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PREFACE

Shotgun proteomics: where do we stand now?

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As a general trend, the pipeline for shotgun proteomics can be outlined in four flashes. First, the complex proteome is treated to digest the sample; second the peptides are separated using Liquid Chromatography (LC); third the peptides are sequenced using tandem-mass spectrometry, and finally, the data sets recorded with the mass spectrometer are interrogated using computational biology and bioinformatics.

Making a sample ready for shotgun proteomics analysis has always been a time-consuming operation. Many little steps involving protein extraction, total protein quantification, protein reduction with dithiothreitol, protein alkylation with iodoacetamide, protein digestion with an appropriate enzyme and clean-up to remove salts used to be a processing workflow taking as long as 24 h. To overcome this problem the proteomics community developed a number of approaches intended to simplify the handling and to shorten the number of steps and the time consumed in each one of them. Of the many technological developments, the application of ultrasonic energy meets the requirements of simplicity, low cost, high sample throughput and easy implementation for online applications [1]. Currently, 96 samples can be prepared in just two hours, matching perfectly clinical applications for treating samples in large-scale.

Liquid Chromatography – MS has also evolved dramatically to answer the challenge to analyze complex proteomes such as whole cell lysates, plasma or serum. Thus, from the outset,

the mainstream was to analyze few samples as deep as possible to find out differences that could enable the discovering of the so-called "Biomarkers." This mainstream had a complexity reflected in approaches that described online systems using dual column chromatography [2] or extensive protein prefractionation[3] as the ways to overcome the large dynamic range of protein abundances. Such approaches resulted in time-consuming, expensive and low throughput workflows. The result has been a reduced number of approved biomarkers, as low as 22 during the period comprised between 1995 and 2010 [4]. However, new technological advances in chromatography are called to change the panorama mentioned above dramatically. Thus, the introduction of the Evosep [5] chromatograph concept has taken biomedical research a level forward as it has allowed high throughput and robustness for the first time in proteomics history. As much as 200 samples can be treated per day, rendering approximately 1500 proteins identified in chromatographic runs as short as 15 min.

The advent of the "Evosep" concept as mentioned above has been possible because the advances in mass spectrometry and computation have allowed the changeover from long chromatographic runs (90-120 min) to short ones (5-15 min) with a relatively high number of features differentiated, about 1000 to 2000 proteins, enough to efficiently profile the proteome of one patient and thus allowing to classify large cohort of patients. But, what kind of advances have prompted mass spectrometry to convert the Evosep concept in a disrupting moment in proteomics?

The introduction of the orbitrap in 2005 [6] was a breakpoint in the history of mass spectrometry and has dominated the market during the last ten years. However, it seems the tide to turn over the reign of the orbitraps after the introduction by the Bruker company [7] of the new trapped ion mobility spectrometry (TIMS) technology for higher-speed, higher-sensitivity and robust shotgun proteomics with outstanding single-shot peptide and protein identification performance. The ion mobility has added a new dimension of separation to shotgun proteomics, which join it to the Evosep chromatography concept has allowed to reach an unprecedented level of throughput to proteomics, almost 200 samples per day.

This new technology needs to be complement with computation and here is where the Parallel Accumulation Serial Fragmentation (PASEF) acquisition method enters to play [8].

(PASEF) enables hundreds of MS/MS events per second at full sensitivity. Also, this is due to the combination of unprecedented MS/MS acquisition speed (> 100 Hz), increased sensitivity (X50), uncompromised resolution performances and the benefit of an orthogonal ion mobility separation.

The advent of the proteomics era in the late 90´s held the promise of a new era in diagnosis and prognosis. Such promise was the perspective of some visionaries. A vision that was established too early and that led some people to believe the promise would never be completed. Now, almost two decades later, the promise seems closer than ever. Calculations done, 200 samples can be now handled in just five working days, from sample treatment to mass spectrometry analyses. The only bottleneck that remains is computation, but no for data acquisition but for data interpretation. Moreover, when the computer scientists had resolved this last piece of the puzzle, then, proteomics will go where no other human knowledge has gone before. Such is the endeavor, we the proteomics, have ahead.

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