KENNESAW STATE UNIVERSITY
OFFICE OF RESEARCH
Undergraduate Research

URCA STUDENT FUNDING CONFERENCE TRAVEL TEMPLATE

The Auto-Inhibitory Loop of Endothelial Nitric Oxide Synthase Kinetic and Structural characterization to p38

Student Name and Department: Emma Henry, Department of Molecular and Cellular Biology

Student Email: ehenry19@kennesaw.edu

Anticipated Student Graduation Date (month, year): December 2019

Faculty Member(s) Name and Department (list all faculty mentors associated with this project):
Jonathan L. McMurry, Department of Molecular and Cellular Biology
Thomas C. Leeper, Department of Chemistry and Biochemistry
Carol A. Chrestensen, Department of Chemistry and Biochemistry

Faculty Mentor(s) Email (list all faculty mentors' emails):
Jmcmurr1@kennesaw.edu
tleeper@kennesaw.edu
cchreste@kennesaw.edu

Name of Conference (no abbreviations):
The Southeastern Regional Meeting of the American Chemical Society (SERMACS)

Travel Dates: October 19th, 2019 – October 23rd, 2019

Travel Location (city, state/province, country): Savannah, GA, USA

Type of Presentation (e.g., Oral, Poster, or Other): Poster

Has the presentation been accepted? YES ☐ NO ☒

Does $1,000 cover all travel expenses? YES ☒ NO ☐

Have all travel arrangements been established? YES ☒ NO ☐

Institutional Review Board (IRB) number for projects involving human or animal subjects (leave blank if not applicable):
N/A

Faculty Member Statement (written by faculty mentor; 300 words max): *Describe your research portfolio (manuscripts, grants, presentations, awards, etc.) as well as your experience incorporating undergraduates into your research activities.*

My research has been continuously funded my entire time at KSU, now 13 years. I have had 4 R15 grants, an NSF RUI, an MRI, and several smaller awards from NIH, the Research Corporation and Georgia Research Alliance. I have not only funded my own projects which have been impactful (24 peer-reviewed papers at KSU, 18 with KSU student coauthors; H-index=15) and created numerous opportunities for students, but also generated hundreds of thousands of dollars in indirects that have supported the research environment in the college and at KSU. I have trained 51 undergraduates and 10 MSIB students. My students or I have given 102 conference presentations. I have been the prime mover on acquisition of **instrumentation that gives CSM a unique-among-non-R1 capabilities** in intermolecular interaction analysis. My efforts have brought the Biacore X100 SPR instrument (via an NSF MRI grant), the ForteBio BLI instrument (via an R15 of mine with college matching) and the TA Instruments NanoITC (R15 & college support). These instruments have been used to generate data used in dozens of papers and grant proposals.

Institutional Impact Statement (written by faculty mentor; 200 words max): *Describe the benefit to the institution related to this research activity (preliminary data for pursuing external funding, plans to publish/present, development of intellectual property, collaboration with reputable institutions, etc.).*

Ms. Henry's project is an unfunded collaboration with Drs. Chrestensen and Leeper to solve a structure for a regulatory domain of endothelial nitric oxide synthase. Nothing is known of the structure whether free or bound by regulatory kinases. Success will not only be a fundamentally important bit of new knowledge about eNOS regulation, but it will open up a new avenue of investigation that will catalyze competitive NIH and NSF grants proposals. Results will be published in peer-reviewed journal.

Student Impact Statement (written by faculty mentor; 400 words max): *Describe how this work will positively impact participating undergraduates immediately and in their future endeavors.*
Ms. Henry is bound for a biomedical doctoral research program upon graduation from KSU (she will be applying this fall). She is gaining cutting-edge training in molecular and cellular biology, learning methods such as mammalian tissue culture, confocal microscopy, optical biosensing, NMR spectroscopy, protein biochemistry, etc. etc. Beyond the training, a presentation at SERMACS will be invaluable experience for her and an important part of her doctoral applications. And lest reviewers think 'McMurry has money, let him pay to send her off of his grants,' I would point out that this project is entirely unfunded and I cannot justify travel expenses to my grant for presenting work that has nothing to do with that which was funded. If an award can't be made, Ms. Henry will have to withdraw her abstract. (Neither can she afford to go on her own – she has significant financial need for school as it is.)

Project Narrative (700 words max across each section below):

DESCRIPTION OF PRESENTATION SESSION

BACKGROUND *(provide brief, relevant scholarly or research context, including citations, that demonstrate how the research makes a unique contribution to the area of inquiry)*

Mitogen-activated protein kinases (MAPKs) ERK and p38 participate in negative feedback signaling networks with endothelial nitric oxide synthase (eNOS). eNOS contains a MAPK binding site associated with the autoinhibitory loop (AI), a major eNOS control element. We previously showed that a pentabasic MAPK binding site and a residue phosphorylated by MAPK, S602, are within the AI and have important enzyme control elements between them. Despite extant crystal structures of homologous NOSs, nothing is known of the structure of the AI beyond secondary structure prediction. To address this lack of knowledge, we constructed a synthetic gene encoding a maltose binding protein (MBP)-AI fusion and expressed and purified the protein. . The results will be incorporated into an updated model of MAPK regulation of eNOS, which may have implications for understanding diabetic physiology, atherosclerosis and cancer.

KEY RESEARCH QUESTION AND/OR CREATIVE PROJECT GOAL

What is the structure of the Auto-Inhibitory loop and how does that structure change when interacting with MAPK?

METHODS

Biolayer interferometry (BLI) , surface plasmon resonance (SPR), ^{1}H, ^{15}N heteronuclear NMR methods. Transverse Relaxation Optimized Spectroscopy (TROSY) and Saturation Transfer Difference (STD).

RESULTS (OR ANTICIPATED RESULTS)

Biolayer interferometry (BLI) showed that MBP-AI bound p38 with low µM affinity, as expected, confirming the presence of the MAPK binding site within the AI. BLI and surface plasmon resonance (SPR) were used to perform a complete kinetic characterization of AI-p38 binding. MBP-AI will be labelled with ^{15}N and characterized with ^{1}H, ^{15}N heteronuclear NMR methods. Transverse Relaxation Optimized Spectroscopy (TROSY) and Saturation Transfer Difference (STD) experiments to determine the p38-bound structure of the AI will be described, along with other NMR and biophysical characterization methods.

CONCLUSION/DISCUSSION

Determination of a kinase-bound structure for the AI will provide novel structural insight into the molecular regulation of eNOS, affording a better understanding of the regulatory mechanisms of an enzyme that is critically important in many cellular signaling pathways.

Budget Table and Justification:

CATEGORY	AMOUNT
REGISTRATION	$70

PER DIEM	$305
AIRFARE (DRIVING)	$150
LODGING	$472.50
TOTAL REQUEST	$997.50

Registration – Fee for undergraduate non-member if registered before September 24th according to the SERMACS website.

Per Diem – The per diem for Savannah, GA is $61 dollars per day multiplied by 5 days is $305 dollars total.

Airfare (Driving)– Driving distance is 294 miles from my house to the conference so there and back is 588 miles and I multiplied it by the 2019 business mileage rate of 0.58 cents per mile for a total of $341. However, I will be carpooling with others to the meeting and I am thus requesting a $150 contribution towards driving expenses.

Lodging – Savannah Marriott Riverfront where the conference is held has a special rate 189/night and I will share my room, so my portion of the room would be $472.50.

References *(include references for the citations provided in the Narrative section)*:

- Salerno JC, Ghosh DK, Razdan R, et al. Endothelial nitric oxide synthase is regulated by ERK phosphorylation at Ser602. *Biosci Rep*. 2014;34(5):e00137. Published 2014 Sep 17. doi:10.1042/BSR20140015