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

1. Name and NetID

   Zach Frevert – ZBF63851

2. Chemistry Faculty Research Director

   Dr. Julie Soukup

3. Title: Characterization of Ornithine Decarboxylase Antizyme RNA Structure and Function

4. Proposal:

   Riboswitches are non-coding segments of messenger RNA that directly bind to metabolites and alter gene expression. Commonly found in bacteria, the Soukup lab has proposed a novel riboswitch in mice with a homologous sequence in humans. We propose riboswitch functionality of a translational frame shift in the ornithine decarboxylase antizyme (OAZ) pseudoknot RNA (PK RNA). OAZ is highly conserved amongst vertebrates and plays a major role in the regulation of polyamine biosynthesis. Polyamines serve a variety of roles in the cellular environment such as nucleic acid stabilization and intracellular signaling; disruption of this pathway would likely lead to activation of apoptotic mechanisms, and could serve as a potential target for antibacterial or antineoplastic purposes.

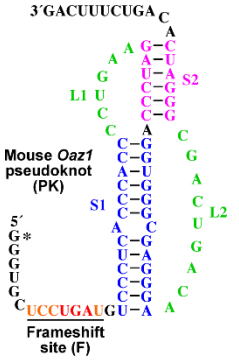

Figure 1. Riboswitch sequence in proposed 2D structure.

   Previous studies in lab have determined apparent binding affinity and specificity for a variety of polyamines using in-line probing (ILP) and equilibrium dialysis (EQ). EQ has shown that spermine has a higher affinity for the PK RNA; and ILP has demonstrated that a conformational change occurs based upon spermine binding. These two effects are important indicators of riboswitch functionality: the specificity prevents other molecules from affecting the system and the conformational change enables regulation of translation by changing availability of ribosomal binding sites.

Isothermal Titration Calorimetry (ITC) is now being used to determine binding affinities of a variety of polyamines, both natural and synthetic (see Figure 1), to help elucidate the mechanism by which these molecules interact with the RNA. ITC provides a range of thermodynamic values, but the value of note is K_d, the dissociation constant. K_d is the concentration of ligand at which half of available binding sites are occupied; lower K_d (nanomolar/picomolar) indicate strong binding, whereas higher K_d (micromolar/millimolar) indicate weaker interaction because more ligand is necessary to fill binding sites. So far K_ds have been determined for Spermine, Spermidine, and analogs A-C, with the values 250 µM, 500 µM, 2 mM, 30 µM, and 15 µM respectively. Further ITC studies are to be performed using other polyamines such as putrescine, cadaverine, and ornithine.

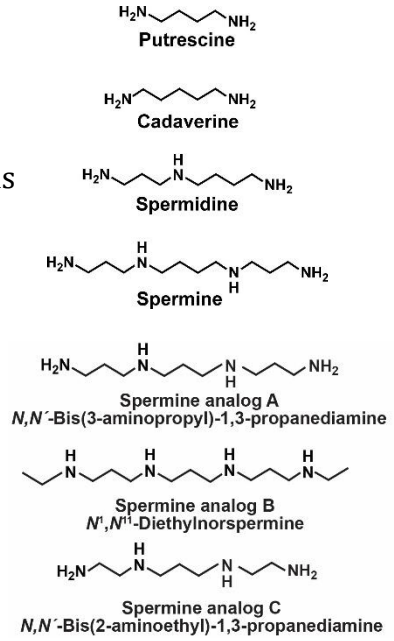

Figure 2. Name and Structure of ligands of interest.

Current studies also include a collaboration with the Borgstahl laboratory at UNMC. The Borgstahl lab focuses on the crystallization of biological molecules in order to determine three dimensional structure using x-ray crystallography. RNA crystallography is not a well-established field, and the flexibility of RNA can make crystallization difficult. Currently large scale screens in which crystals are attempted to be grown in a variety (~400) of solutions are necessary to determine conditions which can promote crystal formation. Further refinement of promising conditions will theoretically lead to crystal growth to the size necessary to perform x-ray scattering on the putative riboswitch to elucidate the structure of the spermine-PK RNA complex.

5. Presentation of research results (past and future conferences, publications, seminars, etc.)

   INBRE Conference 2017,2018; Nebraska Academy of Sciences 2018,2019; Creighton St. Albert's Day 2018,2019

6. Post-graduate plans (job market, graduate school, medical school, etc.)

   Graduate School for PhD in Biochemistry

7. Number of semesters completed in research, including the current semester (summers count as two semesters).

   11 Semesters (3 Summers, 5 Semesters)

8. Anticipated graduation date:

May 2019

Applicant signature

Chemistry research director's signature

Zach Frevert