The production of nucleotides by all cells and tissues is tightly controlled to maintain normal metabolic homeostasis, and the biosynthesis of these critical biomolecules must be adjusted to adapt to various environmental conditions cells in the body routinely encounter. The Cunningham lab focuses on one pair of enzymes, called the phosphoribosyl pyrophosphate synthetases (PRPS), that are the chief regulators of this vital process and whose deregulation is a major contributor to human disease. Mutations that activate one isoform of these enzymes, PRPS1, cause a rare inherited metabolic disorder that manifests in phenotypes resembling gout and these same mutations have also been found to drive chemotherapy resistance in a subset of patients with B-cell acute lymphoblastic leukemia – the most common childhood cancer. Therefore, identifying strategies to suppress the activity of this enzyme are likely to have a significant impact on human health. We believe that we have identified one such strategy that we hope to ultimately exploit for therapeutic benefit. Notably, one important feature of the PRPS enzyme is that it must assemble into a hexameric configuration in order to possess catalytic activity. This means that cellular concentrations of PRPS1 protein must reach a critical ‘threshold’ in order for the higher order enzyme complex to form and become active. Thus, to suppress enzyme activity, all that is necessary is to limit the expression of PRPS1. We have discovered that cells possess a built-in evolutionarily-conserved mechanism that achieves this. Specifically, we have identified a cis-regulatory element within the 5’ untranslated region of the PRPS1-encoding mRNA (the transcript of the PRPS1 gene) that has key features of a protein-coding sequence (e.g. it possesses both a START and STOP codon). These mysterious cis-regulatory elements, termed upstream open reading frames (uORFs), typically suppress translation of the normal (in this case PRPS1-encoding) open reading frame that is positioned downstream. We also have evidence that the ribosome, the cellular machinery that translates PRPS1 mRNA into PRPS1 protein, can occupy
this upstream position. What remains unknown is how, and to what degree, this uORF regulates PRPS1 enzyme expression. The major goal of the proposed WISE project will be to better understand the functionality of this form of regulating PRPS1 expression so that we may use it to suppress the unrestrained nucleotide production caused by PRPS1 superactivity. The WISE student will use an array of CRISPR/Cas9-based genome-editing approaches to specifically mutate the uORF- or PRPS1-encoding sequences in cultured cells followed by western blotting detection methods to assess uORF-dependent control of PRPS1 expression. Additionally, the student will perform in vitro assays to assess the mechanism of uORF-mediated translational suppression. If time permits, we will perform proof-of-concept studies to demonstrate that exacerbating uORF-mediated suppression of PRPS1 mRNA translation limits nucleotide production and ameliorates uric acid output – the culprit responsible for Gout-like symptoms.