**University of Leicester**

**Chemistry GTA Studentship Project 2022**

**Section 1 – *Supervisor Information***

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| **First Supervisor (Name and Title)** | Professor Andrew Hudson |
| **School** | Chemistry |
| **Email** | [ah242@leicester.ac.uk](mailto:ah242@leicester.ac.uk) |

**Section 2 – *Project Information***

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| **Project Title** | Fluorescence lifetime imaging with genetically encoded sensors to unravel the mechanisms controlling haem supply and demand in cells | |
| **Project Highlights:** | 1. | This project has been inspired by recent reports that implicate free (or exchangeable) molecules of haem in signalling activities in the cell; this conflicts with the long held view of haem as exclusively a prosthetic group buried inside a protein |
| 2. | The design of a genetically encoded sensor to quantify the concentration and redox state of exchangeable haem |
| 3. | Determination of the cellular distribution of exchangeable haem using fluorescence lifetime imaging |
| **Project Overview** | | |
| A large number of proteins depend on a small inorganic complex, iron protoporphyrin IX (haem), which is bound by means of metal-ligand coordinate bonds to proteins. These haem proteins are involved in many fundamental processes in the cell: transport and storage of oxygen, movement of electrons and redox chemistry, and chemical catalysis. 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 underpinned by these metal-ligand interactions: for example, haem can influence the amounts of different proteins made, circadian rhythms and the in-going and out-going flux of ions in the cell – all of which are critically important to health. These new discoveries have challenged long-held conceptions of the demand for haem and how haem is supplied to support chemical activity in the cell: the lifecycle of haem must be more complicated than its synthesis (in mitochondria) and degradation (in the endoplasmic reticulum); and more than a stoichiometric amount, relative to haem-binding proteins, is needed. This is because these newly discovered processes require haem intermittently to change the behaviour or function of a sub-group of haem-binding proteins, and there must be a readily available supply of haem to meet the transient requirements of the cell.  Understanding how haem is mobilised by proteins is an important frontier of chemical biology because the availability of haem will have broad consequences on cell physiology (too little, or too much, will have deleterious consequences). There must exist a reservoir of exchangeable haem which can be mobilised by known haem-binding proteins, and other, hitherto unknown, proteins, when and where it is needed, however, the existence of this reservoir of haem has defied explanation because molecular haem is cytotoxic, and so cannot be freely available in the cell. ***This has been a particularly long-standing problem in cell biology (the first reports describing the need to conceptualise a heme pool appeared in the 1970s) but a lack of progress has been made to address the problem due to technical limitations in biophysical approaches and imaging technology.*** | | |
| **Methodology** | | |
| In a recent published article, we reported a breakthrough discovery of the mechanistic basis of how haem is made availability in cells. By fusing a monomeric haem-binding peroxidase to a monomeric form of green-fluorescent protein, we developed a haem sensor that can be expressed recombinantly in different cell lines. By means of fluorescence lifetime imaging (FLIM), the sensor was shown to be responsive to haem availability in the cell, and can be used to precisely calculate the cellular concentrations of free haem. We discovered that there existed and in-built cellular buffering system that sequesters much larger concentrations of exchangeable haem, but retains the capability to mobilise haem when and where it is needed.  In this project, we will extend on this earlier work by designing a new sensor with the capability to reveal both the concentration and redox state of haem. As before, we will use fluorescence lifetime imaging to reveal the spatial location and oxidation-state distribution of haem. | | |
| **Further Reading:** | Gallio, A. E.; Fung, S. S. P.; Cammack-Najera, A.; **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>  Leung, G. C. H.; Fung, S. S. P.; Gallio, A. E.; Blore, R.; Alibhai, D.; Raven, E. L.; **Hudson, A. J.** Unravelling the mechanisms controlling heme supply and demand. *P Natl Acad Sci USA 2021*, 118, e2104008118; <https://doi.org/10.1073/pnas.2104008118> | |