

Project Directory v.7

Rotation 2 – 7 January 2013 to 15 February 2013

Primary Contact	Additional Contact	Mini-Project Title	Additional Information
malcolm.bennett@nottingham.ac.uk		Branching out: discovering and exploiting the genetic regulation of lateral root development	
jon.aylott@nottingham.ac.uk		A high-resolution cell biological approach to investigate root gravitropism using fluorescent nanosensors	
martin.broadley@nottingham.ac.uk		<u>High-throughput screening of a Brassica rapa mutant population for genetic variation in seed size, oil and protein content using an automated NMR platform</u>	Based at Rothamsted
freddie.theodoulou@rothamsted.ac.uk	mike.holdsworth@nottingham.ac.uk	Novel mechanism which control seed oil mobilisation: the role of targeted proteolysis*	Based at Rothamsted
mike.birkett@rothamsted.ac.uk	robert.stockman@nottingham.ac.uk	<u>Elucidating the Biosynthesis of the Aphid Sex Pheromone</u>	Based at Rothamsted
kim.hammond-kosack@rothamsted.ac.uk	john.foulkes@nottingham.ac.uk	<u>Exploring the genetic and mechanistic basis of resistance to Take-all disease in wheat</u>	Based at Rothamsted
steve.thomas@rothamsted.ac.uk	zoe.wilson@nottingham.ac.uk	Understanding the impact of gibberellin signalling on fertility in wheat	
ian.mellor@nottingham.ac.uk		<u>Discovery of pesticide leads from Coccinelid alkaloids</u>	

dylan.sweetman@nottingham.ac.uk		In vitro generation of muscle tissue from genetically diverse strains of pig	
peter.crittenden@nottingham.ac.uk		Surface enzyme activity in lichen	
rita.tewari@nottingham.ac.uk		Understanding molecular mechanism regulating unusual cell division in malaria parasite	
matthew.elmes@nottingham.ac.uk		Unravelling the molecular, electrophysiological and endocrinological mechanisms through which cholesterol decreases myometrial contractile activity	
john.brameld@nottingham.ac.uk		Adipogenic and myogenic differentiation of porcine adult skeletal muscle stem cells	
kostas.tsintzas@nottingham.ac.uk	fran.ebling@nottingham.ac.uk	FGF21 and the role of tancytes in regulating energy balance and body weight	
paul.williams@nottingham.ac.uk		Quinolone-inspired quorum messenger-mimicking antimicrobials - biological activity and mechanistic determinants	
gareth.hathway@nottingham.ac.uk		Age and tissue specificity in signalling mechanisms employed by opioid and cannabinoid receptors: promiscuous pathways providing novel insights into neurodevelopment and pain	
panos.soultanas@nottingham.ac.uk		Regulation of DNA replication by central carbon metabolism in B. Subtilis	
frank.ball@nottingham.ac.uk		Measurement of G protein-coupled receptor dynamics and ligandbinding in membrane microdomains of single living cells	

stephen.coombes@nottingham.ac.uk		Stochastic Neural Network Modelling	
sarah.kuehne@nottingham.ac.uk		Bio-engineering of a non-toxinogenic Clostridium difficile strain to provide prophylaxis via colonisation and the generation of a protective immune response	
nicole.clarke@nottingham.ac.uk		Exploring novel IRF1 protein-protein interactions: can we identify targetable interfaces?	
clive.roberts@nottingham.ac.uk		Unravelling the role of protein molecular assemblies in bacterial DNA replication through microscopy and force spectroscopy	
felicity.rose@nottingham.ac.uk		In-vitro engineering to develop tissue grafts facilitating the regeneration and healing of the human colon	
cinzia.alleglucci@nottingham.ac.uk		Investigating the genetic program of mammalian germ cells development and differentiation*	
lisa.chakrabarti@nottingham.ac.uk		The role of mitochondria in ageing and neurodegeneration*	
freddie.theodoulou@rothamsted.ac.uk	ian.kerr@nottingham.ac.uk	Understanding the targeting and function of the adrenoleukodystrophy protein, a peroxisomal ABC transporter	
peter.shaw@nottingham.ac.uk		Fundamentals of eukaryotic transcription: Regulation of Elk-1 function by ubiquitylation*	
ed.louis@nottingham.ac.uk		Exploring and exploiting natural genetic variation underlying complex traits in yeast	

david.gray@nottingham.ac.uk	tim.foster@nottingham.ac.uk	<u>Sustainable nutrition from green-leaf waste</u>	
klaus.winzer@nottingham.ac.uk		<u>Making biofuels a reality - improving bacterial butanol production through rational metabolic engineering</u>	
angela.karp@rothamsted.ac.uk	greg.tucker@nottingham.ac.uk	<u>The influence of genotype, environment and management on the efficiency of conversion of willow to fuels and industrial products</u>	
gill.stephens@nottingham.ac.uk	rachel.gomes@nottingham.ac.uk	<u>Using laccases to produce chemicals and remediate wastes</u>	

Project title: High-throughput screening of a *Brassica rapa* mutant population for genetic variation in seed size, oil and protein content using an automated NMR platform.

Contact: martin.broadley@nottingham.ac.uk

Rotation: 2

A preliminary visual screen by Kurup (RRes) of a subset of M2 seed batches from the Brassica rapa R-o-18 mutant population grown at Nottingham by the Broadley lab has already led to the identification of two seed size/morphology mutants. In the miniproject students will carry out anatomical and biochemical analysis of these mutants at RRes providing a brief practical introduction to the range of microscopy and analytical chemistry methods that will ultimately be necessary for the full PhD project. The students will also help develop a calibration for chemometric estimation of protein content of R-o-18 seeds by NMR, and use this together with established methods for determination of seed weight, oil and moisture to begin screening seed patches of the population. This will provide initial training in NMR analysis and also allow any mutants identified early in the miniproject to be grown on to subsequent generations and crossed, providing material for genetic analysis right at the start of the full PhD project.

Skills you need		Skills you'll develop	
Computer Skills	<i>Knowledge of basic software</i>	Computer Skills	<i>Use of Statistical software e.g. Genstat</i>
	<i>Spreadsheet manipulation</i>		
Numeracy Skills	<i>Basic stats</i>	Numeracy	<i>Advanced statistical analyses and experimental design (e.g. quantitative genetics)</i>
	<i>Basic concentration/dilution skills</i>		
General Lab. Skills	<i>Understanding of the need for precision and accuracy</i>	General Lab. Skills	<i>Ability to plan and conduct scientific experiments</i> <i>High throughput screening</i> <i>Nuclear magnetic resonance Spectroscopy</i> <i>Mass Spectrometry</i> <i>Near-Infrared Spectroscopy</i> <i>Microscopy</i> <i>Protein extraction & electrophoresis</i> <i>Phenotyping of plants</i>
	<i>Basic equipment handling eg pipettes</i>		
	<i>Record keeping</i>		
Communication Reading/Writing/Presenting Skills	<i>Report-writing</i>	Writing Skills	<i>Scientific paper-writing</i>
	<i>Ability to carry out literature-searching</i>		<i>Ability to write a literature review</i>
	<i>Ability to communicate to colleagues</i>		<i>Ability to communicate to outside audiences</i>
			<i>Ability to represent research group</i>

Project title: Elucidating the Biosynthesis of the Aphid Sex Pheromone

Contact: robert.stockman@nottingham.ac.uk; mike.birkett@rothamsted.ac.uk

Rotation: 2

The aim of the mini-project at Rothamsted will be to provide training in the isolation, analysis and identification of aphid sex pheromones, which is essential to the success of the full PhD project. The following activities will be carried out over a six-week period:

Volatile collection of aphid sex pheromone. Purified air will pass through glass vessels containing bean plants, *Vicia faba*, heavily infested with sexual pea aphids, *Acyrtosiphon pisum*. Volatiles will be collected over 4 days onto a porous polymer (Porapak Q, 50 mg), and after collection will be eluted with redistilled diethyl ether (750 µl). Samples will be stored in a freezer (-22°C) sealed in glass ampoules.

GC Analysis of aphid sex pheromone samples. Collected volatile samples will be analysed by high resolution gas chromatography (GC) using an Agilent 6890 GC fitted with both polar (DB-wax) and non-polar (HP-1) GC columns, cool-on-column injectors, deactivated retention gaps (1 m×0.53 mm inner diameter), and flame ionization detectors (FIDs).

Pheromone identification. Coupled gas chromatography-mass spectrometry (GC-MS) analysis of volatile samples will be performed on a VG Autospec Ultima magnetic sector mass spectrometer coupled to an Agilent 6890 GC fitted with a non-polar HP-1 column. Identifications of aphid sex pheromone components will be made by comparison of MS data with mass spectral databases (NIST) and literature spectra, and confirmed by GC peak enhancement with authentic standards. A multiple-point external standard method will be used to quantify the amount of identified chemical components present in volatile samples.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	Use of advanced data analysis software
	Spreadsheet manipulation		Use of mass spectrometry database/library searches
Numeracy Skills	Basic stats	Numeracy	
	Basic concentration/dilution skills		
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	Volatile collection from plants and insects
	Basic equipment handling eg pipettes		Gas chromatography analysis of collected samples
	Record keeping		Coupled GC-mass spectrometry analysis
			Culturing of insects and plants
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Oral and poster presentations

Project title: the genetic and mechanistic basis of resistance to Take-all disease in wheat

Contact: john.foulkes@nottingham.ac.uk

Rotation: 2

The root performance of four cereal genotypes will be compared in the 4 week pot test done in our biological containment facility at RRes. The genotypes selected are T. monococcum lines MDR31 (highly resistant) and MDR043 (susceptible), the hexaploid wheat Riband (susceptible) and Oats (immune). Pots will be inoculated at seed sowing (6 seeds / pot) with either the Take-all fungus (Ggt), the rice blast fungus (Mg) available in a transgenic Green Fluorescent Protein expressing form or mock inoculated with water. The levels of infection will be explored each week by washing out the entire root systems of 4 pots / interaction type and preparing two separate total DNA samples. The other 2 pots will be visually inspected for disease symptoms using the binocular microscope in both light and UV modes. Using the genome sequence of both fungi available at the BROAD website, species-specific primers will be devised for the genes actin and tubulin and the reporter gene GFP. These primers will first be tested on pure fungal cultures. Then extracted DNA samples will be used in qPCR analysis to quantify the accumulation of the biomass of the root systems over 4 weeks.

Hypothesis: The resistant genotype MDR 31 is effective against both fungal species.

Training: Basic aseptic techniques, culturing fungi, inoculating plants, observing disease symptom formation over time, PCR primer design, DNA extraction, qPCR analyses, statistical analyses and basic microscopy, report writing.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	Use of advanced data analysis software
	Spreadsheet manipulation		Use of molecular modelling software
Numeracy Skills	Basic stats	Numeracy	
	Basic concentration/dilution skills		
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	Centrifugation
	Basic equipment handling eg pipettes		Electrophoresis
	Record keeping		Enzyme assays
			Spectrophotometry
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Ability to represent research group

Project title: Discovery of pesticide leads from Coccinellid alkaloids

Contact: ian.mellor@nottingham.ac.uk

Rotation: 2

The mini project will be closely related to the main project but will focus more on the electrophysiological techniques that will be employed. The students will express mammalian nicotinic acetylcholine receptor subunits that are known to function in *Xenopus* oocytes and measure some of their basic electrophysiological properties using two-electrode voltage-clamp. On successful completion of this part of the project they will investigate the actions of a novel alkaloid alongside an antagonist known to inhibit nicotinic acetylcholine receptors.

The mini project will provide training in preparation of *Xenopus* oocytes and their injection with RNA; two-electrode voltage-clamp recording; measurement, analysis and interpretation of electrophysiological and pharmacological data. It will also provide training in basic laboratory practice and techniques.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	Use of advanced data analysis software
	Spreadsheet manipulation		Use of Bioinformatics software/databases
Numeracy Skills	Basic stats	Numeracy	Curve fitting and estimating potency
	Basic concentration/dilution skills		
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	Culture of cells and nematodes
	Basic equipment handling eg pipettes		Protein expression
	Record keeping		RNA synthesis
			Electrophysiology
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Chemical extractions
	Ability to carry out literature-searching		HPLC separation
	Ability to communicate to colleagues		Scientific paper-writing
			Ability to write a literature review
			Ability to communicate to outside audiences
			Oral and poster presentations

Project title: In vitro generation of muscle tissue from genetically diverse strains of pig

Contact: dylan.sweetman@nottingham.ac.uk

Rotation: 2

Production and characterisation of inducible Myogenic Regulatory Factors.

The Myogenic Regulator Factors (MRFs: Myf5, MyoD, Myogenin and MRF4) are transcription factors regulating muscle formation. They have the ability to convert non-muscle cells into muscle and are thought of as master regulators of myogenesis. They will provide a key part of our strategy to induce muscle development in iPS cells.

The mini project will involve the cloning and initial characterisation of inducible versions of these transcription factors. They will be cloned into a lentiviral vector to generate MRF / estrogen receptor fusions; these proteins can then be activated by the addition of tamoxifen to induce nuclear localisation of the fusion protein.

The cloning will be done using Clontech in-fusion approach, a recombination based method currently being successfully used in my lab.

Once the cloning is done the vector will be tested in cultured pig fibroblast cells. The fusion will contain an HA tag to facilitate detection of the protein. Staining with an anti-HA antibody will be used to determine the efficiency of nuclear translocation following tamoxifen treatment. To determine if muscle specific gene expression has been induced we will use RT-PCR with primers to MRFs and muscle specific myosins as well immunostaining for muscle markers such as Desmin, Myogenin and MHC. All these approaches are established and being currently used in the lab.

This will provide training in basic molecular biology, cell culture and immunohistochemistry.

		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Objective 1:	Cloning MRF-ER constructs	■	■				
Objective 2:	Tamoxifen treatment (α -HA staining)		■	■	■		
Objective 3:	RT-PCR				■	■	■
Objective 4:	Immunohistochemistry				■	■	■

Skills you need		Skills you'll develop	
Computer Skills		Computer Skills	Image capture / analysis
			Genomic database mining
Numeracy Skills	Basic concentration / dilution skills	Numeracy Skills	
General Lab. Skills	Basic molecular biology	General Lab. Skills	Cell culture
	Record keeping		Generation of retroviral expression vectors
			Fluorescent microscopy / immunostaining
			PCR
Communication Reading/Writing/Presenting Skills	Literature searching	Communication Reading/Writing/Presenting Skills	Presentation to colleagues
	Report writing		Writing lit review

Project title: Surface enzyme activity in lichens

Contact: peter.crittenden@nottingham.ac.uk

Rotation: 2

Phosphatase activities in lichens of contrasting ecologies.

This mini project will test the hypothesis that phosphatase activities in lichens are high in species from oligotrophic habitats and low in those from eutrophicated sites. Two lichen species identified as nitrophytic and two thought to be nitrophobic will be selected for study. These designations are based on UK distributional data in relation to nitrogen pollution and on documented qualitative assessments of habitat preferences. Lichens will be collected from the field and the pH optimum for phosphomonoesterase (PME) and phosphodiesterase (PDE) activities determined at 15 °C in the dark using a range of buffered assay media. Enzyme saturating substrate concentrations will then be determined for the most enzyme active species by constructing substrate response curves. PME and PDE activities in the four species will then be compared at the species-specific pH optima and using saturating substrate concentrations. In order to locate the PME activity in the thallus, hand cut sections of each species will be exposed to the enzyme-labelled fluorescent phosphatase substrate ELF 97 phosphate and examined by fluorescence microscopy. To determine the cellular location of PME activity, the fast growing lichen-forming fungi *Cladonia floerkeana* and *Acarospora furcata* will be isolated onto phosphorus- limiting defined media at the start of the project and then, in the final week, mycelia will be exposed to ELF 97 phosphate and examined using fluorescence microscopy (Nikon TiE Eclipse). The project will provide training in microbial ecology, fungal culture, enzyme assays, enzyme location and fluorescence microscopy.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	
	Spreadsheet manipulation		
Numeracy Skills	Basic stats	Numeracy	
	Basic concentration/dilution skills		
General Lab. Skills	Understanding of the need for precision and accuracy		Enzyme assays
	Basic equipment handling eg pipettes		Spectrophotometry
	Record keeping		
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Ability to represent research group

Project title: Understanding molecular mechanism regulating unusual cell division in malaria parasite.

Contact: rita.tewari@nottingham.ac.uk

Rotation: 2

The mini project will train the student to generate the targeting construction for transfection and to understand the different developmental stages of malaria parasites. The genes involved will be some novel molecules involved in signal transduction pathway namely kinases, phosphatases or armadillo repeat proteins. They will be following the molecular techniques like Cloning and DNA isolation, southern blotting, PCR for DNA analyses and Western blotting for protein analyses. In addition they will be exposed to some cell biology techniques using fluorescence microscopy.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	Use of advanced data analysis software
	Spreadsheet manipulation		Use of molecular modelling software
Numeracy Skills	Basic stats	Numeracy	
	Basic concentration/dilution skills		
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	Centrifugation Cell biology
	Basic equipment handling eg pipettes		Electrophoresis
	Record keeping		Enzyme assays
			Spectrophotometry
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Ability to represent research group

Project title: FGF21 and the role of tanycytes in regulating energy balance and body weight

Contact: kostas.tsintzas@nottingham.ac.uk

Rotation: 2

The mini project will characterise further the *in vivo* actions of a FGFR agonist (H7) provided in a collaboration with Eli Lilly that we hypothesise targets tanycytes, and will use immediate early gene markers of neuronal activation (*egr-1*, *c-fos*) to investigate whether tanycytes are activated by H7 treatment.

Adult male hamsters will be treated with H7 or vehicle, and examined for 48 in metabolic cages. At the end of the study period hamsters will be euthanized, and perfused transcardially to obtain a suitably fixed brain. Students will work alongside PI (PIL holder) for this phase of the project.

Sections will be cut on a freezing microtome, and hypothalamic sections immunostained for *egr-1*, *c-fos*. Sections may also be dual stained for tanycyte markers (GPR50, nestin, vimentin). Section will be analysed to determine whether H7 treatment increased *egr-1*, and / or *c-fos* expression, assessed as number of cell nuclei in specific hypothalamic regions expressing these immediate early gene products. Students will work alongside a Research Technician (Dr Maxine Fowler) for this phase of the project.

Students will gain training in animal handling, *in vivo* experimental design, use of metabolic cages (Comprehensive lab Animal Monitoring system), histology and immunohistochemistry, basic microscopy, data analysis.

Skills you need		Skills you'll develop	
Computer Skills	Spreadsheet manipulation	Computer Skills	Data analysis
Numeracy Skills	Some statistical background eg Analysis of variance	Numeracy	Use of statistics software eg Prism
Intellectual skills	Basic understanding of mammalian physiology	Intellectual skills	Greater understanding of brain-body interactions in the regulation of energy metabolism
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	Brightfield and/or fluorescence microscopy
			neuroanatomy of the rodent brain
			tissue sectioning and immunohistochemistry
			animal handling
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Report writing
	Ability to carry out literature-searching		
	Ability to communicate to colleagues		
	Ability to function in a team		

Project title: Quinolone-inspired quorum messenger-mimicking antimicrobials – biological activity and mechanistic determinants

Contact: paul.williams@nottingham.ac.uk

Rotation: 2

Two alternative mini-projects are offered depending on the background of the student:

Solid state NMR mini-project: The objective is to give in a nutshell a flavour of the continuity in biological research from design and sample preparation through analysis to functional characterisation. Week 1-2: Synthetic AQs/QZNs will be modelled computationally and a computational prediction of its NMR spectrum will be obtained. Week 3-4: Model membranes will be prepared incorporating the AQ. The lipid membranes will be studied by solid state NMR to obtain a ^{13}C spectrum from the AQ. Week 5-6: Experimental spectra will be compared to the prediction and used for spectral assignment.

Microvesicle induction mini-project: The objective is to offer a hands-on experience into working with living organisms and at the boundary between chemistry and biology and a brief introduction to preparative techniques allowing visualisation on the microscopic and nanoscopic lengthscale. Week 1-2: Investigate gene regulation in *P. aeruginosa* by PQS: coculture *P. aeruginosa* pqsA and pqsH mutants carrying *lecA::lux* fusion with PQS autoinducer to show restoration of *lecA* expression using bioluminescence. Week 3-4: Investigate *in vitro* vesicle formation in pure LPS incubated with AQ or with inhibitor QNZ. Prepare samples and observe microvesicle formation by electron microscopy. Week 5-6: Investigate *in vivo* vesicle formation in *P. aeruginosa* incubated with AQ or inhibitor, QNZ, using electron microscopy. Compare to *in vitro* LPS microvesicle formation.

Skills you need		Skills you'll develop	
Computer Skills	<i>Knowledge of basic software</i>	Computer Skills	<i>Use of advanced data analysis software</i>
	<i>Spreadsheet manipulation</i>		<i>Use of molecular modelling software</i>
Numeracy Skills	<i>Basic stats</i>	Numeracy	
	<i>Basic concentration/dilution skills</i>		
General Lab. Skills	<i>Understanding of the need for precision and accuracy</i>	General Lab. Skills	<i>Centrifugation</i>
	<i>Basic microbiology and lab equipment handling skills</i>		<i>Spectrophotometry</i>
	<i>Record keeping</i>		<i>Enzyme assays</i>
	<i>Basic theoretical knowledge of DNA manipulation</i>		<i>Reporter gene expression assays</i>
Communication Reading/Writing/Presenting Skills	<i>Report-writing</i>	Writing Skills	<i>Scientific paper-writing</i>
	<i>Ability to carry out literature-searching</i>		<i>Ability to write a literature review</i>
	<i>Ability to communicate to colleagues</i>		<i>Ability to communicate to outside audiences</i>
			<i>Ability to represent research group</i>

Project title: Age and tissue specificity in signalling mechanisms employed by opioid and cannabinoid receptors: promiscuous pathways providing novel insights into neurodevelopment and pain

Please be aware that to undertake the PhD project allied to this lab rotation you will need additional training similar to that which used to be required for a Home Office licence (the HO Licence training is currently in the process of being reviewed.) Details tbc.

Contact: gareth.hathway@nottingham.ac.uk

Rotation: 2

There is great potential for the use of δ -opioid receptor (DOR) agonists in a variety of pain states. DORs appear to be recruited to the plasma membrane of sensory nerves and become available for activation when inflammatory signals increase intracellular calcium concentrations ($[Ca^{2+}]_i$). This is counterintuitive since opioids commonly reduce Ca^{2+} mobilisation via interaction with inhibitory G proteins (Gi). Our preliminary data reveal that some DOR agonists increase $[Ca^{2+}]_i$ in adult dorsal root ganglion (DRG) neurones, but whether this excitation is Gi-mediated is unknown. There is evidence that opioid receptors can be either coupled to Gi or to the stimulatory G protein (Gs) and that the balance can be shifted by age of the host and membrane environment.

This project aims to determine whether DOR agonist effects on DRG can be shifted between excitation and inhibition by modelling inflammation, by aging and by altering the lipid composition of the membrane. Having harvested tissues, the student will measure single cell Ca^{2+} imaging using cultured DRG from adolescent and mature rats challenged with DOR agonists in the presence and absence of pertussis toxin (to test Gi involvement), an “inflammatory soup” of cytokines and the ganglioside GM1. Potential inhibitory effects will be assessed by monitoring DOR effects on bradykinin-stimulated Ca^{2+} -mobilization. Translocation of DOR from cytosol to membrane will be examined using immunohistochemistry and confocal microscopy. The student will have the opportunity to “sit-in” on related electrophysiology and behavioural experiments. Students will be trained and perform all tasks directly associated with the project.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	Use of advanced data analysis software
	Spreadsheet manipulation		Use of advanced data acquisition software
Numeracy Skills	Basic stats	Numeracy	Advanced statistical analysis of electrophysiological data
	Basic concentration/dilution skills		
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	Centrifugation
	Basic equipment handling eg pipettes		Electrophysiology
	Record keeping		Behavioural assessment
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Ability to represent research group

Project title: Regulation of DNA replication by the central carbon metabolism in *B. subtilis*

Contact: panos.soultanas@nottingham.ac.uk

Rotation: 2

The miniproject will involve the initial cloning of the 4 pdh genes (pdhABCD) into pET expression vectors followed by protein expression studies to optimize overexpression conditions. Training will be provided in bacterial culturing, agarose-based gel electrophoresis, PCR-based cloning, genomic DNA preparation, plasmid preparation, IPTG-based heterologous protein expression in *E. coli*, protein analysis by SDS-PAGE and basic bioinformatics analysis of protein properties (theoretical pI, basic sequence comparisons) and a literature review on the structure/function of pyruvate dehydrogenases.

Skills you need		Skills you'll develop	
Computer Skills	<i>Knowledge of basic software (Microsoft office, internet, email)</i>	Computer Skills	<i>Use of photoshop for the production of publication quality figures and Grafpad for data manipulation and graph preparation</i>
	<i>Spreadsheet manipulation</i>		<i>Use of protein structure 2D and 3D visualisation software such as PYMOL and SPDBV</i>
			<i>Use of protein and/or gene analysis web based programs resources such as Protein Data Base, Subtilin, Colibri etc</i>
Numeracy Skills	<i>Basic stats</i>	Numeracy	<i>Standard errors, deviations etc</i>
	<i>Basic concentration/dilution skills</i>		<i>Ability to work out accurately concentrations and make biological buffers</i>
			<i>Ability to plot graphs and bar charts</i>
			<i>Ability to independently interpret and analyze data, design experiments, formulate hypotheses.</i>
General Lab. Skills	<i>Understanding of the need for precision and accuracy</i>	General Lab. Skills	<i>Centrifugation, plasmid preparations, protein purification</i>
	<i>Basic and advanced equipment handling eg pipettes, chromatography columns, PCR machines etc</i>		<i>Protein and DNA Electrophoresis, PCR techniques, site directed mutagenesis,</i>
	<i>Meticulous record keeping, basic safety understanding</i>		<i>Enzyme assays (gel based and radiolabeling), protein-protein</i>

			<i>interaction and protein-DNA interaction assays (supershift assays, chemical linking, Surface Plasmon Resonance), DNA polymerase assays, DNA primase assays, DNA helicase assays</i>
	<i>Analysis, interpretation and presentation of data</i>		<i>Spectrophotometry, chromatography, protein purification, protein handling and manipulation, bacteriology (handling, culturing and manipulating bacteria)</i>
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	<i>Scientific paper-writing</i>
	<i>Ability to carry out detailed advanced literature-searching</i>		<i>Ability to write a literature review</i>
	<i>Ability to communicate to colleagues</i>		<i>Ability to communicate to outside audiences</i>
			<i>Ability to represent research group</i>

Project title: Measurement of G protein-coupled receptor dynamics and ligand-binding in Membrane microdomains of single living cells

Contact: frank.ball@nottingham.ac.uk; stephen.bridson@nottingham.ac.uk

Rotation: 2

The technique of fluorescence correlation spectroscopy (FCS) allows the interaction of drugs with GPCRs to be quantified in small areas of the cell membrane of single living cells. We have developed techniques in Nottingham that allow the interactions of fluorescent drugs with GPCRs to be measured in small membrane microdomains and to provide information on the diffusional characteristics of ligand-bound receptor complexes. The technique allows us to monitor the size of these complexes and the number of them present with a small membrane domain. We have also developed techniques to label the receptors on their N-terminus with different probes under physiological conditions. This mini project will use these techniques to provide data on the diffusional characteristics of a labelled adenosine A3 receptor and an unlabelled A3-receptor bound to different fluorescent agonists and antagonists. In parallel existing mathematical approaches will be used to generate a dynamic simulation (using random walk approaches) of how the ligands can bind and dissociate from receptors within such a small microdomain region. The combination of the two approaches will provide a visual illustration of the power of the techniques and the ability to model the effects of changing key parameters.

Skills you need		Skills you'll develop	
Computer Skills	<i>Handling of large datasets</i>	Computer Skills	<i>Manipulation and handling of multiple large datasets and formats</i>
	<i>Some experience of high level computing language (e.g. Matlab)</i>		<i>High performance computing</i>
			<i>Advanced Matlab scripting</i>
			<i>Advanced non-linear curve fitting</i>
Numeracy Skills	<i>Statistical and probabilistic knowledge equivalent to at least 2nd year undergraduate maths</i>	Numeracy	<i>Development and analysis of stochastic models</i>
			<i>Devising and implementation of Monte Carlo simulations</i>
			<i>Bayesian and classical methods of statistical inference</i>
			<i>Analysing receptor-ligand kinetic interactions</i>
General Lab. Skills	<i>Non required (will be trained)</i>	General Lab. Skills	<i>Cell and solution based fluctuation spectroscopy</i>
			<i>Basic wet lab skills (dilutions/compound handling)</i>
Communication Reading/Writing/Presenting Skills	<i>Report-writing</i>	Writing Skills	<i>Writing of scientific papers and literature reviews</i>
	<i>Literature searching</i>		<i>Ability to write for readers with diverse scientific backgrounds</i>
	<i>Ability to communicate with colleagues from different subject backgrounds</i>		<i>Ability to communicate to outside audiences</i>
			<i>Ability to represent research group</i>

Project title: Stochastic Neural Network Modelling

Contact: stephen.coombes@nottingham.ac.uk

Rotation: 2

- Literature review and written report on existing modelling frameworks.
- Attend and contribute to relevant journal clubs within the Centre for Mathematical Medicine and Biology.
- Programme an implementation of a prototypical model in Matlab.
- Translate this in silico model to the Python programming language and learn to implement in a parallel programming environment.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	Matlab
			Python
			Gnuplot
			LaTeX
Numeracy Skills	BSc mathematics	Numeracy	
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	
	Basic equipment handling		
	Record keeping		
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Ability to represent research group

Project title: Bio-engineering of a non-toxinogenic *Clostridium difficile* strain to provide prophylaxis via colonisation and the generation of a protective immune response.

Contact: sarah.kuehne@nottingham.ac.uk

Rotation: 2

An introduction to engineering *Clostridium difficile*

During the Mini-project the student will learn how to work with anaerobic bacteria, become familiar with the genetic tools used within CRG and how they will be of value for the project.

Within the six weeks the student will

- receive safety inductions to the building and laboratory and be trained on relevant equipment.
- learn how to work in anaerobic cabinets, culturing and manipulating *Clostridium difficile*.
- be trained to work with the cell culture model in order to perform cytotoxicity tests and also adherence assays.
- test the growth characteristics of non-toxinogenic *C. difficile* strains.
- proof that the strains do not produce toxins by Western blot and cytotoxicity.
- Construct a BioBricks to learn the principle and clone this into an appropriate vector.
- be introduced to ACE (allelic coupled exchange) technology.

Within the six weeks the student will acquire the basic techniques required to carry out the proposed project and gain an understanding of the study. They will learn how to culture *C. difficile*, how to introduce DNA into the organism (via conjugation), how to construct BioBricks. Furthermore they will learn how to work with epithelial cell cultures and test toxicity of *C. difficile* in a variety of assays. They will also become familiar with the laboratory setting and some important computer software allowing *in silico* cloning and sequence analysis (GenTle, DNASTAR).

Skills you need		Skills you'll develop	
Computer Skills	Standard knowledge of computer software: office (word, excel, power-point) or similar	Computer Skills	Use of: DNA analysis tools, <i>in vitro</i> cloning, (GenTle, DNASTar), graph pad
	internet searches		next generation sequencing analysis
			Bioinformatics as required
Numeracy Skills	Basic concentration/dilution skills	Numeracy	Basic stats as required
General Lab. Skills	Accurate record keeping	General Lab. Skills	Molecular skills: DNA handling, cloning, mutagenesis, PCR
	Basic equipment handling eg pipettes		Microbiology skills: working with aerobic (<i>E. coli</i>) and anaerobic (<i>Clostridia</i>) bacteria
			Phenotypic assays: sporulation, cytotoxicity, adherence assays
			<i>In vivo</i> infection model
Communication Reading/Writing/Presenting Skills	Literature search	Writing Skills	Write abstracts
	Communicate to colleagues		write a literature review
			Scientific paper-writing
			Present the work in seminars and at conferences, communicate to different audiences

Project title: Understanding the targeting and function of the adrenoleukodystrophy protein, a peroxisomal ABC transporter

Contact: ian.kerr@nottingham.ac.uk; freddie.theodoulou@rothamsted.ac.uk

Rotation: 2

Students will investigate the suitability of Baker's yeast as an expression host for the human long-chain fatty acid transporter ALDP. Students will express completely codon-optimised ALDP (cDNA available) with and without a C-terminal histidine tag using expression plasmids with different strength promoters. ALDP function will be tested by complementation of the yeast *pxa1 pxa2Δ* mutant which lacks the orthologous ABC transporter and therefore cannot metabolise long chain fatty acids. Therefore, ALDP function is determined by the ability of yeast to grow on long chain fatty acid carbon sources. Protein localisation will be confirmed by Western blotting using commercially-available antibodies. Stability of the protein will be tested by Western blotting and, time-permitting, trial purification by immobilised metal affinity chromatography will be attempted, as will ATPase assays. This mini project is assured of a good outcome, since a partially re-coded ALDP is functional in *Saccharomyces cerevisiae* when expressed at low levels (van Roermund et al., 2008 FASEB J. 22, 4201-8).

The rotation project (based at Rothamsted Research – although with regular interaction with the Nottingham lab) will provide training in molecular biology (plasmid construction), yeast transformation, protein and organelle purification and biochemical assays. All biological materials are available.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	Use of advanced data analysis software
Numeracy Skills		Numeracy	Statistical analyses
	Basic lab maths i.e. making up buffers, dilutions etc		
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	Yeast culture Insect cell culture
	Basic equipment handling eg pipettes		Membrane protein purification
	Record keeping		Spectrophotometry
	Some experience of molecular biology useful		Advanced molecular biology
			Fluorescence microscopy
			Experience in academic and "translational" research labs
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
	Willingness to identify and fill your knowledge gaps		Ability to represent research group

Project title: Making biofuels a reality – Improving bacterial butanol production through rational metabolic engineering

Contact: klaus.winzer@nottingham.ac.uk

Rotation: 2

Characterisation of a *Clostridium acetobutylicum* fermentation pathway mutant

This mini project will serve as an introduction to anaerobic microbiology and the biology of the genus *Clostridium*. Its scientific objective will be the phenotypic characterisation of a *C. acetobutylicum* mutant strain.

The student will:

- receive an induction to cover safe laboratory practice and use of relevant equipment within the CBS
- be trained in anaerobic microbiological methods and techniques such as the use of anaerobic cabinets
- be given a *C. acetobutylicum* mutant defective in a fermentation pathway gene; this will be grown in batch culture, together with a wild-type control
- learn to record the growth of these cultures by taking optical density readings, colony-forming-unit counts, and determination of total protein
- microscopically examine these cultures and learn to recognise vegetative cells, clostridial forms, and endospores
- determine the concentration of fermentation products in culture supernatants by gas chromatography
- take cell samples for (i) the preparation of cell extracts, followed by the determination of key enzyme activities, (ii) determination of granulose content, and (iii) endospore counts
- be shown how to critically analyse his data, qualitatively and quantitatively, and in comparison to the literature

Through this project, the student will learn many of the key techniques required to undertake the proposed PhD project. Training includes the anaerobic handling and cultivation of strains, microscopic techniques, gas chromatography, and determination of intracellular enzyme activities. The student will also gain skills in critical data analysis and the application of statistical methods.

Skills you need		Skills you'll develop	
Computer Skills	<i>Knowledge of Microsoft Office software</i>	Computer Skills	<i>Use of data analysis software</i>
	<i>Spreadsheet manipulation</i>		
Numeracy Skills	<i>Basic concentration/dilution skills</i>	Numeracy	<i>Basic statistics</i>
General Lab. Skills	<i>Record keeping</i>	General Lab. Skills	<i>Microscopy</i>
			<i>Handling of anaerobic bacteria</i>
	<i>Basic equipment handling eg pipettes</i>		<i>Use of spectrophotometer</i>
			<i>Gas chromatography</i>
			<i>Enzyme assays</i>
		<i>Centrifugation</i>	
Communication	<i>Ability to carry out literature-searching</i>	Writing Skills	<i>Report-writing</i>
Reading/Writing/Presenting Skills	<i>Ability to communicate to colleagues</i>		<i>Ability to represent to research group</i>

Project title: The influence of genotype, environment and management on the efficiency of conversion of willow to fuels and industrial products

Contact: gregory.tucker@nottingham.ac.uk; angela.karp@rothamsted.ac.uk

Rotation: 2

The aims of the mini project will be to provide training for the student in trait assessment and sampling of willows in the field and in the main technologies required for the biochemical processing. At RRes the student will spend time with a team of staff postdocs and students who are working with willow and will be provided with background training in sampling and trait measurements, the diversity of willow available and use of the willow database that has been developed in BSBEC in which all trait and genetic data are recorded. At Nottingham they will likewise join a team of BSBEC scientists and will be trained in biochemical technologies, including the kinetic analysis of both biomass response to hydrothermal pretreatment and saccharification by enzyme hydrolysis. At the end of their six weeks they will have been not only provided with basic training in the methodologies but they will also have obtained a clear picture of where their own project fits within a much larger framework of research and the strategic goals behind this.

To be assured of an outcome the mini-project will focus on a small set of contrasting genotypes which the student will take through the research process from measuring stem traits, sampling the biomass basic pretreatments (RRes), conversions and kinetics studies (Notts). They will also be trained in the interpretation of the results and will be able to compare the different genotype samples. All materials and facilities are available.

Skills you need		Skills you'll develop	
Computer Skills	Knowledge of basic software	Computer Skills	
	Spreadsheet manipulation		
Numeracy Skills	Basic stats	Numeracy	
	Basic concentration/dilution skills		
General Lab. Skills	Understanding of the need for precision and accuracy	General Lab. Skills	Centrifugation
	Basic equipment handling eg pipettes		Electrophoresis
	Record keeping		Enzyme assays
			Spectrophotometry
Communication Reading/Writing/Presenting Skills	Report-writing	Writing Skills	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Ability to represent research group

Project title: Using laccases to produce chemicals and remediate wastes

Contact: gill.stephens@nottingham.ac.uk

Rotation: 2

The mini project will investigate the use of laccases to depolymerise lignin, using reaction systems developed in our lab. A range of laccases will be tested for their ability to degrade organosolvents, lignin from biofuel processes, lignocellulosic wastes from bioethanol processes (DDGS), and lignin wastes from paper pulping (Kraft lignin). The enzymes will be tested with a matrix of different mediators and reaction conditions (T, pH, buffer composition) to identify optimum conditions for processing. This will demonstrate the methods to handle industrial enzymes and provide an insight into optimisation of enzyme reactions in the process industries. The reaction products will be analysed by HPLC, GCMS, NMR and GPC, providing training in analytical methodologies that are used throughout the biorenewables industries and in academic research labs. In addition to the lab training, the students will gain an insight into the complexity of the product mixtures formed when biosystems operate on complex biorenewable feedstocks, and the need for rigorous experimental designs and data analysis to extract maximum information from the minimum number of experiments. This project will form an excellent springboard for the proposed PhD project using laccases to produce chemicals and remediate wastes, and also provides training in many of the techniques which underpin a broad range of other projects in the areas of biorenewables and bioenergy.

Skills you need		Skills you'll develop	
Computer Skills	<i>Knowledge of basic software</i>	Computer Skills	<i>Use of mass spectral data libraries</i>
	<i>Spreadsheet manipulation</i>		
Numeracy Skills	<i>Basic stats</i>	Numeracy	Analysis of data, including calculation concentrations of chemicals from analytical chemistry experiments
	<i>Basic concentration/dilution skills</i>		Interpretation of mass spectra
General Lab. Skills	<i>Understanding of the need for precision and accuracy</i>	General Lab. Skills	<i>Preparative scale enzyme reactions</i>
	<i>Basic equipment handling eg pipettes</i>		<i>Solvent extraction</i>
	<i>Record keeping</i>		<i>Small scale enzyme assays</i>
			<i>Spectrophotometry</i>
			<i>Gas chromatography</i>
			<i>Mass spectrometry</i>
			<i>HPLC</i>
			<i>Gel permeation chromatography</i>
			<i>Small scale centrifugation</i>
Communication Reading/Writing/Presenting Skills	<i>Report-writing</i>	Writing Skills	<i>Interpreting data from analytical chemistry experiments</i>
	<i>Ability to carry out literature-searching</i>		<i>Ability to write a literature review</i>
	<i>Ability to communicate to colleagues</i>		<i>Ability to communicate to outside audiences</i>
			<i>Ability to represent research group</i>

Project title: *In-vitro* engineering to develop tissue grafts facilitating the regeneration and healing of the human colon

Please be aware that this project is working with human cells. If you are interested in undertaking this lab rotation (or the PhD project allied to it) you will need to discuss your Hep B vaccination status with Occupational Health when you complete registration at the University.

Contact: felicity.rose@nottingham.ac.uk

Rotation: 2

The key objective of this project is to develop *in vitro* cultures of CD24 positive intestinal progenitor cells which can ultimately be used as epithelial grafts to facilitate wound healing and mucosal regeneration *in vivo*. Experiments central to this goal will form the basis of the mini project to introduce the students to many of the skills required for the PhD project itself. In order to ensure adequacy of tissue availability, experimental work will be performed on immortalised colonic cell lines rather than human tissue. Cell lines will be tested for CD24 expression by Western blotting. Cell lines which are negative for CD24 expression will be transfected with a CD24 expression construct (already made and available in our laboratories). The CD24 population will be isolated by fluorescence activated cell sorting (FACS) and will be plated onto a matrigel sandwich. Cells will be initially grown in medium described by Sato et al. containing Wnt3a / EGF / Noggin / R-spondin / Nicotinamide / p38 inhibitor. Cell proliferation, morphology and expression of cell surface markers will be monitored using microscopy and FACS and cultures monitored for intestinal organoid development. This project will introduce students to mammalian cell culture techniques, gene transfection, cell function assays, Western blotting and FACS providing the student with an excellent skills set for any mammalian cell biology focussed PhD project.

Sato, T. et al., Long-term Expansion of Epithelial Organoids from Human Colon, Adenoma, Adenocarcinoma, and Barrett's Epithelium. *Gastroenterology* 2011; 141(5): 1762-1772.

Skills you need		Skills you'll develop	
Computer Skills	<i>Knowledge of basic software</i>	Computer Skills	<i>Use of advanced data analysis software</i>
	<i>Spreadsheet manipulation</i>		<i>Use of statistical analysis software</i>
			<i>Database searching including bioinformatics</i>
Numeracy Skills	<i>Basic stats</i>	Numeracy	<i>More advanced statistical analysis</i>
	<i>Basic concentration/dilution skills</i>		<i>Confidence in calculations and dilutions</i>
General Lab. Skills	<i>Understanding of the need for precision and accuracy</i>	General Lab. Skills	<i>Mammalian Cell Culture</i>
	<i>Basic equipment handling eg pipettes</i>		<i>FACS(Flow Assisted Cell Sorting)</i>
	<i>Record keeping</i>		<i>Microscopy</i>
			<i>Gel electrophoresis and Western Blotting</i>
Communication Reading/Writing/Presenting Skills	<i>Report-writing</i>	Writing Skills	<i>Scientific paper-writing</i>
	<i>Ability to carry out literature-searching</i>		<i>Ability to write a literature review</i>
	<i>Ability to communicate to colleagues</i>		<i>Ability to communicate to outside audiences</i>
			<i>Ability to represent research group</i>
			<i>Writing SOPs and Risk Assessments</i>

Project title: Unravelling the molecular, electrophysiological and endocrinological mechanisms through which cholesterol decreases myometrial contractile activity.

Please be aware that to undertake this lab rotation or the allied PhD project you may require additional training similar to that which used to be required for a Home Office licence (the HO Licence training is currently in the process of being reviewed.) Details tbc.

Please be aware that this project is working with human cells. If you are interested in undertaking this lab rotation (or the PhD project allied to it) you will need to discuss your Hep B vaccination status with Occupational Health when you complete registration at the University.

Contact: matthew.j.elmes@nottingham.ac.uk

Rotation : 2

The mini-project will be used to generate preliminary data from myometrial tissue collected from our previous trials using the high-fat high-cholesterol model, and will be made up of 3 different experiments providing training in different laboratory techniques. The techniques are in routine use in our laboratory and the work proposed is considered feasible for 2 students to achieve within a 6 week period.

Experiment 1. Quantification of contractile associated proteins

One key aspect of the mini project would be to determine the myometrial expression of calcium channels in both human and animal samples. Western Blotting techniques will be used to determine whether cholesterol decreases calcium channel expression in the myometrium. If this evidence can be provided across both species then it will support the translation between the animal model to the human. (Time to complete 3 weeks +)

Experiment 2. Analysis of plasma to determine lipid and hormone profiles.

The 2nd experiment would be to determine plasma lipid profiles and levels of pregnancy and parturition hormones. Commercially available kits would be used to measure triglyceride and cholesterol levels, and colorimetric assays to measure prostaglandins and steroid hormones. (1 -2 weeks).

Experiment 3. Histological analysis of myometrium

Previous findings within the literature have established that myometrial contractile dysfunction is associated with myocyte accumulation of cholesterol esters. Uterine horn samples stored in formalin will be embedded in paraffin and stained with hematoxylin/safran/eosin. This histological stain will identify any differences in uterine structure and lipid accumulation. (1-2 weeks)

Skills you need		Skills you'll develop	
Computer Skills	<i>Knowledge of basic software such as Excel</i>	Computer Skills	<i>Use of advanced data analysis software such as Chart for Windows</i>
	<i>Spreadsheet manipulation</i>		<i>Use of statistical software such as GraphPad Prism and SPSS</i>
			Densitometry analysis
Numeracy Skills	<i>Basic statistics</i>	Numeracy	
	<i>Basic concentration/dilution skills</i>		Concentration/dilution skills.
General Lab. Skills	<i>Understanding of the need for precision and accuracy</i>	General Lab. Skills	<i>Centrifugation, Homogenisation of tissues, and production of protein preps</i>
	<i>Basic equipment handling eg pipettes</i>		<i>Gel Electrophoresis and Western Blotting Techniques</i>
	<i>Record keeping</i>		<i>TAG and cholesterol colorimetric assays</i>
			<i>Histological analysis</i>
Communication Reading/Writing/Presenting Skills	<i>Report-writing</i>	Writing Skills	<i>Scientific paper-writing</i>
	<i>Ability to carry out literature-searching</i>		<i>Ability to communicate to outside audiences</i>
	<i>Ability to communicate to colleagues</i>		<i>Ability to represent research group</i>

Project title: Sustainable Nutrition from Green-Leaf Waste

Please be aware that the PhD project allied to this mini-project may require working with human cells. If you are interested in undertaking the PhD project allied to this lab rotation you will need to discuss your Hep B vaccination status with Occupational Health once you complete registration at the University.

Contact: david.gray@nottingham.ac.uk

Rotation: 2

6 Week Training Period

Laboratory Training will include:

The overall project is essentially split into 4 areas (Extraction; Characterisation; Funtionalisation; Demonstration). The mini project will equip the student in the rudimentary skills required for these different areas:

Microscopy (light and electron)

Lipid Extraction

Spectroscopy – Light; Fluorescence;

Chromatography – HPLC; GC-MS

Experimental Design

Texture Measurements

Sequence of Work over 6 Weeks, joining the PhD students already active within the Biomaterials Group:

1. Repeat established methods to recover chloroplasts and CWM from spinach leaves (model leaf tissue)
2. Assess the integrity of recovered chloroplasts and CWM (light and electron microscopy)
3. Test the impact of osmoticum type and concentration on the integrity of recovered chloroplasts
4. Recover chloroplasts from fresh spinach leaves, extract nutrients of interest [vitamin A (β -carotene), C, E and α -linolenic acid] and measure their concentration in fresh material relative to chlorophyll content, and the physical parameters describing textural attributes of CWM.

Skills you need		Skills you'll develop	
Computer Skills	<i>Knowledge of basic software</i>	Computer Skills	<i>Use of advanced data analysis software</i>
	<i>Spreadsheet manipulation</i>		<i>Reference management software</i>
Numeracy Skills	<i>Basic stats</i>	Numeracy	
	<i>Basic concentration/dilution skills</i>		
General Lab. Skills	<i>Understanding of the need for precision and accuracy</i>	General Lab. Skills	<i>Handling plasma samples from clinical feeding trials</i>
	<i>Basic equipment handling e.g. pipettes</i>		<i>Plant tissue disruption and retention of intact organelles</i>
	<i>Record keeping</i>		<i>Extraction of phytonutrients</i>
	<i>Operate safely using SOPs and GLP</i>		<i>Chromatography: GC-MS; HPLC; TLC</i>
			<i>Spectrophotometry</i>
			<i>Physical measurements e.g. viscosity</i>
Communication Reading/Writing/Presenting Skills	<i>Report-writing</i>	Communication Skills	<i>Scientific paper-writing</i>
	<i>Ability to carry out literature-searching</i>		<i>Ability to write a literature review</i>
	<i>Ability to communicate to colleagues</i>		<i>Ability to communicate to outside audiences</i>
			<i>Ability to represent research group</i>