NUTS AND BOLTS OF ITRAQ-BASED QUANTITATIVE ANALYSIS

Campos, A.[1], Diema C.[2], Odena M.A.[1], Bellido D.[1], Villaseca M.[2], and Oliveira E[1]

[1]Plataforma de Proteòmica. Parc Científic de Barcelona. Serveis Cientificotècnics. Universitat de Barcelona;
[2]Plataforma Científica de Espectrometria de Masses. Institut de Recerca Biomèdica de Barcelona

Deployment of mass spectrometry for peptide-based quantitative analysis has become mainstream technology across proteomics laboratories. In particular, the iTRAQ technology has caught the attention of the scientific community mainly due to its capacity to multiplexing up to eight samples in a single mass spectrometry (MS) experiment. Because relative quantitative information on iTRAQ-tagged peptides is obtained in MS/MS scans, setting up optimal instrument conditions is full of twists and turns.

Accurate iTRAQ quantitation relies largely on instrument particularities such as efficiency of the ionization and fragmentation processes. Since its implementation, iTRAQ analysis has been largely relegated to quadrupole time-of-flight (QTOF) and MALDI-TOF/TOF instruments. More recently, the development of the novel fragmentation method ***P***ulsed-***Q***-***D***issociation (PQD) (Thermo Fisher™) has opened up a new possibility for iTRAQ analysis in linear ion trap instruments such as the high-performance LTQ-FT. In the present study, we have thoroughly explored the impact of a number of different instrument parameters on robustness, accuracy and sensitivity of iTRAQ analysis. We have also assessed the strengths and weakness of different MS instruments (QTOF, MALDI-TOF/TOF, and LTQ-FT) on iTRAQ analysis. Another major challenge in iTRAQ analysis is the computational and statistical analysis of the data. We have compiled and evaluated the different freely available tools for iTRAQ data mining. Finally, to facilitate the implementation of iTRAQ technology, we provide general guidelines for instrument parameter setup and robust data mining.