ICPL-based quantitative MudPIT analyses: novel development and applications

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Abstract

High throughput analysis of complex proteome has become an achievable goal thanks to technologic development mainly in proteins/peptides separation and mass spectrometry. To date, stable isotope labelling procedure is more and more used for high throughput analysis of complex proteome, but substantial improvements are still needed in terms of efficiency. Indeed, even if the number of labelling reagent is growing exponentially none of them has completely resolved all drawbacks in terms of reliability, sequence coverage, complexity reduction, etc.

In this context, our lab has undertaken to compare the cICAT strategy with the more recently developed ICPL technology. ICPL clearly performed better in terms of number of proteins identified as in terms of number of peptides usable for quantification. Using ICPL, no more 80% of the identified proteins are generally quantified. In order to increase the number of obtained quantitative data, we have adapted the amine reactive ICPL reagent to a post digest scheme, in which all N-terminal amines of tryptic peptides are ICPL tagged. This "post-digest" ICPL approach clearly increased the number of proteins that can be identified and quantified. Comparison of the three approaches, cICAT, regular ICPL and "post-digest" ICPL have been realised.

The results of regular ICPL application to analysis of two different growth conditions of a model bacterium (*C. metallidurans* CH34) are also presented. First the effects of cultivation of the bacteria on acetone as sole carbon source were analysed. This stimulus induced drastic changes at the proteome level, as already highlighted by 2D DIGE and µarray analysis. Data obtained with ICPL were compared to those of 2D DIGE and clearly show a high correlation of the results but also an increased number of differentially expressed proteins identified in ICPL analysis. The second example of regular ICPL application concerned the growth of our model bacterium in simulated microgravity. In this case, the stimulus is supposed to induce very slight effect at the proteome level. Indeed, most of the proteins were not affected by these cultivation conditions. However, thanks to ICPL high accuracy, we were able to highlight a very interesting and unexpected shift of the bacterium metabolism to chemolithoautotrophy.

Figure 1

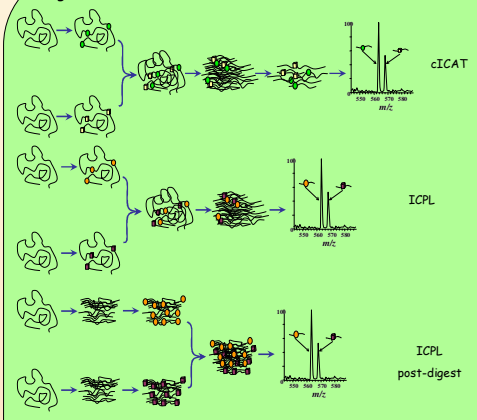
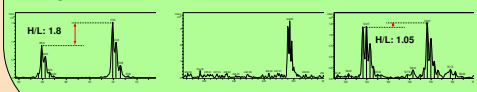


Figure 2



Differential MudPIT strategies comparison

- ✓ cICAT, regular ICPL and the homemade "ICPL post-digest" strategies (fig. 1) were compared
- ✓ ICPL always performed better than cICAT in terms of number of identified and quantified proteins, as well as in terms of number of quantifiable peptides/proteins (fig. 3)
- ✓ However, even while using the SILE based precursor selection option that allow precursor selection only if differentially expressed peptides are detected (fig. 2), +/- 20 % of identified proteins were still not quantified in regular ICPL
- ✓ ICPL procedure was applied to tryptic peptides what increased both number of identified and quantified proteins(fig.3)
- ✓ Incomplete labeling of N-terminal primary amine was nowadays always detected if regular ICPL procedure was just transfer to tryptic peptides
- ✓ A protein standard mixture (3 proteins) was used to optimize the ICPL post-digest procedure
- ✓ We were finally able to get complete labeling of the proteins mixture as can be observed in Maldi-Tof spectrum in which no single pic could be observed! Moreover, LC MS/MS analysis of the labeled proteins mixture (25µg!) failed to detect unlabeled peptides.
- ✓ ICPL post-digest allows high throughput high coverage differential MudPIT analysis!
- ✓ Non isobaric N-terminal labeling is compatible with SILE based precursor selection

Figure 3

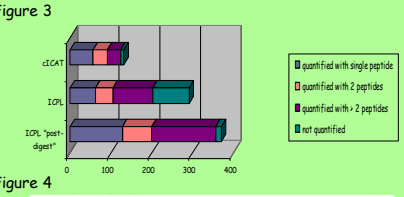
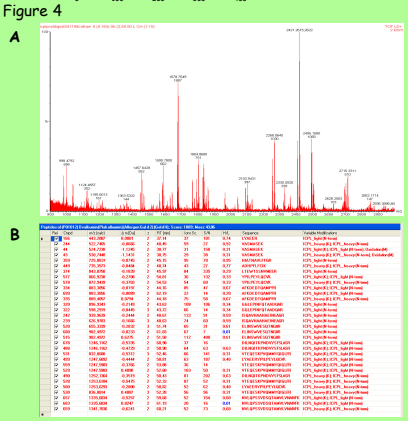
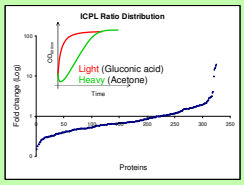


Figure 4



Acetone metabolism

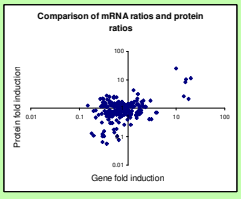
- ✓ *C. metallidurans* cultivated with 25 mM acetone as sole carbon source and compared to gluconic acid growth control
- ✓ Two independent biological replicates were analyzed
- ✓ 25µg of proteins analyzed in SCX-RP MS/MS, SILE based precursor selection



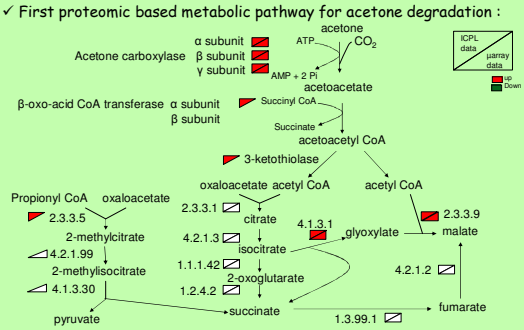
- ✓ 443 different proteins identified (FDR: 0.74%)
- ✓ 325 quantified proteins
- ✓ 115 proteins differentially expressed (threshold: 2; 0.5)
- ✓ maximum fold change: 18X!

	2D-DIGE	ICPL
Acetone carboxylase subunit B		
Acetone carboxylase subunit A		
Acetone carboxylase subunit C		
β-oxo-acid CoA transferase subunit A		
β-oxo-acid CoA transferase subunit B		
PhA serine kinase		
Isocitrate lyase et phosphorylmutase		
NAD+ dependant aldehyde dehydrogenase		
Malate synthase A		
Alkaline phosphatase		
Extracellular ligand-binding receptor		
Periplasmic phosphate binding protein		
Uncharacterized protein UFP0065		
Phosphonate transport system substrate binding protein		
Phosphonate binding periplasmic protein		

- ✓ Main proteomic modification observed in 2D DIGE were also identified in ICPL
- ✓ ICPL detected more than 80 additional highly regulated proteins of unknown role in acetone metabolism!
- ✓ ICPL results were highly correlated with µarray data (0.61)



- ✓ First proteomic based metabolic pathway for acetone degradation :



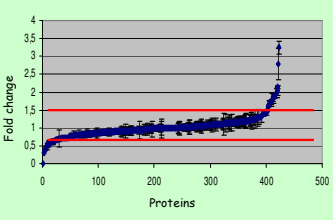
- ✓ Uncolored triangle: identified proteins not differentially expressed

Simulated microgravity

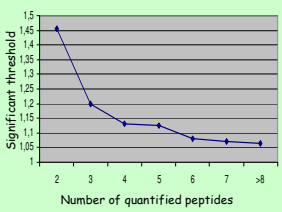
- ✓ microgravity was simulated using Random Positioning Machine (RPM) and compared to 1xg control
- ✓ three pooled biological replicates were analyzed twice (technical replicates)
- ✓ 25µg of proteins analyzed in SCX-RP MS/MS, SILE based precursor selection
- ✓ manual validation of quantitative data
- ✓ t-student based statistical analysis of quantitative data

Protein name	NCBI ID	#	fold	sd
cytochrome d1, heme region	gi|94312103	10	2,78*	0,44
cytochrome c oxidase, cbb3-type, subunit II	gi|94310981	2	1,99*	0,13
cytochrome c, class I	gi|94312355	1	1,89	
ribulose 1,5-bisphosphate carboxylase small subunit	gi|94310442	8	1,81*	0,12
ribulose bisophosphate carboxylase	gi|94310443	10	1,42*	0,06
phosphoribulokinase/uridine kinase	gi|94310454	3	2,04*	0,14
fructose-1,6-bisphosphate aldolase	gi|94310460	7	1,67*	0,08
nickel-dependent hydrogenase, large subunit	gi|94310241	6	1,65*	0,12
hypothetical protein Rmet_5074	gi|94313995	4	1,81*	0,06
hypothetical protein pMOL30_161	gi|56130790	2	1,97*	0,12
hypothetical protein Rmet_6330	gi|94152737	2	3,25*	0,18
hypothetical protein Rmet_0422	gi|94309367	5	1,6*	0,03
hypothetical protein Rmet_0458	gi|94309403	3	1,77*	0,18
hypothetical protein Rmet_1387	gi|94310329	4	1,72*	0,07

- ✓ Among differentially expressed proteins, one third were proteins of unknown function
- ✓ Maximum fold change for Rmet_6330, Plasmidic hypothetical protein!
- ✓ Decrease in redox related proteins (SOD)
- ✓ Important modification of proteins belonging to the respiratory chain were observed (shift to nitrate as terminal electron acceptor)
- ✓ CBB cycle up-regulated! ➡ Autotrophy!
- ✓ Nickel dependent hydrogenase: Hydrogen metabolism ➡ Chemolithoautrophy!
- ✓ Effect of lowered medium homogenization??



- ✓ 612 different proteins identified (FDR: 0.53%)
- ✓ 440 quantified proteins
- ✓ simulated microgravity induced very slight proteomic changes
- ✓ thank to manual validation significant threshold (calculated using t-student statistic) could be set to 1.5 (0.67)
- ✓ 27 and 21 proteins were down- and up-regulated, respectively
- ✓ maximum fold change was 3.4



Conclusions

- ✓ **ICPL demonstrated to be highly efficient**
- ✓ **ICPL can be used in post-digest scheme Optimization crucial!**
- ✓ **ICPL post-digest performed better than regular ICPL**
- ✓ **ICPL well suited for analysis of deep proteomic changes Acetone metabolism pathway highlighted!**
- ✓ **ICPL also efficient for very slight effect analysis Simulated microgravity induced crucial metabolic shift**

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