**BBSRC MIBTP Studentship Project**

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| **Project Title** | The role of haem in plant signalling and regulation |
| **Project Summary**  |
| The molecule, haem, is structurally similar to chlorophyll – both comprise a metal ion chelated by a tetrapyrrole ligand. A large number of proteins depend on haem, which can form additional bonds between the metal ion and the side chains of amino acids. Haem proteins are involved in many fundamental processes: transport and storage of oxygen (the globins), movement of electrons and redox chemistry (the cytochromes), and chemical catalysis (for example, the peroxidases). This breadth of chemical functionality has long been held as a testament to the versatility of haem, and it now appears that this is just the tip of the iceberg. The recent literature has begun to describe a whole new portfolio of other biological processes in which haem acts as either a signalling molecule (for example, haem binds to GUN1 to mediate retrograde signalling) or a regulatory molecule (for example, haem can modulate the affinity of protein binding to enhancer sites on DNA and, by this means, control gene transcription). Haem also has a high affinity to the PAS domains present in clock proteins and, as a consequence, it is also likely to play a role in the maintenance of circadian rhythms in plants.  The availability of haem for either the regulation of gene transcription, or retrograde signalling, will be tightly controlled by cells. This is essential as large concentrations of haem lead to the production of reactive-oxygen species. Until recently, it has been challenging to measure haem in cells but we have developed a sensor technology that is able to reveal changes in haem availability in live cells. This genetically encoded sensor is a chimeric protein that comprises a haem-recognition element fused to green fluorescent protein, which reports on haem binding via changes in the fluorescence emission lifetime. The sensor can be expressed in a wide range of different cell lines, and used to probe changes in haem levels in response to either different environment cues, the circadian time, or stress in plants. Current knowledge of the role of haem signalling in most plant responses is virtually non-existent, thus deployment of this sensor has the potential to provide novel insight into a range of plant cellular processes. Initial focus will be on plant biotic and abiotic stress responses as these integrate a range of environmental signals and have huge impact on both food and nutritional security.    There will be two components to the project work: (1) We will explore changes in haem availability in plant cells in response to biotic and abiotic stressors (informed by public transcriptome datasets) and the well established circadian clock which interfaces these stress responses and in other organisms is modulated by tetrapyrrole synthesis. Findings will be further validated by crossing the reporters into the plethora of characterised *Arabidopsis* mutants, including those predicted to result in excess or deficiency of heam.  (2) We will characterise the interactions (i.e. the binding affinity and the location of binding) between haem and retrograde signalling proteins, initially using GUN1 as an exemplar.  This pioneering research will deliver the first quantitative measurements of haem availability in plants, and provide molecular level understanding of haem signalling and regulation alongside an assessment of the plant phenotype. The research work will involve targeting the chimeric protein constructs (the sensor) to different cellular locations (cytosol, chloroplast, mitochondria and ER in the first instance) using mCherry in place of GFP to provide a robust pH insensitive genetically encoded reporter. These will be introduced into *Arabidopsis* by conventional floral dip methods and homozygous lines selected and characterised prior to undertaking biotic (phytpathogenic bacteria and fungal challenges) and abiotic (high light and drought stress) treatments.   Techniques that will be undertaken during the project:* Design of genetically-encoded haem biosensors
* Genetic modification of plant cell lines
* Biophysical characterisation of proteins
* Multiphoton fluorescence lifetime imaging microscopy

BBSRC Strategic Research Priority: Sustainable Agriculture and Food - Plant and Crop Science |
| **References** |
| Gallio, A. E.; Fung, S. S.-P.; Cammack-Najera, Ana; Hudson, A. J.; Raven, E. L. Understanding the Logistics for the Distribution of Heme in Cells. JACS Au 2021. <https://doi.org/10.1021/jacsau.1c00288>  |