Lectures

The power of modern Biochemistry and Molecular Biology lies in their ability to explain the mechanisms underlying co-ordinated processes in cells and organisms by identifying the specific underlying molecular interactions.

The course considers two main questions:

What are the specific molecular structures and dynamic interactions between nucleic acids, proteins and enzymes on which life is based?
How do these interactions mediate the organisation and regulation of cellular processes?

NST Part IB Biochemistry and Molecular Biology is organised by a small committee, convened by Dee Scadden, who can be contacted in the Biochemistry Department (Network tel: 33671; e-mail: adjs100@cam.ac.uk) and will be pleased to provide more information or answer any questions.

The course is designed to start a little earlier (on the first Wednesday) in the Lent and Easter Terms and to finish a little later (on the last Friday) in the Michaelmas and Lent Terms than is customary, so that there is a clear period at the end of the lectures in the Easter Term for consolidation and revision before the examinations begin. **Please note this in your diaries.**

Below we provide a summary of current teaching in the course. From time to time there may be some modifications to accommodate sabbatical leave.

Michaelmas Term

In this term 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?

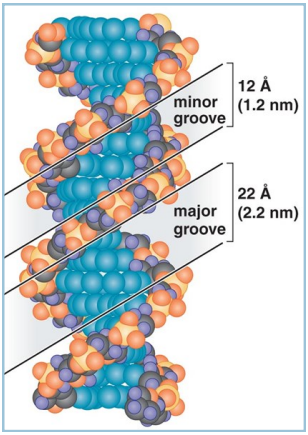
(i) Gene cloning and Manipulation

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.

We first look at the polymerase chain reaction and its various applications, and then consider vectors and hosts that are used in more conventional gene cloning. Once a

clone is obtained, we investigate various ways that this may be used experimentally. For instance, we look at how genes can be expressed to make large quantities of the proteins they encode, and how those proteins may be modified for use in specific experiments (e.g. localization, protein interactions etc.). We conclude by looking at various methods for reducing gene expression (e.g. RNAi, CRISPR-Cas9), and for creating transgenic mice.

(ii) Nucleic Acid Structure, Protein-Nucleic Acid Interactions and Transcription



These 5 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:

1. DNA & RNA structure
2. Prokaryotic transcription mechanisms
3. Prokaryotic transcriptional regulation
4. Packaging of eukaryotic DNA into chromatin
5. Eukaryotic transcription – core promoter and general transcription factors (GTFs)
6. 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.

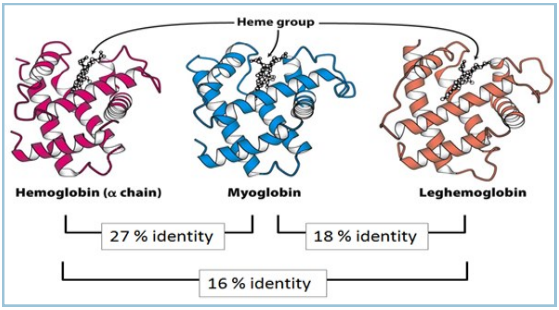
(iii) Post-Transcriptional Control of Gene Expression

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.

(iv) Protein Structure, Function and Evolution

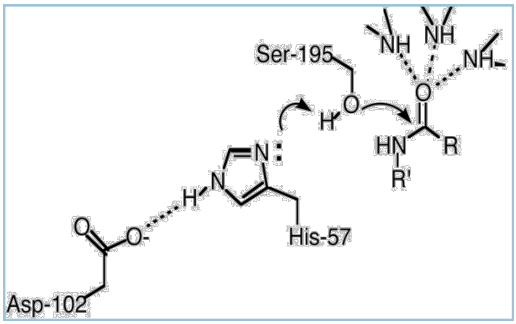
Proteins play most of the effector roles in living organisms. They maintain the structures of cells, of the extracellular matrix and tissues; they catalyze most reactions in cells and generate mechanical force in the muscles; they are involved in information transfer through recognition of other molecules 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. 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.



The three-dimensional structures of haemoglobin, myoglobin and leghaemoglobin: members of the globin family with limited sequence identity but highly similar structures and a conserved binding site for the haem co-factor.

(v) Enzyme Catalysis and Protein Engineering

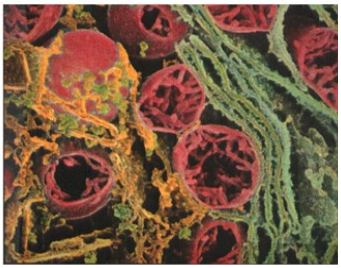


This lecture series focuses on how the peptide and protein structures discussed in the preceding module can assume functions - and on experiments that delineate the mechanisms involved. First we develop ideas about enzyme catalysis, mechanism and kinetics. We look in detail at the co-operative (allosteric) molecular basis of metabolic regulation. Other protein structures that are discussed include immunoglobulins and their binding to specific antigens, and the principles of protein folding and stability. Finally we look at the 'holy grail' of protein engineering and mechanistic enzymology – how to create novel, functional proteins, by rational design, semi-rational approaches, and by directed evolution.

Lent Term

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?

(i) Energy Transduction in Bacteria, Mitochondria and Chloroplasts



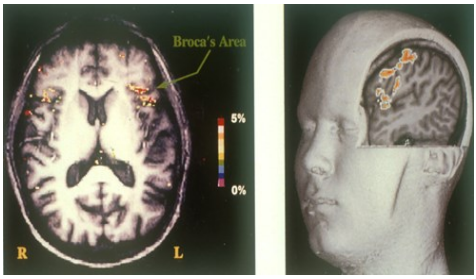
The scanning electron micrograph of a mammalian cell shows (some) cross-sections through the mitochondria that are responsible for our own energy production.

Bioenergetics is the study of how energy is acquired and used in living systems. Recent discoveries of key structures and mechanisms have greatly enhanced our understanding of this process. This knowledge is being applied to medicine, 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.

(ii) 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.



Imaging brain function through changes in cell

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:

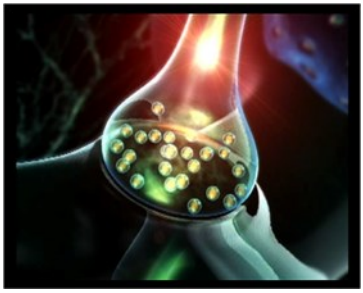
- Metabolic control analysis, which allows for quantitative determination of the importance of any enzyme for flux control *in vivo*.
- Two key non-invasive spectroscopic techniques - fluorescence and NMR - that permit the study of metabolic events in intact cells and tissues.

(iii) 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 extra-cellular 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.



Cartoon of a nerve cell which will transmit information from the incoming action potential to the adjacent cell, using neuro-transmitters stored in the synaptic vesicles. Neuronal signaling is one of the many types of signaling that will be explored in this course.

The lectures will, for example, examine the roles of the 'second messengers' that often mediate part of cell signalling cascades, and will explore how these cascades allow very low concentrations of initiating signals to generate large responses in their target cells. Special attention is paid to G-protein coupled responses, and to the multiple roles played by protein phosphorylation in relaying intracellular signals.

This lecture course will be complemented by two successive practical classes in which students gain hands-on experience of the techniques used to probe the roles of proteins in three different cell signalling pathways.

(iv) Control of Eukaryotic Cell Growth

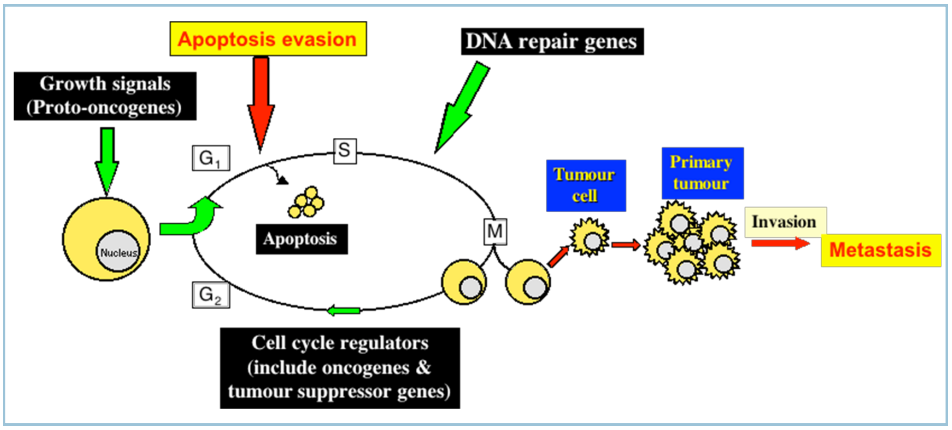
The cell cycle is the term used to describe the succession of events that occur to produce two cells from one. An understanding of the molecular events involved in progression through the cell cycle is central to solving the larger problems of how the tightly controlled expansion of cell populations 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:

a) To give an understanding of the experimental approaches that can be taken to investigate the molecular machinery of a complex biological process.
b) To explain how the molecular components that regulate cell cycle progression were identified and how their function was determined. (3) To discuss a model of how the ordering of transitions that ultimately lead to cell division is regulated.

(v) Oncogenes, Tumour Suppressor Genes and Cancer

The next four lectures build on the story of the cell cycle in eggs and yeasts by describing how normal mammalian cell proliferation is controlled. The focus is 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.



These lectures address the question of what happens in diseases, such as cancer, where control of 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.

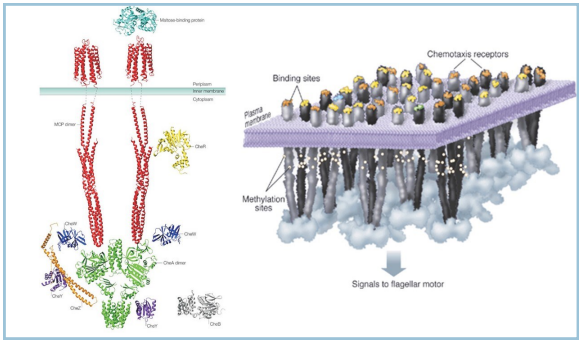
Easter Term

This final group of lectures considers bacteria and protozoa as model systems and the course now brings together the themes explored in the first two terms to examine key questions relating to prokaryotic and protist biochemistry such as motility, chemotaxis and the importance of protein targeting and other systems in virulence and pathogenicity.

(i) 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.



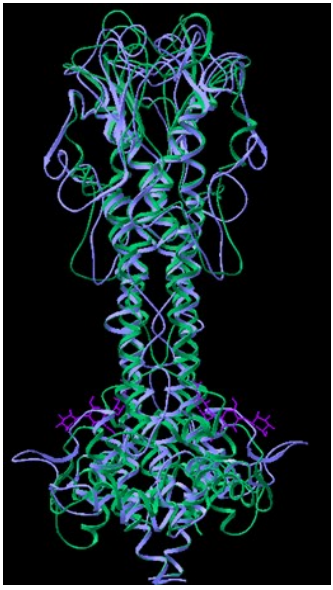
We look at how the signalling pathway is assembled, how it works, and how its output influences the rotational bias of the flagellar motor (and therefore, ultimately, the swimming behaviour of the cell). Finally, we look at what is known about the flagellar motor itself - the world's smallest multi-speed 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.

(ii) Bacterial Secretion Systems

Protein secretion mechanisms are of fundamental importance in bacteria. Prokaryotes are highly tractable model systems for analysis of the basic principles of protein targeting and the ability to manipulate such targeting can be exploited in some biotechnological processes. Furthermore, the ability to actively secrete and regulate the production of structurally diverse proteins involved in bacterial virulence is a key aspect of pathogenesis in plant and animal diseases. The lectures summarise the main protein secretion systems in Gram-negative bacteria, with examples taken from pathogens of animals and plants. Evolutionary connections between secretory machines is highlighted.

The general nature of bacterial cell surfaces is discussed and the exploitation of prokaryotic surface molecules that are parasitized as "receptors" by bacterial viruses (bacteriophages) is highlighted.

In the lab classes associated with these lectures, students conduct experiments on protein targeting using bacterial mutants generated *via* transposon insertions that can generate protein fusions. In addition, global gene regulation and intercellular chemical signalling (quorum sensing) in a bacterium that makes antibiotics are both addressed.



Trypanosome VSGs have divergent primary, but conserved tertiary, structures to function in antigenic variation and as a protective coat on the external surface of the plasma membrane.

(iii) Molecular Biology of Protozoa

Protozoa encompass over 60,000 species of eukaryote including many that are highly divergent from animals, there is more evolutionary diversity within the protozoa than between green plants, metazoa and fungi. The best-studied protozoans are parasites that cause diverse chronic diseases, such long-term infections provide a model for studying the complex molecular interactions between pathogen and host.

Protozoa have evolved a number of novel strategies for overcoming host resistance to infection and, in this context, lectures will address the unusual strategies for regulation of gene expression, especially of the Variable Surface Glycoproteins (VSGs) in trypanosomes. Such studies of parasitic protozoa have provided unique insights into our understanding of basic molecular processes, for example, the structure and biosynthesis of GPI anchors in the context of cell surface architecture.