

## *Supplementary Note*

On a trapping instrument, such as the Q-Exactive, an MS/MS scan consists of three steps: 1) accumulation of precursor ions, 2) fragmentation of precursor ions, and 3) mass analysis of fragment ions. Because the fragmentation of precursor ions (step 2) by HCD is orders of magnitude faster than the other two steps (nanoseconds compared to milliseconds), the MS/MS acquisition rate depends almost exclusively on the amount of time spent on ion accumulation and mass analysis<sup>1</sup>. The Q-Exactive performs ion accumulation and mass analysis in parallel by accumulating ions for scan N+1 while the ions for scan N are being analyzed. The mass analysis takes a fixed amount of time depending on the desired resolving power (17,500 in this protocol). Therefore, the fastest scan rate is achieved when the maximum time spent for ion accumulation is matched to the time spent on mass analysis. Setting the maximum injection time to “auto” for MS/MS scans does this matching automatically to achieve the highest possible scan rate at a given resolving power.

## *References*

1. Gallien, S. *et al.* Targeted Proteomic Quantification on Quadrupole-Orbitrap Mass Spectrometer. *Mol. Cell. Proteomics* **11**, 1709–1723 (2012).