are completed in Year 1, and in most cases, students then select a thesis advisor (typically in May). ***(b) Teaching commitment***. Formal instruction and experience in teaching is a requirement for all graduate students at Emory. The Teaching Assistant Training and Teaching Opportunity (TATTO) Program is administered by the LGS to provide teacher training, and BCDB Trainees fulfill this requirement in Year 2 by *(i)* participation in a 2-day teaching training workshop (TATT 600) covering methods and ethics of teaching, as well as content-specific practice sessions tailored to the discipline of the student, and *(ii)* serving as a Teaching Assistant (TA) for at least one semester, with the quality of the teaching experience being evaluated via reports from the course directors. Several options are available to BCDB students who wish to gain additional teaching experience and t o establish teaching credentials, subject to continued satisfactory progress in their dissertation research subject. ***(c) Qualifying exams (QE)***. The BCDB QE is completed in two parts. **QE1** (May/June Year 1) is a written (essay-type question), closed-book examination administered over two days. QE1 is designed to test foundational knowledge and critical thinking/writing, and thus student preparedness to progress in the Program. **QE2** (to be completed before June 1 of Year 2) is an oral examination designed to assess the student's ability to integrate different aspects of the first two years of graduate training including lab work, data interpretation, hypothesis development, research design, presentation of research, and all other course work. ***(d) Dissertation defense***. The BCDB Program requires students to produce a rigorous body of novel research, including at least one first-author peer-reviewed research paper, published or accepted for publication, before the dissertation defense is scheduled. Before the PhD degree will be granted, the following tasks must then be completed in order: *(i)* submission of the written dissertation, *(ii)* a closed session oral examination (defense) by the student’s thesis committee, and *(iii)* an advertised public oral presentation of the thesis research.

Monitoring and Evaluation of Student Progress. Student progress is monitored from initial entry into the Program via an established, formal process involving academic probation (based on coursework grades and average semester GPA) and the requirements for its resolution through improved performance. Failure to satisfy these criteria is grounds for termination from the BCDB Program. After completion of QE1 and no later than the Spring semester in Year 2, students must form a Dissertation Committee comprising the dissertation advisor and a minimum of four other faculty members, at least three of whom must be BCDB faculty members. The first committee meeting is held no later than six months after passing QE2, and thereafter meetings are required *every six months* (every *four* months after Year 5). Compliance is tracked by the BCDB Executive Committee. The Program requires the following slides in meeting presentations: *(i)* scientific update: techniques learned, papers, grants, presentations, awards, etc., *(ii)* short-term goals, and *(iii)* goals beyond graduate school. **The average time to PhD degree for BCDB students over the last 10 years is 5.8 years.** Sam is on track to graduate within this timeframe.

Provided by Michael Koval, Ph.D. (BCDB Director) & Graeme L. Conn Ph.D. (BCDB Dir. Grad. Studies)

RESOURCE SHARING PLAN

All reagents produced during the course of this research program will be shared in line with the policies of Emory University and the NIH (https://www.nlm.nih.gov/NIHbmic/nih_data_sharing_policies.html). Data will be shared freely between the PI, Sponsor, and Co-sponsor and members of their research teams, with colleagues at meetings and conferences, and through publications. Where appropriate, data will be deposited in a public repository, e.g. the RCSB Protein Data Bank for X-ray crystal structure coordinates and electron density maps.