In proteomics, use of mass spectrometry has become more and more popular. Given the number of peptides coming from digestion of hundreds of proteins and dynamic range of their concentration, it is almost impossible to identify proteins of a very complex sample. The on-line coupling of Reversed Phase Liquid Chromatography with Electrospray (RPLC-ESI) is most frequent setup. The development of numerous devices allowing the collection of peptides in relation of mass flow-rate chromatography allowed the efficient implementation of the coupling between liquid chromatography and MALDI ionization source mass spectrometers. The analysis of samples with the two types of ionization (ESI and MALDI) allows a better coverage of the sequence of proteins of interest. In this work we have compared the results obtained combining these 2 ionization modes in order to established some workflow for a better proteome coverage of the samples.

### Optimization of the LC- MALDI coupling

#### Triflc digest:

A tryptic digest of 6 proteins from 11 kDa to 155 kDa (Cytocrom C, Lysozyme, Alcohol dehydrogenase, Bovine serum albumin, Serotonin and 6-Galactosidase, Dionex, Amsterdam, The Netherlands) is used at different concentrations to estimate the identification for each proteins.

**Chromatography liquid phase:**

The peptides are separated by a RP HPLC (U3000 Dionex): desalting and concentration are realized with a precolumn (C18 PepMap100, 3 μm, 300μm i.d., 5 mm length, Dionex). The separation of peptides is realized with an analytical column (C18 PepMap100, 5 μm, 300μm i.d., 15 cm length, Dionex) and a linear gradient from 0 to 60% solvent B in 35 min at 220 nL/min (buffer A: HACN/HFA, 98/2, v/v, buffer B: HACN/HFA, 10/90, 1, v/v).

**Microfraction collector**

The microfraction collector Probot® (Dionex) collects eluted peptides starting 15 min after the injection of the sample, to prepare on-line the spot for mass spectrometry analysis. For each run, 240 spots (1 spot every 10 seconds) are collected with a coaxial micro matrix rate at 0.43 μml/min.

**MALDI-TOF/TOF Analysis**

MS analyses are obtained with a mass spectrometer tandem 4800 MALDI-TOF / TOF Analyzer (Aplisys Applied Biosystems Inc., USA), in automatic mode switching between MS and MS/MS. Analysis MS is realized in positive reflection mode on a mass range 700 to 4000 Da, with an intensity of the laser above the description threshold. A precursors list is generated from MS analyses according to two major criteria: minimum signal-to-noise ratio at 40 for the top 7 precursors. Peptides are automatically selected in the spot where the signal is most intense for MS/MS analyses in positive mode (2V-TOF200 with an intensity of the laser approximately of 150% superior to that in MS mode).

### Results of the optimization

20 fmol of the tryptic digest is first analyzed with the system, as illustrated below:

#### Figure 1: schematic representation of the LC-MALDI system

The results obtained during the different analyses in LC MALDI allow us to validate our experimental set-up. This method turns out to be a technique of choice for the identification of proteins in complex mixtures. Furthermore, as previously stated, it gives a more extensive analysis of peptides. Several global analyses in MS and MS/MS can be successively realized on the same LC separation with different parameters on the opposite of electrospray analysis where the sample is definitively injected and is not any more available afterwards. Thus it is interesting to observe the differences between both methods of ionization.

#### Figure 2: 20 fmol tryptic digest chromatogram

#### Figure 3: Result of Mascot search for 20 fmol tryptic digest

#### Figure 4: Tryptic digest of 3 fmol Result of Mascot search

### Comparison between ESI and MALDI ionization modes

To compare both types of ionization, two LC-MSMS series have been realized: a first comparison between RPLC-MALDI-TOF and RPLC-ESI-QTOF and a second between RPLC-MALDI-Orbitrap and RPLC-LIT-FTICR. The use of MyProMs® (Proteome Analysis using Mass Spectrometry with MYSQL Database) developed by Curie Institute (Paris, France) allows to visualize the results with a schematic representation of the sequence coverage for each protein and for the two ionization modes.

Here is the example of the D-ribose-binding periplasmic protein precursor Escherichia coli. 3 peptides are identified for each ionization mode, and the combination of ESI and MALDI increases the sequence coverage from 29.7% to 44.6% (a global 50% increase).

#### Comparison RPLC-ESI-QTOF versus. RPLC-MALDI-TOF/TOF analysis

The use of MyProMs® (Proteome Analysis using Mass Spectrometry with MYSQL Database) developed by Curie Institute (Paris, France) allows to visualize the results with a schematic representation of the sequence coverage for each protein and for the two ionization modes.

#### Comparison RPLC-MALDI-ORBITRAP versus RPLC-ESI-LIT-FTICR analysis

Analysis of 100 fmol of E. coli tryptic digest with RPLC-MALDI-ORBITRAP and RPLC-ESI-LIT-FTICR allows to identified 44 common proteins. Here is the example of the DNA-directed RNA polymerase subunit alpha.

#### Figure 7: Comparison of the RPLC-MALDI-ORBITRAP and RPLC-ESI-LIT-FTICR analyses for Trigger factor protein precursor

In spite of the different dynamic ranges of the mass spectrometers it is interesting to combine the various results to observe the specificity of peptides according to the ionization used. The schematic representation show the specificity of peptides to appears only with a ionization mode.

Coupling the chromatography and mass spectrometry is important for the identification of complex samples. However the coupling between RPLC and one single ionization mode not sufficient for an extensive identification of the peptides. The use of one alternative ionization mode method contribute to a better coverage of the sample (the proteome coverage). Furthermore the use of both ionization modes allows to increase significantly the protein sequence coverage.

We intend to set-up a post LC split to analyze the same sample separation with both methods to maximize sequence coverage which is crucial for post translational modifications studies. This study will also be applied to 2D-LC for very complex samples.