**Presented at the 14th Annual Frederick Conference on Capillary Electrophoresis**
**November 3-4, 2003**
**Frederick, MD**

# Global Analysis and Targeted Phosphorylation Status of the Cortical Neuron Proteome

**Li-Rong Yu[1], Thomas P. Conrads[1], Takuma Uo[2], King Chan[1], David A. Lucas[1], Carl F. Schaefer[3], Kenneth H. Buetow[3], Josip Blonder[1], Haleem J. Issaq[1], Richard S. Morrison[2], and Timothy D. Veenstra[1]**

[1]Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., National Cancer Institute at Frederick, P.O. Box B, Frederick, MD 21702-1201; [2]Department of Neurological Surgery, University of Washington School of Medicine, Box 356470, Seattle, Washington 98195; [3]Center for Bioinformatics, National Cancer Institute, Bethesda, MD 20892

## ABSTRACT

If proteomics is to achieve the goal of providing a high throughput platform by which to define active functions within the cell, it is necessary to identify significant numbers of proteins in a single study. A multidimensional fractionation approach combined with tandem mass spectrometry (MS/MS) has been used to characterize proteins expressed within primary cultures of mouse cortical neurons. Proteins extracted from the cortical neurons were digested with trypsin followed by fractionation using strong cation exchange chromatography (SCX). Each of these SCX fractions was analyzed by microcapillary reversed-phase liquid chromatography MS/MS (μRPLC-MS/MS). This analysis resulted in the identification of over 15,000 unique peptides that originate from almost 5,000 unique proteins. A systematic evaluation of the number of false positive peptide identifications was assessed by searching the entire MS/MS dataset against a database containing the sequences of over 12,000 proteins from different members of the archaea kingdom. This analysis was used to determine the level of confidence in the identification of peptides of different charge states based on the use of SEQUEST cross correlation scores. For targeted phosphoprotein identification, those proteins that were classified by gene ontology as having signal transduction activity were used to construct a database against which the raw MS/MS data was searched. This targeted phosphopeptide analysis resulted in the identification of over 200 possible phosphorylation sites within these proteins. The results presented here provide the broadest proteome coverage for a cell to date and show that MS-based proteomics, as it continues to develop, has the potential to provide high coverage of proteins expressed within a cell.