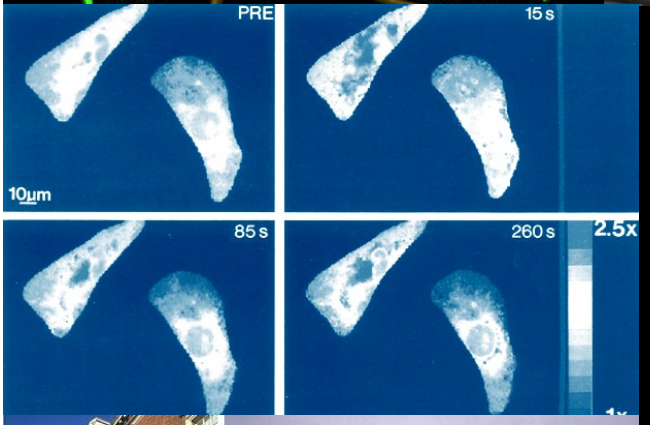
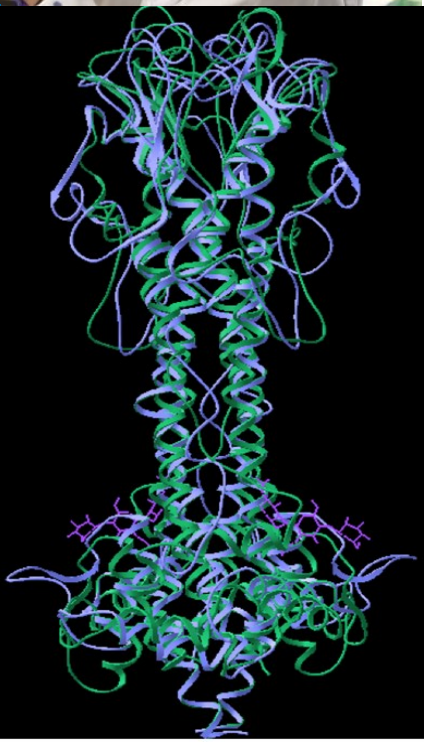
UNIVERSITY OF CAMBRIDGE

Department of Biochemistry

Natural Sciences Tripos
Part IB Biochemistry and Molecular Biology
2018-2019





UNIVERSITY OF CAMBRIDGE

CONTENTS

Overview of the Natural Sciences Tripos Part IB Course in Biochemistry and Molecular Biology

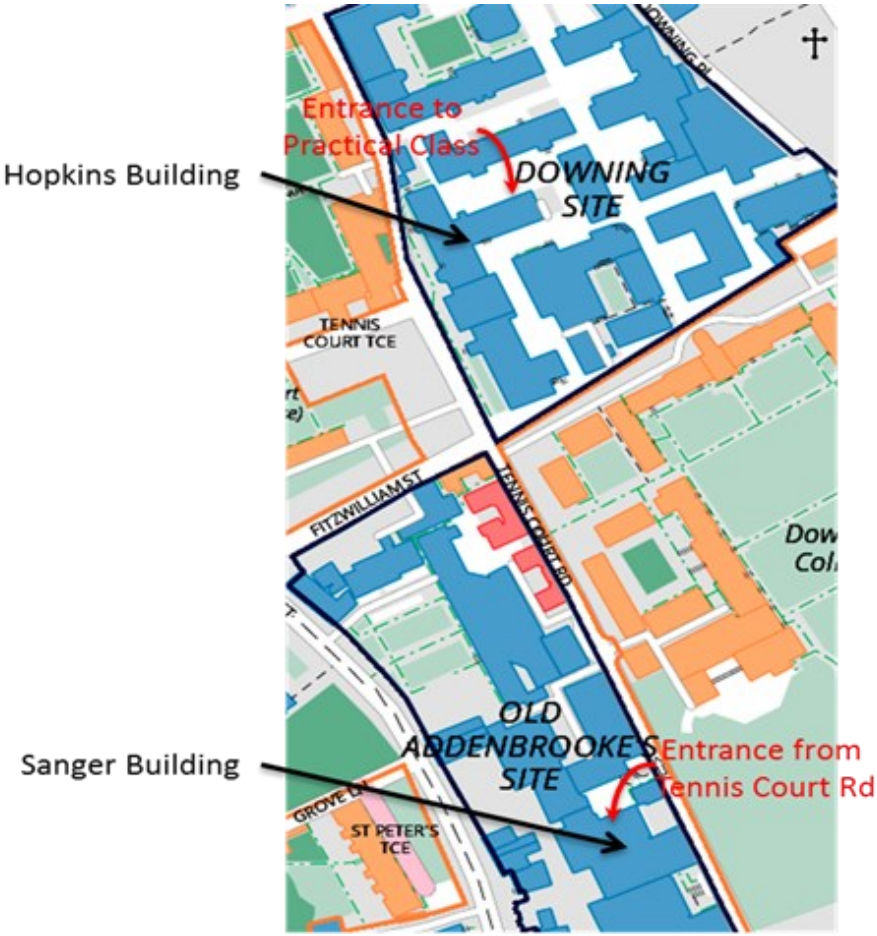
Biochemistry and molecular biology are the fundamental disciplines that underpin the study of living organisms. Both fields are developing rapidly, providing fascinating insights into the assembly and function of biological molecules, machines, cells and tissues. Equally important, the theoretical background and underlying experimental strategies provide the foundation for the current exciting developments in molecular genetics, cell biology, neurobiology, developmental biology, medical science and biotechnology.

The Part IB Biochemistry and Molecular Biology (BMB) course offers an in-depth understanding of biological molecules and processes that is essential for proper comprehension of all modern biomolecular sciences. The course introduces state-of-the-art concepts of molecular structure and function, cellular development and metabolic control, and builds naturally on the foundations that will be familiar to you from Part IA Biology of Cells. Between 100 and 150 students take the IB BMB course each year. IB BMB can be combined successfully with many other subjects in both biological and physical sciences. It complements Part IB Cell and Development Biology (CDB) particularly well, providing the molecular insights that underpin and explain the breadth of phenomena described in that course. Equally, it adds the biological dimension to courses more focused on Chemistry. In recent years, Part IB students have combined BMB with CDB, Chemistry A, Chemistry B, Pathology, Experimental Psychology, Animal Biology, Physiology, Plant Sciences, Pharmacology, Ecology, History and Philosophy of Science, Mathematics, Advanced Physics and Fluid Mechanics.

The Biochemistry Department is a large teaching and research institution with some 45 independent research groups and around 300 post- and pre-doctoral researchers, between them studying physiological, pathological, cellular and molecular processes in all types of organism: animals, plants and microbes. Laboratories in the department employ the whole spectrum of cell and molecular biological techniques, together with state-of-the-art facilities in biophysics, computational biology, advanced cell and whole body imaging, electrophysiology and genetically engineered mouse models. Our research portfolio is summarized at: http://www.bioc.cam.ac.uk/research.

The Department is especially strong in systems biology (bioinformatics; mass spectrometry in metabolomics and proteomics), structural biology (protein NMR and x-ray crystallography) and drug design (computational, fragment-based, and antibody engineering), stem cell biology (developmental biology; cell signalling), cancer biology (cell cycle, DNA damage sensing, mouse models, whole body NMR and PET imaging of tumours to monitor therapeutic mechanism and efficacy), cardiovascular research (live cell imaging of thrombosis, and electrophysiology of cardiac arrythmias and other channelopathies), microbiology (bacterial virulence, quorum sensing, bacterial viruses and antibiotics), parasitology, chemical biology and biofuel development. Arguably our greatest strengths in research and teaching lie in the cross-fertilisation between research fields that this rich diversity provides.

Practical classes are held in the basement of the Department's Hopkins Building on the Downing Site, which faces onto Tennis Court Road about 100 metres from Downing Street. Lectures take place in our Sanger Building, also on Tennis Court Road, closer to the Chemistry Department on Lensfield Road. You are very welcome to visit the buildings when choosing your Part IB subject.



See Downing Site and Old Addenbrooke's Site on University map:

http://map.cam.ac.uk/

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: 33671/66012; 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.

Since the 2016/17 academic year, the Department has been involved in a University-wide pilot for lecture capture, so the BMB lectures have been recorded and available via Moodle.

Michaelmas Term

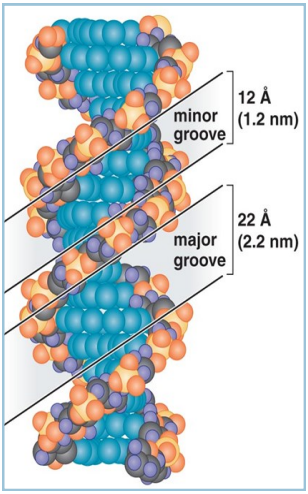
In this term the course examines the molecular biology of DNA and protein structure. How is DNA packaged in cells? How does chromatin structure affect gene expression? How is genetic engineering actually carried out? How are transcription and translation regulated? What are the principles of protein 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

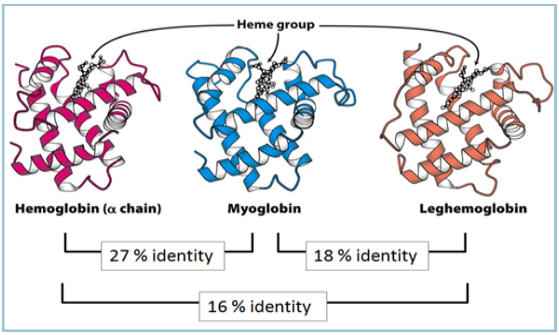
The overarching theme of DNA-protein interactions - both sequence-specific and non-specific - runs through all of these topics. At appropriate points, relevant experimental approaches and techniques will be highlighted.

(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.

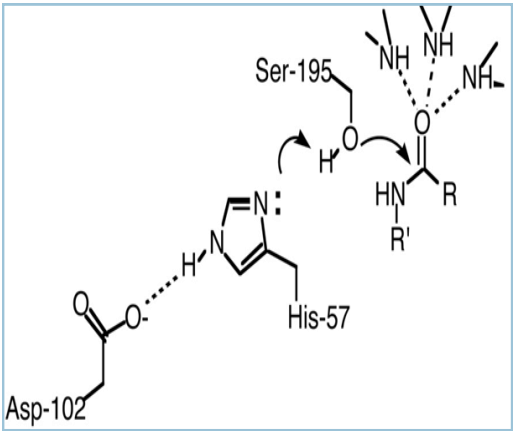
(iv) Protein Structure, Function and Evolution

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.

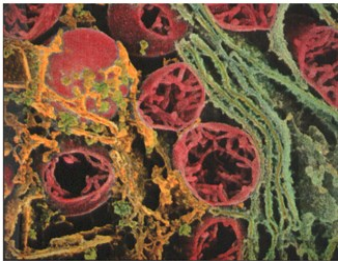
Finally we look at the 'holy grail' of protein engineering and mechanistic enzymology – how to create novel, functional proteins, by rational design, semi-rational approaches, and by directed evolution.

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

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.

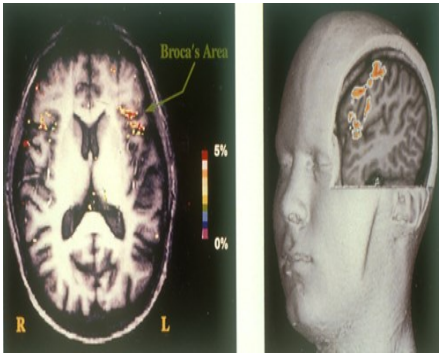


The scanning electron micrograph of a mammalian cell shows (some) cross-sections through the mitochondria that are responsible for our own energy production.

(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 metabolism.

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:

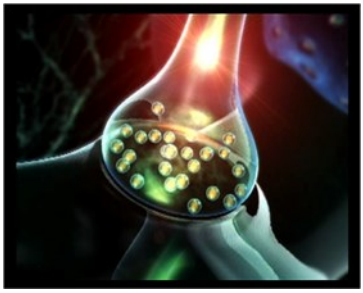
- Metabolic control analysis, which allows for quantitative determination of the importance of any enzyme for flux control *in vivo*.
- Two key non-invasive spectroscopic techniques - fluorescence and NMR - that permit the study of metabolic events in intact cells and tissues.

(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 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

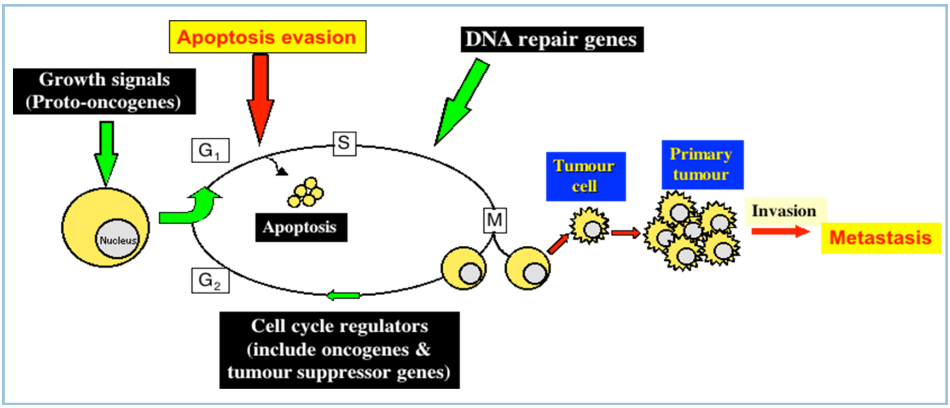
The cell cycle is the term used to describe the succession of events that occur to produce two cells from one. An understanding of the molecular events involved in progression through the cell cycle is central to solving the larger problems of how the tightly controlled expansion of cell populations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

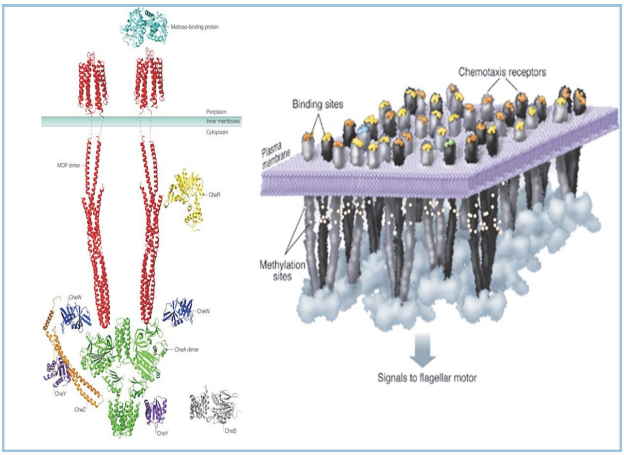
This final group of lectures considers bacteria and protozoa as model systems and the course now brings together the themes explored in the first two terms to examine key questions relating to prokaryotic and protist biochemistry such as motility, chemotaxis and the importance of protein targeting and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

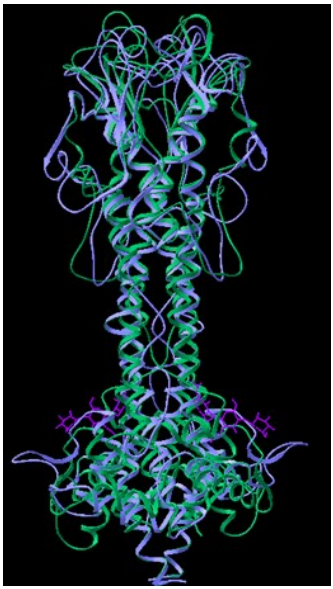


(ii) Bacterial Secretion Systems

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.

Practicals & Examinations

Practicals

The practical classrooms are in the basement of the Hopkins Building, accessed from the Downing Site car park, *via* steps at the north-east corner of the building between Genetics and Biochemistry (see map on Page 5).

In most weeks, experimental work is scheduled for one day, which involves informal classes of about 20 students working in pairs. Practical classes provide a unique opportunity for you to experience at first hand the techniques and experimental strategies of modern biochemical and molecular biological research, and give you another chance to consolidate and expand the lecture material. Our aim is to provide interesting practicals that work and are closely integrated with the lecture course. The senior demonstrator is often the course lecturer as well, so the practicals are also a good opportunity for you to discuss with your lecturers and other demonstrators (who are usually post-docs or research students working on a relevant problem) interesting aspects of the course or any questions that may have arisen in the lectures. In each of the Michaelmas and Lent Terms one practical class takes the form of a "Journal Club" in which students read, analyse and discuss a particular research paper. There is also an interactive session on Experimental Design in the Lent Term.

Topics covered include PCR, cloning, *in vitro* protein synthesis, biochemical analysis of protein-DNA interactions, protein engineering, investigation of subcellular enzyme localisation, metabolic control analysis, signal transduction, protein targeting, bacterial gene regulation, and hands-on computer analysis of DNA and protein sequences with interrogation of databases. Methods you will use include purification of macromolecules, gel electrophoresis, chromatography, use of computers in molecular biology, cell fractionation, micro-scale handling of biological materials and spectrophotometric, electrode, polarographic and enzymatic assays.

Examinations

The course is examined through two written papers dealing with the lecture material and one data handling paper based on the content of the practical course. Past exam papers can be obtained from Moodle.

Timetables

Last year's timetable as an example of the course this year:

NST PART IB BIOCHEMISTRY & MOLECULAR BIOLOGY

LECTURE TIMETABLE 2017-18

Course Organiser: Dr A D J Scadden (adjs100@cam.ac.uk)

MICHAELMAS TERM Genes and proteins; macromolecules in action
First Lecture of the Term is on **Friday**, October 6; Last Lecture on **Friday**, December 1

Date	No.	Title	Lecturer
Oct 6	5	Introduction to the course (in lecture 1)	Dr A D J Scadden
Oct 6, 9, 11, 13, 15	5	Gene cloning and manipulation	Dr A D J Scadden
Oct 18, 20, 22, 25, 27	5	Nucleic acid structure, protein-nucleic acid interactions and transcription	Prof C W J Smith
Oct 30, Nov 1, 3, 6, 8,	5	Post transcriptional control of gene expression	Dr J Mata
Nov 10, 13, 15, 17, 20	5	Protein structure, function and evolution	Dr M Hyvonen
Nov 22, 24, 27, 29, Dec 1	5	Enzyme catalysis and protein engineering	Prof F Hollfelder

LENT TERM Energy transduction, cell signalling and cell proliferation
First Lecture on **Wednesday**, January 17; Last Lecture on **Friday**, March 16

Date	No.	Title	Lecturer
Jan 17, 19, 22, 24, 26, 29	6	Energy transduction in bacteria, mitochondria and chloroplasts	Prof C J Howe
Jan 31, Feb 2, 5, 7, 9, 12	6	Control of metabolism	Prof K M Brindle
Feb 14, 16, 19, 21, 23, 26	6	Transmembrane signalling: molecules and mechanisms	Dr D Owen
Feb 28, Mar 2, 5, 7	4	Control of eukaryotic cell growth	Prof D M Carrington
Mar 9, 12, 14, 16	4	Oncogenes, tumour suppressor genes and cancer	Prof G I Evan & Dr T D Littlewood

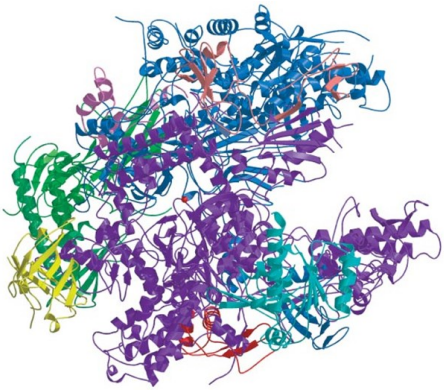
EASTER TERM Biochemistry of Microorganisms First Lecture on **Wednesday**, April 25; Last Lecture on **Monday**, May 14			
Date	**No.**	**Title**	**Lecturer**
Apr 25, 27, 30	3	Bacterial chemotaxis	Dr M Welch
May 2, 4	2	Bacterial signalling and secretion systems	Prof G P C Salmond
May 7, 9, 11, 14	4	Molecular biology of protozoa	Prof D M Carrington

PRACTICAL TIMETABLE 2017-18

MICHAELMAS TERM 2017		
Dates	**Practical**	**Senior Demonstrator**
Week 1 Oct 5 -11	Safety. (1) Cloning of Oct1 POU domain gene into *E. coli* plasmid. Restriction mapping of plasmid.	Dr A D J Scadden
Week 2 Oct 12 -18	(2) Expression of POU domain in *E. coli*. Melting properties of DNA.	Prof B Luisi
Week 3 Oct 19 - 25	(3) Purification of POU domain	Prof N Gay &
Week 4 Oct 26 - Nov 1	(4) Electrophoresis mobility shift assay	Dr N M Standart
Week 5 Nov 2 - 8	(5) Using online databases as tools **Venue:** Craik Marshall Building **1.30**	Dr A D J Scadden
Week 6 Nov 9 - 15	(6) Discussion in lab of (1, 2, 3, 4) 11.00 a.m. (7) Journal Club (2.00) **Venue:** see Moodle	Dr D Owen Staff: Dr M Welch
Week 7 Nov 16 - 22	(8) Protein structure by molecular graphics **Venue:** Craik Marshall Building **1.30**	Dr R W Broadhurst
Week 8 Nov 23– 29	(9) Reactivity and enzyme catalysis. **Venue:** Teaching rooms 1 and 2 **See Moodle**	Prof F Hollfelder

LENT TERM 2018		
Week 1 Jan 18 - 23	(10) Subcellular fractionation	Dr M de la Roche
Week 2 Jan 25 - 31	(11) Kinetic analysis of catalysis by chymotrypsin	Dr D Nietlispach
Week 3 Feb 1 - 7	(12) Mitochondrial oxidative phosphorylation	Prof G Brown
Week 4 Feb 8 - 14	(13) Experimental design 11.00-1.00 **Venue**: see Moodle (14) Metabolic Control *in silico* 2.00-4.30 **Venue**: Craik Marshall Building	Staff: Prof D M Carrington Prof J Griffin
Week 5 Feb 15 - 21	(15) Cell signalling (1): Tyrosine phosphorylation in platelets	Dr A Git/Dr D Owen
Week 6 Feb 22 - 28	(15) Cell signalling (1): Tyrosine phosphorylation continued and discussion	Dr A Git/Dr D Owen
Week 7 Mar 1 - 7	(16) Cell signalling (2): Thromboxane production by platelets	Dr M Hyvonen
Week 8 Mar 8– 14	(16) Cell signalling (2): Thromboxane production by platelets: data analysis & discussion (11.00) (17) Journal Club (2.00) **Venue:** see Moodle	Dr M Hyvonen Staff: Dr M Welch
EASTER TERM 2018		
Week 1 Apr 26 - May 2	(18) Microbial Biochemistry	Prof G P C Salmond
Week 2 May 3 - May 9	(18) Microbial Biochemistry	Prof G P C Salmond

Notes



Part IB BMB Brochure 2018-2019

Department of Biochemistry

Tennis Court Road

Cambridge CB2 1QW

Tel: 01223 (3)33600