

I hope to pursue Ph. D. studies in Experimental Medicine with Dr. Juergen Kast in his study of causes and treatments for cardiovascular disease through examination of relationships between platelets, monocytes, and the endothelium. I believe that I offer a solid analytical foundation that would compliment Juergen's research group. The aim of my research would be to develop new mass spectrometry tools to better understand fundamental cellular processes and investigate molecular mechanisms and relationships with the underlying aim of developing therapeutic treatments. By tracking post translational modifications (PTM's) we hope to understand underlying processes that lead to and test treatments for, the number one cause of death in the Western world, cardiovascular disease.

Mass spectrometry has been a tremendous tool for analyzing protein expression in cells and defining structure- function relationships in proteomics. We hope to develop two distinct but intimately related areas of mass spectrometry: A.) Multiplexed mass spectrometry; Using multiplexed methods and post acquisition data analysis to dramatically increase the MS duty cycle to near 100%, allowing the possibility of analyzing transient, sub-stoichiometric and time/ location specific PTM's. B.) Alternative modes of fragmentation: It has been found that different modes of fragmentation produce different fragmentation patterns in proteins. As PTM's can be identified through fragmentation patterns, different patterns may reveal potentially useful information about complex biochemical systems. Last summer at the American Society for Mass Spectrometry conference, Juergen, Dr. Damon Robb (Blades research group of Chemistry) and I discussed in detail collaborating on these projects. We've mapped out how to investigate atmospheric pressure ionization - electron capture ionization, a technique developed at UBC, for use with biological samples.

Binding of platelets to the endothelium leads to inflammation resulting in cardiovascular disease. Using mass spectrometry we hope to understand the activation of platelets and expression of adhesion molecules. Currently, tools using collision induced dissociation (CID) give an incomplete picture of the interactions of GTPase Cdc42 and integrin beta 1 that lead to platelet adhesion. We hope to develop electron capture dissociation (ECD) as a compliment to CID to more fully analyze the products of formaldehyde cross-linking to identify of specific mechanisms of platelet adhesion. Characterizing biochemical pathways identifies the path to developing treatments. Treatments that potentially reduce inflammatory reactions could be first screened for effectiveness by mass spectrometry. Accordingly, MS could play a significant role in all steps leading to treatment: understanding the complex system, suggest routes of attack, evaluate treatment for efficacy and screen for unexpected side reactions.

We also hope to extend work on nitroxyl (HNO) treatment of platelets to inhibit platelet aggregation. It has been shown that several proteins are modified by HNO but little is known about the mechanism. We propose to further investigate the mechanism of HNO and evaluate its therapeutic potential with regard to cardiovascular disease. We seek to expand upon current CID to use both CID and ECD to provide more information on how

HNO donor, Angeli's salt, modifies various proteins that direct metabolism and signal transduction providing further insights. In addition to treating platelets, dosing monocytes and endothelial cells with HNO will be done to measure any effect.

Lastly, we'd like to look at effects on protein activation/ deactivation by phosphorylation. Using alternative fragmentation modes we seek to examine phosphopeptides isolated by metaloxide chromatography via tandem mass spectrometry. Through comparison with isotopically labelled treatments changes in phosphorylation can be associated with drug treatment.

I hope that my experience from my master's, where I am examining fundamental mechanistic and application based mass spectrometry, is very much applicable to Juergen's work. I believe the hardware development skills that I've learned will position me will to be an analytical chemist in the Kast Lab. Juergen and I have talked in detail of how my analytical skill set would fit into his interdisciplinary team. If accepted into the Experimental Medicine program, I hope to learn much and believe I have much to offer. I am excited with the opportunity to work in the Kast lab.