Automated Quantitative Phosphopeptide Enrichment using Titania-packed Capillary Columns and Its Application to Human Tissues for Label-Free Phosphoproteomics

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ABSTRACT

Bioanalytical and phosphoproteomics approaches are useful tools for the study of post-translational modifications (PTMs), such as phosphorylation, as these modifications typically affect protein function. The presence of PTMs on proteins can be detected using a variety of proteomic approaches. Phosphoproteomics is a specific type of proteomics that focuses on the study of phosphoforms, i.e., phosphorylated protein species. Phosphoproteomics is a challenging field due to the wide range of dynamic PTMs and highly complex proteomes. The use of label-free methods for phosphoproteomics is increasing due to its high reproducibility and sensitivity. Automated enrichment setups are gaining popularity due to their reproducibility and automation.

** Materials and Methods: **

To achieve reproducibility and label-free quantification of phosphopeptides, we developed a workflow using automated enrichment setups. Our workflow included the use of Titania beads and a capillary column. We performed experiments on the Tissue cores from the left ventricle of seven individuals during implantation of a Left Ventricle Assist Device (LVAD). Tissue cores were harvested from the same seven patients during heart transplant surgery. The total amount of protein for each sample was 1 mg. A total of 637 phosphopeptides, corresponding to 300 phosphoproteins, were identified across the entire dataset. Using each patient as their own control, only 31 phosphopeptides were significant in at least 3 patients at a fold change greater than 2 and p-value less than 0.001.

** Results: **

The histogram at left, the analytical reproducibility of the technique yields an average variability of just over 10%. The histogram at right contains the combined biological and technical variability for enrichment of heart tissues. The shift toward higher FDR in this study can therefore be attributed to the biological variability of the tissue samples.

** Conclusion: **

We demonstrate that the use of label-free methods in combination with automated enrichment setups is well suited for the study of phosphoproteomes.

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** Experimental Workflow **

- ** Spin Column Enrichment **
  - Sample Preparation
  - Automated Enrichment
  - Post-enrichment Sample Processing

- ** LC and MS Experimental Details for Method Evaluation **

- ** LC: NanoAcquity UPLC System **
  - Column: 75um x 150mm BEH110 C18, 1.7 um particle size
  - Gradient: 3-80%ACN in 90 minutes

- ** MS: Waters Synapt G2 Max Mass Spectrometer **
  - Operated in DDA mode, submitting up to 3 ions from a single survey scan to MS/MS

- ** LC and MS Experimental Details for Heart Study **

- ** LC: Waters 2D NanoAcquity UPLC System **
  - 1st Dimension pH 3-10 Peptide Map: 3% buffer, 2% acetonitrile, 1.5% formic acid
  - 2nd Dimension: 30 minute gradient in the second dimension after bumping peptides off of the first dimension with increasing amounts of acetonitrile

- ** MS: Waters Synapt G2 Max Mass Spectrometer **
  - Operation in sensitivity mode and DDA mode, submitting up to 3 ions from a single survey scan to MS/MS

** Standard Peptide/Protein Identification and Relative Quantitation Workflow **

- ** Peptide identification was performed by search DDA runs against a 1x-reversed species-specific database **
  - Zebrafish samples: NCBI Zebrafish
  - Rat Brain samples: NCBI RatBrain
  - Heart samples: SwissProt Human

- ** Quantitation was performed within Rosetta Eludator by following a Top Taller retention time alignment and appropriate intensity scaling. **

- ** Peptide intensities were reported as the sum of all features belonging to peptides above a Mascot Ion Score that resulted in a 1.5 spectral FDR determined by hits to reversed sequences. **