A QUALITATIVE AND QUANTITATIVE ION MOBILITY ENABLED DATA INDEPENDENT SILAC WORKFLOW

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INTRODUCTION

SILAC experiments employ metabolic incorporation of genetically labelled amino acids into proteins for LC-MS based quantitative proteomics studies and have predominantly used MS data dependent acquisition (DDA) strategies to date. Due to the multiple nature of a SILAC experiment and the accompanying increase in sample complexity, co-fragmentation of multiple precursor ions challenges the specificity of the assay and thereby the quantitative and qualitative outcome. High resolution data independent acquisition (DIA) methods, especially using in-line ion mobility separation, have the potential to overcome a number of acquisition related issues in these additionally complex samples. Here, we demonstrate proof of principle of DIA and IM LC-MS acquisition strategies, evaluating concurrently the overall performance of the workflow.

METHODS

Sample preparation

Three SILAC sample types were investigated following reversal, acylation and trypsin digestion:
1. 15N-labeled mouse cells comprising a l2K167T cell line were labeled on +13C, +12C, +12C, +13C media (LC).
2. Non-labeled UPS2 dynamic range standard was spiked into +12C, +12C, +13C labeled and +12C, +12C, +13C labeled cell media
3. Bovine heart mitochondrial membrane to detect light and heavy AA labeled UPS2 proteins in 200 ng SILAC medium (cyan, green and red tails of one of the quantified proteins. In combining unlabeled

RESULTS

Protein quantification results DIA SILAC quantitation was explored by spiking non labeled and heavy isotope labeled UPS2 proteins in 200 ng SILAC medium (cyan, green and red tails of one of the quantified proteins. In combining unlabeled

CONCLUSIONS

Data-independent (DIA) MS acquisition strategies, incorporating high peak capacity and sensitivity, enable more precise, taking advantage of both SILAC labeling experiment [4] of which the principle and results are published, and database searched SILAC based quantification measurements are accurate and precise, taking advantage of both SILAC

References

1. Absolute quantification of proteins by LCMS: a virtue of parallel MS separations can be routinely applied for SILAC experiments. OMICS. 2012;16:0

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