Mass Spectrometric Large-Scale Proteome Analysis

Mass spectrometers have become essential for the analysis of complex proteomes. Ion traps mass analysers are advantageous in proteomics applications due to their high duty cycle for a combined MS and MS/MS analysis, allowing successful protein identification by peptide fragmentation analysis.
However, the detection of low abundant proteins is limited in highly complex samples by dynamic range and acquisition speed of the instrument. Thus proteins of particular interest may be lost.

Raise the Limit for Protein Identifications

Besides intelligent tools for MS/MS selection routines during auto-MS/MS enabled by elevated sensitivity, the limits for low abundant peptides analysis is raised significantly by increasing the number of achievable MS/MS spectra per time, namely by increasing speed.

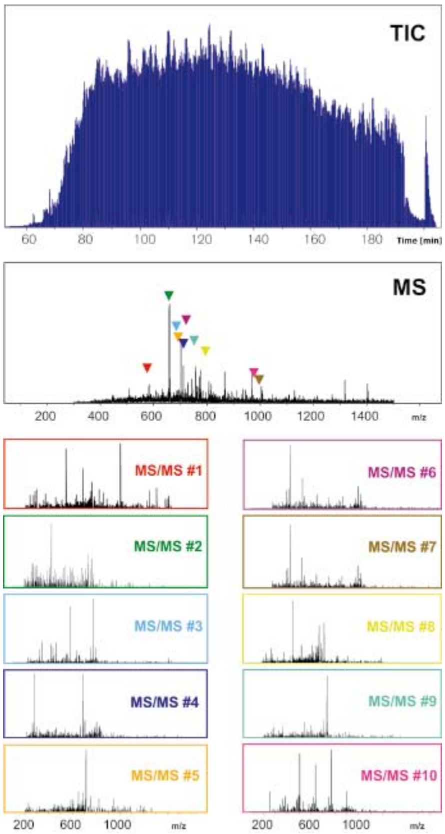

Fig. 2: TIC, MS-, and MS/MS-spectra. The spectra were acquired on the HCTultra within 3,4 seconds.

Fig. 1: HCTultra ion trap

Sensitivity and speed are two important parameters improved considerably in the new HCTultra ion trap mass spectrometer (Fig. 1). By refined high capacity ion trap technology and speed optimization by electronics and software design, the HCTultra increases the sequence coverage for protein identifications and allows the discovery of low abundant proteins from complex samples, permitting more MS/MS identifications in real-time from LC runs.

Complex Protein Samples

Using digested *E. coli* lysates separated on capillary LC, the HCTultra acquires about 200 MS/MS spectra per minute in data dependant intelligent AutoMS/MS mode [1]. This multiplies by more than a factor of two even to the previous fast speed high capacity ion trap, the HCTplus. These data also imply that low abundant proteins are detected with more confidence and the sequence coverage in protein identifications is increased dramatically with the HCTultra.

In a further experiment a limited number of human cells (10^4), kindly provided by H. E. Meyer, Medical Proteome Center, Bochum, Germany, was analyzed for the identification of proteins. A fast 1D nanoLC-MS/MS run took only 3,5 hours for proteome analysis. Applying conservative criteria, a total of 292 proteins could be identified. Figure 2 shows the high complexity of the sample expressed by the Total Ion Current (TIC), from which the high-speed analysis of the HCTultra revealed 10 MS/MS spectra from a single MS spectrum within 3.4 seconds, even under these ambitious separation conditions.

However, running a 2D-nanoLC-MS/MS analysis with the HCTultra unfolds the instruments full performance: Here, a total of 635 proteins were unambiguously identified [2], demonstrating the necessity to appropriately spread highly complex samples in LC runs, even with the extremely high analysis speed of the HCTultra.

Conclusion

The new HCTultra dramatically increases the number of identified proteins compared to the renowned HCTplus in any large-scale proteomics application. Outstanding detection rates of low abundant peptides and high sequence coverage of the entity of proteins is a result of the renowned excellent mass accuracy as well as remarkable improvements in speed and sensitivity in the HCTultra.

References

[1] A Novel High-Speed Duty Cycle of Ion Traps for a more detailed Analysis of Complex Protein Mixtures. Markus Lubeck *et al.*, Bruker Daltonik GmbH, Poster THp192 53rd ASMS Conference on Mass Spectrometry.

[2] Proteome Analysis of a limited Number of Human Cells. Markus Lubeck & Arnd Ingendoh; Bruker Daltonics Technical Note #16.

BRUKER DALTONICS®

Ralf Ketterlinus
Bruker Daltonik GmbH
Fahrenheitstrasse 4
28359 Bremen, Germany
rak@bdal.de