Improve knowledge of complex samples by multiplying dimensions of analyses: Complementarity of LC-MALDI and LC-ESI

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Introduction

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 set-up. The development of numerous devices allowing the collection of peptides in relea of nano 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 a 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 samples.

Optimization of the LC- MALDI coupling

The coupling between chromatography and the microfraction collector is first optimized.

Tryptic digest:
A tryptic digest of 6 proteins from 11 kDa to 135 kDa (Cytochrom C, Lysozyme, Alcohol dehydrogenase, Bovine serum albumin, Serotransferrin and ß-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 d.i., 5 mm length, Dionex). The separation of peptides is realized with an analytical column (C18 PepMap100, 3 µm, 300µm d.i., 15 cm length, Dionex) and a linear gradient from 0 to 50% solvent B in 35 min at 220 nL/min (buffer A: $H_2$0/ACN/FA, 98/2/0.1,v/v/v, buffer B: $H_2$0/ACN/FA, 10/90/0.1, v/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 matrix flow rate at 0.436 µl/min.

MALDI-TOF/TOF Analysis
MS analyses are obtained with a mass spectrometer tandem 4800 MALDI-TOF / TOF Analyzer (Applera Applied Biosystems Inc., USA), in automatic mode switching between MS and MS/MS. Analysis MS is realized in positive reflectron mode on a mass range 700 to 4000 Da, with an intensity of the laser above the desorption 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 (2kV-TIS200 with an intensity of the laser approximately of 150 % superior to that in MS mode).

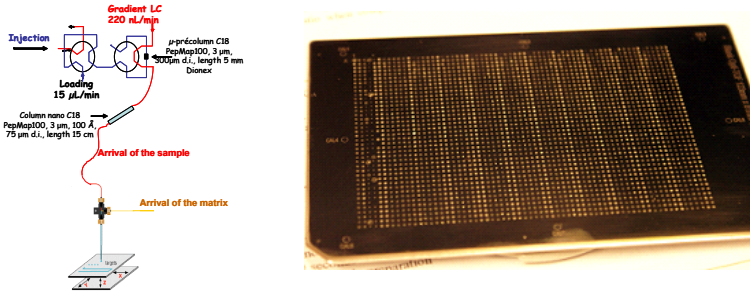
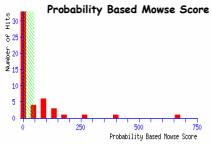


Figure 1: schematic representation of the LC-MALDI system

Results of the optimization

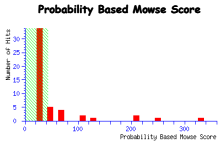
20 fmol of the tryptic digest is first analyzed with the system, as illustrated below:

Figure 2: 20 fmol tryptic digest chromatogramm



PROTEIN NAME	ACCESSION NUMBER	NUMBER OF PEPTIDES	PROTEINE SCORE
Serotransferrin precursor	Q29443	30	664
Serum albumin precursor	P02769	26	405
Cytochrome c	P00015	6	179
Lysozyme C precursor	P00698	9	280
Beta-galactosidase	P00722	10	151
Alcohol dehydrogenase 1	P00330	9	133

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



PROTEIN NAME	ACCESSION NUMBER	NUMBER OF PEPTIDES	PROTEIN SCORE
Serotransferrin precursor	Q29443	8	330
Serum albumin precursor	P02769	7	247
Cytochrome c	P00015	3	208
Lysozyme C precursor	P00698	3	111
Beta-galactosidase	P00722	1	54
Alcohol dehydrogenase 1	P00330	1	44

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

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.

Comparison between ESI and MALDI ionization modes

To compare both types of ionization, two LC-MS/MS series have been realized: a first comparison between RP MALDI-TOF/TOF and RPLC-ESI-QTOF and a second between RPLC-MALDI-Orbitrap and RPLC-LIT/FTICR. realize this comparison the studied sample is a tryptic digest of 100 fmol protein extract from Escherichia coli. The combination of the two ionization modes allows to increase the sequence coverage of each protein.

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

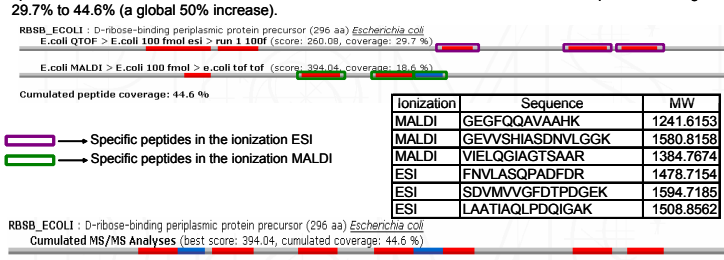
To study the complementarities of analysis, proteins identified in both analyses are selected. The associat identified peptides are listed together with the percentage of sequence coverage within one single analysis a within both combined analyses.

Proteins	MW (kDa)	Description - Organism	coverage %MALDI	coverage %ESI	Percentage of cover with a combined anal
RPOA_ECOLI	36.5	DNA-directed RNA polymerase subunit alpha Escherichia coli	24.9	32.2	43.2
RBSB_ECOLI	31	D-ribose-binding periplasmic protein precursor Escherichia coli	18.6	29.7	44.6
HNS_ECOLI	15.5	DNA-binding protein H-NS Escherichia coli	29.9	23.4	39.4
EFTU_ECOLI	43.3	Elongation factor Tu Escherichia coli	11.9	5.8	11.9
DNAK_ECOLI	69.1	Chaperone protein dnaK Escherichia coli	5.5	2.4	7.8
RL25_ECOLI	10.7	50S ribosomal protein L25 Escherichia coli	30.9	9.6	30.9
RL7_ECOLI	12.3	50S ribosomal protein L7/L12 Escherichia coli	29.9	37.2	47.1
TIG_ECOLI	48.2	Trigger factor Escherichia coli	8.3	10.2	18.5
MALE_ECOLI	43.4	Maltose-binding periplasmic protein precursor Escherichia coli	5.8	4.5	10.4
AHPC_ECOLI	20.8	Alkyl hydroperoxide reductase subunit C Escherichia coli	14.4	15	29.4
FKBB_ECOLI	22.2	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase Escherichia coli	11.2	6.3	17.5
ENO_ECOLI	45.7	Enolase Escherichia coli	4.6	11.1	13.2
CSPC_ECOLI	7.4	Cold shock-like protein cspC Escherichia coli	17.4	42	42
RS1_ECOLI	61.2	30S ribosomal protein S1 Escherichia coli	2	7.9	9.9
DBHA_ECOLI	9.5	DNA-binding protein HU-alpha Escherichia coli	11	57.8	57.8
CSPA_ECOLI	7.4	Cold shock protein cspA Escherichia coli	17.1	31.4	31.4

Figure 5: Sequence coverage for each protein of E.coli tryptic digest

The use of MyProMs® (Proteome Analysis using Mass Spectrometry with MYSQL Database) developed Curie Institute (Paris, France) allows to visualized the results with a schematic representation of the sequen 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 a specific to each ionization mode, and the combination of ESI and MALDI increase the sequence coverage fro 29.7% to 44.6% (a global 50% increase).

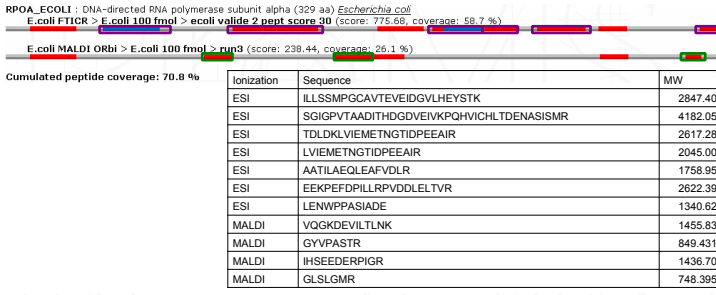


Ionization	Sequence	MW
MALDI	GEGFQQAVAAHK	1241.6153
MALDI	GEVVSHIASDNVLGGK	1580.8158
MALDI	VIELQGIAGTSAAR	1384.7674
ESI	FNVLASQPADFDR	1478.7154
ESI	SDVMVVGFDTPDGEK	1594.7185
ESI	LAATIAQLPDQIGAK	1508.8562

Figure 6: Comparison of the RPLC-MALDI-TOF/TOF and RPLC-ESI-Q-Tof analyses for D-ribose-binding periplasmic protein precursor

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

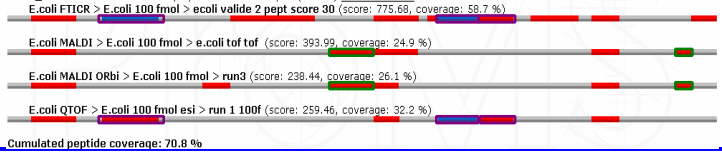
Analysis of 100 fmol of E. coli tryptic digest with RPLC-MALDI-ORBITRAP and RPLC-ESI-LIT-FTICR allows to identified 44 common proteins. Here is the example of the DNA-directed RNA polymerase subunit alpha.



Ionization	Sequence	MW
ESI	ILLSSMPGCAVTEVEIDGVLHEYSTK	2847.40
ESI	SGIGPVTAADITHDGDVEIVKPQHVICHLTDENASISMR	4182.05
ESI	TDLDKLVIEMETNGTIDPEEAIR	2617.28
ESI	LVIEMETNGTIDPEEAIR	2045.00
ESI	AATILAEQLEAFVDLR	1758.95
ESI	EEKPEFDPILLRPVDDLELTVR	2622.39
ESI	LENWPPASIADE	1340.62
MALDI	VQGKDEVILTLNK	1455.83
MALDI	GYVPASTR	849.431
MALDI	IHSEEDERPIGR	1436.70
MALDI	GLSLGMR	748.395

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

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 identificati 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 the sample (the proteome coverage). Furthermore the use of both ionization mod allows to increase significantly the protein sequence coverage.
We intend to set-up a post LC split to analyze the same sample separation with bo methods to maximize sequence coverage which is crucial for post translati modifications studies. This study will also be applied to 2D-LC for very complex samples