Improved *N*- and *O*-glycopeptide identification using FAIMS

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Mass spectrometry is the premier tool for identifying and quantifying site-specific protein glycosylation globally. Analysis of intact glycopeptides often requires an enrichment step, after which the samples remain highly complex and exhibit a broad dynamic range of abundance.

Here, we evaluated the analytical benefits of high-field asymmetric waveform ion mobility spectrometry (FAIMS) coupled to nano-liquid chromatography mass spectrometry (nLC-MS) for analyses of intact glycopeptide devoid of any enrichment step. We compared the effects of compensation voltage on the transmission of N- and O-glycopeptides derived from heterogeneous protein mixtures using two FAIMS devices. We comprehensively demonstrate the performance characteristics of the FAIMS device for glycopeptide analysis and recommend optimal electrode temperature and compensation voltage (CV) settings for N- and O-glycopeptide analysis.

Under optimal CV settings, FAIMS-assisted gas-phase fractionation in conjunction with chromatographic reverse phase separation resulted in a 31% increase in the detection of both N- and O-glycopeptide compared to control experiments without FAIMS. Overall, our results demonstrate that FAIMS provides an alternative means to access glycopeptides without any enrichment providing an unbiased global glycoproteome landscape. In addition, our work provides the framework to verify 'difficult-to-identify' glycopeptide features.