Assessment of current mass spectrometric workflows for the quantification of low abundant proteins and phosphorylation sites

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The data described here provide a systematic performance evaluation of popular data-dependent (DDA) and independent (DIA) mass spectrometric (MS) workflows currently used in quantitative proteomics. We assessed the limits of identification, quantification and detection for each method by analyzing a dilution series of 20 unmodified and 10 phosphorylated synthetic heavy labeled reference peptides, respectively, covering six orders of magnitude in peptide concentration with and without a complex human cell digest background. We found that all methods performed very similarly in the absence of background proteins, however, when analyzing whole cell lysates, targeted methods were at least 5-10 times more sensitive than directed or DDA methods. In particular, higher stage fragmentation (MS3) of the neutral loss peak using a linear ion trap increased dynamic quantification range of some phosphopeptides up to 100-fold. We illustrate the power of this targeted MS3 approach for phosphopeptide monitoring by successfully quantifying 9 phosphorylation sites of the kinetochore and spindle assembly checkpoint component Mad1 over different cell cycle states from non-enriched pull-down samples. The data are associated to the research article 'Evaluation of data-dependent and data-independent mass spectrometric workflows for sensitive quantification of proteins and phosphorylation sitesó (Bauer et al., 2014) [1]. The mass spectrometry and the analysis dataset have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the dataset identifier PXD000964.
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