# Improved Protein Coverage and Throughput in Proteomics using On-Line Multiplexed Enzyme Digestions and Targeted MS/MS with a Modified LTQ-FTICR

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## Overview

- The use of pressurized systems for protein digestion in proteomics was demonstrated with the non-specific enzyme pepsin.
- The performance of the different pressurized systems, pressure cycling technology (PCT) and fast on-line digestion system (FOLDS), was characterized for pepsin digestions.
- The use of "Smart" MS/MS software improved the quality of the data by at least an order of magnitude.
- The coupling of FOLDS with a modified hybrid instrument improved the overall quality of the experiment in a reduced amount of time compared to standard protocols.
- Using this strategy we have, for example, been able to identify platinum-containing peptides in a matter of minutes.

## Introduction

- Protein digestion for bottom-up proteomics has traditionally been performed using a relatively lengthy incubation period (6-12 h).
- In current proteome workflows, digestion is one of the most time consuming steps in the analytical process and usually trypsin is the most widely used enzyme.
- The use of non-specific-cleavage (NSC) enzymes, such as pepsin, has the potential of providing full protein coverage, allowing for digestion experiments that can occur at a low pH.
- LC-MS/MS experiments followed a pseudorandom strategy in which the most intense ion in the survey scan was subjected to fragmentation, even if it was not a peptide.

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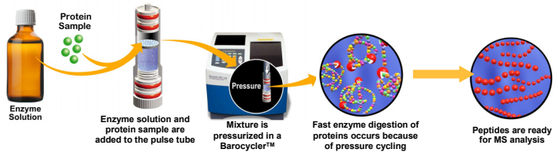
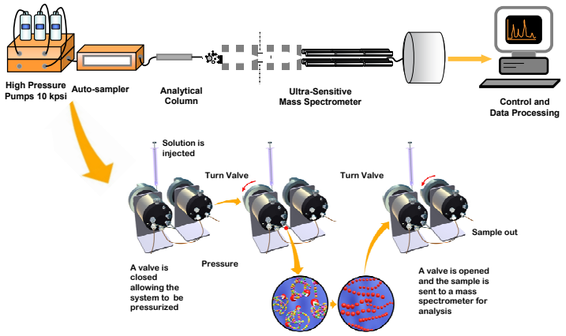
## Methods

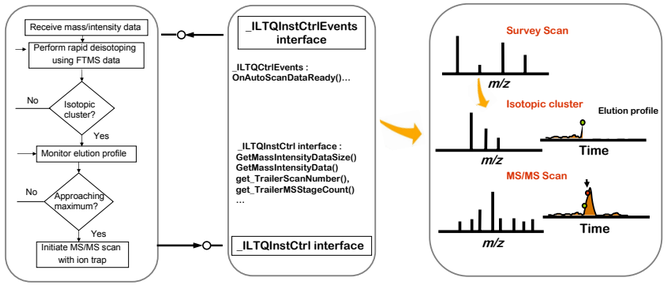
### Pressure Cycling Protein Digestion System (Off-line method[1])




### High-Pressure Enzymatic Protein Digestion System (On-line method[2,†])



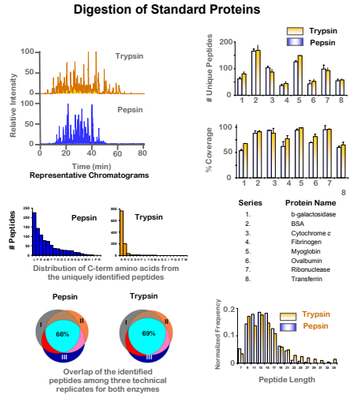

### Smart MS/MS Strategy with Hybrid Instruments




## Results

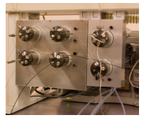
### Characterization of Pepsin-PCT-assisted digestion

#### Comparison between Pepsin and Trypsin Digestions in 2 min at 20 kpsi



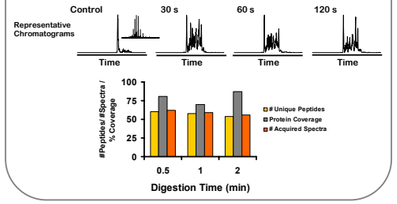

#### Improved Throughput and Decreased Sample Handling by Pepsin On-line Digestion

Custom built platform consisting of a modified LTQ-FT and automated Fast On-line Digestion System



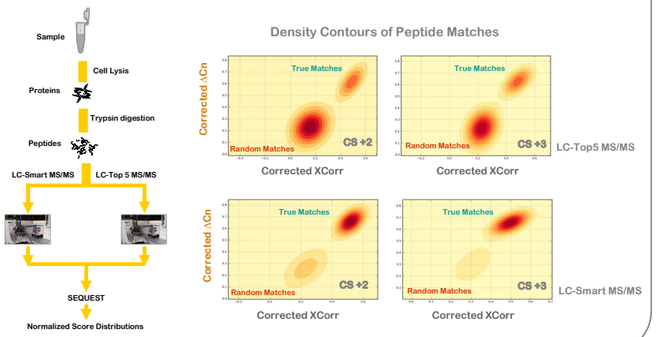
Detailed picture of the Fast On-line Digestion System coupled to a 2-column LC-system

#### Digestion of 100 ng of Enolase at 10 kpsi using the Fast On-line Digestion System

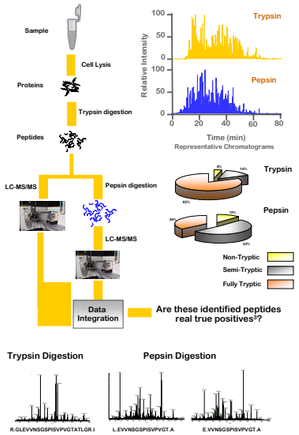



### Application of SMART MS/MS for targeted MS/MS

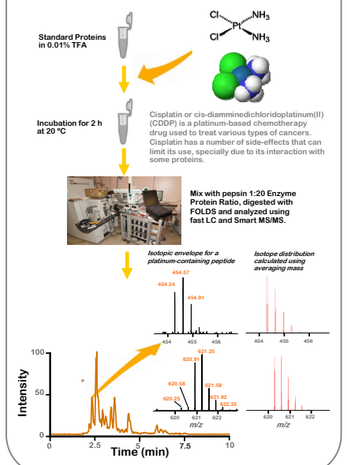
#### Comparison between Smart MS/MS and Top 5 MS/MS with dynamic exclusion




#### Improved protein coverage and confidence by sequential multiplexed fast enzyme digestions




#### Detecting the characteristic isotopic distributions of the platinum containing peptides




## Conclusions

- The use of either PCT or high pressure in protein digestion using pepsin allows for short reaction times (1-2 min).
- An on-line micro-reactor integrated into a capillary LC system coupled to a cutting-edge mass spectrometer has been developed.
- Although it has been shown that pepsin has no specificity, it cleaves in a specific pattern that is as reproducible as tryptic digests, which could prove valuable for the next generation of MS/MS search engines.
- FOLDS coupled to Smart MS/MS in a hybrid instrument analysis platform with the capacity to rapidly detect a large number of peptide ions, which is useful for protein characterization or monitoring of special isotopic envelopes.
- The use of multiplexed ultrafast digestions and targeted MS/MS adds confidence to peptide and protein identifications.
- We demonstrate for the first time the application of pepsin in pressurized systems for complete protein digestion for LC-MS/MS proteomics experiments.

## Acknowledgements

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†A utility patent covering the technology with respect to its application in proteomics and on-line embodiment of the system was filed with the USPTO in August 2008.

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## References

1. López-Ferrer et al., *J. Proteome Res.* 2008, 7: 3276-3281
2. López-Ferrer et al., *Anal. Chem.* 2008, 80: 8930-8936
3. Pevzner et al., *Science* 2008, 321 (5892):1040b.