The Optimization of *In Vivo* Fast Photochemical Oxidation of Proteins (FPOP) and the Structural Characterization of Neuronal Proteins within the *C. elegans* Nematode

Stephanie Akanoh\(^1,2\), Christina Williams\(^2\), Lisa Jones, Ph.D.\(^2\)

Department of Chemistry & Biochemistry, University of Maryland-Baltimore County, Baltimore MD, USA\(^1\)

Department of Pharmaceutical Sciences, University of Maryland, Baltimore MD\(^2\)

Neuronal proteins are targets for a variety of neurological and psychological therapeutics against prevalent conditions such as clinical depression, Alzheimer’s disease, and schizophrenia among others. Unfortunately, these proteins can be harder to study, so it is essential to innovate new methods to investigate their structural properties to gain a better understanding of their important interactions in biological systems.

The lab of Dr. Lisa Jones has done extensive work using the protein footprinting technique, fast photochemical oxidation of proteins (FPOP) coupled to mass spectrometry (MS), to study protein structure and dynamics. In FPOP, laser irradiation of hydrogen peroxide forms hydroxyl radicals that oxidatively modify the side chains of solvent accessible amino acids; this modification induces a mass shift that can be detected by MS. Tandem MS/MS is used to identify which amino acids are modified and to quantify the extent of labeling. FPOP can capture changes in solvent accessibility due to protein-protein interactions, ligand binding or other conformational changes. For example, a protein will have a higher level of solvent accessibility than its ligand-bound complex. *In vivo* FPOP (IV-FPOP) was developed to take the variable environment of a live organism into account when characterizing various proteins. Using *C. elegans* as the animal model to conduct IV-FPOP to study neuronal proteins was an apt choice as \(\sim 80\%\) of its proteins are conserved between humans according to genome sequencing. The current aim of this project is to develop and optimize the procedures for the IV-FPOP workflow, which are as follows: synchronization of *C. elegans*, IV-FPOP, larval cell dissociation, cell sorting, protein extraction and digestion, then MS analysis.

The majority of optimization lies in IV-FPOP conditions, flow system procedures, and the dissociation and isolation of neuronal proteins.

This research was partially funded by the USM LSAMP program, supported by NSF LSAMP Award “#1619676”.