

## STRATEGIES FOR DE NOVO SEQUENCING OF PROTEINS FROM EIMERIA TENELLA

Nicola Leeds<sup>1</sup>, Liz Bromley<sup>2</sup>, Emma M<sup>c</sup>Gregor<sup>1</sup>, Malcolm Ward<sup>1</sup>, Helen L. Byers<sup>1</sup>, James Campbell<sup>1</sup>, Mike Dunn<sup>3</sup> and Fiona Tomley<sup>2</sup>

<sup>1</sup>*Proteome Sciences plc, South Wing Laboratory, Institute of Psychiatry, King's College, London, SE5 8AF, United Kingdom.*

<sup>2</sup>*Institute for Animal Health, Compton, Berkshire, RG20 7NN, United Kingdom.*

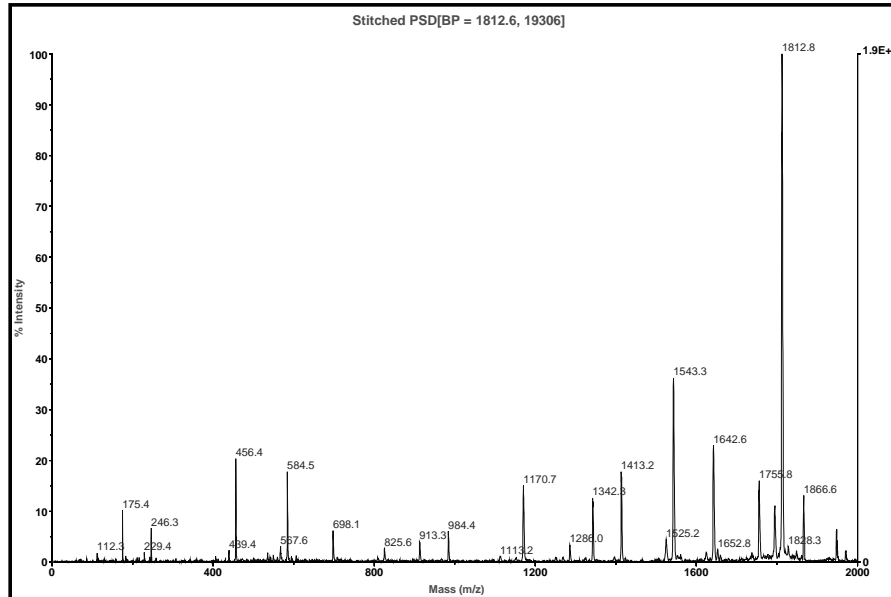
<sup>3</sup>*Institute of Psychiatry, King's College, London, SE5 8AF, United Kingdom.*

The apicomplexan protozoan, *Eimeria tenella*, is a constant threat to poultry welfare, and thus there is an urgent need to identify molecules that are rational targets for drug design, such as those involved in host cell invasion. To date, only a small number of organelle proteins have been identified and characterised and their precise functions are largely unknown.

We have purified two organelles, micronemes and rhoptries, from *E. tenella* sporozoites. These organelles are specialised secretory systems that are essential for the invasion of host cells by apicomplexan parasites. Two-dimensional gel electrophoresis (2DE) has been used to separate these proteins and to create specific maps for each organelle.

We have obtained a large set of Peptide Mass Fingerprints (PMF's) using Matrix Assisted Laser Desorption Ionisation Time of Flight (MALDI-TOF) mass spectrometry and in addition several peptides have been fully sequenced using chemically assisted fragmentation (CAF) in conjunction with MALDI and Post Source Decay (PSD). The PSD spectrum of a CAF labelled peptide is shown to exemplify the quality of the data (Figure 1). Here, the full sequence of the peptide was determined.

We have also analysed several proteins by LC/MS/MS to provide sequence information for a number of peptides within each sample.



**Figure 1. PSD spectrum of CAF-labelled peptide at m/z 1947.05**

Here we present the results for a preliminary set of microneme and rhoptry proteins and contrast the two mass spectrometry based methods for *de novo* sequencing of proteins from the *E. tenella* organism. The sequences generated have been compared with the *E. tenella* EST and genomic databases to locate the genes encoding these proteins. The cDNAs of selected proteins can now be sequenced and their subcellular localisation verified using antibodies raised against expressed recombinant proteins. We now intend to identify the full protein repertoire of micronemes and rhoptries in *E. tenella* using these methods.