

Submitter name: Pawel Ciborowski

Submitted email: pciborowski@unmc.edu

### **Investigation of Macrophage proteome exposed to methamphetamine: A Multiple Reaction Monitoring (MRM) approach**

Sarah Zieschang<sup>1</sup>, Katarzyna Macur<sup>1,2</sup>, Andrew Schissel<sup>1</sup>, Shulei Lei<sup>1</sup>, Emma Harwood<sup>1</sup>, Brenda Morsey<sup>1</sup>, Howard Fox<sup>1</sup>, Pawel Ciborowski<sup>1</sup>

<sup>1</sup> University of Nebraska Medical Center, Omaha, NE; <sup>2</sup>Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, University of Gdansk, Gdansk, Poland

Macrophages are essential cells of the innate immune system. Exposure to toxic substances such as methamphetamine (Meth) impairs their protective function. We used a targeted proteomics approach to determine the effects of Meth exposure on HIV-1 infected macrophages. Our model uses human monocyte-derived macrophages (hMDM) *in vitro* infected with HIV-1 (Control-Infected-Control or CIC), exposed to Meth after infection (Control-Infected-Meth or CIM), and exposed to Meth before and after infection (Meth-Infected-Meth or MIM). This experimental setting allows us to investigate the direct effect of Meth on HIV-1 infected cells. The trypsin-digested samples were analyzed using a Multiple Reaction Monitoring (MRM) mode for quantitative proteomics. Protein selection was based on previous studies of the HIV-1 infected hMDMs proteome. Five replicate injections of three concentrations of whole-cell lysates were measured for three conditions: CIC, CIM, and MIM. The linearity of concentrations of spiked-in BSA peptides showed no interferences in the presence of the complex biological matrix in contrast to endogenous  $\beta$ -actin, galectin-1, and galectin-9. Galectin-1 expression increased in MIM at all tested concentrations when compared to either CIC or CIM. Experimental verification of *in silico* predefined peptides were investigated in cell lysates for quantification of, actin, Galectins 1, 3 and 9, HSP7c, Profilin-1, Peptidyl Prolyl Isomerase A (PPIA). MRM-based targeted proteomics appears to be a useful method to track quantitative changes in the proteome; nevertheless, there are significant obstacles in measuring some proteins. We also attempted to validate MRM data using immune histo-staining approach, which seems complementary to mass spectrometry-based quantification of proteins.