**Sensitive MS analysis of single-cell proteome and N-glycosylation**

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Single-cell analysis has received much attention in recent years for elucidating the widely existing cellular heterogeneity in biological systems. However, the ability to measure the proteome in single cells is still far behind that of transcriptomics due to the lack of sensitive and high-throughput mass spectrometry methods. Herein, we report an integrated strategy termed “SCP-MS1” that combines fast liquid chromatography (LC) separation, deep learning-based retention time (RT) prediction and MS1-only acquisition for rapid and sensitive single-cell proteome analysis. In SCP-MS1, the peptides were identified via four-dimensional MS1 feature (m/z, RT, charge and FAIMS CV) matching, therefore relieving MS acquisition from the time consuming and information losing MS2 step and making this method particularly compatible with fast LC separation. By completely omitting the MS2 step, all the MS analysis time was utilized for MS1 acquisition in SCP-MS1 and therefore led to 65%–138% increased MS1 feature collection. Using this strategy, more than 2000 proteins were obtained from 0.2 ng of peptides with a 14-min active gradient at a false discovery rate (FDR) of 0.8%.

As one of the major post-translational modifications, protein N-glycosylation plays crucial roles in various important biological processes. However, comprehensive N-glycoproteome profiling for single cells has not been achieved due to the extremely limited sample amount and incompatibility with the available enrichment strategies. Here, we have developed an isobaric labeling-based carrier strategy for highly sensitive intact N-glycopeptide profiling for single cells or a small number of rare cells without enrichment. In our strategy, a carrier channel using N-glycopeptides obtained from bulk-cell samples significantly improved the “total” signal of N-glycopeptides and therefore, promoted the first quantitative analysis of averagely 260 N-glycopeptides from single HeLa cells.

**Keywords:**

Single-cell proteomics/ N-glycosylation/ LC-MS/MS/ MS1-only acquisition/ Isobaric Labeling