

## LARGE SCALE PROTEIN IDENTIFICATION USING AUTOMATED 2D-GEL SPOT EXCISION, DIGESTION AND DATA DEPENDENT ONLINE LC ESI-MS/MS

*St. John Skilton<sup>1</sup>, James Langridge<sup>1</sup>, John Hoyes<sup>1</sup>, Christopher Hughes<sup>1</sup>, Ronan O'Malley<sup>1</sup>, Jyoti Choudhary<sup>2</sup>, Malcolm Ward<sup>2</sup>, Alicia Ma<sup>2</sup>, Helen Byers<sup>2</sup>, Lu Yu<sup>2</sup>, Christina Nock<sup>2</sup>, Walter Blackstock<sup>2</sup> and Robert Bordoli<sup>1</sup>*

2 Glaxo Wellcome Research and Development Ltd, Stevenage, SG1 2NY, UK

1 Micromass, Wythenshawe, Manchester M23 9LZ, UK.

### Introduction.

The use of mass spectrometry and the information contained within protein and EST databases to accurately identify proteins is now widely demonstrated and accepted. However, the concept of characterising a proteome under a certain set of conditions is less secure and requires that a very large number of proteins be rapidly identified in a consistent and reproducible way. If 2D-gels are used, much of the sample preparation and analytical work is tedious and therefore prone to error. Robotics may alleviate some problems, allow sample tracking and by reducing contamination allow access to lower sample levels.

### Methods and Instrumentation.

1D- and high-resolution 2D-gels on human embryonic kidney (HEK) cell lysates were prepared using commercial equipment (BioRad) or by the method of Klose. After image analysis, a sample cutting robot reproducibly excised multiple 1mm diameter cores from each spot at up to 1020 cores per hour and deposited them into a microtiter plate with 100% fidelity. The bar-coded plate is transferred to a microfluidic chemistry station for consistent and reproducible chemical and enzymatic processing. The digested samples are analysed by automated MALDI and by automated LC ESI-MS/MS using data dependent scanning on a Q-ToF and automated data processing.

### Preliminary Data.

Not all proteins readily enter 2D-gels, and there will be occasions when limited fractionation on 1D-gels, or even no fractionation will be preferable. To establish guidelines for when to use a given approach, and simultaneously to better understand the critical points of automated analysis, we have analysed a high resolution 2D-gel of HEK cell lysate by MALDI and fast LC/MS/MS and compared this to analysis of 1D-gel bands from the same sample using 1 hr LC/MS/MS runs on a Q-ToF. This allows us to compare shifting the emphasis from detailed protein separation (2D-gels) to detailed on-line LC/MS/MS analysis of complex mixtures (1D-bands), and to see if proteins are 'missed' by working on 2D-gels.

Comparable amounts of a crude fractionation of the soluble HEK proteins were separated by both 1D- and 2D- PAGE. In the region between 30-50kDa approximately 200 protein spots were detected by mass spectrometry compatible silver staining. Following digestion these protein spots were analysed by MALDI-MS as well as by rapid (15 minute) chromatography ESI-MS/MS. After gel staining, all sample processing and

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MS analysis were carried out as automated processes. From a 1D-gel, 25 protein bands were excised in the same molecular weight region. The resulting tryptic digestion mixtures from each band were analysed by online capillary LC-MS/MS using 1hr gradients with fully automated data acquisition and processing on a Q-Tof. 197 Proteins were identified; these include metabolic enzymes, ribosomal subunits, kinases and phosphatases, cell adhesion and cytoskeleton, mitochondrial, nuclear, endoplasmic reticulum proteins.

Somewhat counter-intuitively, MALDI is not necessarily the higher throughput technique, in terms of proteins convincingly identified per unit time. Fast LC with mass and some sequence information is a powerful alternative to peptide mass fingerprinting and allows EST and genome database searching. Automated data reduction of long run time LC/MS/MS can readily identify 40 -50 proteins per hour.

**Novel Aspect:**

Decisions on efficient approaches to automation and choice of MALDI or LC/MS/MS using 1D- and 2D-gel have been quantified.

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