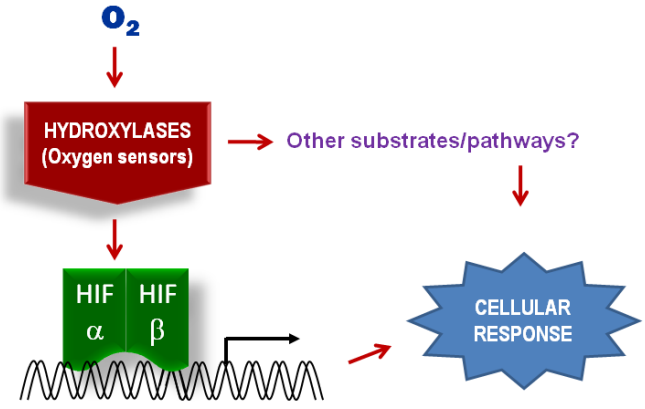
HONOURS PROJECTS IN THE PEET LAB 2012

Dan Peet
Room 3.41 MLS Building
Email: dan.peet@adelaide.edu.au
Web site: https://www.adelaide.edu.au/mbs/research/peet/

These two projects (listed below) are part of the broad field of understanding how cells sense oxygen. Oxygen sensing, and more importantly the response to low oxygen or hypoxia, is essential for survival, with direct relevance to normal physiology and development, ischaemic (eg. heart attack, stroke, etc.), vascular, and metabolic diseases, and cancer. Many of the response to hypoxia involve changes in gene expression, and this response is mediated by a novel class of oxygen-sensing hydroxylases which regulate the activity of the HIF transcription factors. We and others have recently shown that these hydroxylases can also modify other proteins and pathways, although the function and consequence of these modifications remains a mystery. The research focus of the Peet laboratory is to understand the different parts of this cellular response to hypoxia, including the regulation of specific proteins and genes, in the context of normal physiology and disease.



Cellular oxygen sensing and response

The two Honours projects for 2012 are independent research projects, but remain integrated with other research currently being performed in the laboratory, and Honours students will be an integral part of the research team. Both projects address cutting edge questions in this field, with the long term aim of publication and presentation at conferences. Each project will involve a number of different techniques, to provide students with a range of skills and experience. Please note that this is quite a competitive international field of research so details of the projects are not included here, but I would be more than happy to discuss the projects with anyone who is interested.

Project 1: Investigating the role of oxygen-sensing hydroxylases in infection.

We have some very interesting preliminary evidence for the oxygen-sensing hydroxylases and HIF transcription factors being involved in a specific infectious disease. This is of particular interest because the infection is not obviously linked to hypoxia. The project will follow up these initial observations, analysing the components of the HIF pathway in response to infection. The aim will be to determine if the HIF pathway is specifically targeted by the infection, what effect this has on the activity of the pathway, and ultimately how important this is in the progression of the disease. Techniques will include the analysis of proteins by western blotting, changes in expression of known HIF targets and other genes by real-time PCR, and the generation and analysis of functional mutants.

Project 2: Characterising a novel substrate of the HIF asparaginyl hydroxylase, FIH.

In addition to the HIF transcription factors, we and others have shown that a number of proteins that contain ankyrin repeat structural motifs are also substrates of one of the HIF hydroxylases, the asparaginyl hydroxylase FIH. However, in most cases we have been unable to find a function or consequence of this modification in the ankyrin repeat proteins. We have recently identified a new substrate, and cell-based activity assays strongly support a role for this hydroxylase in regulating the activity of this new substrate. This would be the first ankyrin repeat substrate where hydroxylation has an obvious function. The Honours project is to follow up the characterisation of this substrate, including the specific site of modification both in vitro and in a cellular context. The experimental techniques will include expression and purification of proteins from bacteria and cultured mammalian cells, in vitro enzyme assays, protein analysis by mass spectrometry, and site-directed mutagenesis to develop non-functional mutants.