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Comparison of Variants of the Limited-Proteolysis Approach for Drug Target Deconvolution and Structural Biomarker Discovery including FAIMS-DIA and TMT-Pro

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Introduction

Phenotypic screening is a major focus of drug development, largely due to high attrition rates in target-centric approaches. Limited proteolysis coupled with mass spectrometry (LiP-Quant) is a powerful new target deconvolution technique exploiting drug-induced structural alterations in drug target proteins. Utilizing a sequence-unspecific but structure-specific digestion step, LiP exploits these alterations via the identification of unique 'conformotypic' peptides by proteomic analysis. The introduction of new workflows in LC-MS hardware like ion mobility and 16-plex TMT-Pro have the potential to significantly improve LiP-Quant. Here we compare the LiP-Quant workflow in sample preparation, mass spectrometric acquisition and data analysis using a broad kinase inhibitor. We find that by implementing novel workflows we can significantly improve target identification.

Methods

Two LiP-Quant experiments were performed with the kinase inhibitor staurosporine, the first at room temperature and second at 37°C. The resulting samples were aliquoted for repeated mass spectrometry acquisition on an Exploris 480, both with and without a FAIMS unit. The data was recorded in DIA at 2h and 4h gradient length. Additionally, an aliquot was labelled with the TMT-Pro kit and then fractionated (30 fractions) using HPRP, followed by 2h FAIMS-DDA acquisition. Following this, the data were analyzed with Spectronaut and SpectroMine (Biognosys). Subsequent LiP data processing was further done using an in-house algorithm that computes a peptide and protein-specific LiP score based upon several factors including the correlation of peptide abundance across treatment concentrations to expected dose response curves.

Preliminary Data

To compare and improve the performance and coverage of the LiP-MS approach, we optimized the reaction temperature and evaluated different MS acquisition strategies.

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To compare the performance of the LiP-Quant reaction at different temperatures, a staurosporine experiment was executed at room temperature and in parallel at 37°C. Then the samples were acquired on the MS using identical settings. Interestingly, the analysis of the two experiments showed, that at 37°C 107% more kinases were in the filtered candidate list ranked by LiP score (LiP score >1.8).

Next, we performed a large LiP-Quant experiment and aliquoted it for acquisition using five different MS strategies. First and second, we acquired the experiment by 2h or 4h standard DIA. Third and fourth, we acquired the experiment by 2h or 4h FAIMS-DIA. We developed FAIMS-DIA methods for maximal peptide and protein coverage of the LiP reactions. Fifth, we isobarically labelled an aliquot of the LiP-Quant reaction using TMT-Pro. These samples were separated in 30 HPRP fractions and acquired with 2h FAIMS-DDA methods. To compare the acquisition strategies, the number of known drug target proteins in the filtered candidate list ranked by LiP score was assessed (LiP score >1.8).

Comparison of the results from the five strategies demonstrated the clear benefit of longer gradients for identification of drug targets. Namely, 107% improvement from 2h to 4h in DIA and 64% improvement in FAIMS-DIA 2h to 4h. When comparing DIA to FAIMS-DIA at equal gradient length, we found an improvement of 215% at 2h gradients (52% for 4h versions). Finally, we compared the best strategy from the DIA/FAIMS-DIA set, the 4h FAIMS-DIA against the TMT-Pro analysis. The 4h FAIMS-DIA approach clearly outperformed the TMT-Pro approach by 146%.

On the LiP data analysis side, we introduced improvements of the scoring and ML framework of the analysis algorithm based on the LiP-Quant publication and found it to be on average 40%.

Collectively, these results demonstrate that LiP-Quant improves dramatically when using ion mobility-based FAIMS-DIA and that the experiment performed at 37°C yields more targets than the one at room temperature.

Novelty aspect

Significant improvement of the limited proteolysis-based structure probing by evaluation of novel MS solutions and algorithmic improvements.