LF208-18 Protein Biochemistry

20/21

Department
Life Sciences
Level
Undergraduate Level 2
Module leader
David Roper
Credit value
18
Module duration
11 weeks
Assessment
100% exam
Study location
University of Warwick main campus, Coventry

Description

Introductory description

The module introduces students to the complexity of protein structure. The emphasis is on the understanding of the molecular basis for how structure determines protein function. The determinants of specificity for ligand binding and catalysis are dissected. Students will become familiar with the basic methods of studying enzymes in order to understand the mechanisms whereby enzymes are able to catalyse reactions, and to how individual reactions are controlled and integrated into the metabolic pathways of the cell.

Module web page

Module aims

This module is essential core material for Biochemists, to extend introductory enzymology that was covered in BS125 Proteins, Genes and Genetics, and to lay a foundation for final year modules on Biophysical Chemistry. Content in this module will also support both second and final year laboratory work.

Living organisms are continually degrading and synthesising organic compounds. To effect the conversion of simple organic substrates into the complex polymers of the cell (and vice versa) requires the concerted use of a wide range of enzymes with many different functions. The student learning objectives of this module are to become familiar with the basic methods of studying

enzymes, to understand the mechanisms whereby enzymes are able to catalyse reactions, and to appreciate how individual reactions are controlled and integrated into the metabolic pathways of the cell.

Outline syllabus

This is an indicative module outline only to give an indication of the sort of topics that may be covered. Actual sessions held may differ.

C. Smith

Lectures 1-2 Assay of enzymes. General principles, and varied examples e.g. continuous and fixed-point assays, spectrophotometric and radioisotope methods, coupled assays. Purification of enzymes and quantitation of purification.

Dr. C. Corre

Lecture 1 Introduction to enzyme catalysis.
Features of enzyme catalysis.
Concept of enzyme active site.
Substrate binding via non-covalent interactions.
Energetics and kinetics of enzyme catalysis.
Free energy profiles for enzyme-catalysed reactions.
Michaelis-Menten kinetics for enzyme-catalysed reactions.

Lecture 2 Importance of transition state stabilisation.
Proximity effects in intramolecular and enzyme-catalysed reactions.
Types of catalysis observed in enzyme-catalysed reactions.
Acid-base catalysis (triose phosphate isomerase, ketosteroid isomerase).
Covalent catalysis (haloalkane dehalogenase, acetoacetate decarboxylase).
Strain in enzyme catalysis (carboxypeptidase A, lysozyme).
Stereospecificity – prochiral selectivity.

Lecture 3 Serine proteases.
Selectivity of proteases.
Alpha-chymotrypsin: evidence for acyl enzyme intermediate; elucidation of catalytic triad by protein crystallography; oxyanion hole; specificity pocket; role of histidine, aspartate (site-directed mutagenesis). Comparison with trypsin.
Role of serine proteases in blood coagulation cascade.

Workshop 1 Enzyme kinetics calculations; insight into enzyme mechanisms from kinetic analysis of site-directed mutant enzymes.

Lecture 4 Other protease families.
Cysteine proteases: His/Cys pair, mechanism.
Metalloproteases: carboxypeptidase A, thermolysin; role of metal ion; possible mechanisms.
Aspartyl proteases: renin, pepsin, HIV protease; mechanism; inhibitors of HIV protease.

Lecture 5 Phosphoryl transfer and NAD-dependent dehydrogenases
Use of adenosine triphosphate (ATP): chemistry, energetics; examples of phosphotransfer reactions; kinases; amide bond formation (glutamine synthetase); phosphatases (alkaline

phosphatase).
NAD/NADH as a redox agent; stereospecificity of alcohol dehydrogenase.
Mechanisms of alcohol dehydrogenase, lactate dehydrogenase.

C. Smith

Lecture 3-4 Identification of key residues; homology modelling, chemical modification, site-directed mutagenesis. Rapid reaction methods and the determation of enzyme reaction rate parameters.

Workshop 2 Calculations on enzyme purification and on sub-cellular localization.

Lecture 5 Regulation of enzyme activity; allostery, the T to R transition, ATCase case study. Regulation of enzyme activity by phosphorylation and other reversible covalent modifications. Regulation of enzyme activity by proteolysis; zymogen activation and proteolytic cascades.

Protein Structure (Dr David Roper)

1-2 The basic facts and principles: The importance of proteins as the executive agents of genes; the basics of protein structure as polymers of amino acids; representations of protein structures to show end results of protein folding and the dimensions involved. Amino acids; structure and properties of the 20 protein amino acids, including dissociation of charged groups, the hydrophobic interaction and the importance of side chain variation for the properties of proteins.

3 The peptide bond; chirality and planarity; phi and psi angles and the Ramachandran plot.

4 Secondary structure elements in proteins and the forces that drive proteins to fold. Regular structures in proteins; secondary structures; the alpha helix and beta strand and their characteristics, loops and their importance.

5 Protein super secondary structures; motifs and super secondary structures; domains built from structural motifs. Alpha, alpha/beta and beta structures, intrinsically unstructured proteins.

6-7 Principles of protein engineering. Why engineer proteins? For functional study, to improve or change biological properties. E.g. addition of cysteines for disulphide bond thermostability of lysozyme; site directed mutagenesis; effects of adding glycine and proline residues to protein chains. The addition of tryptophan residues as fluorescent probes, incorporation of Aza-tryptophan in the study of protein-protein complexes. Methods to increase protein evolution by exon shuffling and error prone PCR. Biotechnology and industrial applications; applications of protein engineering, Biosteal and de-novo design.

From Structure to Function (Dr Yuriy Pankratov)

8-9 Protein folding; energetic and kinetic considerations; folding pathways, folding intermediates, roles and action of molecular chaperones, roles of protein disulphide isomerase and peptidyl-prolyl isomerase.

10-11 Overview of protein function and architecture: The many levels of protein function from ligand binding to catalysis; molecular switches and structural proteins. How structural features dictate the ability of proteins to bind a wide variety of ligands and catalyse the wide variety of chemical transformations on which life depends.

12 The molecular mechanism that control protein function in the cell. Regulation of protein function

by pH, cellular location, redox environment and protein degradation. Structural/function relationships of phosphorylation, glycosilation lipid and other covalent protein modifications.

13-14 Illustrations of protein function from a protein structural level prospective. The structural basis of mechanisms of protein switches based on nucleotide binding and hydrolysis. Case studies include activation mechanisms of different types of protein kinases, GTPases and ATPases.

Workshop in Pymol (VF)

15 Molecular Graphics program workshop with example files held in the ICL (see www.pymolwiki.org) and module page for details.

Learning outcomes

By the end of the module, students should be able to:

- Understand and be able to discuss how protein structure determines function.
- Develop specific understanding of how enzymes, such as proteases, catalyse reactions in living cells.

Indicative reading list

From Structure to Function (Dr Allister Crow)
8 Protein folding; Evidence that sequence determines structure - Afinsen's experiments; Levinthal's paradox; protein folding pathways and folding landscapes; peptidyl-prolyl isomerase. How many folds are there? Can we predict protein structure from sequence?
9 Chaperones; How chaperones assist folding. Two general mechanisms (i) Afinsen cage and (ii) Iterative Annealing mechanisms; Hsp70 structure and mechanism (example of IAM); the Hsp100 'unfoldase' motor; GroEL and TriC (examples of the Afinsen Cage) What happens when protein folding goes wrong (example, Amyloid beta structure) 10-11 Overview of protein function and architecture: The many levels of protein function from ligand binding to catalysis; molecular switches and structural proteins. How structural features dictate the ability of proteins to bind a wide variety of ligands and catalyse the wide variety of chemical transformations on which life depends.
12 The molecular mechanism that control protein function in the cell. Regulation of protein function by pH, cellular location, redox environment and protein degradation. Structural/function relationships of phosphorylation, glycosylation lipid and other covalent protein modifications.
13-14 DNA-binding proteins; How proteins recognise and bind DNA. Functions of DNA binding proteins; DNA structure; Recognition via the DNA minor and major grooves; Common DNA binding motifs (Zn fingers, Helix-turn-helix motif); Specific and non-specific DNA binding (Restriction enzymes); RNA-guided DNA binding (CRISPR, Telomerase); The Tal Effectors and their beautiful DNA binding code; DNA packing Workshop in Pymol (DR and AC) 15 Molecular Graphics program workshop with example files held in the ICL (see www.pymolwiki.org) and module page for details.

Students are directed to the current literature for an up-to-date appreciation of developments in this area.

Subject specific skills

Understand the basic facts behind protein structure.

Understand how proteins fold, and how secondary structure is determined.

Understand the molecular mechanism for the control of protein expression.
Understand how to assay enzymes and the features of enzyme catalysis.
Understand the mechanism of action of proteases and their role in the body.
Understand the mechanism of phosphoryl transfer
Understand the functioning of dehydrogenases
Understand the role of key residues involved in protein function and how this information can inform protein engineering.

Transferable skills

Adult learning, self directed learning, team based learning and quantitative skills.

Study

Study time

Type	Required
Lectures	30 sessions of 1 hour (17%)
Practical classes	2 sessions of 1 hour (1%)
Private study	148 hours (82%)
Total	180 hours

Private study description

148 hrs private study (self directed learning and revision)

Costs

No further costs have been identified for this module.

Assessment

You must pass all assessment components to pass the module.

Assessment group B1

	Weighting	Study time
Online Examination 1.5 hour examination (June)	100%	

- Online examination: No Answerbook required

Feedback on assessment

Pastoral meetings with personal tutors

Past exam papers for LF208

Availability

There is currently no information about the courses for which this module is core or optional.