Betty A. and Donald J. Baumann Family Scholarship Fund Application Form

1. **Name and NetID**
   Rhiannon McCracken
   rbm71897

2. **Chemistry faculty research director**
   Dr. Juliane Soukup

3. **Proposal title**
   Structural and Functional Analysis of *Crassostrea gigas* OAZ-PK RNA

4. **Proposal description. Please limit the proposal to about 500 words and include figures as appropriate. Your proposal should briefly outline the overall project and its goal(s). If you have previous results related to your proposed project, concisely summarize those results and describe what you expect to accomplish during the time frame of the scholarship.**
   Please see proposal below!

5. **Presentation of research results (past and future conferences, publications, seminars, etc.)**
   Past Presentations
   1. 2022 ACS Midwest Regional Meeting: October 19-21, 2022 (Iowa City, Iowa)
   2. Rustbelt RNA Meeting 2022: October 14-15, 2022 (Cleveland, Ohio)
      ***OUTSTANDING UNDERGRADUATE POSTER PRESENTATION AWARD**
   3. 20th Annual NE-INBRE Conference: August 7-9, 2022 (Nebraska City, Nebraska)
      ***AWARDED FIRST PLACE FOR POSTER PRESENTATION**
   4. 27th Annual Meeting of the RNA Society: May 31-June 4, 2022 (Boulder, Colorado)
   5. Nebraska Academy of Sciences Annual Meeting: April 22, 2022 (Lincoln, Nebraska)
   6. Creighton University Research Week: April 19-20, 2022 (Omaha, Nebraska)
   7. 45th West Coast Biological Sciences Undergraduate Research Conference: April 9, 2022 (San Diego, California)
   8. Inaugural BIG EAST Undergraduate Research Poster Symposium: March 12, 2022 (New York City, New York)
   9. Twelfth Annual Conference for Undergraduate Women in Physical Sciences: October 21-23, 2021 (Lincoln, Nebraska)
      ***BEST POSTER AWARD**
   10. NE-INBRE Annual Conference: August 9-11, 2021 (Virtual)
   11. 2021 IDeA Central Region "Zoom" Conference: July 26-27, 2021 (Virtual)
   12. Creighton University Research Week: April 19-23, 2021 (Virtual)

Future Presentations

1. Fusion 7th Nucleic Acids Conference: February 9-12, 2023 (Cancun, Mexico). Abstract accepted.
2. CURAS Research and Scholarship Fair (Spring 2023)
3. Creighton University Research Week (Spring 2023)
4. Nebraska Academy of Sciences Annual Meeting (Spring 2023)

6. **Post-graduate plans (job market, graduate school, medical school, etc.)**
   I am planning on attending graduate school and pursuing a Ph.D. in biochemistry next year.

7. **Number of semesters involved in research, including current semester (summers count as two semesters)**
   12 semesters

8. **Anticipated graduation date**
   May 2023

Structural and Functional Analysis of *Crassostrea gigas* OAZ-PK RNA

Introduction

Riboswitches are non-coding RNA sequences that regulate downstream gene expression when bound to a metabolite. In order for an RNA to be classified as a riboswitch, it must have three key features: binding specificity, induced structural change, and modified gene expression.[1] Over forty classes of riboswitches have been identified in prokaryotes, but none have been found in higher eukaryotic organisms.[1,2,3]

My project focuses on studying a potential eukaryotic riboswitch in the Ornithine Decarboxylase Antizyme pseudoknot (OAZ-PK) RNA.[4,5] More specifically, I am analyzing the structural and gene expression changes of this segment of RNA in *Crassostrea gigas*, a species of oyster, when it interacts with various concentrations of natural and non-natural polyamine metabolites (Figure 1). One outcome of this project is identifying OAZ-PK RNA as a riboswitch, which could provide a new target for developing non-natural ligands that can bind to the RNA and ultimately disrupt its gene expression. This would open up possibilities for novel antibiological agents.

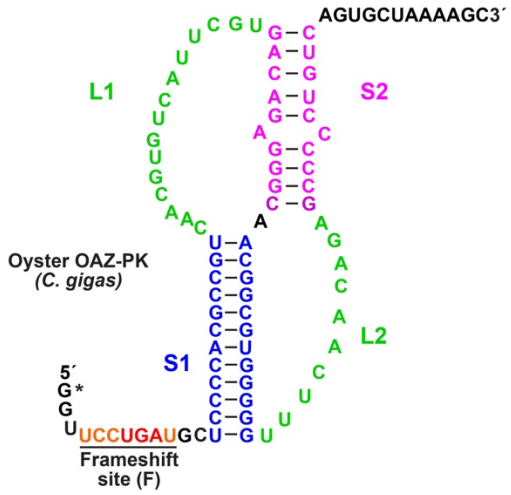

Figure 1. Secondary structure of *Crassostrea gigas* OAZ-PK RNA. Stem (S) and loop (L) regions are identified.

Preliminary Results

The primary technique I use in my project is in-line probing (ILP). This assay allows me to analyze the conformational changes a sequence of RNA undergoes when it is incubated with various concentrations of natural and non-natural polyamines under slightly basic conditions.[6,7] My in-line probing results so far suggest that the OAZ-PK RNA in oyster undergoes conformational changes in the presence of the natural polyamine spermine (Figures 2A and 2B). Various bands on the ILP gels change intensity when the concentration of spermine is altered, indicating that the RNA is changing shape upon ligand binding. These structural changes do not happen when other polyamines are introduced.

I am also using a Dual Luciferase Reporter Assay (DLRA) to study whether the binding of a ligand causes a frameshift in oyster OAZ-PK RNA and if this frameshift alters downstream gene expression (Promega). Preliminary results show that the presence of spermine causes a 2.1 fold increase in the luciferase ratio (Figure 2C). This means that a change in downstream gene expression is occurring. These results are promising because induced structural changes and altered gene expression are two requirements for OAZ RNA to be classified as a riboswitch.

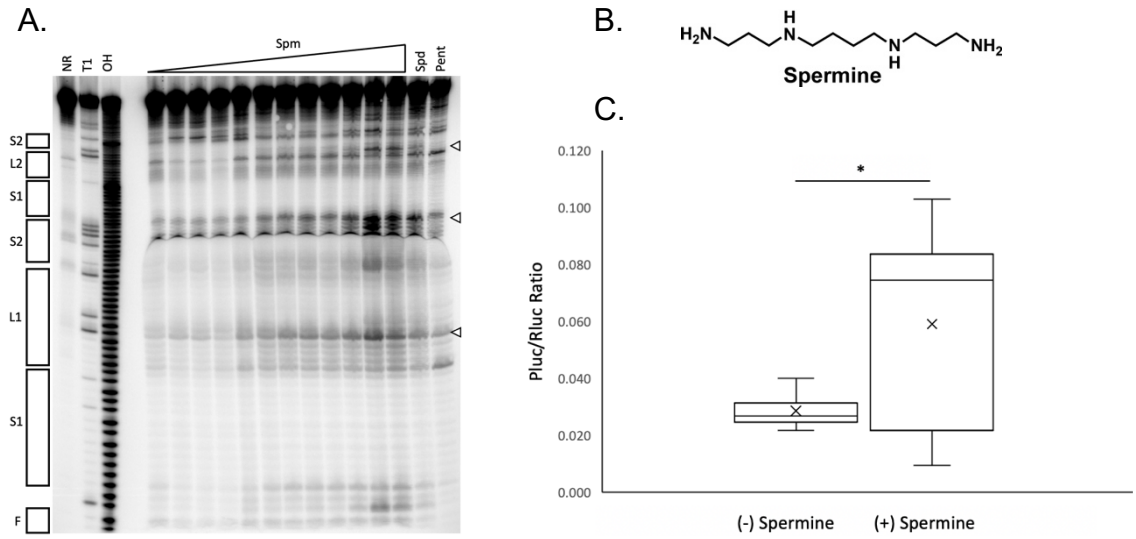

Figure 3. (A) ILP gel result that contains OAZ-PK RNA that was unreacted (NR), partially digested with RNase T1 (T1), or underwent OH cleavage (OH). The spermine (Spm) concentration ranged from 0-1mM. Two additional 1mM polyamine lanes contained spermidine (Spd) and pentamine (Pent). Some of the spermine induced structural changes are indicated by the white arrowheads. Stem (S) , loop (L), and frameshift (F) regions are identified. (B) Structure of the natural polyamine spermine. (C) DLRA results. * Indicates a $p<0.05$.

Future Research

During the time frame of this scholarship, I plan to continue performing the in-line probing and Dual Luciferase Reporter Assays with natural and non-natural polyamines. In addition, the ILP images will be quantitated using ImageQuant in order to determine apparent K_d values for OAZ-PK RNA binding to polyamines. These two techniques will allow me to further validate that oyster OAZ RNA is a potential eukaryotic riboswitch that undergoes conformational changes and has altered gene expression in the presence of spermine.

References

1. Barrick, JE & Breaker, RR. *Genome Biol.* **8**, R329 (2007).
2. Wachter, A. *RNA Biol*. **7**:1, 67-76 (2010).
3. McCown, PJ., Corbino, KA., Stav, S., Sherlock, ME., Breaker, RR. *RNA*. **23**(7):995-1011 (2017).
4. Ivanov, IP & Atkins, JF. *Nucleic Acids Res*. **35**, 1842-1858 (2007).
5. Pegg, AE & McCann, PP. *Am. J. Physiol.* **243**, C212-C221 (1982).
6. Breaker, RR & Regulski, EE. *Methods Mol Biol.* **419**, 53-67 (2008).
7. Mandal, M & Breaker, RR. *Nat Rev Mol Cell Biol.* **5**, 451-63 (2004).