Universal Unbiased pre-MS Clean-Up Using Magnetic HILIC Microparticles for SPE

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Introduction

The human proteome is a complex and dynamic system, containing millions of proteins and from which the regulation of cellular functions is derived. There is a need for methods that allow the rapid, accurate and unbiased discovery of protein biomarkers. The use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for protein identification has become the standard method in proteomics. The current workflows of sample preparation for LC-MS/MS are mainly based on reversed-phase liquid chromatography (RPLC), due to the high abundance of hydrophobic peptides. However, a high number of hydrophilic peptides are lost during the purification process, which limits the discovery of protein biomarkers. HILIC LC-MS/MS has emerged as a powerful alternative method due to its high retention of hydrophilic peptides, allowing for a more unbiased and comprehensive analysis of the proteome.

Methods

An automated sample preparation workflow using MagReSyn® HILIC SPE microparticles was developed. The workflow involves the following steps: cell lysis, sample equilibration, and magnetic precipitation of hydrophilic peptides for single-phase extraction (HILIC SPE). Followed by a bead to bead application, and dried MALDI MS analysis. Analysis of the workflows was performed using three different peptide sets: inclusion of abundant peptides, neutral peptides and low rank peptides.

Results & Discussion

The workflow was optimized by testing different combinations of method parameters such as pH, acetonitrile and ammonium hydroxide concentrations, and solubilization time. The optimized workflow allows for the unbiased analysis of the proteome, with recovery rates of hydrophilic peptides reaching up to 90%. The workflow is reproducible and can be scaled up to handle large volumes of samples.

Conclusions

This work describes an automated workflow for quantitative proteomic sample preparation using MagReSyn® HILIC SPE microparticles. The performance of the workflow was validated using recombinant human proteins. The workflow is reproducible and can be scaled up to handle large volumes of samples. This work opens up new possibilities for the unbiased analysis of the proteome, allowing for the discovery of new protein biomarkers.

References


Figure 1: An outline of the sample preparation workflow tested in this study is illustrated. Introduction

Figure 2: Automated sample preparation workflow, using magnetic HILIC functionalized microparticles for SPE. Particles are collected and eluted into a SP3 or FASP, or protease and workflow.

Figure 3: Comparative assay for the sample preparation workflow using HILIC (HILIC), SP3 (SP3) and FASP workflows.

Figure 4: Compositional representation of HILIC (HILIC), SP3 (SP3) and SPE workflow.

Figure 5: Automated sample preparation workflow for the peptide enriched fraction. Samples were collected using HILIC, SP3 and FASP workflows. The peptide enrichment recovery is presented using a bar graph. Figure 5(a) shows the retention time distribution of peptides and proteins as compared to SP3 and FASP workflows.

Figure 6: Peptide and protein vector distribution as a function of vector labels that samples processed with HILIC (HILIC), magnetic microparticles results in higher peptide and protein scores compared to four replicates (four replicates).