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Maximizing throughput, analytical depth and sensitivity for clinical proteomics discovery using PASEF on a TIMS-equipped UHR-Q-TOF

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The achievable depth in high throughput proteomics analyses is most often limited by the sequencing speed of mass spectrometers, while the nano-liquid chromatography (nano-LC) turnover rate limits the sample throughput. Here we describe the combination of a trapped ion mobility spectrometry (TIMS) QTOF capable of parallel accumulation serial fragmentation (PASEF) acquisition to a front end allowing efficient use of short gradients, the Evosep One to achieve, high depth at high throughput analysis. The use of short nano-LC gradients reinforces the need of a fast MS instrument to guarantee reasonable analytical depth. TIMS provides a further dimension of separation in addition to the retention time and m/z ratio that increases peak capacity and it releases the ions from the TIMS cell as concentrated ion packets. By applying the PASEF scan mode on those packets we obtain almost 100% duty cycle, increased sequencing speed (>100 Hz) and high sensitivity leading to a deep coverage of the proteome over 4 orders of magnitude dynamic range. To test if we can profit from the high speed and sensitivity of PASEF we set up very short gradients on the Evosep system with a total runtime of 14.0 min (11.5 min gradient) and 7.1 min (5.6 min gradient), resulting in 100 samples/day and 200 samples/day, respectively. Evotips were loaded with only 50 ng of HeLa tryptic digest and analyzed. Using the 200 sample per day turnover we found more than 1200 protein families and 8000 peptide matches, with a very high sample to sample reproducibility.

Biography

Pierre Olivier Schmit graduated from the National High School of Chemistry of Montpellier in 1997 and from the University of Montpellier 1 (Medical Sciences) in 1998. He has been involved in measuring proteins mobility in NMR before starting proteomics in 1999. He joined Bruker in 2000 where he had to set up and manage the French mass spec demo lab, running MALDI TOF and TOF TOF, ion traps and ESI-Q-TOF for various applications, with a strong focus on proteomics, clinical proteomics and protein characterization. He took a Proteomics Market Manager position in 2011 and joined Bruker's global proteomics Business Unit in 2014 with a strong involvement in collaborations setup and solution design.

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