Research Background: Combating drug resistance has become a wide spread topic of study as more pathogens are becoming resistant to current therapies. In *Mycobacterium tuberculosis* (*Mtb*), the infective agent in tuberculosis (TB), resistance continues to rise and lead to 1.4 million deaths worldwide in 2010.[1] Previous work focusing on understanding and overcoming resistance in extensively drug-resistant TB (XDR-TB) in the Garneau-Tsodikova laboratory has shown the unique aminoglycoside acetyltransferase Eis (enhanced intracellular survival) protein to inactivate a wide range of aminoglycosides through a novel multi-acetylation mechanism.[2]

Part of my thesis research focuses on screening and evaluating Eis inhibitors that could stop the action of the Eis resistance enzyme and allow for use of the currently available anti-TB drugs, kanamycin and amikacin, that Eis inactivates. We have used high-throughput screening (HTS) to identify inhibitors of Eis. To date, out of 123,000 compounds screened, we have identified and confirmed >50 inhibitor scaffolds that show inhibition (nM to low μM) against Eis. The breadth of data obtained provides a great opportunity for successful crystallization of Eis-inhibitor complexes. Visualizing how these inhibitors bind to Eis will provide valuable information for the improvement and design of more potent, novel inhibitors *via* medicinal chemistry.

Proposed Sabbatical: For my sabbatical, I would like to work in our collaborator's laboratory (Oleg V. Tsodikov, University of Michigan) to gain knowledge, skills, and expertise in determination of protein structures by X-ray crystallography. This area of study is new to me and would make me a more well-rounded chemical biologist. This valuable experience would also allow me to propel the development of Eis inhibitors forward. What stimulated my interest in pursuit of expanding my biochemical knowledge to structural biology is the work that I have done so far with the identified Eis inhibitors. Performing docking studies of these inhibitors to Eis and structure alignments of *Mtb* Eis with its homologs from other bacteria, using techniques previously foreign to me to delve into structural details, has really piqued my curiosity to learn X-ray crystallography. The Tsodikov and Garneau-Tsodikova laboratories have collaborated to determine the first crystal structure of Eis[2] that I will use for molecular replacement to solve the structures of my Eis-inhibitor complexes. Previous students in the Garneau-Tsodikova laboratory have learned how to set and grow crystals. However, all previous processing of crystals and structure determination has been done in the Tsodikov laboratory. Therefore, by completing this sabbatical I would be the first student from our laboratory to set trays, grow, optimize, and harvest crystals, and determine structures, thereby bringing valuable knowledge back to our group that I could pass on to future students.

The Specific Aims of this project are to (1) learn how to grow, optimize, harvest, and process crystals of Eis-inhibitor complexes, (2) collect diffraction data at the Advanced Photon Source at the Argonne National Laboratory (Argonne, IL), (3) process the collected diffraction data to determine the structure of Eis-inhibitor complexes. From previous experience with Eis crystals, the Tsodikov laboratory has suggested a sabbatical broken into several periods. The growth of Eis crystals usually takes 1-2 months such that I could begin the growth of crystals and resume my sabbatical at the point where crystals have been obtained to work on subsequent Aims (2-3). With Eis-inhibitor complex structures, we will be able to perform further medicinal chemistry studies to improve the activity and properties of the inhibitors. The discovery and thorough understanding of Eis inhibitors is a medically relevant topic that will allow me to further my knowledge of chemical biology and one of its integral techniques, X-ray crystallography.

Reference cited:

1. World Health Organization. http://www.who.int/mediacentre/factsheets/fs104/en/ (accessed May 21, 2012).
2. Chen, W., Biswas, T., Porter, V. R., Tsodikov, O. V., & Garneau-Tsodikova, S. (2011). Unusual regioversatility of acetyltransferase Eis, a cause of drug resistance in XDR-TB. *Proc. Nat. Acad. Sci. U.S.A.*, *108*, 9804-9808.
3. Green, K. D.; Chen, W.; Garneau-Tsodikova, S. (2012). Identification and characterization of inhibitors of the aminoglycoside resistance acetyltransferase Eis from *Mycobacterium tuberculosis*. *ChemMedChem*, *7*, 73-77.

Note to Committee: I realize that the specifications for the student sabbatical say that it is preferred to have a sabbatical outside of the University of Michigan. If this sabbatical does not appeal to the committee, as it is with a very closely related laboratory, I am also very interested in working with Dr. James E. Posey, one of our collaborators located at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. As XDR-*Mtb* strains are BSL3, we do not test our inhibitors against these strains at the University of Michigan. Dr. Posey who has access to BSL3 facilities for TB tests the Eis inhibitors discovered by the Garneau-Tsodikova laboratory on *Mtb* strains, including a strain with an upregulation of Eis that results in extensively drug-resistance (XDR) in this organism. Learning the assays done in this facility as well as the techniques involved in working with BSL3 bacterial cell cultures would be a valuable experience for me as I aspire to have an academic career. Obtaining the experience of working with an expert in the TB field would allow me to pursue the use of *Mtb* in my own research when obtaining an academic position. If this proposal is more appealing to the committee, I would gladly re-write this sabbatical plan.