Synopsis of the BMB lecture course

***Note**: This information is provided at the beginning of the year for your guidance and that of your supervisors. It is not intended to be a comprehensive list of contents. Lecturers will all issue their own handouts and may vary the topics and the order in which they are presented.*

Michaelmas Term: Genes and proteins – macromolecules in action

In Michaelmas, the course examines the molecular biology of DNA and protein structure. How is DNA packaged in cells? How does chromatin structure affect gene expression? How is genetic engineering actually carried out? How are transcription and translation regulated? What are the principles of protein design and how can we exploit them through protein engineering?

***Dee Scadden**: Gene cloning and Manipulation*

These lectures introduce the techniques of gene cloning and manipulation that underpin much of the work described in the rest of the course. Building on material covered in the Part IA Biology of Cells lectures, we look at the use of various techniques to ask specific experimental questions. **Examples of topics covered include**:

- The polymerase chain reaction and its various applications
- A brief overview of vectors and hosts that are used in conventional gene cloning
- Investigating how clones may be used experimentally to investigate protein function. e.g. preparing recombinant fusion proteins, making RNA for *in vitro* studies, investigating protein localization, protein-protein interactions etc.
- Methods for reducing gene expression (e.g. RNAi, CRISPR/Cas9), and for creating transgenic mice.

***Chris Smith:** Nucleic acid structure, Protein-Nucleic acid Interactions and Transcription*

These lectures cover the first step in gene expression – transcription of RNA using genomic DNA as template. How do RNA polymerases recognise the correct locations at which to initiate transcription, and how can this be regulated? Six main topics will be covered:

- DNA and RNA structure
- Prokaryotic transcription mechanisms
- Prokaryotic transcriptional regulation
- Packaging of eukaryotic DNA into chromatin
- Eukaryotic transcription – core promoter and general transcription factors (GTFs)
- Eukaryotic transcription – activating transcription factors and enhancers

The overarching theme of DNA-protein interactions – both sequence-specific and non-specific – runs through all of these topics. At appropriate points, relevant experimental approaches and techniques will be highlighted.

Alex Borodavka and Ben Luisi: *Post-Transcriptional Control of Gene Expression*

The production of functional proteins involves multiple processes in addition to transcription. Although these steps are usually referred to as post-transcriptional, many of them occur concurrently with transcription. These lectures will introduce the processes required for the formation of a mature RNA in eukaryotic cells (capping, splicing and 3' end processing), translation (in both prokaryotes and eukaryotes) and RNA decay. The basic machinery that carries out these processes, as well as the mechanisms by which this machinery is modulated in a gene-specific manner, will be addressed.

Nick Gay: *Protein Structure, Function and Evolution*

Proteins play most of the effector roles in living organisms. They maintain the structures of cells, of the extracellular matrix and tissues; they catalyse most reactions in cells and generate mechanical force in the muscles; they are involved in information transfer through recognition of other molecules and can act as ligands, as receptors, as messengers, and as transcription factors; they act as receptors, gates and channels in membranes. The aim of these lectures is to understand the unique principles of protein structure from primary structure to formation of large oligomeric complexes and molecular machines and to introduce the methods that are used to study protein structures from optical spectroscopies through X-ray crystallography and NMR to cryo-electron microscopy and computational modelling. We will also discuss how proteins have evolved and how analysis of protein structure can help us to understand the evolutionary relationships between different proteins and their function. Finally, we discuss how the use of structural biology helps us to develop better and more specific drugs.

Florian Hollfelder: *Enzyme Catalysis and Protein Engineering*

This lecture series focuses on how the peptide and protein structures discussed in the preceding module can assume functions – and on experiments that delineate the mechanisms involved. We develop a framework for describing enzyme catalysis quantitatively, as a basis for investigations of mechanism by kinetics, and review the principles of protein folding and stability. Then we ask the question how functional proteins can be created: the 'holy grail' of protein engineering and mechanistic enzymology. Starting with antibody binders several approaches will be discussed: rational design, semi-rational methods and directed evolution.

Lent Term: Energy transduction, cell signalling, cell proliferation and cancer

The course now builds on the molecular foundations laid in the Michaelmas Term to develop an integrated view of cellular processes. How do cells make a continuous supply of energy available for transcription, translation, ion pumping, biosynthesis and a host of other processes? How is metabolism regulated according to the varying needs of the cell? What are the mechanisms by which hormones regulate intracellular processes? How is normal eukaryotic cell growth controlled, and what goes wrong when such control is pathologically disturbed in cancer?

Bill Broadhurst: *Control of Metabolism*

The aims of these lectures are:

- To examine the different ways in which enzyme activity may be controlled.
- To consider the benefits these different modes of control offer for the regulation of flux in metabolic pathways.

This discussion takes place in a wider context, as these various modes of control are employed throughout biological systems. Textbook descriptions of control in the metabolic pathways tend to assume that the enzymes involved are 'soluble' and homogeneously distributed in the cell cytoplasm. We will see how this is not the case: rather, a high degree of spatial organisation is critical to the control of these pathways.

Various experimental approaches are described for studying how metabolism is controlled, with particular emphasis on methods that may be used to study intact systems. These include:

- Metabolic control analysis, which allows for quantitative determination of the importance of any enzyme for flux control *in vivo*.
- Two key non-invasive spectroscopic techniques – fluorescence and NMR – that permit the study of metabolic events in intact cells and tissues.

Chris Howe: *Energy Transduction in Bacteria, Mitochondria and Chloroplasts*

These lectures look at how energy is acquired and used in living systems – bioenergetics. Recent discoveries of key structures and mechanisms have greatly enhanced our understanding of this process. This knowledge is being applied to medicine, plant biology, nanotechnology, and the energy industries, informing our attempts to develop renewable biological energy sources. The six lectures explore how bacteria, plants and animals use light, electrons, protons and ATP to transduce energy from the sub-molecular to the cellular level. The lectures use an evolutionary emphasis to make it easier to understand the diversity of bioenergetics systems in nature.

Sarah Lummis: *Transmembrane Signalling: Molecules and Mechanisms*

Cells are continuously bombarded by many different types of signal; the ability of these cells to respond appropriately to such signals is critical for cell survival, adaptation, and specification of function, whether they are individual amoebae or components of a large, complex organism such as a human. This lecture course explores how cells monitor the presence of specific extracellular signalling molecules and how these signals then instigate and drive complex and interwoven intracellular responses. The course will focus on:

- The diversity of signals carrying information to cells; these range from single photons and small molecules to complex proteins.
- The relatively few mechanisms, usually involving plasma membrane receptors, by which the cell perceives the signal.
- The means by which the cell decodes 'the message', a process which may be very rapid, as in neurotransmission, or much slower, as in the signals that regulate gene expression and control growth.

Mark Carrington: *Control of Eukaryotic Cell Growth*

The cell cycle is the term used to describe the succession of events that occur to produce two cells from one. An understanding of the molecular events involved in progression through the cell cycle is central to solving the larger problems of how the tightly controlled expansion of cell populations during the development and growth of any organism occurs and how the loss of regulation of the cycle results in disease – not just cancer but also the inappropriate growth of normal cells.

The aims of the lectures are:

- To give an understanding of the experimental approaches that can be taken to investigate the molecular machinery of a complex biological process.
- To explain how the molecular components that regulate cell cycle progression were identified and how their function was determined.
- To discuss a model of how the ordering of transitions that ultimately lead to cell division is regulated.

Dr Marc de la Roche: *Immunology: the basics*

The aim of "Immunology, The Basics" is to introduce the immune system as a society of professional cells with myriad functions in our bodies – preventing the spread of infection, eliminating pathogens, wound healing, even guiding neural synapse development. The lecture will take a bottom-up approach by considering the evolution of the immune system, its cell types and their functions, and ending with a discussing of immune responses to infection and cancer.

Topics covered will include:

- Evolution and development of the immune system
- The innate immune system
- The adaptive immune system
- Infection by pathogens and how we deal with it
- Immunology's evil, pro-cancer twin

There will be opportunity to engage in supplementary material for those students who have not previously taken immunology. There will be a second Immunology lecture that will take place as part of the Lent term Week 8 practical, to build upon your knowledge and application of antibodies gained during the practical sessions.

Trevor Littlewood and Marc de la Roche: *Oncogenes, Tumour Suppressor Genes and Cancer*

The next four lectures focus on the mechanisms of normal signalling pathways – growth factors and mitogens, their receptors and the mitogenic signals they generate inside the cell, and the pathways that then transduce such mitogenic signals to the various intracellular effectors that precipitate cell growth and replication. The principal effector responses to mitogenic signalling are transcriptional activation of proliferation-associated and cell survival genes and repression of growth suppressing genes, activation of RNA and protein synthesis, and an abrupt shift of metabolism to biosynthesis and aerobic glycolysis. To maintain tissue

and organ homeostasis and function, the pathways responsible for these processes are normally tightly regulated by complex feedback mechanisms.

These lectures address the question of what happens in diseases, such as cancer, where control of pathways controlling cell fate decisions (cell growth, proliferation, survival and migration) is lost through activating mutations in proto-oncogenes and inactivating mutations in tumour suppressor genes. This introduction to molecular oncogenesis sets the scene for a more comprehensive analysis of cancer biology in one of the Part II Biochemistry courses.

Easter Term

How diverse are eukaryotic organisms? What are the molecular mechanisms that underpin bacterial chemotaxis?

Chris Howe: *How the other three quarters lives - how protists break the rules of biochemistry*

Most of what you have heard so far in the course about the biochemistry and molecular biology of eukaryotes is based on a limited number of model organisms, such as *Saccharomyces*, *Drosophila*, humans, and *Arabidopsis*.

However, there is an enormous range of eukaryotic diversity outside those model lineages. This range includes organisms that are tremendously important as pathogens (such as trypanosomes) or ecologically (such as dinoflagellates that underpin coral reefs). Many of these organisms – most of them single celled protists - are strikingly different from the model ones in their basic biochemistry.

These lectures will start with a broad survey of eukaryotic diversity, to see what is out there, and then consider some examples of how some protists break the 'rules' of biochemistry. These include whether DNA is always the repository of genetic information, how cells can do away with histones for DNA packaging, how RNA is processed, and how some proteins pass across as many as five membranes to get to their final destination. At the same time, this will provide an opportunity for revision of earlier parts of the course.

Martin Welch: *Bacterial Chemotaxis and Signal Transduction*

The field of bacterial chemotaxis and motility encompasses perhaps the best-understood prokaryotic signalling pathway. We start by using video footage of motile *E. coli* cells to define the basic swimming behaviour of bacteria in the unstimulated state. We then look at how this behaviour is altered when the cells are challenged with chemostimuli and demonstrate that the observed changes correlate with the sense of flagellar motor rotation. The altered bias in flagellar motor rotation brought about by exposure to chemostimuli causes structural changes in the architecture of the flagellar filaments, and we examine how these subtle molecular alterations can give rise to substantial changes in the behaviour of the whole cell.

We also look at how the molecular components of the chemotaxis and motility apparatus of the cell were discovered, and at the techniques that have been used to piece together the complex signal transduction pathway that is involved in integrating the multiple chemosensory inputs received by the cell at any given time into a single output. This signal transduction pathway involves multiple protein components, transient protein-protein

interactions, phospho-transfer events and other chemical modifications, and its workings are now beginning to be understood at the atomic level.

We look at how the signalling pathway is assembled, how it works, and how its output influences the rotational bias of the flagellar motor (and therefore, ultimately, the swimming behaviour of the cell). Finally, we look at what is known about the flagellar motor itself – the world's smallest multi-speed motor, incorporating both forward and reverse gears. The ingenious methods that have been developed to study this remarkable device are discussed, including some video footage of the motor in action. Moreover, the study of chemotaxis and motility is not simply an esoteric branch of microbiology. With the recent completion of many eukaryotic genome sequences (including the human genome), it has become clear that homologues of the chemotaxis proteins are widespread in "higher" organisms, so these findings are likely to yield valuable insights into the function of many other organisms.

