LF208-15 Enzymology

20/21

Department
Life Sciences
Level
Undergraduate Level 2
Module leader
Corinne Smith
Credit value
15
Module duration
5 weeks
Assessment
Multiple
Study location
University of Warwick main campus, Coventry

Description

Introductory description

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 aims

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.

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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.

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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.

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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.

Learning outcomes

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

- Level 5 understanding of the 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
- Level 5 understanding of research tools and techniques used to measure enzymes and enzyme kinetics
- Level 5 understanding of how enzymes catalyse reactions
- Level 5 understanding how individual reactions are controlled and integrated into the metabolic pathways of the cell

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 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	15 sessions of 1 hour (10%)
Supervised practical classes	3 sessions of 6 hours (12%)
Private study	42 hours (28%)
Assessment	75 hours (50%)
Total	150 hours

Private study description

private study (self directed learning and revision)

Costs

No further costs have been identified for this module.

Assessment

You do not need to pass all assessment components to pass the module.

Assessment group D

	Weighting	Study time
Enzyme Laboratory	30%	30 hours
3 x 6 hr laboratory class- measurement of substrates through measuring enzymic kinetics and intermediate product formation		
Online Examination	70%	45 hours
45 min short answer paper / 45 min essay paper		

	Weighting	Study time

- Online examination: No Answerbook required

Assessment group R

	Weighting	Study time
In-person Examination - Resit 45 min SAQ paper / 45 min essay paper	100%	

- Answerbook Green (8 page)
- Students may use a calculator

Feedback on assessment

Pastoral meetings with personal tutors

Past exam papers for LF208

Availability

Courses

This module is Core for:

- Year 2 of UBSA-C700 Undergraduate Biochemistry
- ULFA-C1A2 Undergraduate Biochemistry (MBio)
  - Year 2 of C1A2 Biochemistry
  - Year 2 of C700 Biochemistry
- Year 2 of ULFA-C702 Undergraduate Biochemistry (with Placement Year)
- Year 2 of ULFA-C1A6 Undergraduate Biochemistry with Industrial Placement (MBio)