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ADVANCED LC-MS-BASED APPROACHES FOR THE INVESTIGATION OF PEPTIDES AND PROTEINS

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In proteomic analysis, scientists are more and more challenged in implementing separation systems capable to provide enhanced separation power, as well as specificity/sensitivity of detection for adequate identification/quantification of the separated compounds. Gel-free, LC based separation techniques come along with the benefits of higher throughput, relative speed, capability of quantitation, and easiness of full automation. From the detection standpoint, the advent of ESI and MALDI ionization techniques have definitely concurred to make LC–MS and LC–MS/MS emerge to a central role in modern proteomics. Furthermore, several two-dimensional comprehensive LC platforms (LCxLC) have been successful in delivering an exponential increase in terms of separation efficiency, the latter in turn allowing for more reliable identification of low-level sample constituents and potential biomarkers.
This presentation will focus on different approaches for high resolution front-end separation of intact and digested proteins, relying on the use of RP-LC due to its amenability of direct linkage to MS. Moreover, in LCxLC approaches the use of RP-LC in both the dimensions alleviated major technical challenges, and allowed for greater flexibility in the choice of column dimensions and operation modes. High efficiency was achieved through the use of fused-core (2.7 μm d.p.) stationary phases (4.6 or 2.1 mm I.D. columns), and the selectivity adjusted by careful selection of the mobile phase pH (basic or acidic buffered solution), and column temperature (35-60 °C). In contrast to shotgun proteomic approaches, the complexity of the ions entering the MS is reduced, avoiding to overwhelm the limited dynamic range per spectrum of the mass detector.

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