The development of mass spectrometric techniques for the characterization of neuropeptide receptors in the American lobster, *Homarus americanus*

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Neuropeptides are a large class of signaling molecules in the nervous system that regulate animal physiology and behavior. These short chains of amino acids initiate a response and regulate behavior by interacting with their respective cell-membrane receptors. One particular group of neuropeptides, C-type allatostatin (AST-C), has been found to have cardiac function in a number of insects and crustaceans. When AST-C is perfused into the heart of the lobster *Homarus americanus*, heart contraction amplitude increases for some animals while it decreases for others.\(^1\)\(^,\)\(^2\) The underlying mechanism responsible for differences in neuronal network responses to a given modulatory input is not fully understood. A working hypothesis to explain the differential response is that there is a change in the expression of AST-C receptors. There could be a change in the number of receptors or a change in post-translational modifications (PTMs) to the receptors. Currently, there is genetic evidence for three AST-C receptors, all of which are suggested to be present in the *H. americanus* brain. Using mass spectrometry, our ultimate goal is to characterize AST-C receptors in the brain of *H. americanus* to provide direct evidence for the receptors (as expressed proteins) and to determine if the receptors undergo post-translational modifications (PTMs) that may impact activity.

Mass spectrometry (MS) is an analytical technique that allows for the inspection of a mixture of proteins at the same time and permits identification of unknown proteins. The MS spectrum provides a mass-to-charge ratio of each component in the sample. Tandem MS/MS sequencing then determines the amino acid sequence and PTMs or matches the MS/MS information against a protein database.\(^3\) Prior to MS analysis, these large cell-membrane proteins have to be extracted from the brain and digested into smaller peptide fragments. Additionally, neuropeptide receptors such as the AST-C receptors are embedded in the cell-membrane so they are largely hydrophobic. Proteins that are mainly hydrophobic tend to group together and do not easily solubilize in water. Detergents, which have both a hydrophilic (water-loving) and hydrophobic region, can aid in solubilizing these receptor proteins in water.

Thus far, I have tested various detergents to improve extraction and digestion of cell-membrane proteins, using the detection of a *H. americanus* sodium-potassium ATPase, a known membrane protein. In particular, a neuronal protein extraction reagent containing the detergent Triton has been found to be effective in extracting *H. americanus* brain proteins. For the sodium-potassium ATPase, a high degree of coverage was detected by MS/MS and included significant portions of the transmembrane domain. Moving forward, I will continue to optimize the method for extraction and digestion of cell-membrane proteins. Additionally, we will compare MS/MS data with *H. americanus* genetic information provided by transcriptomics in order to identify unknown receptor proteins in the brain.\(^4\)

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References:


