Applications of High Pressure for Automated High Performance Proteomics

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

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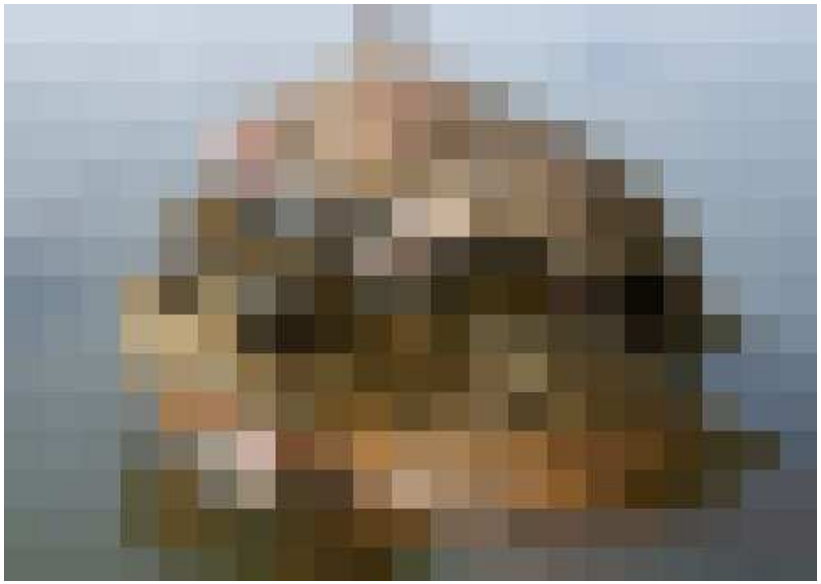
CAPRION PROTEOMICS - INTRODUCTION

- Located in:
  - Montreal, Canada
  - Menlo Park, California (recent acquisition of PPD Biomarkers)
- Founded in 2000
- 42 employees (36 scientists)
- Leading supplier of proteomics-based discovery technology: Cell*Carta*®
- Strategic partnership with Covance, a major international CRO
- Rapidly growing service business
  - Biomarker identification
  - Biomarker assays
  - Mechanism of action
  - Target discovery

Maximizing MS Sensitivity and Throughput—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

Effect of pressure on enzymatic activity

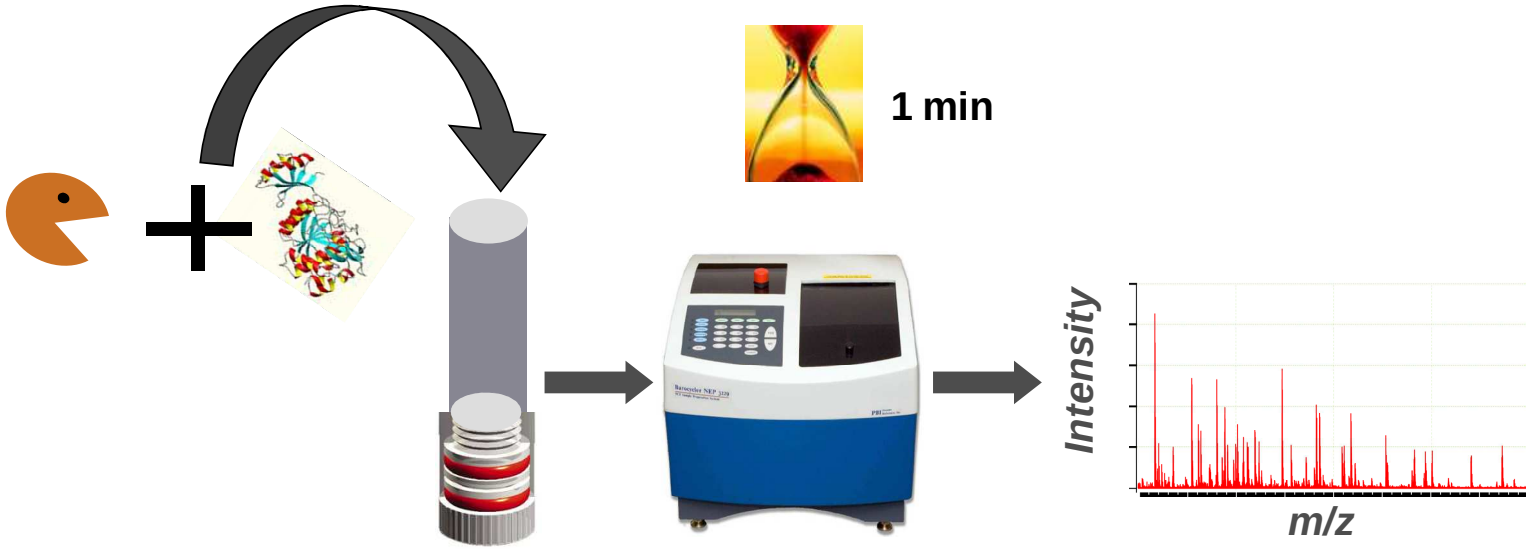
Ultrasounds accelerates chemical or enzymatic reactions due to cavitation, bubbles which collapse

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

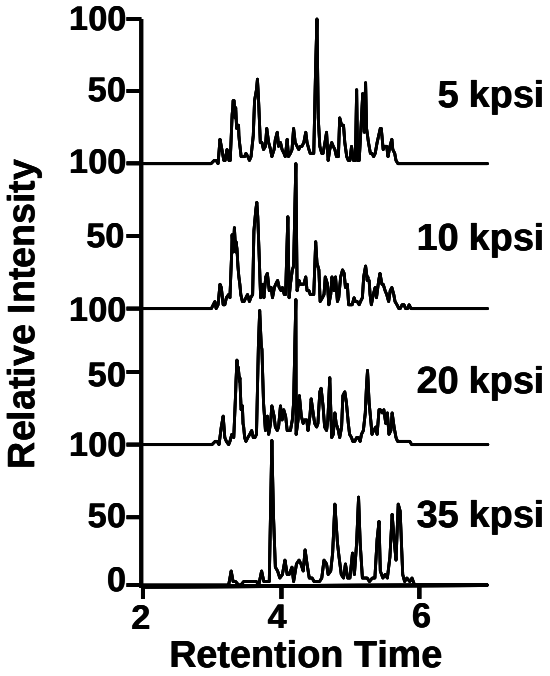
energy

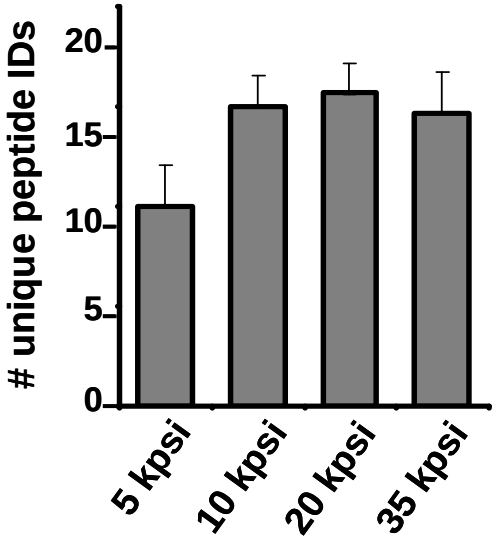
mixing

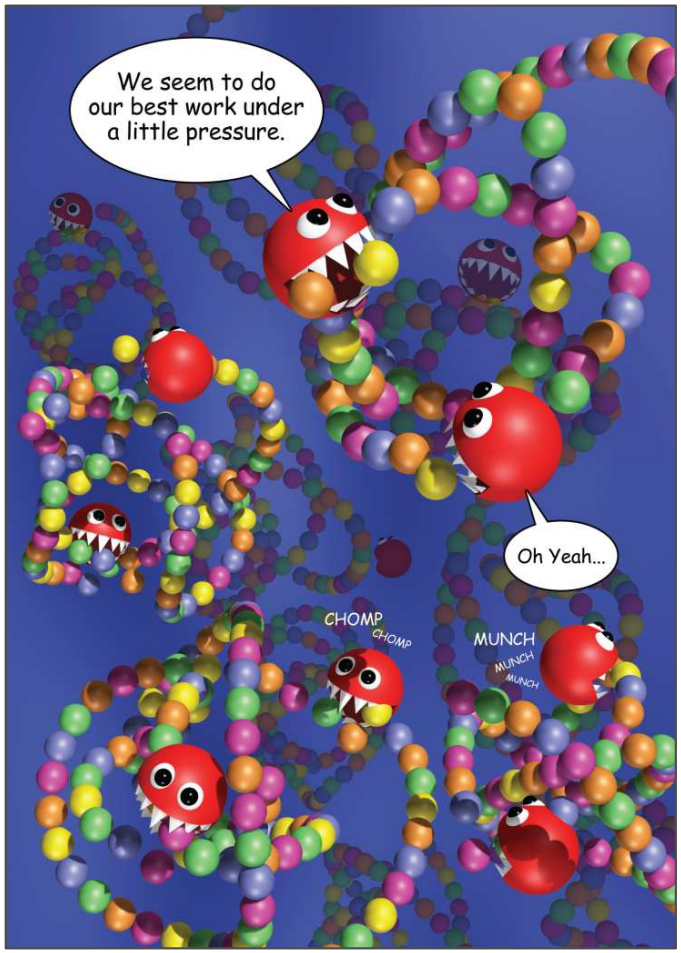
We have Pressure Cycling Technology in the lab…



Complete sample protein digestion in as short a time as 60s with sufficient pressure







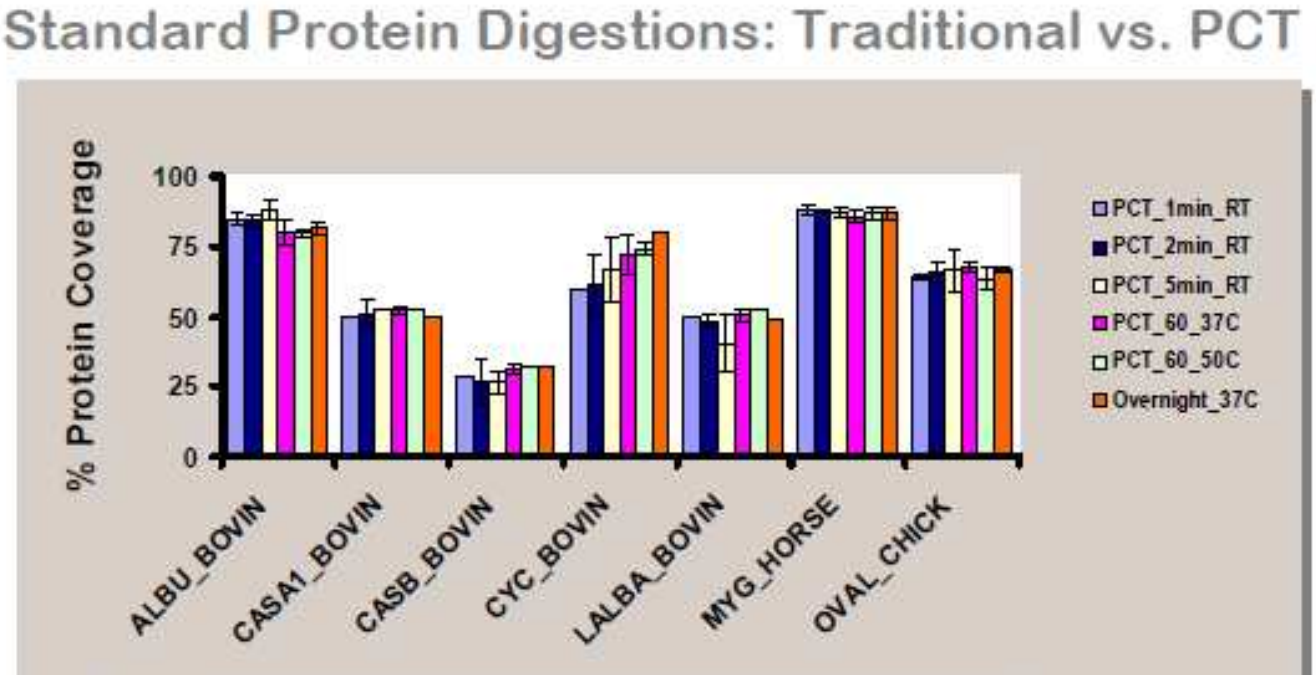
Lopez-Ferrer, D., et al. *J Proteome Res* 2008, 7, 3276-3281

Development of micro tubes for PCT digestions of small samples

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

→ µLC-MS/MS

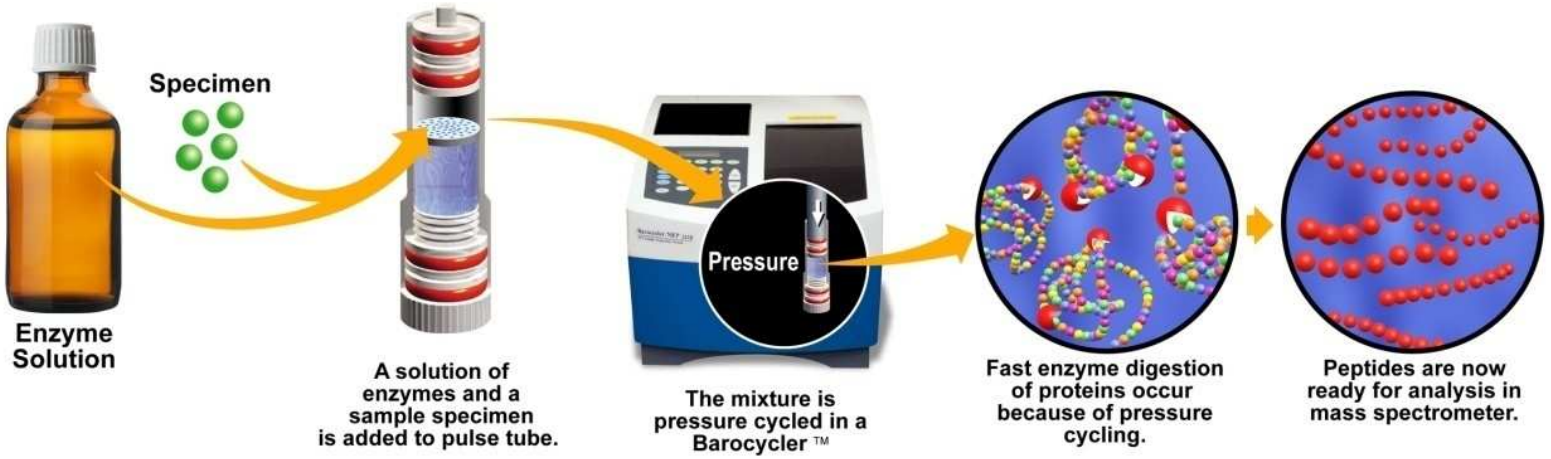
150 µL PCT MicroTubes
3 replicates at 20,000 psi



*The development was a cooperative effort between PNNL and PBI under the research agreement.

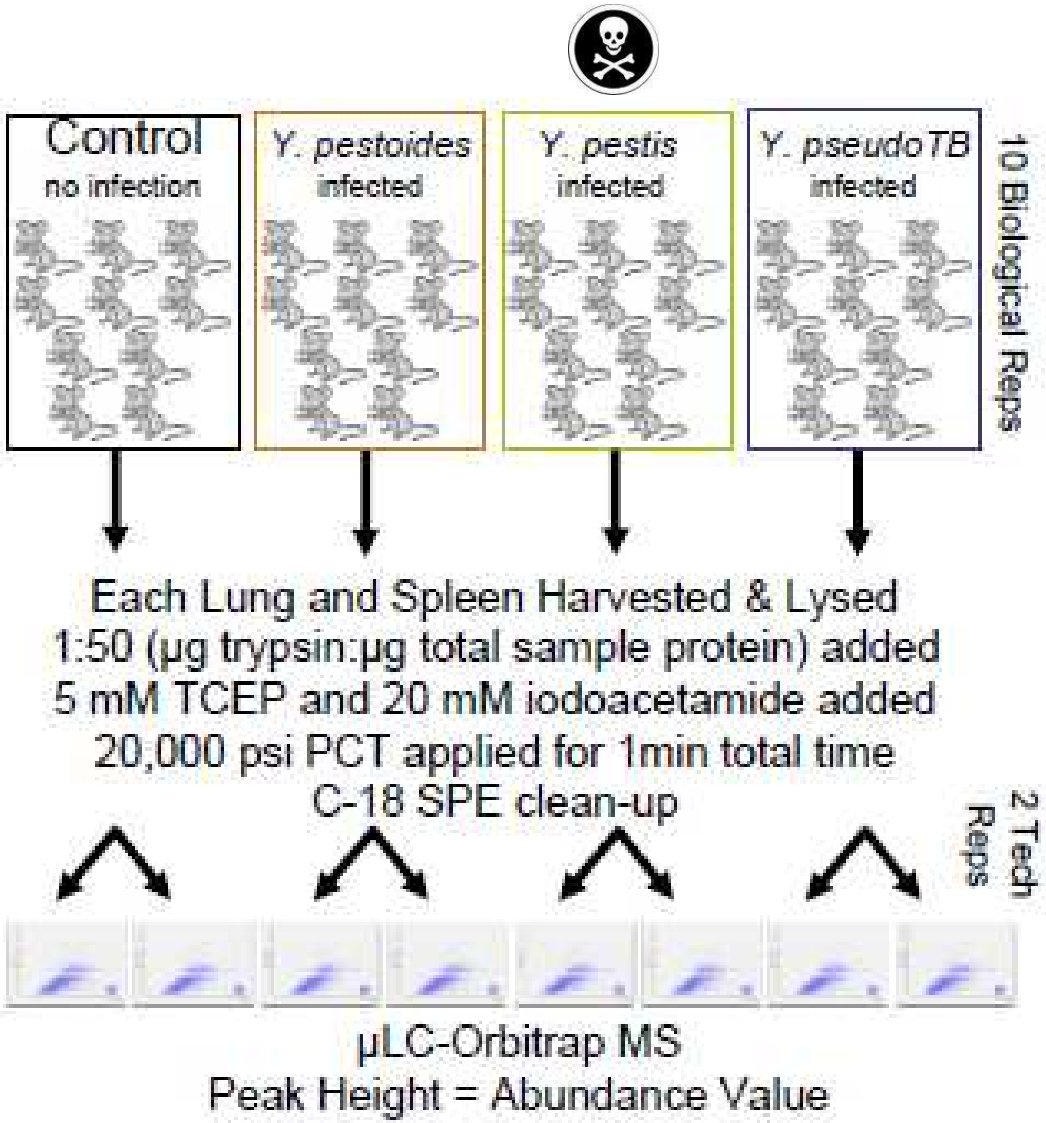
Traditional Protein Digestive Systems

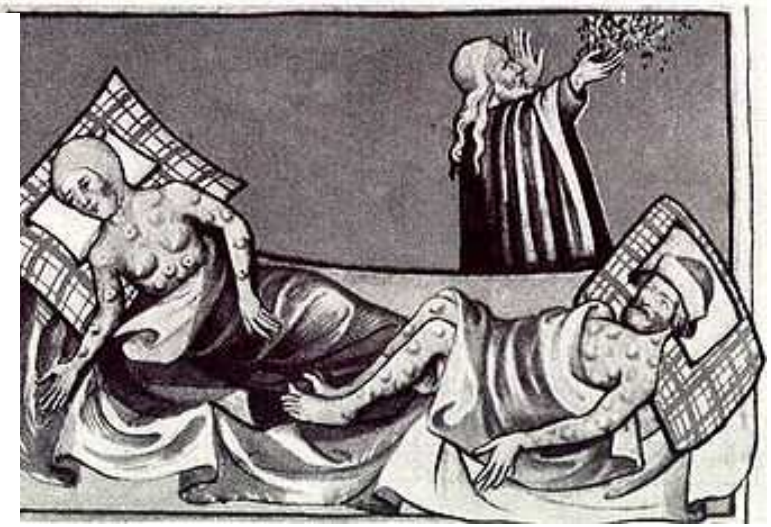
Presure Cycling Protein Digestive System



Initial PCT application: 20,000 psi

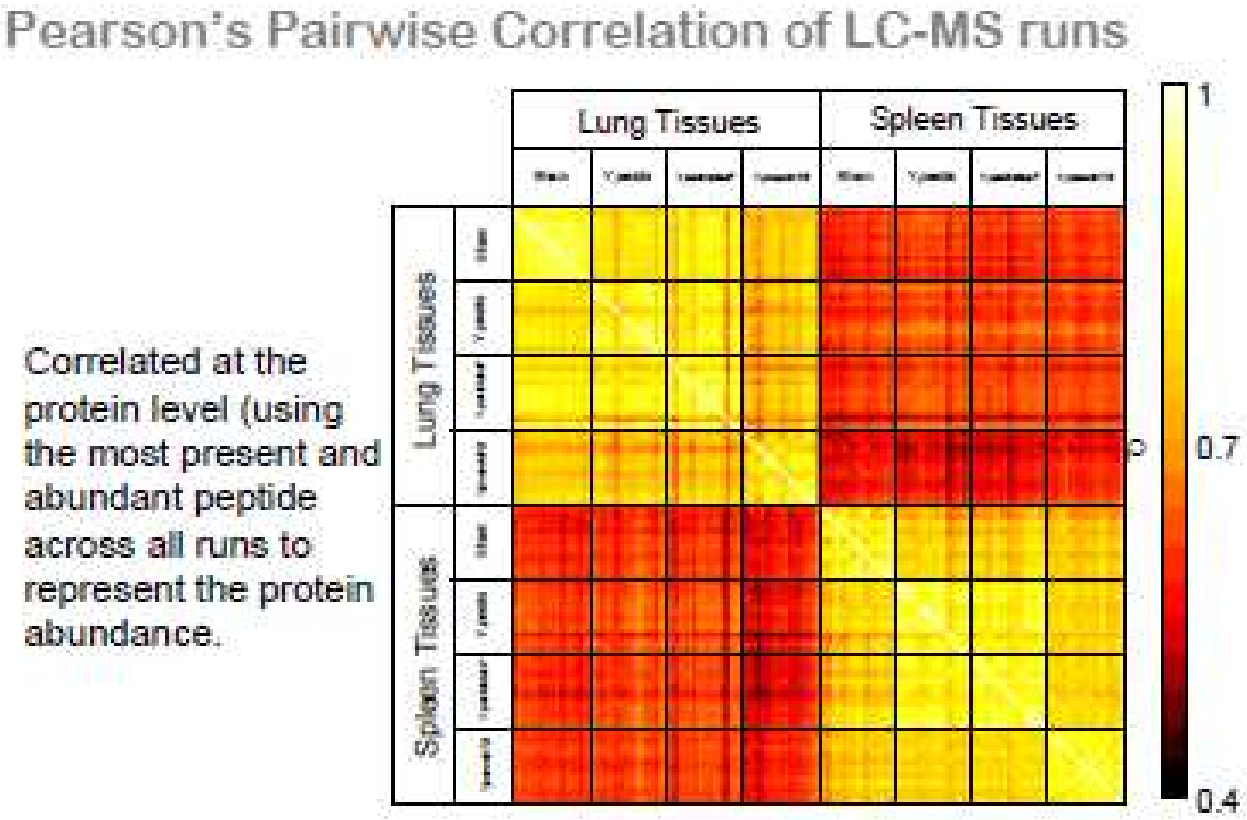
Yersinia pestis infection (relevant to biodefense)





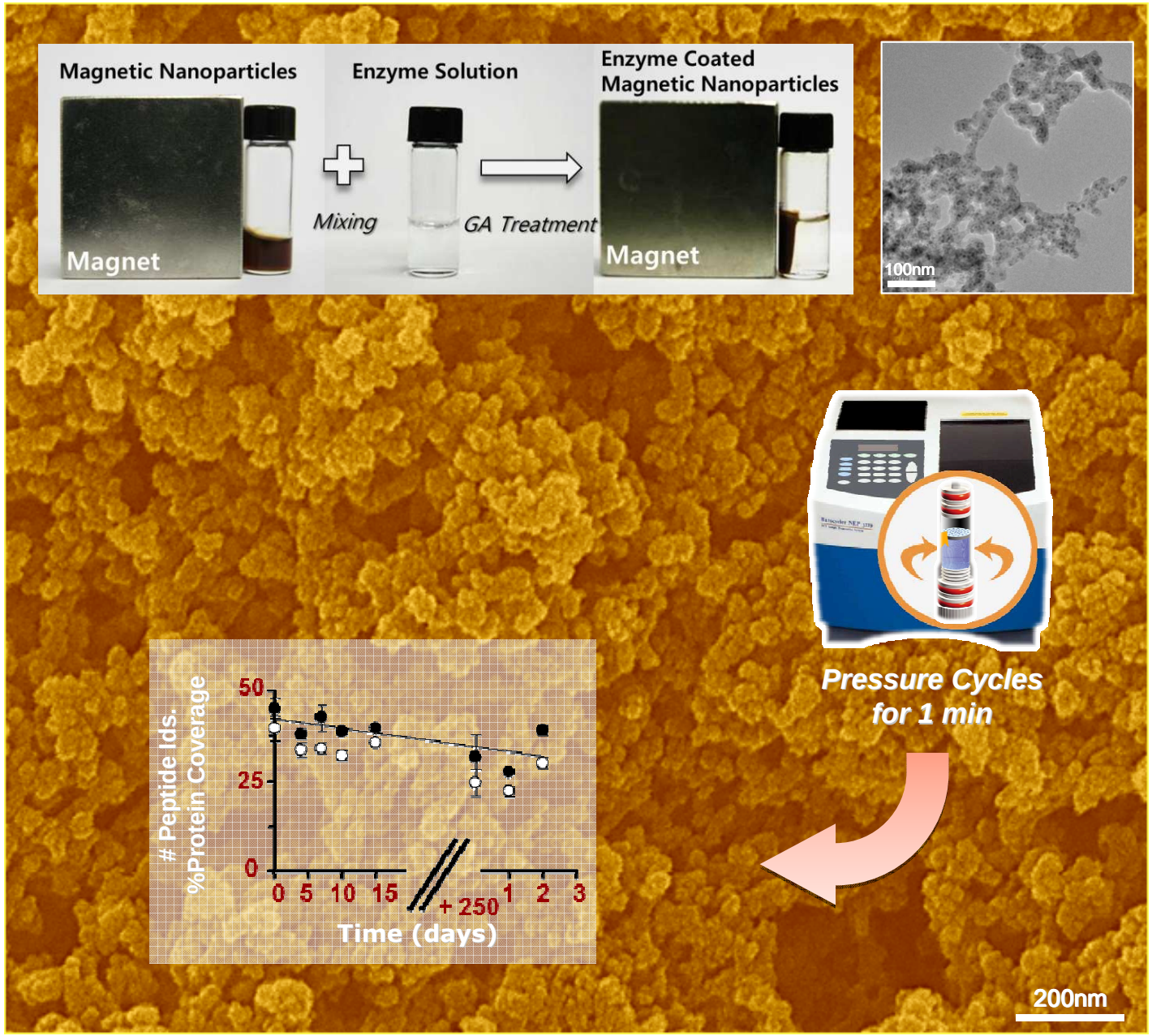
Y. pestis: **Bubonic Plague or Black Death**

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



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



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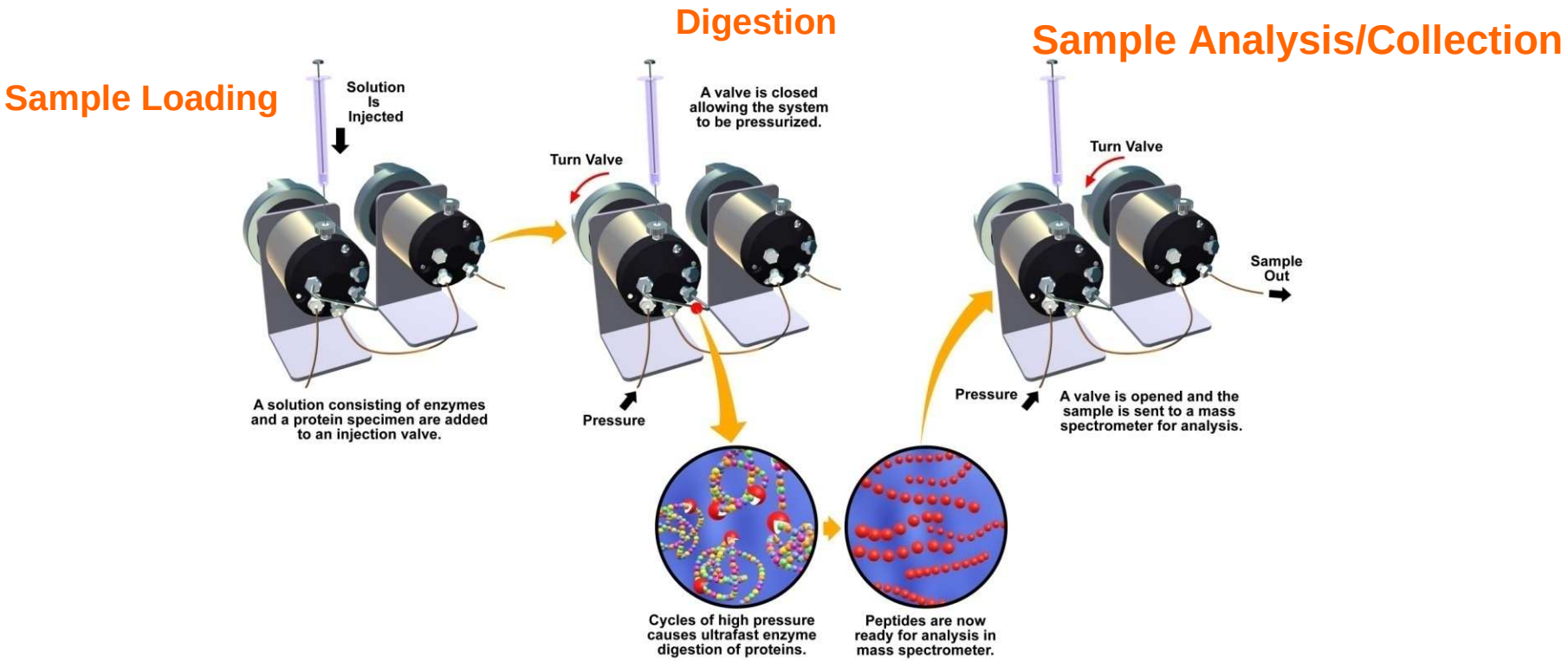
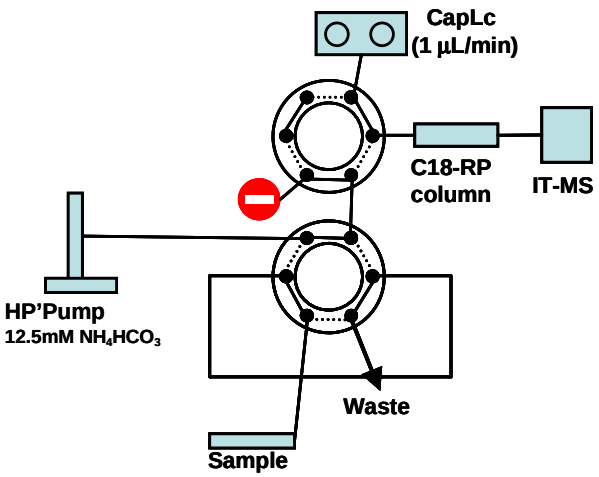
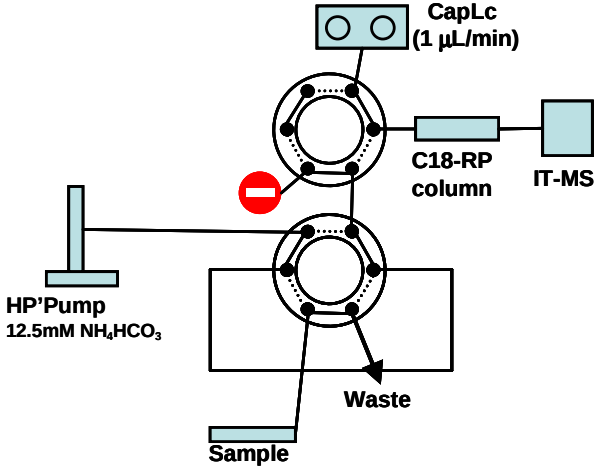
Development of a fast online pressure digestion system

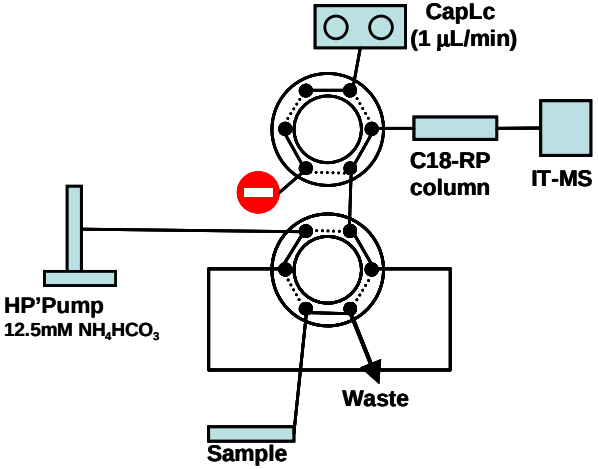


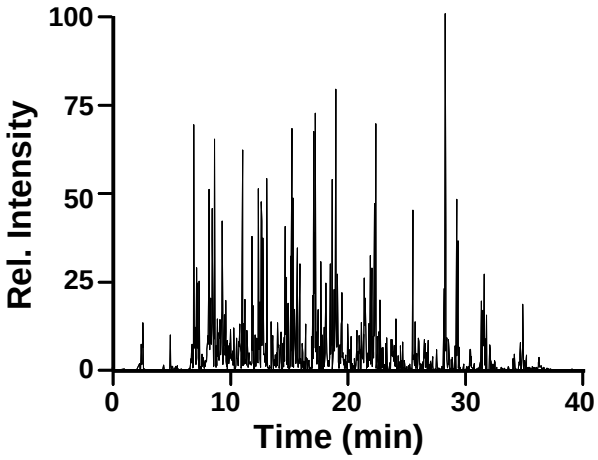
Lopez-Ferrer, D., et al. *Anal. Chem.* 2008, 80, 8930-8936

Coupling fast on-line digestion system with LC







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  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
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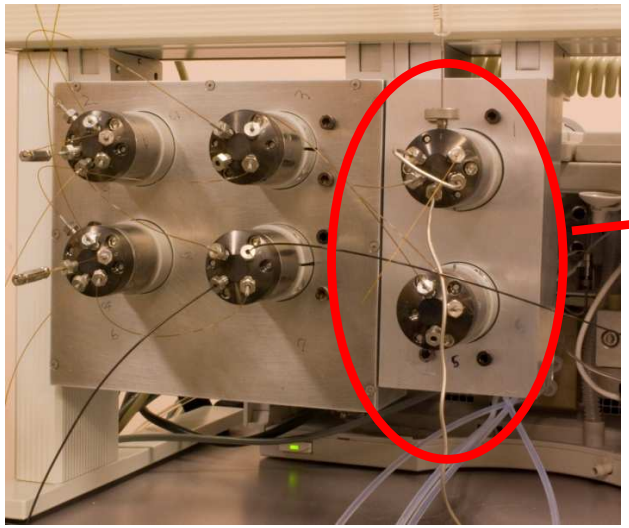
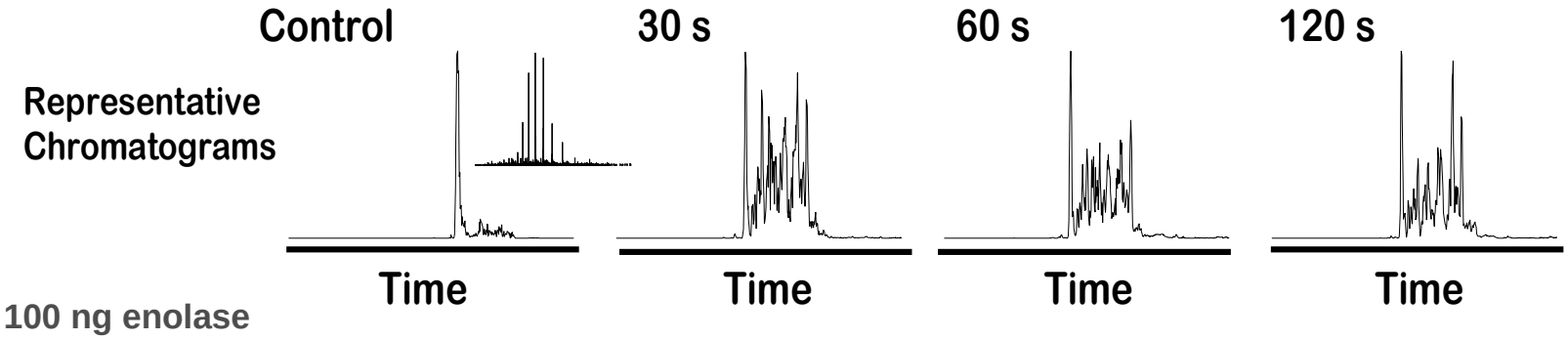
BSA 1 min digestion

Automated fast on-line digestion system



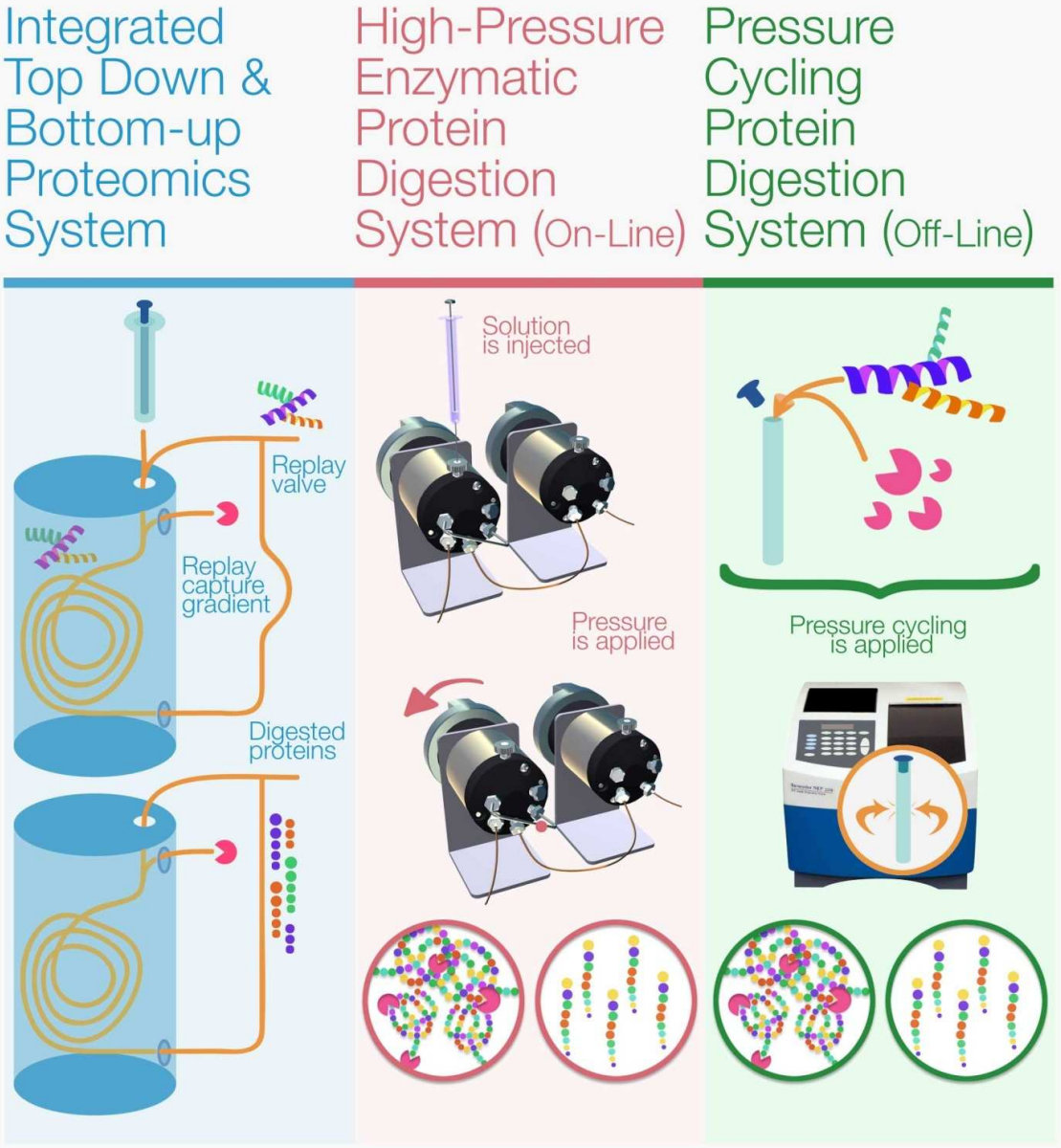
Fast Online Digestion System coupled to 2-column LC-system

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



Complete digestion at 10 kpsi

The next level will be the XstreamPCT Digestion Module



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