Development of a high-throughput mass spectrometry-based method for the detection and localisation of protein tyrosine sulfation, in proteins extracted from human cell lysate

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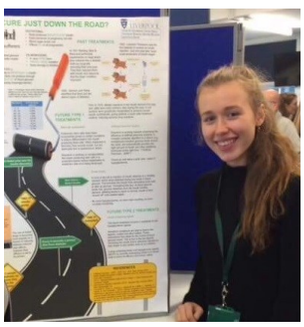

Cells have extensive regulatory flexibility that enable them to survive dynamic environmental changes. Their ability to sense and adapt to extracellular signals is fundamental to the maintenance of their homeostasis. Whilst modulation of gene expression plays a key role, this mechanism alone is too slow. Cells therefore rely on additional switches to ensure fast and reversible regulation. Protein post-translational modifications (PTMs) fulfil this requirement. Their biological importance is reflected in the large number of corresponding regulatory enzymes encoded in the human genome, performing over 200 types of modifications. Whilst the best-studied PTM is phosphorylation, there has been recent interest in tyrosine sulfation, a less common yet biologically significant modification. It is believed that sulfation may be necessary for the bioactivation of many secreted and membrane-bound proteins, perhaps critical for chemokine signalling and hemostasis. Despite this, the extent of this PTM remains unclear, most likely due to there being no conventional high-throughput method to distinguish phosphate groups from sulfate. With a mass difference of only 0.009 daltons, it is possible that tyrosine sulfation is being mistaken for phosphorylation. Since targeting protein sulfation pathways could offer potential strategies to treat inflammatory diseases and steroid hormone-dependent cancers, this less understood modification is rightfully attracting attention in proteomic research. The aim of this project is to develop a high-throughput mass spectrometry-based method that will successfully detect and localise tyrosine sulfation, in proteins extracted from human cell lysate. Until an optimised approach is found, the complex sulfoproteome will remain unexplored. So with some luck, this project will provide the essential foundation for many future studies.

Glutathione transferase responses to ivermectin in *Haemonchus contortus*

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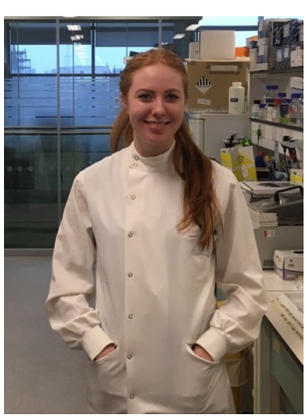

The most economically important gastrointestinal nematode of sheep is *Haemonchus contortus* (the Barber's Pole Worm). This is a parasitic nematode which can cause severe clinical manifestations in the sheep host. Symptoms can include severe anaemia and 'bottle jaw', an accumulation of fluid under the jaw, whilst sheep suffering from high intensity infections of the parasite can succumb and die. It has become increasingly difficult to control infections of *H. contortus* within the global sheep industry due to the rapid development of widespread anthelmintic resistance against multiple drug classes. There are concerns that the worldwide resistance observed against the macrocyclic lactone ivermectin in particular may significantly threaten the feasibility of the sheep industry. Ivermectin is also widely used to treat a range of human parasitic infections including lymphatic filariasis and concerns over resistance in these worms are also reported. However, the mechanisms for anthelmintic drug resistance are poorly understood and prior studies have focused on a range of potential mechanisms and drug targets. Amongst these potential mechanisms are the glutathione transferases (GSTs), a superfamily of detoxification enzymes which may play a role in aiding nematode survival against anthelmintic drugs. However, little is known of the biochemical profile of GSTs in *H. contortus*. This project aims to biochemically characterise the GSTs present in *H. contortus* and establish the potential role GSTs may play in resistance mechanisms. Thus, the findings from this investigation can be applied to combat the inevitable development of resistance in parasitic nematodes infecting both livestock and humans.