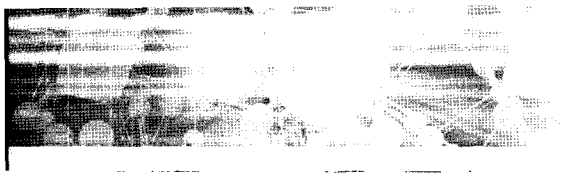

S4-2

Assessment of an Accurate Mass and Time tag strategy in the context of biomarkers discovery

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There is a growing body of evidence supporting the view that peptides and proteins abundances constitute reliable indicators of abnormal biological processes occurring in diseased states. Presently, clinical proteomics is mostly done using either classical 2D-PAGE based approaches followed by one-protein-at-a-time identification using peptide mass fingerprinting or MS/MS, or by SELDI-TOF. However, current methodologies suffer from significant drawbacks in terms of depth of coverage and either throughput or information content. There is therefore a crucial need in clinical biology to move to new technologies that will allow high throughput identification and quantification of thousands of proteins in very short time frame.

The Accurate Mass and Time (AMT) tag strategy consists in a two-phased approach in which peptides identified by MS/MS in the first phase can be elucidated during the second phase based only on their accurate mass and retention time measurements. The first phase of an AMT tag experiment consists in the accumulation of MS/MS evidence and LC retention time information for every peptide originating from a given biological system, cell, fluid or compartment. This generates a very large amount of qualitative data about the system under study, which is compiled into a so-called AMT tag database to allow easy referencing and retrieval during the second phase of the study. The second phase involves the acquisition of nanoHPLC/MS data, in which great attention is paid to the accuracy of the mass and retention time measurements. This information is all that will be required to identify a peptide in the AMT tag database. This non-reliance on MS/MS data during the second phase is the basis for much higher throughput, since all previously identified species can potentially be recognized in a single nanoHPLC-MS analysis, and better sensitivity, as species with abundances close to the limit of detection of the mass spectrometer can be monitored.

Over the past few years, the Protein Chemistry at CEA Grenoble has built a comprehensive analytical and data processing platform for AMT based proteomics with the stated goal to apply the methodology to disease biomarkers discovery in biological fluids and tissues. Based on our preliminary findings and the results of others, we ascertain the potentials of the AMT tag methodology for biomarkers discovery.