871 Automated protein identification using MALDI mass spectrometry


For organisms whose genomes are completely sequenced it is possible to begin investigating the genome function by studying the role of proteins and their complex in cell communication and regulation. This requires a large scale, high throughput facility to identify proteins that have usually been separated and visualised by one or two dimensional polyacrylamide gel electrophoresis. Since, if the genome is completely sequenced, all the proteins are known, peptides mass fingerprinting using matrix assisted laser desorption ionization (MALDI) time of flight mass spectrometry provides an ideal primary screening method for protein identification. MALDI is tolerant to the salts and by products of gel separation, requires only a small (ca 0.5 uL) amount of the protein digest and the acquisition and processing of the data of the can be easily automated. We have designed and constructed a robotic sample preparation device with the capacity to process hundreds of daily samples.

The key stages of the process include; excising a portion of the spot from the gel and removing to a microtitre plate, performing the necessary chemistries associated with enzymatic digestion, separating the digest supernatant from the excised gel spot and finally spotting the sample to a MALDI target plate. The target plates containing up to 384 prepared spots are manually transferred from the robot to a ToFSpec+2E mass spectrometer. The MALDI mass spectra from each spot are then acquired automatically. After acquiring data from each spot, the data are automatically processed into a database input query and the results collated into an identification list. The key stages for this include; validating the spectra for minimum and maximum intensity limits, combining valid spectra into Solutions. Cambridge C53

Albena, Dept of Cell 5iobgy, Edmonton, Canada, T6G 2HZ. 3MaxEnt ways which are scored against the spectral data has been developed. The confidence of the assignment of each residue in the proposed sequence. cations, to the database search engine. The output from the database search is robot to the mass spectrometer all other processes are automated. At these variation of a protein. This sequence information can be used directly to search databases for matching sequences leading to protein identification. An added advantage is that the expressed sequence tag (EST) databases, which are expanding more rapidly than either protein or genome databases, are also useful when using this sequence information. The increasing number of expressed gene products being studied, which fall into this latter category place an increasing requirement for automated high throughput protein identification by ESI-MS/MS. We have previously presented data illustrating high sensitivity MS/MS (1) and data dependent MS to MS/MS switching (2) using a quadrapole time-of-flight (Q-ToF) hybrid tandem mass spectrometer. More recently we have developed a fully integrated low flow rate Capillary HPLC system (Waters CapLC) as a sample introduction device. Automation of post processing software tools allows filtering and processing of multiple MS/MS data sets and searching, using a client/server system, of the resultant output against sequence databases. The MS/MS data and corresponding results, for each sample, are collated and viewed in an interactive browser allowing further investigation and refinement of the results.

Examples of fully automated protein identification using this system will be presented.

(1) Bordoli et al; Natene HPLC-MS and HPLC-MS/MS using a nanoflow ESI interface and Q-ToF hybrid mass spectrometer; Proc. 45th ASMS Conf. On Mass Spec. and Allied Topics, Palm Springs, Calif. June 1-5, 1997.

(2) Hoyes et al; Automated recording of MS and MS/MS spectra during a single HPLC separation on a Q-ToF mass spectrometer; Proc 45th ASMS Conf. On Mass Spec. and Allied Topics, Palm Springs, Calif. June 1-5, 1997.

872 Q-ToF ESI MS/MS and automated software interpretation for de novo sequencing of tryptic peptides from gel separated proteins.

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Mass spectrometry has rapidly become the method of choice for the identification and characterisation of proteins. Electrospray ionisation tandem mass spectrometry (ESI-MS/MS) is an ideal technique since it can be used to provide high quality sequence data from individual peptide masses produced by the enzymatic digestion of a protein. This sequence information can be used directly to search databases for matching sequences leading to protein identification. An added advantage is that the expressed sequence tag (EST) databases, which are expanding more rapidly than either protein or genome databases, are also useful when using this sequence information. The increasing number of expressed gene products being studied, which fall into this latter category place an increasing requirement for automated high throughput protein identification by ESI-MS/MS. We have previously presented data illustrating high sensitivity MS/MS (1) and data dependent MS to MS/MS switching (2) using a quadrapole time-of-flight (Q-ToF) hybrid tandem mass spectrometer. More recently we have developed a fully integrated low flow rate Capillary HPLC system (Waters CapLC) as a sample introduction device. Automation of post processing software tools allows filtering and processing of multiple MS/MS data sets and searching, using a client/server system, of the resultant output against sequence databases. The MS/MS data and corresponding results, for each sample, are collated and viewed in an interactive browser allowing further investigation and refinement of the results.

Examples of fully automated protein identification using this system will be presented.

(1) Bordoli et al; Nanoflow HPLC-MS and HPLC-MS/MS using a nanoflow ESI interface and Q-ToF hybrid mass spectrometer; Proc. 45th ASMS Conf. On Mass Spec. and Allied Topics, Palm Springs, Calif. June 1-5, 1997.

(2) Hoyes et al; Automated recording of MS and MS/MS spectra during a single HPLC separation on a Q-ToF mass spectrometer; Proc 45th ASMS Conf. On Mass Spec. and Allied Topics, Palm Springs, Calif. June 1-5, 1997.

874 DETECTION OF PROTEIN-PROTEIN INTERACTIONS BY VARIATIONS OF THE YEAST TWO-HYBRID SYSTEM

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Interactions between proteins regulate nearly every cellular process. The most powerful in vivo method in the identification of protein-protein interactions is the yeast two-hybrid system and variations thereof (Stagglar et al., Proc. Natl. Acad. Sci. USA, 95, 5187-5192, 1998). Since its original description in 1989, we are the witnesses of an enormous interest for these yeast two-hybrid techniques. Scientists and researchers working in different fields encounter yeast as a model system very often for the first time. Newcomers are therefore often puzzled and experience difficulties when they try to establish a specific yeast system in their laboratories. This procedure can be very time consuming and also cost intensive.

We plan to use our experience and long year experience to found a spin-off company. The company's concept is to provide a yeast one- and two-hybrid based technology platform for the functional characterization of protein-protein interactions in basic and medical applied research. We plan to assist mainly scientific laboratories and life science companies. Our goal will be to bridge effectively the gap between genetic information and drug discovery for human diseases.