Increasing observability of the phosphoproteome in brain tissue

Norah Brown (norahbr@stanford.edu), Stanford University Mass Spectrometry, **Norah Brown**, Stanford University Mass Spectrometry, **Garvey McKenzie**, Stanford University, **Fang Liu**, Stanford University Mass Spectrometry, **Ryan Leib**, Stanford University Mass Spectrometry, **Allis Chien**, Stanford

Mass spectrometry proteomics is a powerful tool for understanding cell biology, protein expression, and their interactions. Many post translational modifications have a low stoichiometric abundance in cells and require techniques like sample enrichment in order to more readily observe these targets. Enrichment of phosphorylation has typically been accomplished using metal-oxide particles composed of titanium dioxide (TiO2) or iron oxide (Fe3O4). More recently, products like immobilized metal affinity chromatography (IMAC) beads with either zirconium (Zn), titanium (Ti), or iron (Fe) metal coordination are used for enrichment because of their increased performance and lower sample input requirement. Phospho-enrichments using IMAC beads have been studied and optimized using mostly cell cultures, bovine serum album, and casein; however there is little information about IMAC bead enrichment performance in brain tissue. Biological tissues have variation in composition, and different species and tissue types vary greatly in the natural abundance of protein phosphorylation. In addition, brain tissue is high in lipid content. When using a standard phospho-enrichment protocol with Zn-IMAC HP beads (Resyn Biosciences) on a mouse brain digested by S-trap mini spin columns (Protifi), we observed low enrichment efficiencies, as measured by comparing the number of spectral matches for phosphorylated peptides to the total number of overall peptide spectral matches. We similarly observed low enrichment efficiencies in brain tissue when using titanium metal affinity beads (Ti-IMAC HP, Resyn Biosciences). Following these results, we tested Zn-IMAC and Ti-IMAC beads with enrichment buffers using a 0.1M glycolic acid additive during sample binding steps and a high organic, 50% methanol buffer for the wash steps of the enrichment procedure. In addition to modifying steps of our enrichment protocol, we also tested cleaning up brain tissue samples before enrichment by using chloroform and methanol (1:1 ratio) solvent systems during protein precipitation and washes followed by a C18 peptide purification. By implementing these techniques for lipid removal, we observed a four-fold increase of phosphorylated spectral matches in brain tissue, indicating increased enrichment efficiencies. These results support that optimizing enrichment protocols to tissue type -- in this case, incorporating chloroform/methanol washes and additional C18 cleanup for lipid removal from brain tissue -- can reduce undesired matrix effects and increase phosphopeptide enrichment efficiencies.