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Impact of ion mobility separation on optimal collision energies from a bottom-up proteomics point of view

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Ion mobility spectrometry (IMS) is a widely used separation technique that can enhance the performance of liquid chromatography – mass spectrometry (LC-MS/MS) methods by providing an additional dimension of separation.
In bottom-up proteomics, the collision energy (CE) applied greatly influences the information content of the obtained MS/MS spectra from peptides. The highest identification scores and the greatest number of successful identifications can be achieved with an instrument-specific optimal setting. When IMS is applied, significant ion heating can occur in the IMS cell as a result of collisions with the buffer gas, which can affect the optimal CE for MS/MS experiments.
The objective of this study was to examine the impact of ion mobility separation on the energetics of peptide ions through the investigation of the optimal CE choice for MS/MS experiments. LC-MS/MS measurements were performed using varied CE settings both with and without IMS on a Waters Select Series Cyclic IMS mass spectrometer, which has two consecutive collision induced dissociation (CID) cells; one before (trap) and one after (transfer) the ion mobility cell. Over a thousand tryptic peptides from HeLa tryptic digest standard were analyzed in terms of CE dependence of identification score using Byonic search engine. The trap cell and transfer cell were also compared as pre and post ion mobility fragmentation modes.
Results indicate that IMS significantly energizes peptides, and the use of lower CE is recommended when IMS is applied. On average, the optimal CE is 6.3 V lower when IMS is applied. This difference also manifests in the optimum CE value versus *m*/*z* trends. It was also determined by comparing the pre and post ion mobility fragmentation modes, that it is necessary to adjust the CE when the trap cell is used for activation instead of the transfer cell.