1: Project Overview

The goal of this project is two fold (1) to identify the pH sensitive residues in *E. coli* mechanosensitive channel of small conductance (MscS) that are responsible for the rapid closing of the channel and (2) to train undergraduate students as biophysical researchers in experimental design and execution and presenting at a conference. The identification of the pH sensor in MscS will allow us to pursue using MscS as a novel antibiotic target for infectious bacterial strains.

2: Project Description

*Project Plan:* **General ion channels:** Ion channels are found in all cells, from neurons in the brain to small bacterial cells like *E. coli*. They are responsible for opening small holes in the cell membrane that allow water, ions, and small molecules to flow from one side of the membrane, or shell, to the other. One large class of ion channels is mechanosensitive ion channels; these channels respond to touch or applied pressure. In humans, these channels open in response to physical contact as well as constant pressure from material objects such as clothing. Bacteria have similar mechanosensitive channels; however these channels sense environmental pressure instead of physical contact. Bacterial mechanosensitive channels function as pressure relief valves, opening in the event of high pressure inside the cell that would lead to cell death if not relieved (Figure 1).

This process is similar to the filling of a water balloon; as water enters the balloon the balloon swells if too much water is added to the water balloon, and then it pops. If perhaps, a small hole or channel was in the water balloon itself then a small amount of water would be expelled from the water balloon prior to the balloon bursting.
exploding. In an ideal world the small hole would close after releasing the proper amount of water allowing the water balloon to survive.

**Bacterial mechanosensitive channels:** Mechanosensitive channels function as these pores (ie. small holes) in the cell membrane and allow water, ions, and small molecules out of the cell to prevent the cell from exploding and closing after reducing the pressure in the cell. In *E. coli*, the mechanosensitive channel of small conductance, called MscS, is analogous to the small pore in a water balloon that opens in response to pressure and closes when that pressure has been relieved.

**E. coli MscS:** In the early studies of *E. coli* MscS it was noted that at physiological pH (7.5, neutral) MscS does not rapidly close, however at lower pHs (6, slightly acidic) MscS rapidly closes (Figure 2). Some researchers attributed this rapid closing to a small cluster of unknown amino acids that sense the pH of the environment. To identify the pH sensor we will (1) determine if the pH sensor is on the inside or outside of the cell by changing the pH of the buffers on either side of the cell membrane and seeing if the rapid closing is observed (2) using the published structures and sequence we will identify regions of the protein that are similar to known pH sensors (3) alter these regions to non-pH sensitive regions and see if the rapid closing of the channel is abolished in patch clamp electrophysiology. This approach will allow for the identification of the pH sensor in MscS, which is essential for the creation of specific antibiotics for bacteria expressing this channel such as *E. coli*, which can cause food poisoning or bacteria expressing similar channels. UNF undergraduate students in the Malcolm Lab will conduct all of these experiments with guidance from Dr. Malcolm.

**Long-range goals:** In recent years a significant number of antibiotic resistant bacteria have been identified, new methods for killing these bacteria are essential. MscS-like channels are found in all bacteria, but not in humans, so understanding their function is critical for utilizing these
channels as antibiotic target. The lack of MscS-like channels in humans suggests that when these channels are targeted for cell death they can be removed precisely, where current broad-spectrum antibiotics kill all bacteria indiscriminately. Understanding channel function will direct the future research into using these channels as a target.

**Potential Impact:** The identification of the pH sensor in MscS will allow scientists to utilize MscS as a novel antibiotic or therapeutic target. MscS and MscS-like channels present a unique target as they are found in all bacterial strains, but not in humans. Some antibiotics kill bacteria by preventing cell growth, these antibiotics would cause the cell to explode, which causes cell death. A greater understanding of exactly how and when MscS opens and closes will allow us to better attack infectious bacteria.

**Benefit for UNF Students:** This fellowship will provide reagents for the research conducted by students in my research group (currently 2 students). These students will conduct the majority of the experiments proposed. Additionally, the DLC fellowship will allow me to send these two students to the Gordon Research Conference: Ion Channels to present a poster. This conference is an intimate gathering of the leading scientists in the field, held at Mount Holyoke College in July of 2018. Students will have personal interactions these leading scientists and exposure to with to the forefront of biophysical research, which will encourage them to continue in science as a post-graduate.

**Benefit to scholarly agenda:** The DLC Faculty Fellowship course release would allow me to train my research students in electrophysiology and help identify the pH sensor in MscS. This research will be the basis for a publication that will be submitted to a high impact journal in late 2018. This fellowship will support preliminary research that will be used as the foundation of a grant submitted to the National Institute of Health (NIH) for external funding of my lab.

**3: Award History:**
I have not been awarded a DLC Faculty Fellowship.

**4: Project Budget**

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<tr>
<th>Item</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Electrophysiology Consumables (reagents, DNA oligos, detergents, plastics)</td>
<td>$2000</td>
</tr>
<tr>
<td>Student Travel to Gordon Research Conference: Ion Channels (for 2 students)</td>
<td>$2000</td>
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**Total:** $4000

Other support sources: I currently have start-up funds from Academic Affairs for the starting of my research group, no additional funds will be required for this project.
Hannah R. Malcolm, Ph.D.
University of North Florida, Department of Chemistry
Building 50/3534, 1 UNF Drive
Jacksonville, FL 32224
Office Phone: (904) 620-1963, Fax: (904) 620-3535, Mobile Phone: (979) 324-9916
Email: hannah.malcolm@unf.edu

Education
Washington University in St. Louis Department of Chemistry, St. Louis, MO
Ph.D. in Chemistry, Defended May 2012, Advisor: Joshua A. Maurer, Ph.D.
Thesis Title: Understanding the structure-function relationship in the Mechanosensitive Channel of Small Conductance (MscS) and Bacterial Cyclic Nucleotide Gated (bCNG) Ion Channels

Texas A&M University Department of Chemistry, College Station, Texas.
B.S. in Chemistry, Graduated May 2006

Professional Experience/Appointments
2015- present Assistant Professor of Chemistry, University of North Florida, Jacksonville, Florida
2012-2015 Postdoctoral Researcher, University of Texas Southwestern Medical Center, Department of Physiology, Dallas, Texas
2006-2012 Graduate Student, Washington University in St. Louis, Department of Chemistry, St. Louis, Missouri
2002-2006 Research Technician, Advisor: Susan S. Golden, Department of Biology, Texas A&M University, College Station, Texas

Teaching Experience at University of North Florida
General Chemistry I (CHM2045)
General Chemistry I Laboratory (CHM2045L)
Biochemistry II (BCH4034)
Biochemistry I Laboratory (BCH4033L)

Research Interests at University of North Florida
Undergraduate research studying how bacteria sense their environment, specifically bacteria with ion channels that are members of the MscS superfamily of channels. To study how bacteria sense their environment we will utilize patch clamp electrophysiology, in vivo cell based assays, and molecular cloning to test our hypothesis. To understand how the bacterium employs these channels during different growth conditions we will utilize RT-PCR.

Current Research Collaborators
**Undergraduate Research Students at University of North Florida**

- Jordyn Veres, Spring 2016-Present
- Maggie McGovern, Spring 2017- Present
- Amanda Southall, Spring 2017-Present
- Carly Mills, Summer 2016- Spring 2017
- Jada Clinton, Fall 2016-Spring 2017
- Casey Lanier, Fall 2016

**Professional Activities and Service at the University of North Florida**

- Jacksonville American Chemical Society Local Section: Chair-Elect, 2017
- ACS Award Coordinator, 2016-2017
- Senior Seminar Mentor
- UNF Natural Sciences Poster Session Judge, 2015, 2016
- Grant Reviewer for Research Councils UK, Biotechnology and Biological Sciences Research Council, 2015

**Selected Publications (Corresponding authors are underlined)**


**Selected Presentations**


3- Malcolm, H.R., October 2015. Exploring the Molecular Motions in the MscS Superfamily of Ion Channels, Jacksonville, FL. Department of Biology, University of North Florida, (talk)