Development of new technology approaches for high-throughput proteome analysis

Capitalizing on thermodynamic principles to reduce sample preparation times from hours to minutes

Daniel López-Ferrer

Biological Separations & Mass Spectrometry Group

Pacific Northwest National Laboratory

May 21, 2010

Pacific Northwest
NATIONAL LABORATORY

- OMICS

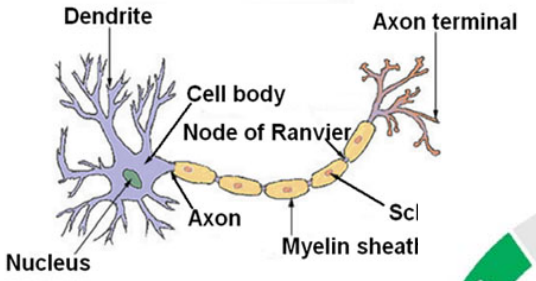
- Genomics
- Proteomics
- Peptidomics
- Glycomics
- Lipidomics
- Metabolomics

ECONOMICS

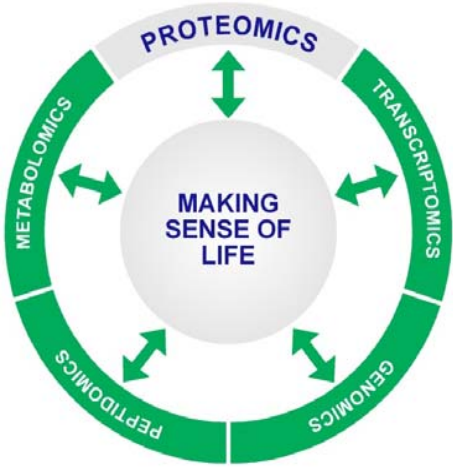
Pacific Northwest
NATIONAL LABORATORY

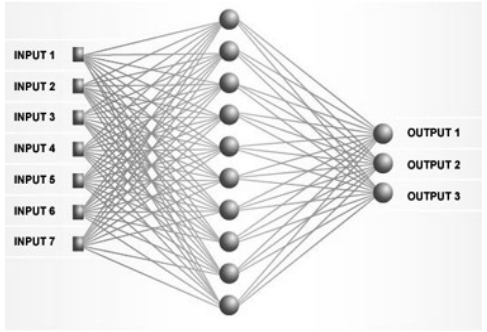
Making sense of life

- The ultimate aim of the modern movement in biology is to explain all biology in terms of physics and chemistry
- Information from proteomics studies increases understanding of biological interactions and processes



Reductionism





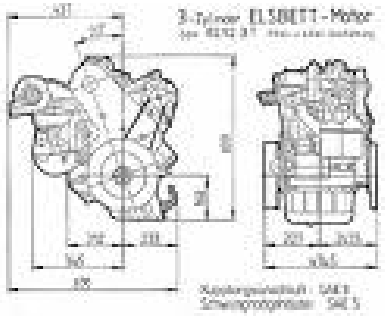
Systemic View

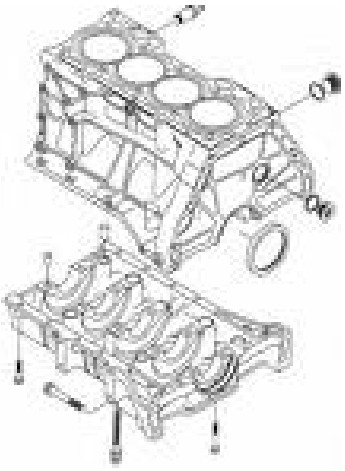
Important to advancing applications in bioremediation, diagnosis/ treatment of diseases, alternative energy systems, industrial processes, etc.

Pacific Northwest
NATIONAL LABORATORY

Proteomics: The study of the entire complement of proteins expressed at a given time in a cell, tissue, organ or organism under a specific set of conditions.

The Proteome





The Genome

Static

Protein Sequences

Static

The Proteome

Dynamic!

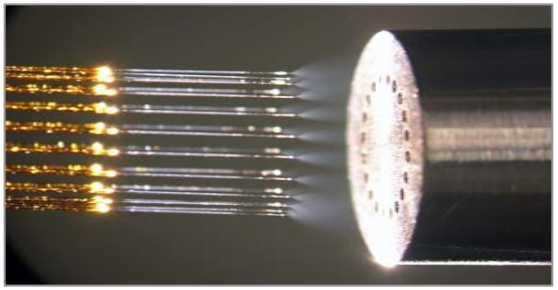
Pacific Northwest
NATIONAL LABORATORY

Liquid chromatography – mass spectrometry: key PNNL technologies for advanced proteomics

Pacific Northwest
NATIONAL LABORATORY

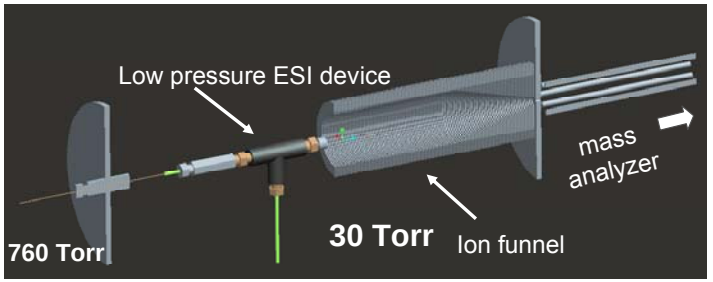
High Sensitivity Technologies Developed at PNNL

Multi emitters

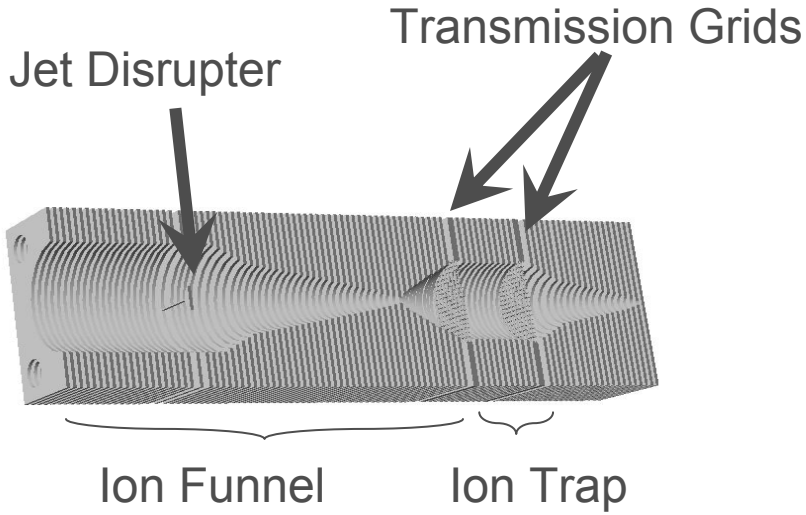


Kelly et al. *Anal. Chem.* **2008,** 5560-5565.

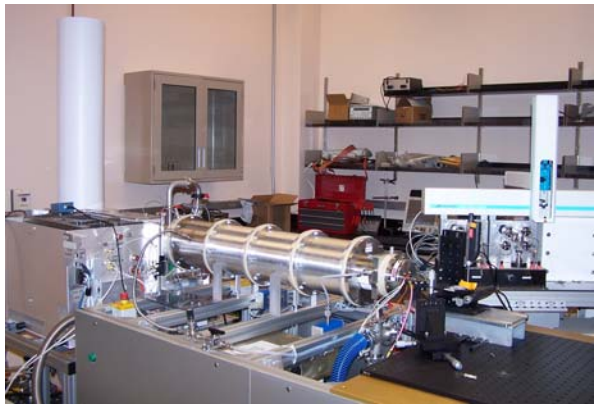
High Performance Interface
(~100% Ion Transmission)



Page et al. *Anal. Chem.* **2008,** 1800-1805.



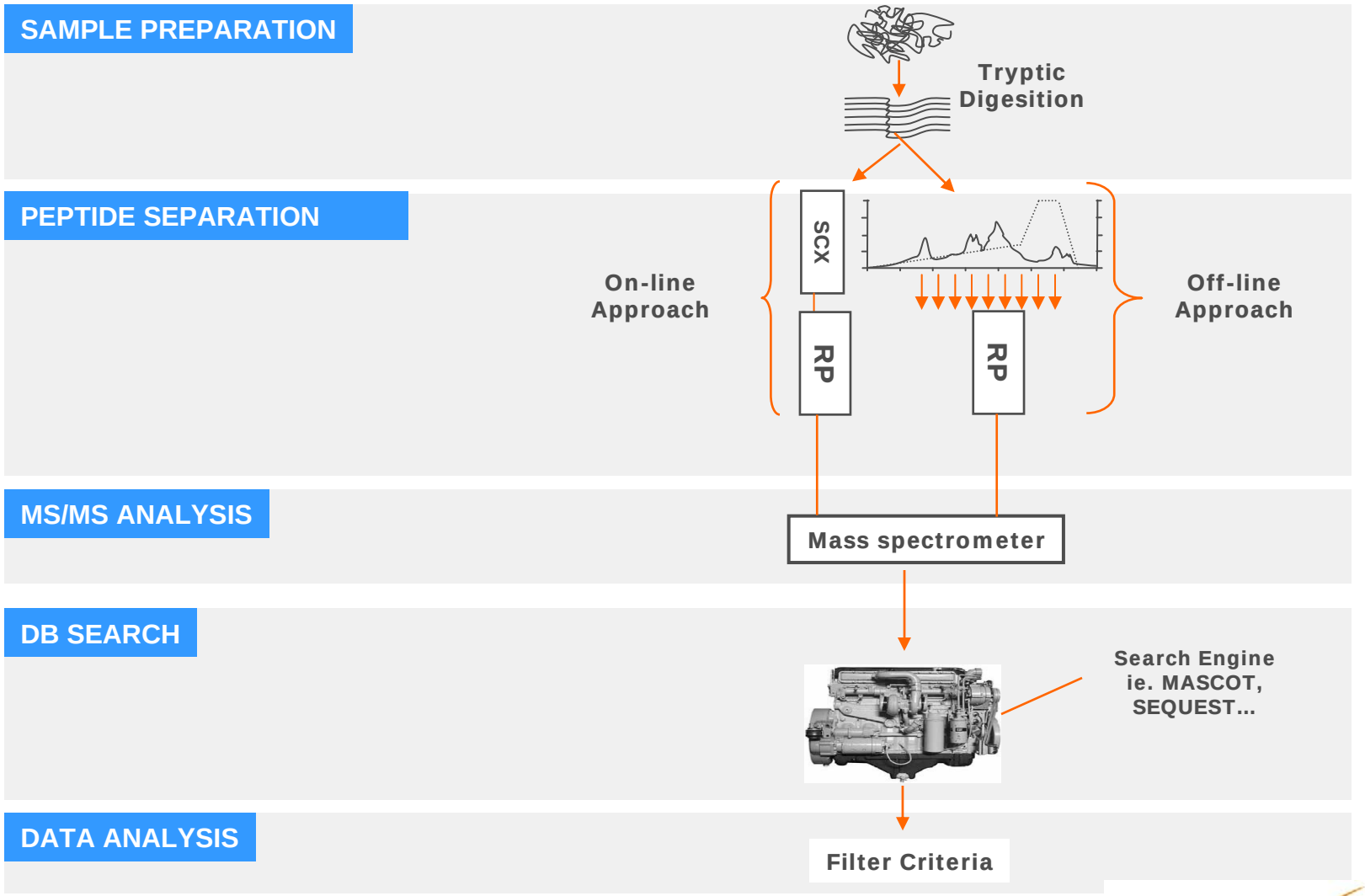
Clowers et al. *Anal. Chem.* **2008,** 5367-5376.



Ion mobility Time of Flight Mass Spectrometer

Belov et al. *Anal. Chem.* **2007,** 2451-2462.

Traditional “bottom up” proteomics



Pacific Northwest
NATIONAL LABORATORY

Effect of pressure on enzymatic activity

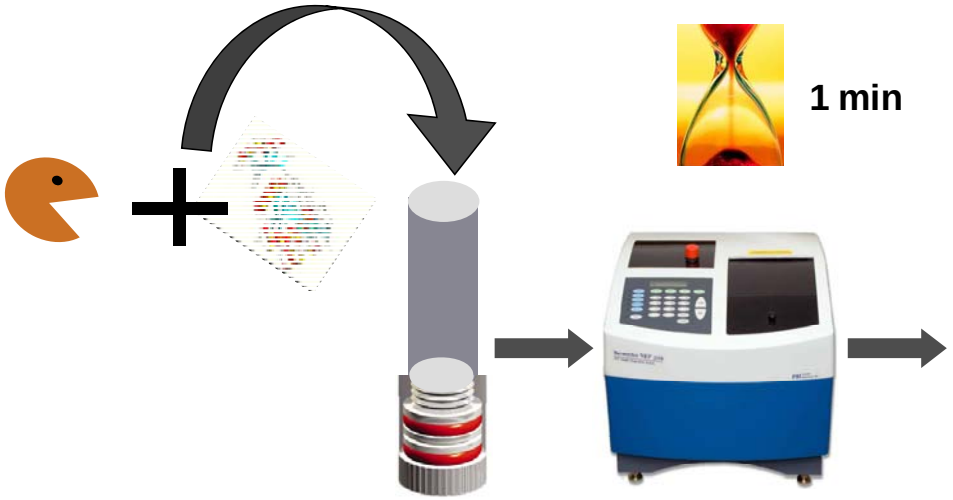
Ultrasounds accelerates chemical or enzymatic reactions due to cavitation, bubbles which collapse

- Localized high pressures
- Localized high temperatures
- Shear forces
- Jets
- Shock waves

energy

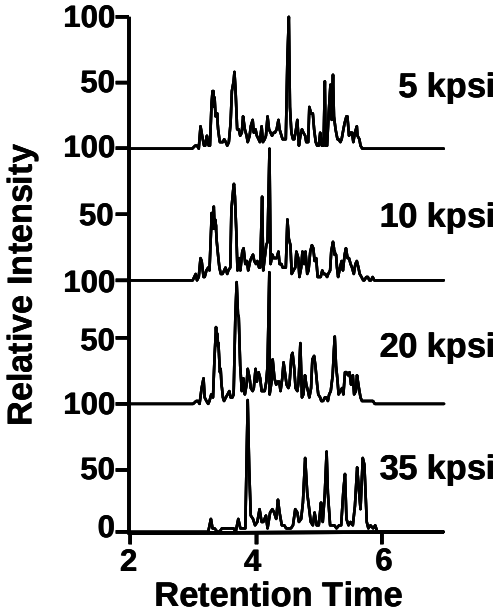
mixing

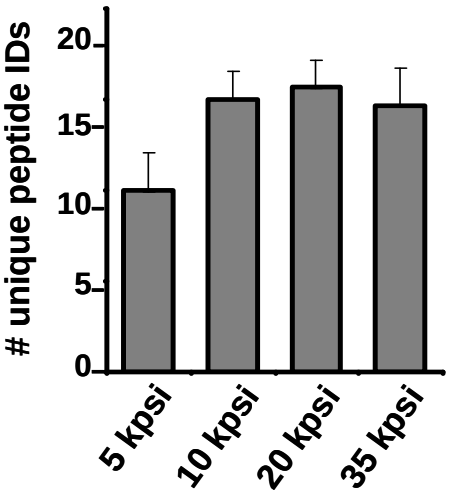
We have Pressure Cycling Technology in the lab…

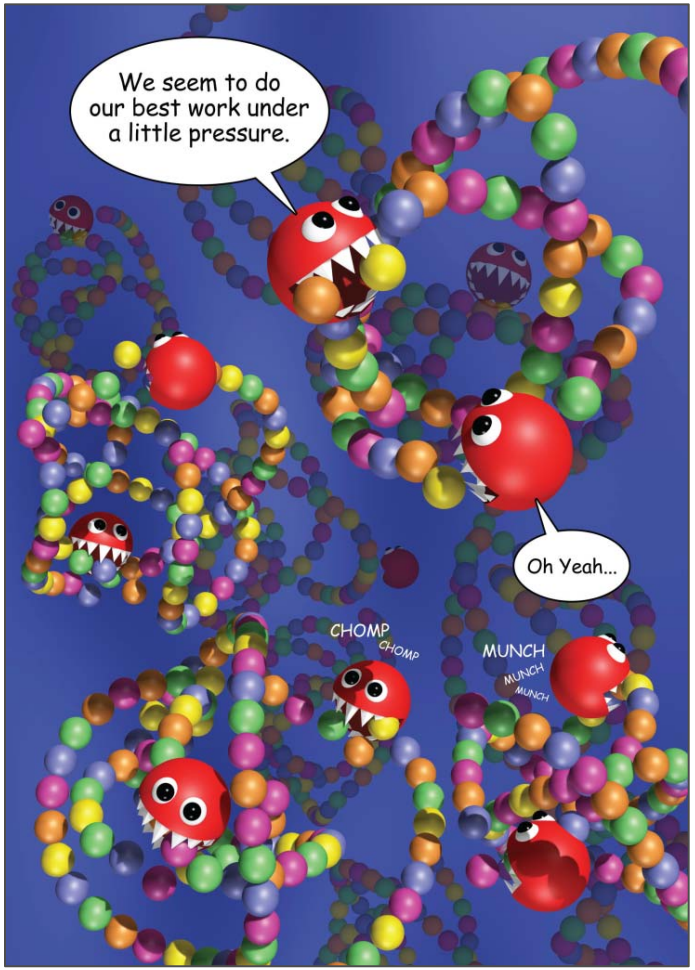


Pacific Northwest
NATIONAL LABORATORY

Complete sample protein digestion in 60 s with sufficient pressure







Lopez-Ferrer, D., et al. *J Proteome Res* 2008, 7, 3276-3281

Pacific Northwest
NATIONAL LABORATORY

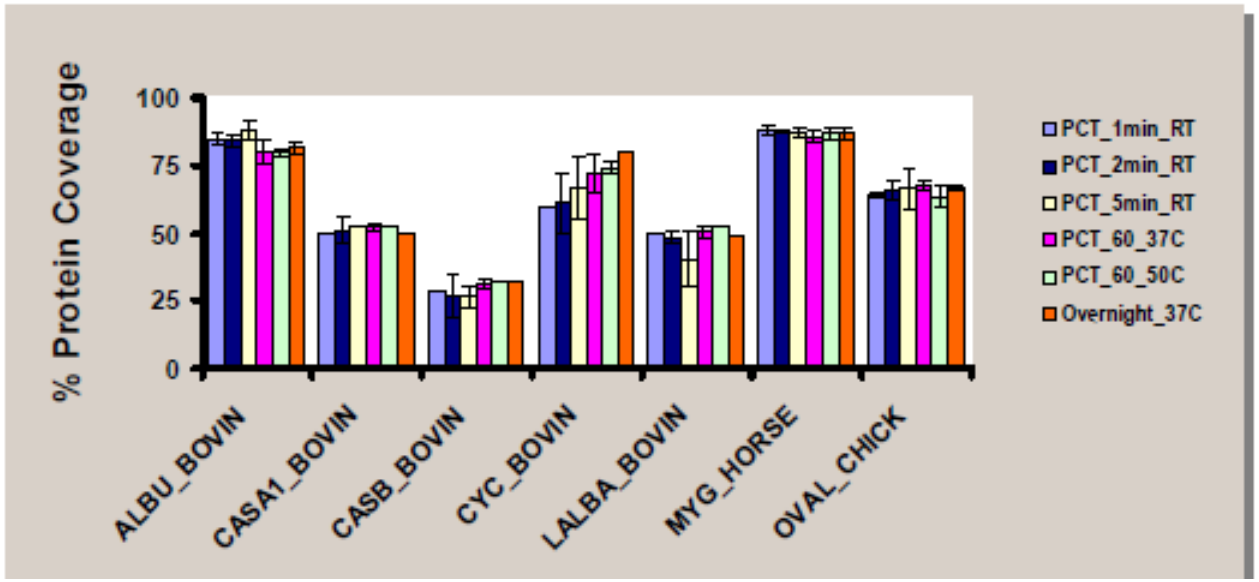
Development of micro tubes for PCT digestions of small samples

Albumin
α-Casein
β-Casein
Cytochrome *c*
Lactalbumin
Myoglobin
Ovalbumin

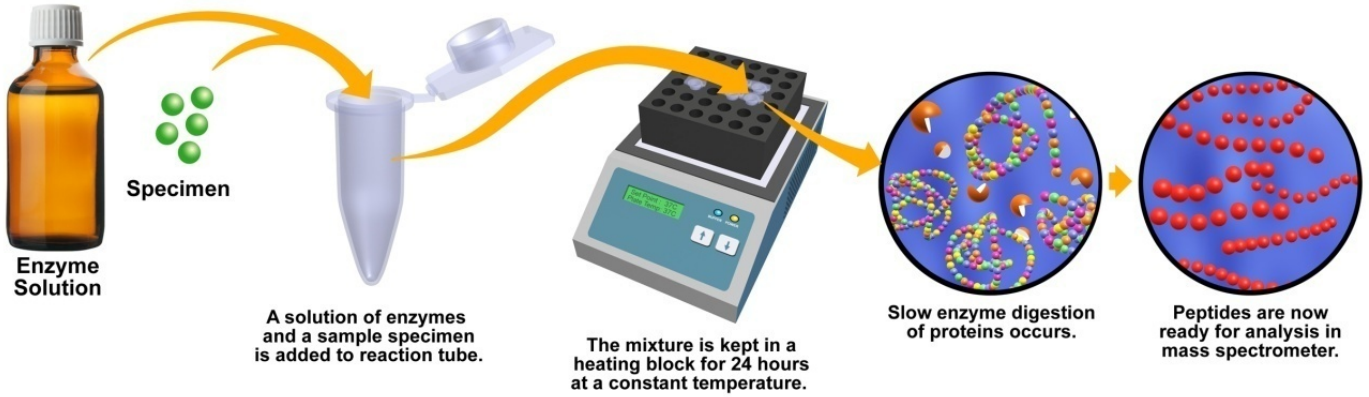
→ µLC-MS/MS

150 µL PCT MicroTubes
3 replicates at 20,000 psi

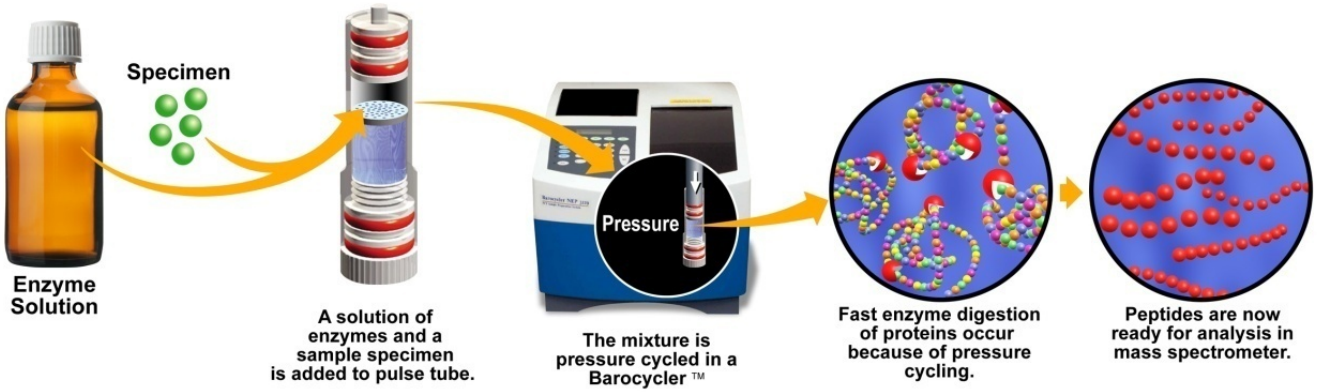
Standard Protein Digestions: Traditional vs. PCT



Traditional Protein Digestive Systems



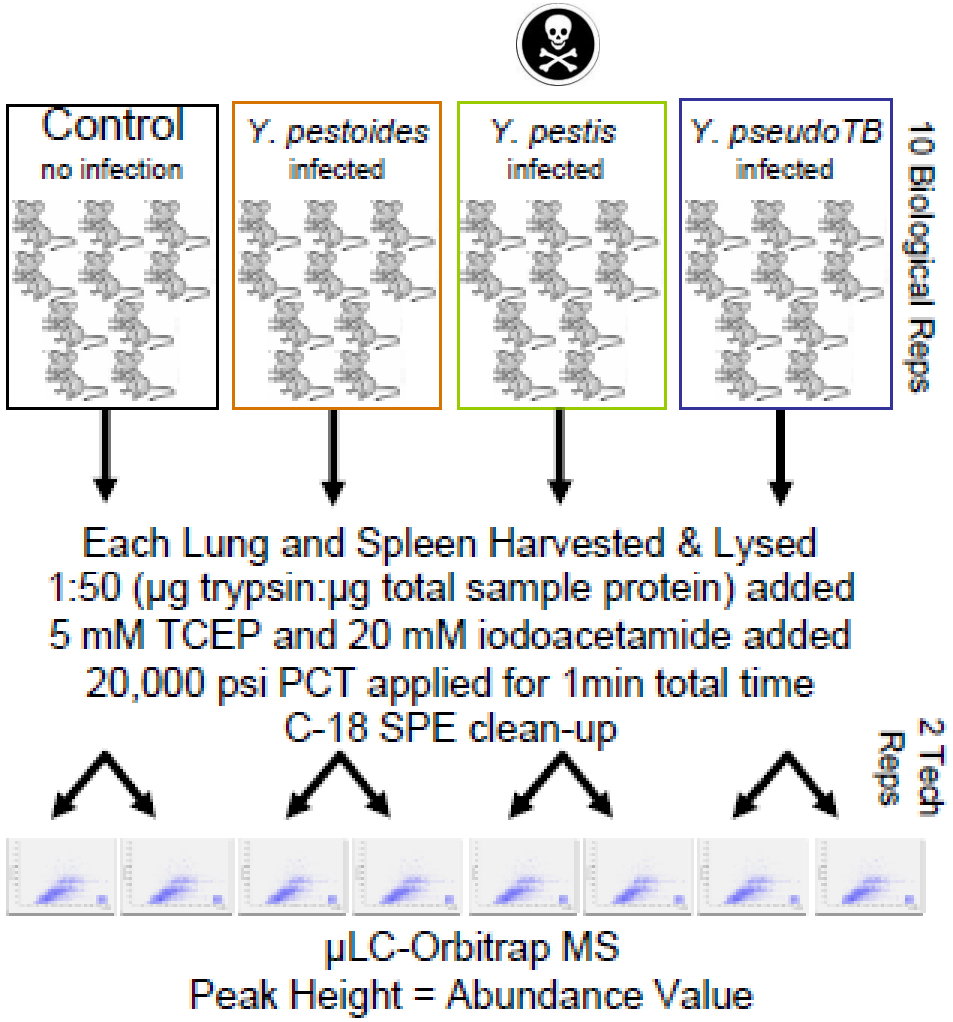
Presure Cycling Protein Digestive System

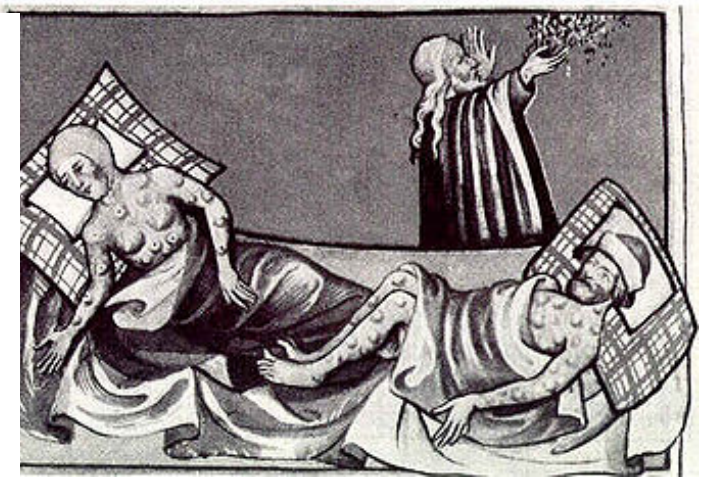


Pacific Northwest
NATIONAL LABORATORY

Initial PCT application: 20,000 psi

Yersinia pestis infection (relevant to biodefense)





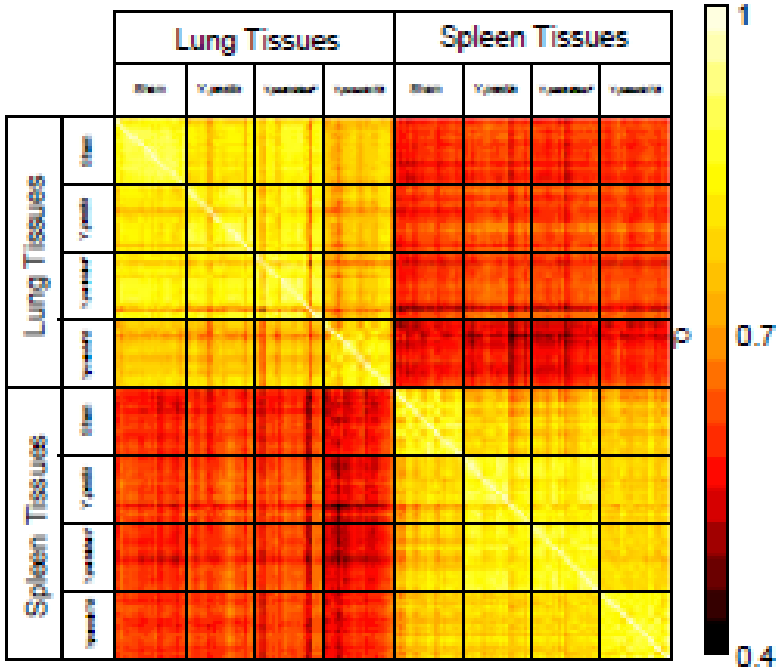
Y. pestis: **Bubonic Plague or Black Death**

Pacific Northwest
NATIONAL LABORATORY

Reproducibility of 20,000 psi PCT digestion: *Yersinia pestis*

Pearson's Pairwise Correlation of LC-MS runs

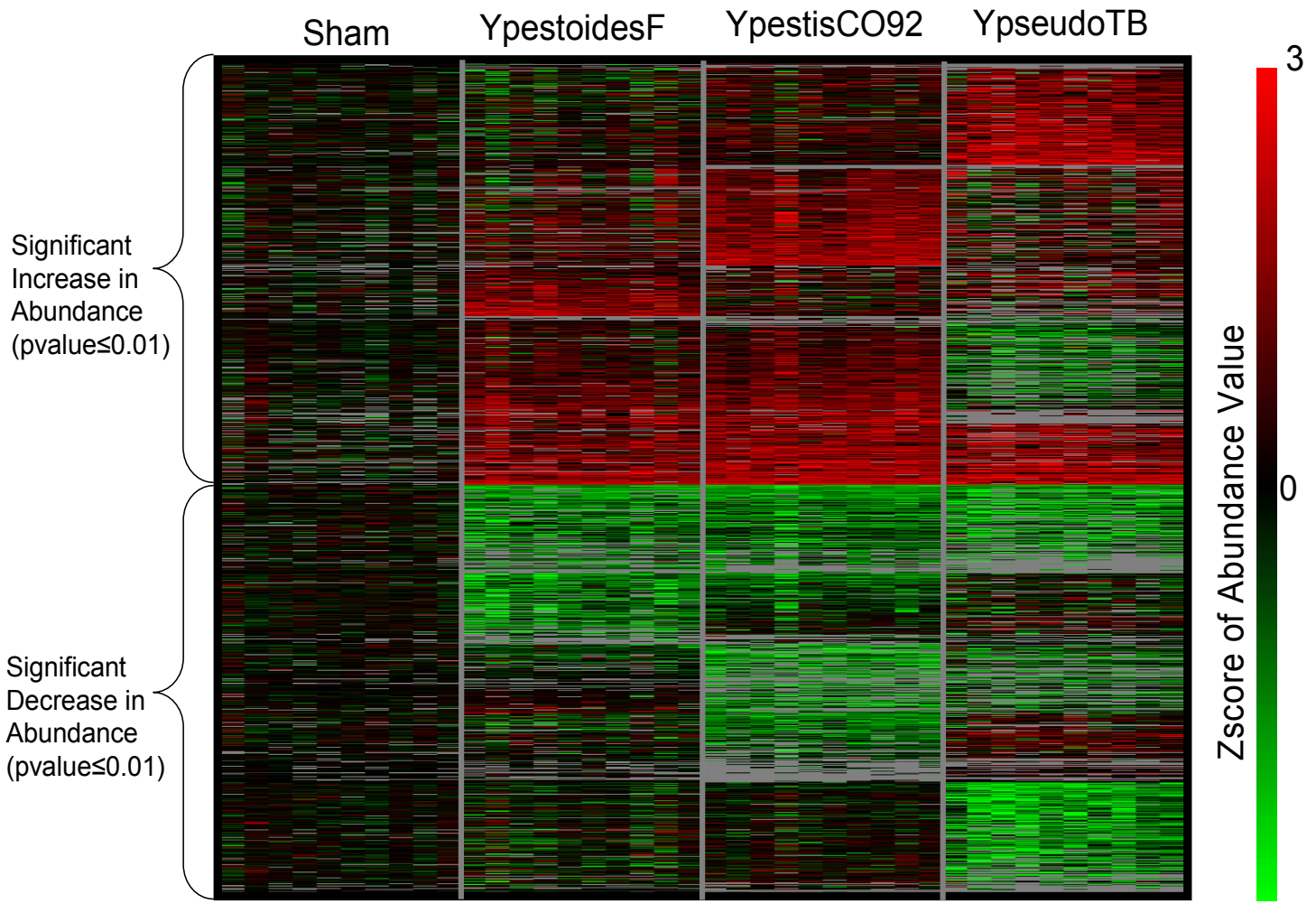
Correlated at the protein level (using the most present and abundant peptide across all runs to represent the protein abundance.



	Lung	Spleen
Avg. Biological Replicate Correlation	0.85	0.86
Avg. Instrument Replicate Correlation	0.89	0.91

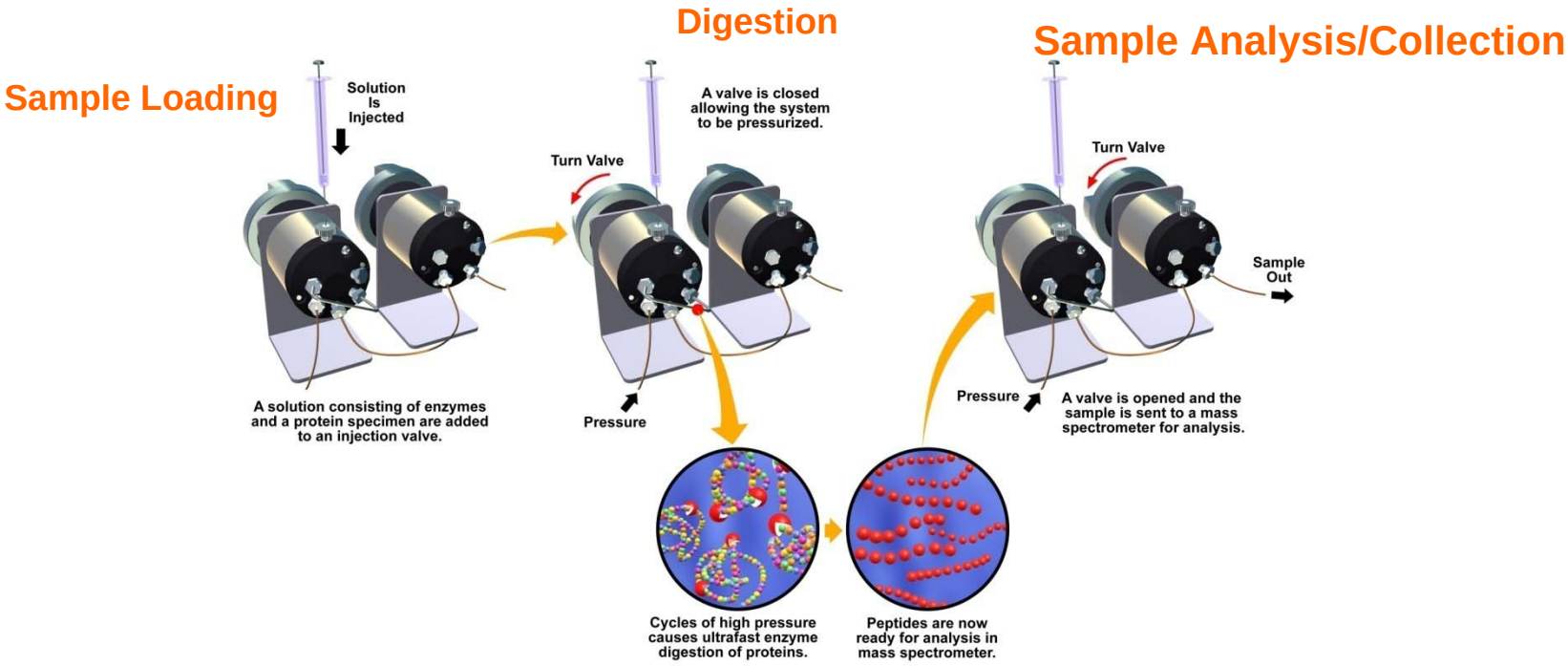
Pacific Northwest
NATIONAL LABORATORY

Reproducible sample preparation enables high quality quantitative proteomics



Pacific Northwest
NATIONAL LABORATORY

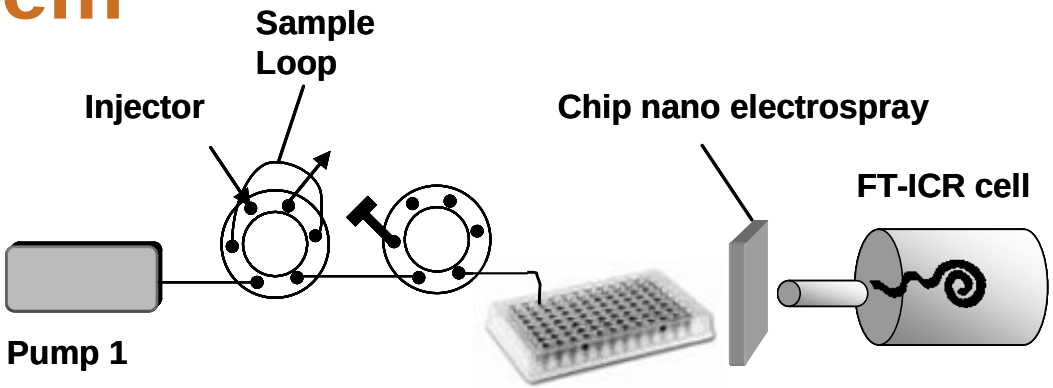
Development of a fast online pressure digestion system

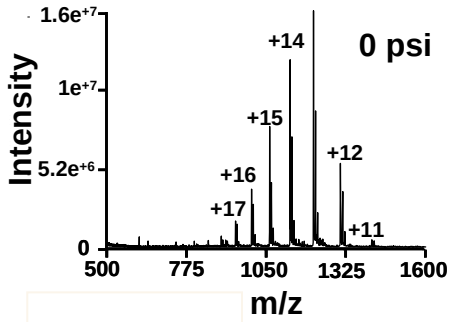


Lopez-Ferrer, D., et al. *Anal. Chem.* 2008, 80, 8930-8936

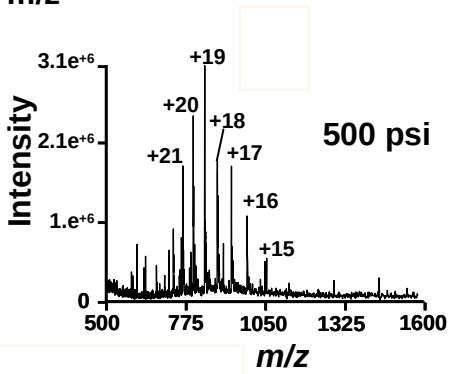
Pacific Northwest
NATIONAL LABORATORY

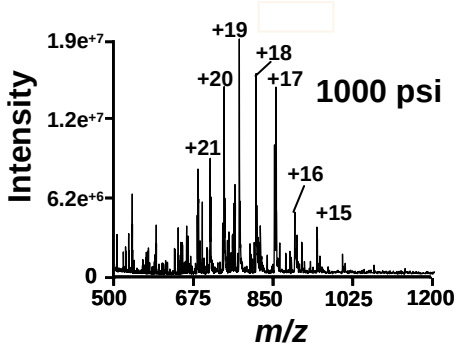
Initial application of fast on-line digestion system

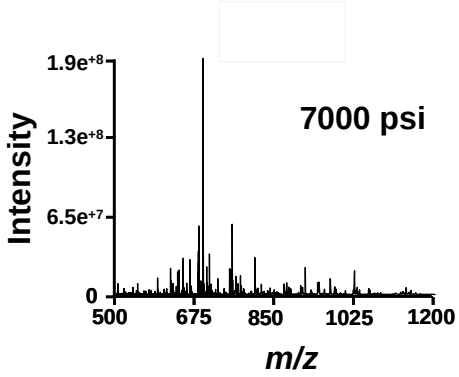




Myoglobin digestion in 1 min!!!!

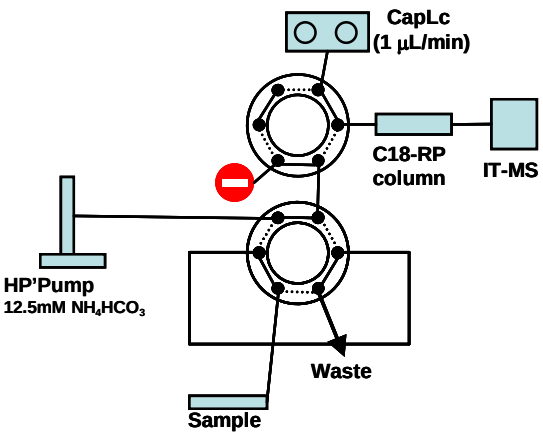


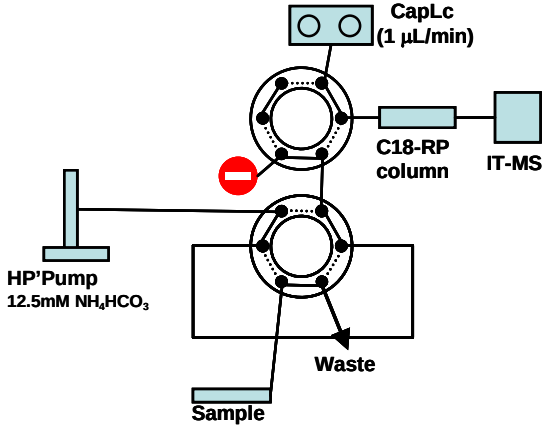


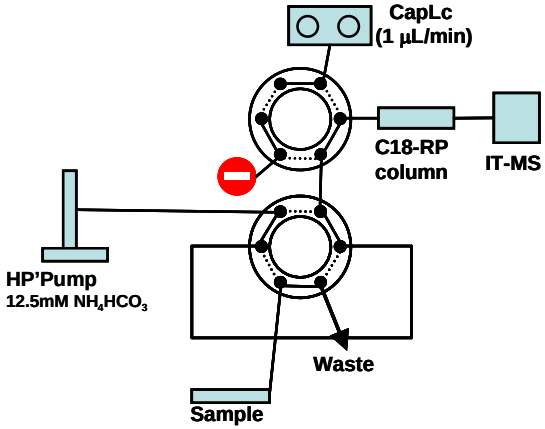


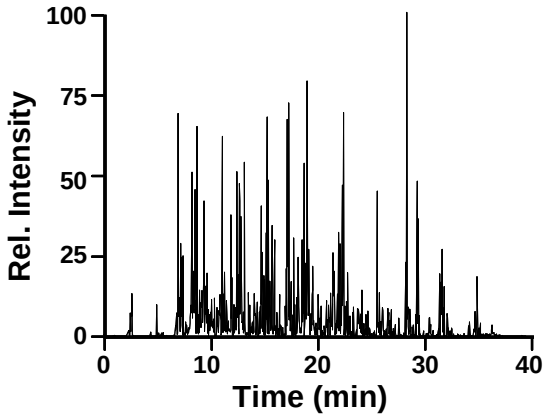
Pacific Northwest
NATIONAL LABORATORY

Coupling fast on-line digestion system with LC







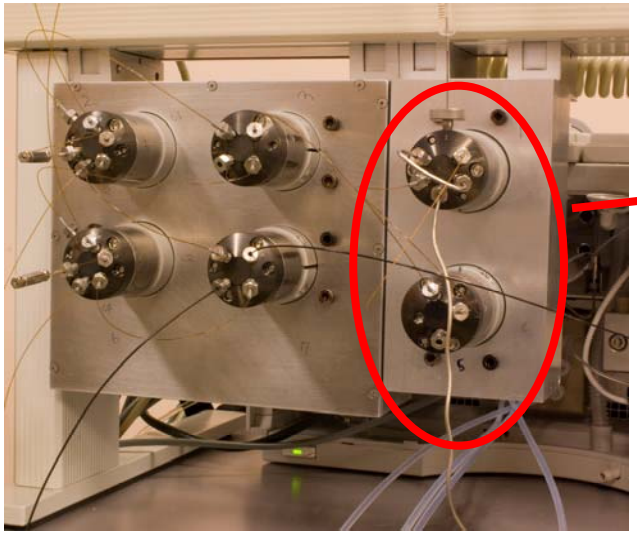



```
  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EQFKGLVLIA  50
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK 100
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF 150
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQD CCQAEDKGAC 200
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE 250
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE 300
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL 350
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL 400
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS 450
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC 500
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT 550
551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV 600
601 STQTALA                                              607
```

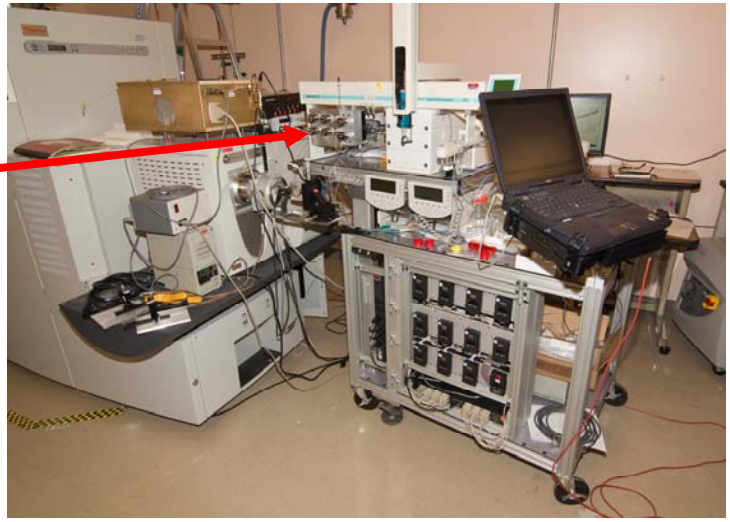
BSA 1 min digestion

Pacific Northwest
NATIONAL LABORATORY

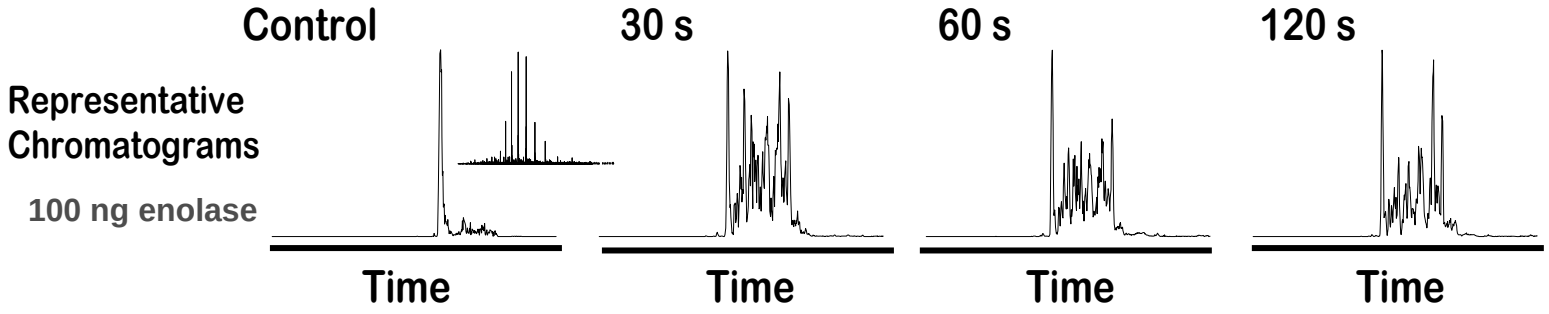
Automated fast on-line digestion system



Fast Online Digestion System coupled to 2-column LC-system



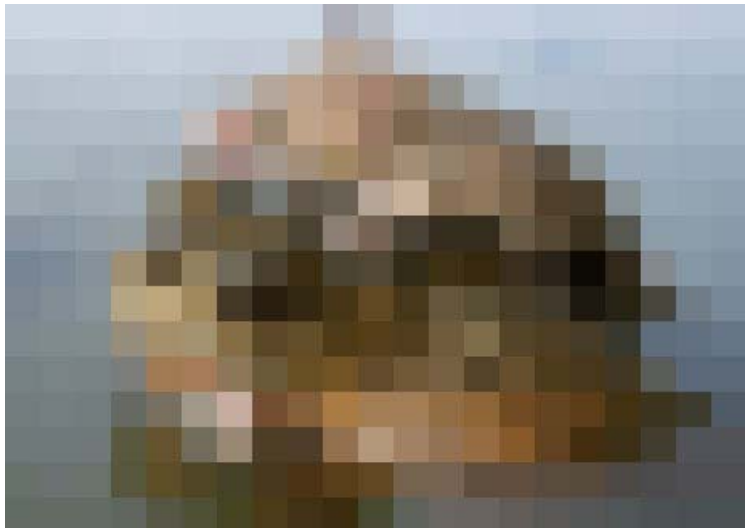
Custom built platform consisting of a modified LTQ-FT and automated Fast On-line Digestion System



Complete digestion at 10 kpsi

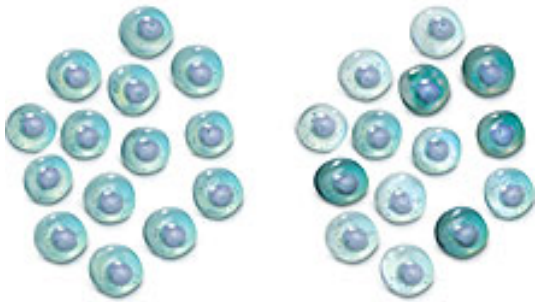
Pacific Northwest NATIONAL LABORATORY

Maximizing MS Sensitivity—Motivation



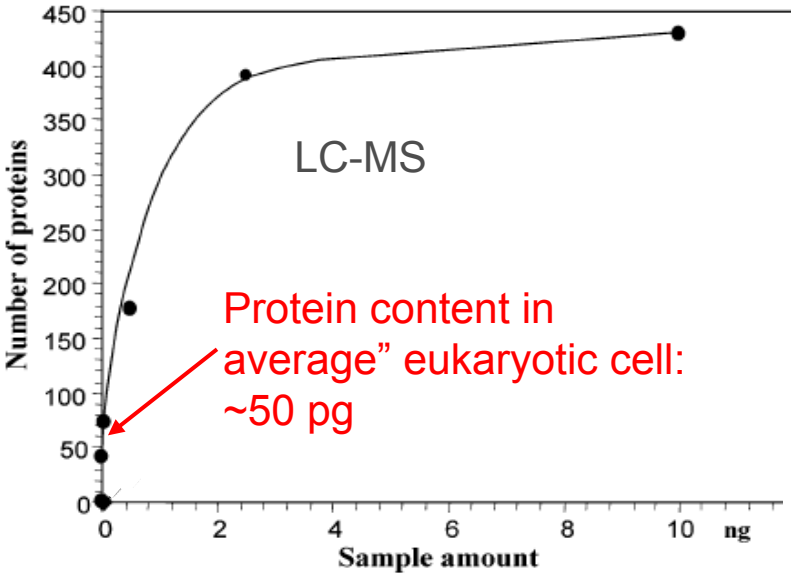
Complex Biological System

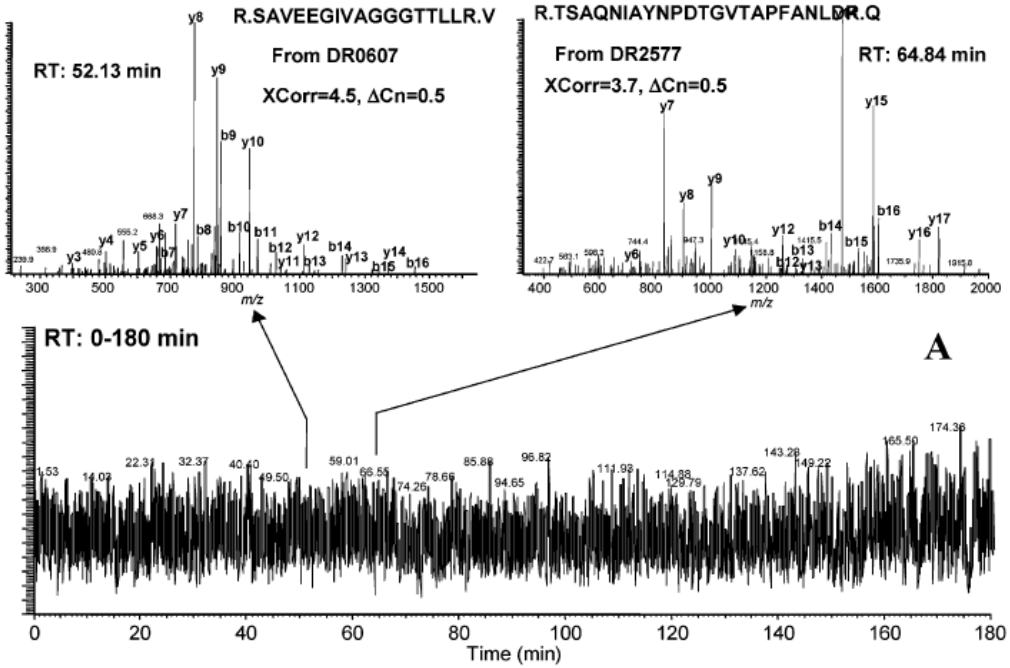
- Interest in cellular processes, composition, function
- Much larger samples generally required
- Heterogeneity obscured



http://beta.technologyreview.com/read_article.aspx?ch=specialsections&sc=emerging&id=18296

Maximizing Sensitivity



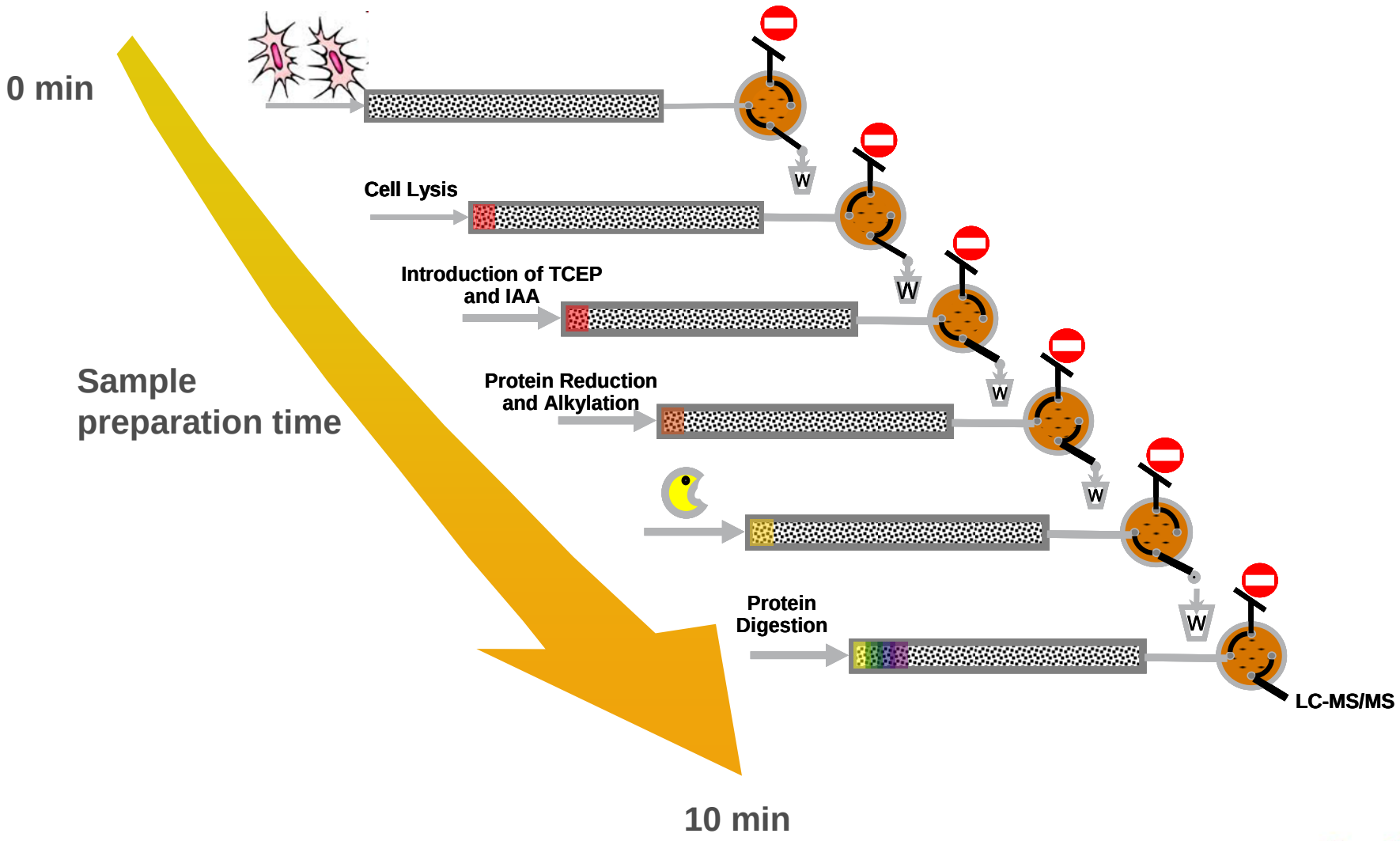


Y. Shen, et al. *Anal. Chem.*, **2004,** *76,* 144–154.

Have pg analytical sensitivity, but need µg–mg samples

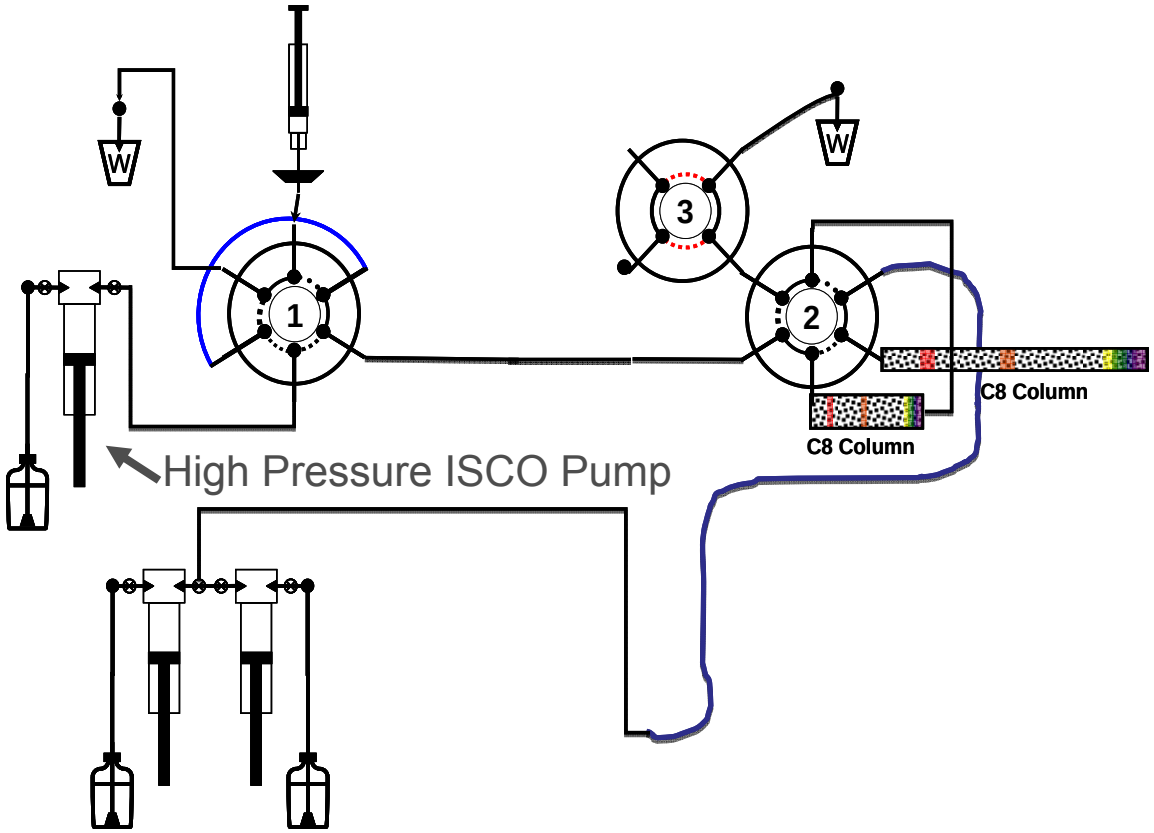
Pacific Northwest
NATIONAL LABORATORY

In-column pressure digestion

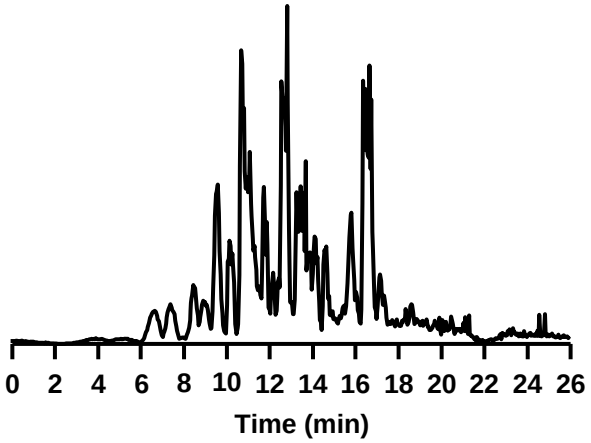


Pacific Northwest
NATIONAL LABORATORY

Pressure assisted in-column digester



Agilent 1200 Capillary Pump

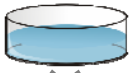


Characterization of Bovine Serum Albumin

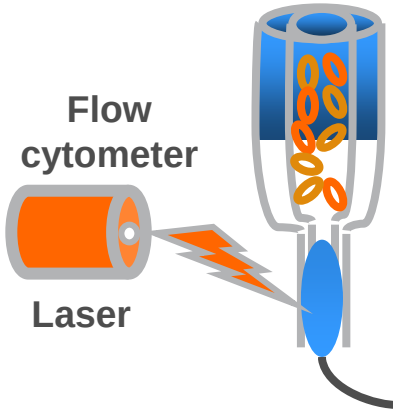
Pacific Northwest NATIONAL LABORATORY

Pressure assisted in-column digester

Mouse

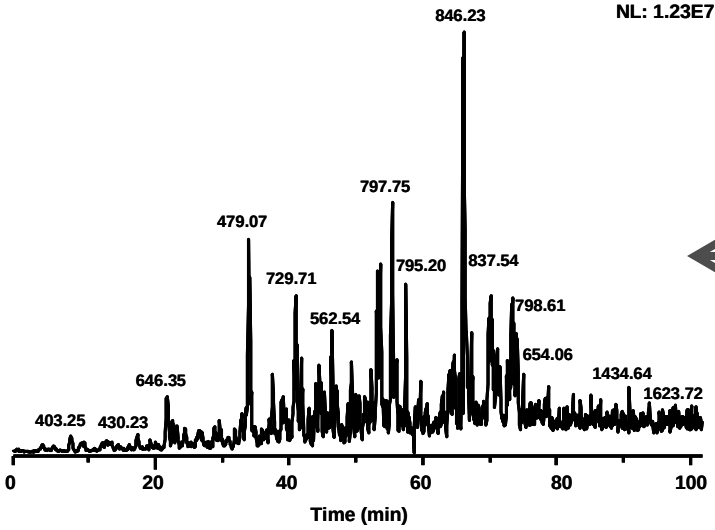


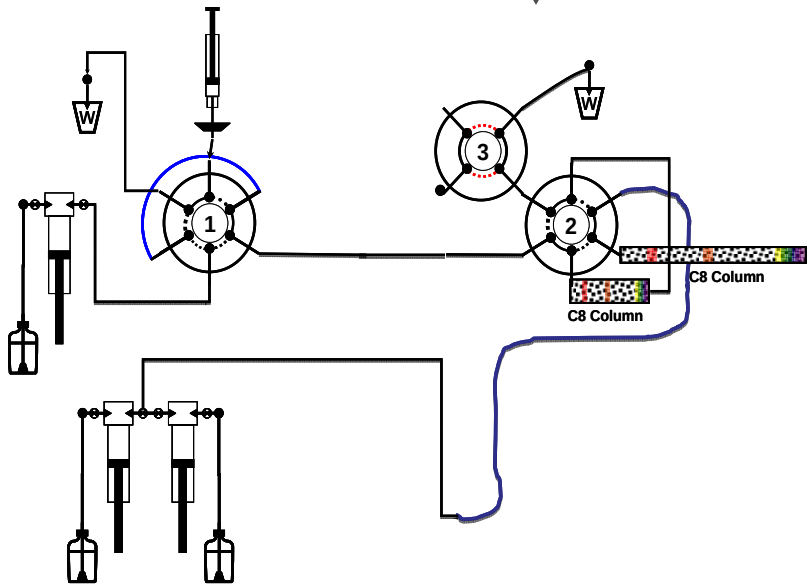
Bone marrow cells



Counted cells dentritic

3000 Cells



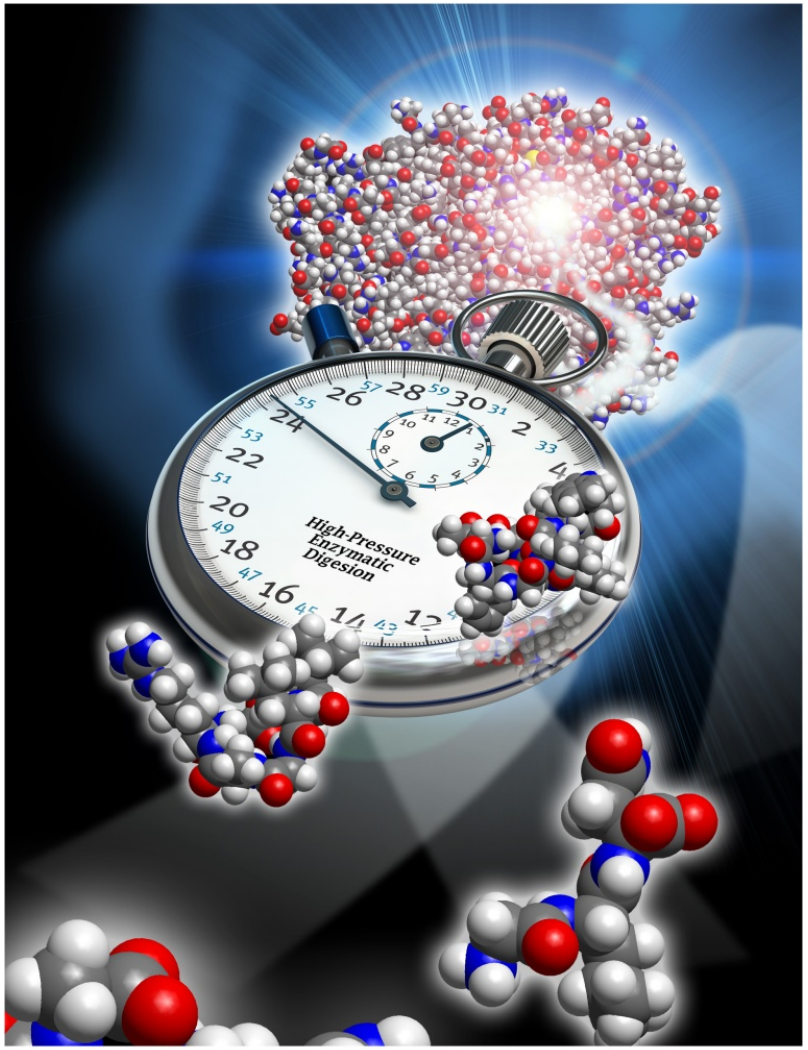


# of cells	# unique peptides	# unique proteins
3000 →	1023 →	421

Pacific Northwest NATIONAL LABORATORY

Summary

- High pressure (HP) plays an important role in enzyme kinetics.
- The use of HP in protein digestion allows for short reaction times (1-2 min) without losing selectivity.
- HP helps denature proteins allowing better access for the enzyme to digest them.
- An on-line micro-reactor integrated into a capillary LC system coupled to cutting-edge mass spectrometer has been developed.
- The in-column pressure digestion represents a unique capability to allow for the automation of proteomics workflows, as well as proteomics analysis of small samples.



The ***High-Pressure Enzymatic Digestion System for Ultrafast Protein Characterization*** breaks proteins into peptides in 60 seconds or less. It is the first protein digestion system that enables fast, high-throughput protein analyses in virtually limitless biological research applications in proteomics, genomics, peptidomics, lipidomics, and metabolomics.

Pacific Northwest
NATIONAL LABORATORY

Acknowledgements

BIOLOGICAL SEPARATIONS & MASS SPECTROMETRY GROUP

Pacific Northwest
NATIONAL LABORATORY