

## Pressure Cycling Technology Accelerates Trypsin Digestion from Hours to Minutes For Use in Mass Spectrometry Analysis

Featured at the 56th ASMS Conference on Mass Spectrometry



### Dr. Daniel Lopez-Ferrer

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#### **Application of pressurized solvents for ultra fast proteolysis: Proteomics on the fly**

**Poster presented Monday June 2, 2008**

**Session:** Proteomics: New Approaches for Sample Preparation  
- Poster Hall: Poster #570

#### **Introduction:**

Protein digestion for bottom-up proteomics has traditionally been performed using a relatively lengthy incubation period (6-12 h), making digestion one of the most time consuming steps in the analytical process. Recently, microwave and ultrasonic assisted digestions have been shown to accelerate enzymatic reaction rates and achieve complete proteolytic digestion of complex matrices within minutes or even seconds. In this study, we investigated the influence of pressure (up to 35,000 psi) on tryptic digestion of simple and complex mixture of proteins. We observed complete tryptic digestions could be obtained in 60 sec.

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#### **Seven Presentations by Two Independent Research Groups Highlight Advantages of Pressure Cycling Technology (PCT) at Scientific Symposia in Philadelphia, Boston, and New York City**

Pressure BioSciences, Inc. announced that scientists from the New York University (NYU) School of Medicine, the Brooklyn Hospital Center, and the Harvard School of Public Health made presentations highlighting the advantages of the Company's patented pressure cycling technology (PCT) at three different scientific meetings earlier this week. There were three oral presentations and four poster presentations, all relating to research studies focused on cancer, stroke, obesity, biomarker discovery, and in-vitro fertilization.

**BIOMARKER WORLD CONGRESS 2008** (Philadelphia, PA) – Dr. Paul Pevsner (NYU School of Medicine) gave an oral presentation on Monday, May 19th on how PCT was used by to extract proteins from colon tissue samples prior to analysis.

**GETTING OPTIMIZED™ TOOLS FOR DIAGNOSTICS** (GOT Summit 2008 – Boston, MA) – Dr. Alexander Ivanov (Harvard School of Public Health) gave an oral presentation on Tuesday, May 20th on the use of PCT and PCT-dependent reagents (ProteoSolve-LRS Kit) for the efficient recovery of proteins from lipid-rich adipose (fat) tissue.

**ACS MID-ATLANTIC REGIONAL MEETING** (MARM 2008 – New York, NY) – Dr. Pevsner gave an oral presentation on Tuesday May 20th. Dr. Pevsner's talk centered on the importance of sample preparation, and how the quality of sample preparation directly affects the quality of downstream results. On the same day, Dr. Pevsner and his colleagues delivered four poster presentations, which described the use of PCT in the extraction of proteins from breast and colon cancer tissue samples, as well as from the nutrient media used with in-vitro fertilization study procedures.

#### **ASMS Poster Presentation**

**Dr. Shane A. Wyatt**

*Department of General Services, Division of Consolidated  
Laboratory Services, Richmond, VA*

#### **High Pressure Trypsin Digestion of Proteins for Proteomic Analysis** (Continued on page 2)

### CALENDAR OF EVENTS

#### ASM 108<sup>TH</sup> GENERAL MEETING

BOSTON, MA  
JUNE 1-5, 2008

#### 56<sup>TH</sup> ASMS CONFERENCE

DENVER, CO  
JUNE 1-5, 2008

#### ADA 68<sup>TH</sup> ANNUAL SCIENTIFIC SESSIONS

SAN FRANCISCO, CA  
JUNE 6-10, 2008

#### BIO 2008

SAN DIEGO  
JUNE 17 – 20, 2008

#### 22<sup>ND</sup> ANNUAL SYMPOSIUM OF THE PROTEIN SOCIETY

SAN DIEGO, CA  
JULY 19-23, 2008

#### IBC'S 13<sup>TH</sup> ANNUAL DDT

BOSTON, MA  
AUGUST 4-7, 2008

#### 7<sup>TH</sup> ANNUAL WORLD HUPO

AMSTERDAM, NETHERLANDS  
AUGUST 16-20, 2008

#### 5<sup>TH</sup> INTERNATIONAL HPBB

SEPTEMBER 15-19, 2008

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## Dr. Daniel Lopez-Ferrer

Pacific Northwest National Laboratory, Richland, WA

### Application of pressurized solvents for ultra fast proteolysis: Proteomics on the fly

#### Methods:

The use of pressure assisted digestions for increased enzymatic reaction rates was demonstrated on-line and off-line. The off-line application used a Barocycler(TM), which uses pressure cycling technology (PCT) in the range of 0 to 35 kpsi. To further enhance the method for high-throughput applications, an on-line digestion system was developed. This on-line system utilized a modified high pressure liquid chromatography (LC) system with a pressurized sample loop where sample and trypsin are loaded. Following digestion, peptides were collected and infused into a 12 T FTICR-MS, analyzed on-line using electrospray ionization ion mobility spectrometry-orthogonal time-of-flight MS (ESI-IMS-oTOF-MS), or using a liquid chromatography (LC) system coupled to an ion trap mass spectrometer (ITMS).

#### Preliminary Results:

The effect of pressure on trypsin digestion was investigated off-line using BSA as the substrate and the Barocycler(TM) operated at different constant pressure (5, 10, 20 and 35 kpsi), as well as alternating pressures (cycling between ambient and high pressure, 0 to 35 kpsi). The total digestion time was kept constant at 60 s. Protein digests were subsequently analyzed by LC-MS/MS. In all cases digestion was complete in 60 s contrary to control experiments performed with no pressure. These results indicate that enhanced enzyme activity is achievable at elevated pressure and that multiple high-low pressure cycles do not negatively influence trypsin activity. To enable rapid pressure assisted enzymatic protein digestion in conjunction with MS, we modified a high pressure LC system for on-line digestion. The sample loop was loaded with both sample and protease (i.e., trypsin) and then pressurized at 7 kpsi for 60 s, at which time the digested sample was directed to the mass spectrometer by way of valve switching. Standard proteins and a complex *Shewanella oneidensis* proteome were digested using both the new pressured solvent and the traditional tryptic digestion methods, and then analyzed using ESI-IMS-oTOF-MS or ITMS. Comparable results, in terms of number of peptide identifications at 1% false discovery rate (FDR), were obtained when pressure digestion and traditional overnight digestion were compared. However, for the pressure experiments there was a noticeable improvement in the number of identifications. We hypothesize that this elevated improvement is a combination of two effects: pressure enhanced enzyme kinetics and pressure denaturation of proteins allowing better access for enzymatic cleavage. This new pressurized solvent method looks promising for ultra-high-throughput applications, i.e., "proteomics on the fly".



## Dr. Shane A. Wyatt

Department of General Services, Division of Consolidated Laboratory Services, Richmond, VA

### High Pressure Trypsin Digestion of Proteins for Proteomic Analysis

Poster presented Thursday, June 5, 2008

Session: Proteins - General, Methods - Poster Hall: Poster #514

#### Introduction:

Within the field of Proteomics, the ability to digest whole proteins is crucial. Typically, digestion of a protein is carried out by the addition of a proteolytic enzyme, or proteinase, such as trypsin, pepsin, or chymotrypsin which attack specific peptide bonds at predictable locations. For complete digestion, the protein is unfolded, allowing access of the proteinase to all potential cleavage sites. Typically unfolding the protein is done by heating the protein/proteinase solution, or chemically denaturing the protein. Both of these methods have drawbacks; they are time consuming, and can involve several steps. The digestion of proteins under ultra high pressure can dramatically reduce this time, and simplify the procedure to a few steps.

#### Methods:

Proteins were obtained to cover a broad range of molecular weights (6 – 66 kDa). Solutions of 0.2 mg/ml were prepared for each protein in 50 mM NH<sub>4</sub>CHO<sub>3</sub>. Trypsin was added to the protein mixtures at 20:1. High static pressures were used to apply mechanical stress to the protein utilizing a Barocycler NEP 3229 (Pressure Biosciences; West Bridgewater, MA). This stress unfolds the protein, allowing trypsin to attack both the interior and exterior cleavage sites. Digested proteins were spotted onto MALDI/TOF target plates, with HCCA as a matrix, and analyzed in reflectron mode. The spectra collected were compared to theoretical digests of the proteins, purchased digestion standards, and proteins digested using traditional methods.

#### Preliminary Results:

The intent of this work was to develop a simple and rapid digestion process for proteins. Rapid identification of proteins is critical for outbreak scenarios and response to biological threat agents. Current methods can require several sample preparation steps, as well as long incubation times for complete digestion to occur. Typically, standard digestion procedures require one to several hours at a higher temperature and chemical pretreatment of globular proteins. Using high pressure we were able to mechanically unfold the proteins and rapidly digest these model proteins in approximately 20 minutes at room temperature. Mass spectrometric analysis of the digested proteins revealed that the proteins were digested to completion, with relatively little sample preparation. Comparison of these results with digested proteins from more common means indicated that complete digestion was achieved in less time than by traditional methods. These results point to the applicability of this technique to the rapid analysis of biological agents where identification is paramount.