HIGH-RESOLUTION 1D AND 2D NANOLC SEPARATIONS FOR DEEP DIVE LC-MS/MS PROTEOME PROFILING

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Introduction

After the start of the human proteome project 10 years ago nanoLC-MS/MS analysis became the main tool for studying human proteome, the search for new protein biomarkers, discovery, and quality control of biotherapeutics. The human genome sequences are responsible approximately for the coding of ca. 20K proteins. This, however, doesn't include post-translational modifications and protein isoforms that make the complexity even larger. The improvement in speed, dynamic range, and sensitivity of modern high-resolution accurate-mass (HRAM) mass-spectrometers (MS) made it possible to identify and quantify thousands of peptides and proteins within a single-shot experiment. However, the highly efficient separation and simplification of the complex sample by orthogonal fractionation techniques are needed to achieve the deepest proteome profiling.

Results and Conclusions

During recent years several nanoLC-MS/MS approached have been developed to achieve the deepest proteome profiling. The application of short columns and capillary flow rates in the range of several µL/min allows to analyze hundreds of samples per day including complex biological fluids like serum Fig. 1 [1,2]. On the other hand, the single-short analysis using very long analytical columns (75 cm x 75 µm) at nano-flow rates allows achieving the unmatched depth of proteome profiling [3] due to high sensitivity, highest peak capacity, and resolution power.

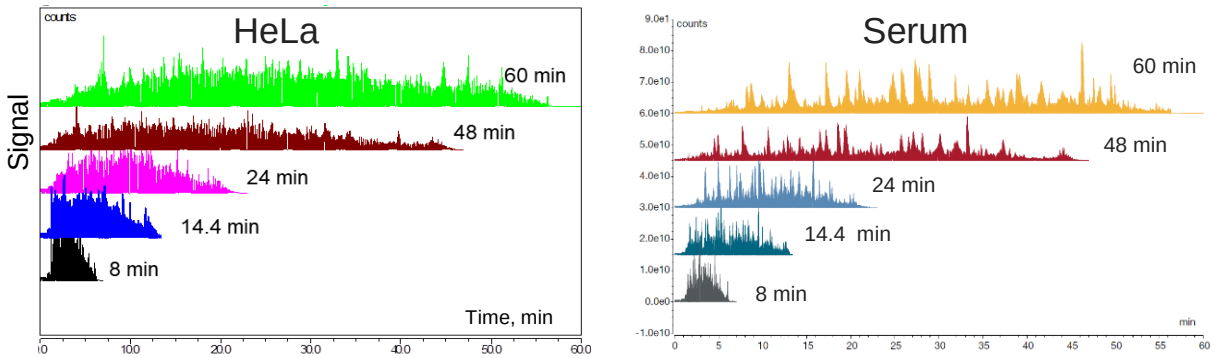

Figure 1. Typical TIC profiles of HeLa protein digest and crude serum protein digest with 8, 14.4, 24, 48, and 60 min standardize methods. TN-73208: Tailored high-throughput low-flow LC-MS methods for large sample cohort analysis

While the standard 1D nanoLC-MS/MS experiments are getting more powerful the fractionation methods, e.g. high-pH reversed phases x low-pH reversed phase techniques allowing to increase loading capacity and dynamic range that translates into more peptides and protein identifications [4]. This is caused by high orthogonality and more time that is used to analyze the single sample.

In this work, a comprehensive analysis of the pros and cons of available techniques for high-sensitivity nanoLC-MS/MS proteome profiling will be conducted to reveal the recommendations for the most suited techniques for each type of scientific task.

References

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