ACCREDITATION EVIDENCE

Title: Microbiology INBRE SPREM Grant; Professor Josh Holmes

Evidence Type: Clear

Date: 1 November 2021

WAN: 22-0442

Classification: Resource

PII: No **Redacted:** No

Dr. R. Scott Seville, INBRE Principal Investigator/Program
Dr. Florence Teulé-Finley, INBRE Program Coordinator
NIH Wyoming IDeA Networks for Biomedical Research Excellence (INBRE)

TO: Joshua Holmes, PhD
DATE: November 1, 2021
RE: WY INBRE SPREM proposal

Dear Dr. Holmes:

I am pleased to inform you that following review, your Wyoming INBRE Phase 4 Scaled Participatory Research and Education Model (SPREM) proposal titled "Identification of binding determinants of PopZ binding partners" has been selected for support in the amount of $9,950.

In accepting the award please review carefully the following requirements.

1. You will be required to submit an Annual Progress Report for this project through the Piestar WY INBRE reporting system. Details will be provided shortly.
2. If you have not already done so you must register with eRA Commons to establish an eRA Commons Username. Contact your institutional eRA Commons officer so they can assist in this process.
3. All individuals receiving support from NIH are required to have conflict of interest (COI) training. If your school does not have a COI policy policy (in compliance with 42 CFR Part 50) you should follow UW's Conflict of Interest policy available at the following URL: http://www.uwyo.edu/research/Compliance/Conflict%20of%20Interest/conflict-of-interest.html . In addition, UW's COI policy has a training component that will need to be completed by any faculty and staff (including part-time student employees) who are engaged in research related to this award. The online training should be completed prior to engaging in the activity and must be completed at least every 4 years. When logging into the CITI website, if you have not already done so, create an account specifying your institution as University of Wyoming.
4. The Wyoming INBRE Phase 4 Year 2 budget period is May 1, 2021 through April 30, 2022. **There are no provisions for carryover of unexpended funds so all funds must be encumbered by April 30, 2022.**
5. The dollar amount allocated for your project reflects direct dollars to support the activities proposed in your application; there is no indirect cost recovery money allocated for your use – only the direct dollars indicated above.
6. Awardees are required to attend and present results of the INBRE project at the annual Wyoming INBRE Conference, and should try to attend the biannual Western Regional and National NISBRE/INBRE meetings. INBRE supported students and postdocs should also attend the annual conference and other INBRE events as requested.

7 With your approval, the best way to allocate the funds would be to add this award to the WWCC INBRE subcontract and you can manage your award internal to WWCC. Please let me know if this is acceptable.

Congratulations and I look forward to hearing the results of your supported activities.

Please reply by email to accept the award and terms stated above.

Cheers, SS

R. Scott Seville, Ph.D.
Professor and Department Head, Department of Zoology and Physiology, http://www.uwyo.edu/zoology/
Director, NIH IDeA Wyoming INBRE, www.uwyo.edu/wyominginbre
Assoc. Director, NIH IDeA Mountain West CTR-IN Pilot Grant Program, https://ctrin.unlv.edu/
Vice President, National Association of IDeA Principal Investigators (NAIPI), http://www.naipi.org/
University of Wyoming- Laramie, 1000 E. University, BS 428B and HH 234, Laramie, WY 82071
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Wyoming INBRE is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant # P20GM103432. Content is solely the responsibility of the authors and does not necessarily represent the official views of NIH.

Table of Contents

Application Summary

Competition Details

Competition Title:	Fall 2021 Wyoming INBRE Scaled Participatory Research and Education Model (SPREM) Grant Program
Category:	Internal/External Funding Opportunity
Cycle:	WY INBRE SPREM Program
Submission Deadline:	10/15/2021 5:00 PM

Application Information

Submitted By:	Josh Holmes
Application ID:	700
Application Title:	Identification of binding determinants of PopZ binding partners
Date Submitted:	10/14/2021 3:59 PM

Personal Details

Applicant First Name:	Josh
Applicant Last Name:	Holmes
Applicant Degree(s):	PhD
Email Address:	jholmes@westernwyoming.edu
Phone Number:	(180) 166-9351
Primary Appointment Title:	Assistant Professor
Contact Person's Name:	Josh Holmes
Contact Person's Email Address:	jholmes@westernwyoming.edu
Contact Person's Phone Number:	8016693517

Application Details

Proposal Title

Identification of binding determinants of PopZ binding partners

Proposal Abstract

Polar organizing protein Z (PopZ) has been shown to interact with over 10 different binding partners. This is odd for such a small protein of 177 amino acids. It has been shown to facilitate these interactions being an intrinsically disordered protein. This is a type of protein that natively has no structure and only adopts one in response to environmental cues or protein-protein interactions. Two of the PopZ binding partners (DivL and CckA) have had small truncations to break the interaction of PopZ. This work will expand upon these two truncations. Our first goal is to determine if these truncated regions alone are sufficient for interaction with PopZ. The second goal is to work to identify if there are any sharded traits between these two regions and the other PopZ binding partners. If there is, this would suggest a universal binding determinant. This work could provide novel insight into intrinsic disordered protein-protein interactions.

Comments to the Administrator(s)

Acknowledgment

Additional Requirements

[Acknowledged] Recipients of SPREM/SEED Grants are required to:

1. Submit project updates when requested and results of support via the Wyoming INBRE Piestar reporting database and/or the Annual Progress Report. Details will be provided.
2. Attend and participate (students and faculty) in Wyoming INBRE-supported events including the spring Wyoming INBRE Conference and Wyoming Undergraduate Research Day and the annual Fall Wyoming INBRE Network Retreat.
3. Establish a research collaboration with a University of Wyoming or other western INBRE state faculty researcher for collaborative research and/or education projects.

NOTE: Any product resulting from support from Wyoming INBRE, from the use of INBRE equipment, or an INBRE sponsored student must acknowledge Wyoming INBRE. This includes publications, presentations, press releases, requests for proposals, bid invitations, or any other documents or applications that describe projects or programs that were supported by INBRE. For additional information and examples go to: http://www.uwyo.edu/wyominginbre/articles/cite_inbre.html.

2021-22 WYOMING INBRE SCALED PARTICIPATORY RESEARCH AND EDUCATION MODEL GRANT PROGRAM

Project Title: Identification of binding determinants of PopZ binding partners

Principal Investigators: Joshua Holmes, jholmes@westernwyoming.edu

Other involved faculty: Bud Chew, bchew@westernwyoming.edu

Project summary

The primary goal of this project is to teach Western Wyoming Community College students basic microbiology, molecular biology, and fluorescent microscopy techniques. Students working under Dr. Josh Holmes, will work with the intrinsically disordered protein (IDP) Polar Organizing Protein Z (PopZ). This project aims to understand the interaction of PopZ with its binding partners and understanding how or what disordered proteins recognize. Intrinsically disordered proteins are often crucial to many human diseases specifically cancer. The p53 tumor suppressor gene is the most mutated gene in all of human cancer. This central protein has been shown to be an IDP. Many of the mutations that affect this protein are found within its disordered regions. Having a better understanding of the basic mechanics of IDP and binding partners could lead to a new understanding in cancer progression due to mutations in p53 disordered regions.

This project will involve creating truncations of the various binding partners and testing for loss of interaction with PopZ. These interactions will be tested via two methods. Our primary method will be an in vivo system using *E. coli* as a living test tube. This will use a fluorescent microscopy co-localization assay. The secondary method will be a bacterial adenylate cyclase two-hybrid system as an in vitro system. Using two systems will provide us with the upmost confidence in our results. Students conducting this research will learn skills in basic bacterial culture and fluorescent microscopy. Students will learn how to extract and purify DNA, advanced cloning techniques and DNA sequence analysis. Students will be required to learn how to analyze and quantify data. The students will be required to present this research to the scientific community. All these skills would give a student an excellent opportunity to understand how research is done. Students learning these skills would also be well prepared to join nearly any biological lab.

Microbiology research is well suited for a community college environment. There is a great deal of research that can be done that does not require expensive equipment. Microbiology experiments are well suited for undergraduate researchers. While many experiments will require students to come in 3-5 times a week, it is rare for any experiment to take more than 2 hours. Most costs with this project will be on consumable reagents such as DNA extraction kits and molecular cloning enzymes. Upgrades to our fluorescent microscope will enable higher quality experiments for this project and expand the research possibilities for other labs and classes here at Western Community College.

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only*.					
CONSULTANT COSTS					
EQUIPMENT	$500				
SUPPLIES	$8,450				
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES					
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*					
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS					

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD **$ 9,950**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Itemized Equipment List

Vortex	$500
Synthetic DNA constructs	$2500
Cloning reagents	$1000
DNA extraction kits	$800
Microscope upgrades and software	$3000
Culture reagents and supplies	$1500
New glassware	$250
Induction and 2-hybrid reagents	$400

Joshua Holmes Ph.D Curriculum Vitae

2152 Century Blvd
Rock Springs WY 82901
Email jholmes@westernwyoming.edu
Cell: 801-669-3517

Education

2017-2018	**University of Colorado Medical Campus** Postdoctoral Fellow Department of Immunology and Microbiology Laboratory of Dr. Kelly Doran	Aurora, CO
2012–2017	**University of Wyoming** Ph.D. Department of Molecular Biology Laboratory of Dr. Grant R. Bowman	Laramie, WY
2008–2012	**Utah Valley University** B.S. Biotechnology Laboratory of Dr. Heath Ogden	Orem, UT

Skills

Molecular biology: Plasmid extraction and purification, Recombinant DNA techniques, ligation, transformation, isothermal cloning, site directed mutagenesis, bacterial transfection

Microbiology: Bacterial culturing techniques with E. coli, Agrobacterium, Brucella, Shigella, Vibro, Streptococcus and Bacillus. Biosafety level 2 training

Cell culture: Growth and maintenance of human cell lines. Pathogen adherence and invasion assays. Live cell imaging, Cell lysis techniques, Immunofluorescence

Microscopy: Fluorescent microscopy, confocal microscopy, light microscopy, förster resonance energy transfer (FRET), Fluorescence Loss in Photo bleaching (FLIP), Fluorescence recovery after photo bleaching (FRAP), immunofluorescence

Protein: Protein purification, western blot, bacterial protein expression, column chromatography, ELISA, bacterial adenylate cyclase two-hybrid assay (BACTH)

Teaching Experience

2010	Tutor for School Community University Partnership (SCUP) Utah Valley University
2011	Biology 1010 Utah Valley University (Teaching assistant)
2013	MOLB 4170/5170 DNA cloning Laboratory (Lab instructor)
2013	MOLB 4260/5260 Quantitative Microscopy University of Wyoming (Teaching assistant/lab instructor)
2013-2017	Undergraduate research mentor (Numerous students)
2014	Wyoming SRAP high school internship mentor
2014	MOLB 4400/5400 Immunology University of Wyoming (Lab instructor)
2015	Wyoming SRAP high school internship mentor
2015	MICR 4321 Microbiology Capstone (Guest lecturer)
2015	Bioinformatics Boot Camp (Instructor)
2015-2017	Volunteer Undergraduate to Graduate program advisor
2016	MOLB 3000 Introduction to Molecular Biology (Teaching assistant)
2016	INBRE undergraduate mentor
2018	GEMS undergraduate reviewer
2018-Current	Assistant Professor of Microbiology Western Wyoming Community College Teaching General Biology 1010, General Microbiology 2020, and Introduction to Science Research

Research Presentations and publications

2012	National Conference on Undergraduate Research presentation at Weber State University, Ogden, Utah
2013, 2014	Molecular and Cellular Life Sciences Symposium University of Wyoming, Laramie, Wyoming
2013-2017	Rocky Mountain Branch American Society for Microbiology presentations at various locations annually 2013-2017
2014-2016	Monday molecular biology seminar series presentations at University of Wyoming, Laramie, Wyoming
2015	American Society for Microbiology tri-branch conference presentation at Ft Lewis College, Durango, Colorado

2015 Wind River Conference on Prokaryotic Biology presentation at Estes Park, Colorado 2015
2016 Wyoming INBRE conference presentation Jackson Hole, Wyoming
2017 American Society for Microbiology national conference presentation at New Orleans, Louisiana
2017 Wind River Conference on Prokaryotic Biology presentation at Estes Park, Colorado 2015
2017 Front Range Cytoskeleton presentation at Colorado University Anschutz Medical Campus, Aurora, Colorado

Peer-reviewed publications

Holmes JA, Follett SE, Wang H, Meadows CP, Varga K, Bowman GR. Caulobacter PopZ forms an intrinsically disordered hub in organizing bacterial cell poles. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(44):12490-12495. pnas.org/content/113/44/12490
*Article recommended by faculty of 1000

Liwen Deng, Brady L. Spencer, **Joshua A. Holmes**, Rong Mu, Sara Rego, Thomas A. Weston, Yoonsung Hu, Glenda F. Sanches, Sunghyun Yoon, Nogi Park, Prescilla E. Nagao, Howard F. Jenkinson, Justin A. Thornton, Keun Seok Seo, Angela H. Nobbs, Kelly S. Doran. The Group B Streptococcal surface antigen I/II protein, BspC, interacts with host vimentin to promote adherence to brain endothelium and inflammation during the pathogenesis of meningitis. PLOS Pathogens doi: 10.1371/journal.ppat.1007848 2019

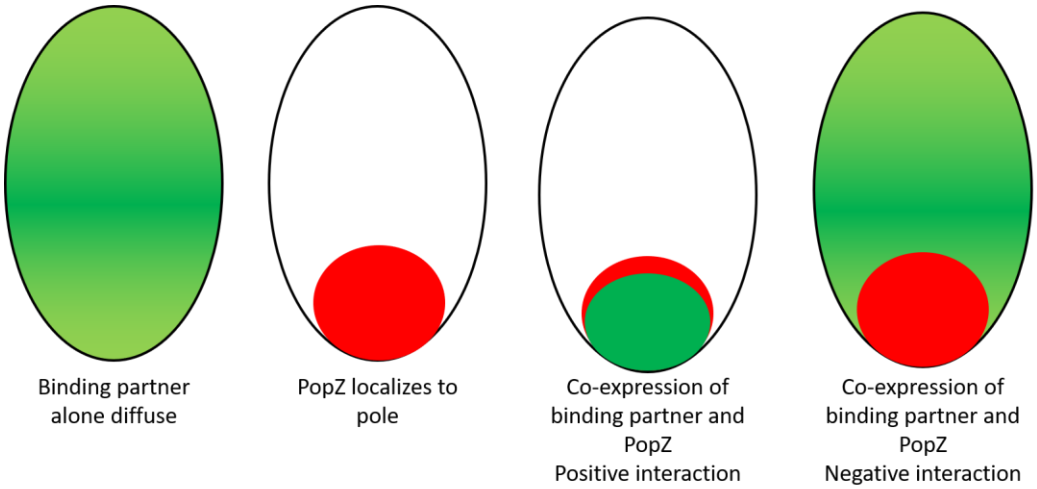
Fellowships and awards

2015 Eldon and Josephine Johnston Family Graduate Fellowship
2016 Science Initiative (SI^2) graduate teaching fellowship
2020 Scaled Participatory Research and Education Model (SPREM) “Assay development for bacterial protein-protein interaction screening” $15,200 award

Specific Goals and Research Plan

1. Specific project goals
    a. Create and test truncations of the binding partners of Polar Organizing Protein Z (PopZ), to identify binding determinates for protein-protein interactions with PopZ. The binding partners DivL and CckA will be the main focus, however this list could be expanded to all binding partners. There is also potential for testing binding partner homologues in other *alphaproteobacteria.*
    b. Optimize a fluorescent co-localization assay for detection of protein-protein interactions and subcellular localization patterns. Western Wyoming Community College recently purchased an Nikon ni U fluorescent microscope that will be used for this work.
    c. Teach students the theory and practice of using a research fluorescent microscope.
    d. Develop a standard protocol for fluorescent microscopy techniques as well as methods of quantification of microscopy images.

2. Achieving goals
    a. Truncations of binding partners will be primarily done using PCR reactions to remove specific sections of DNA. This will result in specific amino acids being cut from that binding partner. These constructs will be cloned into the inducible plasmids pBAD and pACYC. These two plasmids can be independently controlled using arabinose for pBAD and IPTG for pACYC. If these truncations are too difficult to create via PCR, synthetic DNA sequences will be used instead. Synthetic sequences will primarily be used for any gene coming from pathogenic bacteria.
    b. Testing of protein-protein interactions will be done using a fluorescent microscopy co-localization assay. This assay works based upon the ability of PopZ to localize to the cell pole in our living test tube of *E. coli*. PopZ is fused to the protein mCherry and binding partners are fused to msfGFP. If a protein interacts with PopZ it will co-localize with PopZ at the cell pole. This will show the red foci of PopZ co-localizing with the green foci of the binding partner. If a Binding partner does not, it will remain diffuse which will be shown by msfGFP being diffuse through the entire bacterial cytoplasm. See diagram below for a visual graphic showing this.



c. Truncations that result in loss of PopZ binding will be further tested. These truncated regions alone will be used to test if this region is sufficient for interaction with PopZ. These truncations will be fused with msfGFP to be tested in our co-localization *E. coli* assay
d. These interactions will all be quantified using fluorescence intensity measurements using Nikon microscopy software analysis tools.
e. A secondary in vitro method of testing these interactions will be done using the bacterial adenylate cyclase two-hybrid system we developed for use at Western Wyoming Community College.

3. Enhance teaching and training of students pursuing biomedical-related degrees
    a. In order to accomplish the goals of this project students will have to learn many basic and advanced molecular biology skills. Basic skills will be learned such as bacterial culture techniques, aseptic technique and DNA extraction and purification. Advanced techniques such as molecular cloning, PCR primer design and fluorescent microscope skills will be taught. Students will be introduction to reviewing primary literature as well as navigating the NCBI website to utilize DNA and protein alignment tools. Rigorous quantification will be taught for data analysis. This work will be presented to the scientific community by students working on this project yearly.
    b. These techniques will be useful for students that pursue an education and career in the biomedical sciences. Many of the skills learned are universal and can be applied to a wide range of fields. This will allow students to be better prepared and have a larger toolbox of skills starting their various careers. This will allow students joining new research labs to jump into their research faster. Students will also be more competitive for securing research positions in nearly any biomedical lab.

4. How this project will enhance the preparation and number of students transferring to the University of Wyoming
    a. Students coming from community colleges can be at a disadvantage for biomedical careers. This is due to losing out on two years of research experience. This grant will enable students to do research and gain core laboratory skills during their first two years of college. This experience will allow students transferring to UW to jump right into nearly any lab at UW. This will increase the likely hood of them succeeding in both coursework and labs. Students will develop public speaking skills through presenting research at scientific conferences. These skills will be important for anyone pursuing a career in the biomedical sciences. Through the INBRE network students will have the chance to meet and talk to potential labs at University of Wyoming and other states participating in INBRE.

5. How the project is related to prior funding
    a. This project is independent from other projects funded by INBRE

6. How the project will be sustained
    a. This funds from this proposal are intended to purchase equipment and reagents necessary to complete this project. With support from the Division of Math and Sciences and other collaborators will be able to continue this work for years to come.

7. Planned collaborations
    a. We foresee a productive collaboration with Dr. Grant Bowmans lab at UW.

Institutional Support Letter

As Math and Science School Chair at Western Wyoming Community College, I fully support the application for SPREM Grant funding for the laboratory of Dr. Josh Holmes. Dr. Holmes is doing exciting microbiology research and is working with two students in his lab. He continues to build on his research work at Western and his involvement with INBRE. I look forward to seeing that continue.

I approve of Dr. Holmes' proposed budget and feel that the request is reasonable. Western Wyoming Community College will not be able to provide matching funds for this proposal; however, Western's commitment to INBRE research is significant. Dr. Holmes has dedicated research space, which is now a suitable INBRE research lab, thanks to INBRE funding. While all equipment in the lab is INBRE funded, Western provides utilities and basic lab supplies, and supports/maintains the equipment. The college asks for no indirect funding from INBRE. Without this institutional support, INBRE research at Western would not be possible.

Thank you for your consideration of Dr. Holmes' SPREM Grant proposal. It is with highest confidence and enthusiasm that I support the proposal and encourage full funding of the request.

Sincerely,

Sarah Pauley
Chair, School of Math and Science
Western Wyoming Community College

No IACUC materials are required for this project