
MULTIDIMENSIONAL LC-MS APPROACHES IN QUANTITATIVE PROTEOMICS

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During more than a decade, two-dimensional electrophoresis dominated the field as the method of choice for separating and quantifying complete proteomes. Despite its power to separate a hundreds of proteins in a single experiment, its limited dynamic range, its failure to cope with hydrophobic membrane proteins and its stress on sample preparation forced proteomic researchers to search for alternative separation methods. Today, it is clear that multidimensional liquid chromatography combined with state-of-the-art mass spectrometric tools, are driving the discoveries in the biomarker and proteomic field.

In this lecture, we will address the trends in the separation techniques applied in this gel-free proteomics. While the field relied so far on a combination of strong cation exchange chromatography and reverse phase chromatography, building on the original Mudpit concept, introduced by John Yates et al.¹, novel orthogonal approaches are getting introduced. We will discuss the features of the alternative 2D-LC separations, in particular the combination of two reverse phase chromatography separations at high and low pH alternatively. We will illustrate this by examples from proteomic analyses performed in the laboratory.

Secondly, we will discuss the different approaches for quantitation, implementing both stable isotope labelling strategies as well as label-free methods, the latter relying on the quantitative nature of the mass spectral data. Indeed, over the last years, numerous methods appeared and we will present an overview of their general principles and demonstrate their specific features by comparing data on differential proteomic analyses performed using different alternative methods.