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# Toward Treatment of Rocky Mountain Spotted Fever

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**Toward Treatment of Rocky Mountain Spotted Fever**
**A Proposal for the Research and Creative Experience for Undergraduates (RCEU) Program, Summer 2015**

## Faculty Mentor:

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## Project Summary:

Bacterial peptidyl-tRNA hydrolase (Pth1) is an essential enzyme that is responsible for recycling peptidyl-tRNA generated from protein biosynthesis. It is highly conserved in bacteria yet has no essential human homolog. Thus Pth1 is a promising candidate for antibiotic development. To further antibiotic development against this promising new target, we will express the Pth1 from a phylogenetically distinct bacterial species, *R. ricksettii*. We will characterize natural products that inhibit its activity and also try to crystallize this protein and determine its high resolution structure. This project fits into our group's work on Pth1, adding an unusual enzyme to our arsenal of Pth1s under study. Being phylogenetically distinct, Pth1 from *R. ricksettii* will provide a better idea of how structurally different Pth1s can be. To date, only high resolution structures from *E. coli*-like Pth1s have been reported. Also, screening of natural products may uncover novel lead compounds that will be effective against *R. ricksettii*, the cause of Rocky Mountain spotted fever. Current antibiotics are losing efficacy towards this debilitating disease. Lastly, having another Pth1 enzyme will allow for better characterization of small molecule selectivity against bacterial Pth1. That will contribute to understanding the potential for broad range inhibition as well as narrow spectrum and even species specific inhibition.

## Student Duties:

### *Express and Purify R. ricksettii Pth1.*

Pth1 from *R. ricksettii* has already been synthesized and cloned into a bacterial expression vector. The RCEU student will transform W311 bacteria with the *R. ricksettii* expression Pth1 plasmid and determine conditions for soluble expression. We anticipate it will be similar to our other Pth1 constructs, expressed at 30 °C using 0.5 mM IPTG added and an $OD_{600}$ of 0.8. The protein will then be purified by metal chelation chromatography. Once recombinant protein is in hand, the RCEU student will screen natural products for inhibitory activity using an assay previously developed in our lab. The RCEU student will also try to crystallize *R. ricksettii* Pth1 using standard vapor diffusion techniques.

### *Inhibitor Screening.*

The activity of Pth1 can be monitored using a gel shift assay. The migration of the substrate, peptidyl-tRNA, is tracked using methylene blue staining. The gels are imaged and ImageJ software is used to analyze the migration. Cleavage is quantitated by comparing to uncleaved and completely cleaved controls. We can use this assay to screen natural products for inhibitory activity, as we have previously reported. We will use a library of aquatic fungal extracts provided by a collaborator. The RCEU student will, under supervision of Dr. McFeeters, run extensive screening and quantitation of natural product inhibiton. The data from this Pth1 will be compared to other Pth1 data already in hand.

***Manuscript Preparation.***
All undergraduate student researchers are encouraged to write up their results in the form of a manuscript for publication. Under the supervision of Dr. McFeeters, the RCEU student will help prepare the manuscript, which may include data from other undergraduate or graduate students.

***Expected Results and Deliverables.***
It is expected that this project will produce Pth1 from *R. ricksettii*. Also, the screening results from inhibitor activity of aquatic fungal extracts will provide information about how Pth1 from *R. ricksettii* compares to Pth1 from other species. It is also expected that crystallization conditions will be determined for *R. ricksettii* Pth1, x-ray diffraction data collected, and a high resolution structure calculated, though potentially as an extension of the RCEU summer project (i.e. at a later date).

***Benefits to the Student.***
The RCEU student will gain experience with routine biochemistry laboratory techniques well beyond any laboratory course. The student will also gain experience with protein crystallization and the specialized equipment and machinery that goes along with gathering data. It is expected that the student will join the mentor in a trip Argonne National Laboratory to collect diffraction data at the synchrotron source. Thus the student will be exposed to a non-academic working environment, further contributing to their experience and career development.

**Mentor Supervision and Interaction:** All protein expression and purification will be supervised by Dr. Robert McFeeters and his senior Research Associate. Inhibitory screening will be conducted more independently, with analysis supervision from a graduate student in the lab. The RCEU student will participate in weekly lab meeting and will regularly present research results. The RCEU student will be responsible for manuscript preparation that will be supervised by Dr. Robert McFeeters. The student will present the research work as a poster as well.