PROJECT TITLE: Using a Systems Approach to Investigate the Impact of *in vitro* Culture Conditions on Cellular Energetics and Biochemistry

Project Overview

Mitochondria are critical to the normal biochemical function of most mammalian cells *in vivo.* However, when cells are cultured *in vitro* there is often a fundamental shift in their dependence on mitochondria. Also, cellular and mitochondrial function is dramatically influenced by *in vitro* culture conditions which are not static and change over time as cells grow and respire in a fixed body of medium.

This dynamic interplay between culture conditions, mitochondrial function and biochemistry has profound implications for commonly used *in vitro* cell systems but is poorly understood. Two scenarios where this is of clear significance to the pharmaceutical industry are 1) the use of *in vitro* cultures to study mechanisms of drug action on mitochondrial function (for both toxicological and pharmacological applications) and 2) the use of transfected cells for the commercial production of proteins, e.g. bioengineered therapeutic antibodies.

This collaborative project will utilise expertise within the groups of Dr Karl Morten (Nuffield Department of Obstetrics and Gynaecology) and Associate Professor James McCullagh (Department of Chemistry) at the University of Oxford and GSK's Biopharmaceutical and Toxicology departments to develop fundamental understanding of how mitochondrial function and biochemistry are modified by culture conditions *in vitro*, and how this might be manipulated to improve cell models for drug screening (to avoid toxicity and discover potential new drugs) and to improve the production of therapeutic antibodies.

Mitochondrial biochemistry involves many interconnected pathways which feedback and compensate each other, producing a complex metabolic web. Given this complexity, we plan to integrate data from multiple different assay platforms; for example, Seahorse cellular respirometry data (and other key mitochondrial parameters) will be used to provide a detailed view of cellular respiration and mitochondrial function over time. Culture media samples and cell pellets will be analysed for biochemical changes using metabolomics and label free mass spectrometry. Adaptive changes in gene expression will be monitored by transcriptomic analysis of the cells. A highly novel perfused cell culture system being developed in Oxford may also be of value. Finally, the analysis would be supplemented with state of the art *in silico* modelling. These "virtual mitochondrion" computer models include hundreds of embedded biochemical reactions and enable us to simulate the effects of changing cellular or biochemical conditions on mitochondrial function, and vice versa.

The overall aim of this studentship is to provide significant new insights into the function and biochemistry of mitochondria in commonly used cell systems and to highlight how we might manipulate these to impact key cellular processes *in vitro*, such as recombinant protein production and adaptive stress responses (e.g. when challenged with developmental drug). This in turn will enhance the value of *in vitro* systems for our specific applications; investigating mitochondria-drug interactions or improving commercial therapeutic antibody production.

1. F Zagari, et al. Lactate metabolism shift in CHO cell culture: the role of mitochondrial oxidative activity. New Biotechnology vol 30 (2): 238-245 (2013).
2. A. Broom et al. Effects of mid-respiratory chain inhibition on mitochondrial function in vitro and in vivo. Toxicol. Res., 2015, Advance Article DOI: 10.1039/C5TX00197H

Training Opportunity

Students on Industrial CASE Studentships carry out their research with co-supervision from the University of Oxford and an industrial partner (GSK in this case). This gives students the opportunity to experience both an academic and an industrial research environment during their DPhil. Students will spend a minimum of 3 months working at the premises of the industrial partner during their 4-year project.

This collaborative project will afford a unique opportunity for the student to be exposed to excellent academic and industrial research environments. The successful candidate will:

- develop extensive cell culture skills along with analytical skills in the biochemical and gene expression space (e.g. using metabolomics and transcriptomics etc.). There will also be an option to develop skills in the highly innovative field of in silico modelling if the student was interested.

- become expert in the use of cellular systems in an industrial context; specifically, for toxicological and pharmacological assay development and the production of commercial protein products. These skills are highly marketable within industry but are also central to many basic academic research projects too.

GlaxoSmithKline (GSK) is a large pharmaceutical company providing pharmaceuticals, vaccines, and consumer health products to millions of people around the world; helping people to do more, feel better and live longer. The successful student will spend at least 3 months (but up to 2 years) at GSK and be exposed to both an academic science environment and to the process involved in developing new drugs and therapeutics for patients.

Theme

Bioengineered therapeutic antibody production, cell-based assay development (for toxicological and pharmacological applications), cellular bioenergetics and biochemistry.