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Top-down analysis of a FMN-binding membrane protein (FMP) using a multiplexing FTMS data workflow combined with an in-house web-free access ChemInfo.org tool

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Top-down protein structural analysis, increasingly used to assess drug-protein binding sites, benefits from high-resolution and high mass accuracy offered by Fourier transform MS/MS. To improve localization of post-translational modifications and FMN-binding sites on a 19 kDa membrane protein (FMP), we implemented the following workflow on Orbitrap FTMS platforms. First, to increase sensitivity and dynamic range, we acquire a number (up to 10) of consecutive LC-MS/MS experiments (using HCD) from the same sample. Second, we record in parallel both standard mass spectra in raw format using original on-board data processing system (Thermo Scientific) and time-domain signals (transients) using an external high-performance data acquisition system (FTMS Booster X1). The transients are summed across all LC-MS/MS runs, processed with absorption mode FT, the mass spectra are re-calibrated and baseline corrected to generate an accurate peak list (using Peak-by-Peak software). Finally, we apply the in-house developed free-access ChemInfo.org algorithms for predicting and matching the experimental tandem spectra to theoretical fragment ions, in particular internal fragment ions which proved to be crucial for precise localization of FMN binding sites. This versatile tool allows fast and automatic matching of thousands of peaks from complex mass spectra. The localization of the FMN-binding site on the FMP protein was restricted to a string of four residues, among which the threonine was presumably the binding site. The results in terms of number and confidence of fragment ion assigned, similarity scores and localization of FMN binding sites were favorably compared to the classical top-down analysis using a single LC-MS/MS run.

Biography

Laure Menin completed her PhD in Biochemistry, Microbiology and Cellular Bioenergetics in 1997. She worked as a Project Manager at different companies in Switzerland, first in the field of Large-Scale Proteomics then in Drug Discovery and peptidomics analysis of insect hemolymph and venoms of poisonous animals. She is currently managing the Mass Spectrometry Facility of the Institute of Chemical Sciences and Engineering (ISIC) in EPFL, Lausanne.

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