AS Biology Syllabus 9700
Unit 3: Enzymes, DNA and Protein Synthesis

Recommended Prior Knowledge
Students will need to have studied Units 1 and 2 before beginning this Unit

Context
An understanding of enzyme function will be required in order to understand how DNA controls cell function. DNA and protein synthesis will be revisited if students continue to A2 level.

Outline
This Unit builds on knowledge of protein structure from Unit 2, in describing and explaining enzyme activity. DNA and protein synthesis leads on from work in Unit 2 on molecules. This Unit, with links to biotechnology, will provide many opportunities to use knowledge and understanding in new situations or to solve related problems.

C Enzymes
- Mode of action of enzymes
- Factors that affect enzyme action

F Genetic control
- Structure and replication of DNA
- Role of DNA in protein synthesis

There are many opportunities for practical work, and this provides an excellent opportunity for students to develop their practical skills, relating to Assessment Objectives in Group C (Experimental skills and investigations) including their ability to plan and evaluate investigations (assessed in Paper 5). In investigations involving factors affecting enzyme action, students can still be given the opportunity to work on their own, with results pooled to enable data presentation and analysis.

AO	Learning outcomes	Suggested Teaching activities	Learning resources
C	(a) explain that enzymes are globular proteins that catalyse metabolic reactions; (b) explain the mode of action of enzymes in terms of an active site, enzyme/substrate complex, lowering of activation energy and enzyme specificity;	Use questioning to check students' knowledge of enzymes; it is likely that some will associate them only with digestion, and it is important to correct this mistake at an early stage. Revise the meaning of the term *catalyst*. Ensure that students understand that there are many types of catalyst other than enzymes. Students will have already covered protein structure in Unit 2, so it should be a relatively small step forward to explain enzyme structure, including the active site. Emphasise the crucial role of the R groups of amino acids at this site in binding with the substrate. **Class activities** 1. Looking up key terms in the index of a variety of Biology books. 2. Brief written and diagrammatic explanation of polar/non-polar and hydrogen bonding and its importance. 3. Use paper cut out models, simulations, and whole class discussion to develop understanding of mode of action of enzymes, and the importance of complementary shape and fit 4. Give a brief written description and annotated 'boulder analogy' graph to make the point that although the energy content of substrate and products is not changed, the reaction pathway follows a lower energy course.	http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html simple animation of how enzymes work http://www.sumanasinc.com/webcontent/animations/content/enzymes/enzymes.html animation which goes into more detail, including introducing ATP, dealing with activation energy, lock and key and induced fit http://www.learnerstv.com/animation/animation.php?ani=161&cat=Biology interactive animation of activation energy ***AS and A Level Biology*** (Chapter 3) and other textbooks cover this topic thoroughly. ***Bio Factsheet 163:*** *Answering Questions: enzyme activity* Useful for the other learning outcomes ***Bio Factsheet 31:*** *Enzyme control of metabolic pathways* Extension reading for interested students

AO	Learning outcomes	Suggested Teaching activities	Learning resources
C	(c) follow the progress of an enzyme-catalysed reaction by measuring rates of formation of products (for example, using catalase) or rates of disappearance of substrate (for example, using amylase);	Practical work should illustrate the change in the rate of product formation, or substrate disappearance, as an enzyme-catalysed reaction runs its course. Many students will be familiar with the way in which other reactions, such as the production of carbon dioxide by the action of hydrochloric acid on marble chips, proceed and this will help them here. It should be emphasised that a rate measurement is given per unit time. Students will need reminding that they are using iodine in potassium iodide solution to show the loss of starch from the reaction mixture. **Class activities** 1. Use a suitable source of catalase (e.g. yeast, potato, celery, lettuce) and measure the rate of release of oxygen (product) from hydrogen peroxide. The volume of oxygen (e.g. collection over water using inverted glass measuring cylinder or using a gas syringe or manometer), can be measured at regular time intervals and the change in rate calculated. Alternatively determine loss of mass in the reaction vessel (stand it on the pan of an electronic balance). Plot results (mass loss or volume produced) and use the graph to calculate initial rate. 2. Explain the initial steep release of product, which then flattens out, in terms of the behaviour of the enzyme and substrate. 3. Use amylase to break down starch, finding the time taken to remove all the starch. Samples have to be taken at regular intervals and tested with I in KI solution. It is more difficult to produce quantitative results using this method, but it can be done using a colorimeter (trials required beforehand to ensure that the colour of resulting solutions are not too intense for accurate readings to be given). 4. Discuss as a whole class, and then make a brief written explanation, in terms of initial rate of reaction, why measuring the rate of formation of products is a more reliable measure of rate of enzyme reaction that rate of disappearance of substrate (e.g. to form maltose)	http://www.practicalbiology.org/areas/advanc /bio-molecules/factors-affecting-enzyme-activity/investigating-an-enzyme-controlled-reaction-catalase-and-hydrogen-peroxide-concentration,47,EXP.html straightforward catalase practical with downloadable student question sheet and links to other useful practical sites www.csub.edu/~kszick_miranda/Enzymes%20part2.doc amylase activity experiments which include factors affecting enzyme action The theory is explained in ***AS and A Level Biology*** (Chapter 3, pp. 43-45). Apparatus that could be adapted for this investigation is shown in ***Practical Advanced Biology***.***Comprehensive Practical Biology for A Level*** has several protocols that could be used here. ***Advanced Biology: Principles and Applications. Study Guide***, Clegg and Mackean, pub. John Murray (out of print, but 'used' copies available), also has a number of suitable protocols to follow. ***Bio Factsheet 130****: Investigating catalase*

AO	Learning outcomes	Suggested Teaching activities	Learning resources
C	(d) investigate and explain the effects of temperature, pH, enzyme concentration and substrate concentration on the rate of enzyme-catalysed reactions;	Discuss with students why, ideally, initial rates should be calculated when comparing enzyme activity under different conditions. Measuring time taken for complete removal of substrate can sometimes lead to confusion, and is completely unsuitable if trying to measure the effect of substrate concentration (it gives seemingly 'contradictory' results, because with more substrate it actually takes longer for it all to disappear, even though the rate of reaction is faster!). Students often confuse the experiment where they follow the course of an enzyme-catalysed reaction with the effect of increasing substrate concentration on the rate of a reaction. This is probably because the curves are the same shape. This is a good opportunity to improve students' skills of planning an investigation in which several variables need to be controlled. You could initially discuss with the whole group the design of one experiment (which is then carried out by the whole class). **Class activities** 1. Plan and carry out an investigation (in groups, pairs or individual) into the effect of temperature on rate of an enzyme catalysed reaction (other variables controlled) e.g. the catalase experiment in C(c). 2. Carry out an investigation into the effect of pH on rate of an enzyme catalysed reaction (other variables controlled) e.g. trypsin digesting protein in a suspension of milk powder 3. Contribute to question and answer / whole class discussion followed by written explanation and drawing of annotated graphs showing the key impact of; • rate of collisions (e.g. at low temperatures, in relation to concentration of enzyme and substrate (at low substrate • concentrations) • hydrogen bonding, tertiary structure, shape of active site and complementary fit of substrate (e.g. at high temperatures and in relation to pH)	http://www.ncbe.reading.ac.uk/NCBE/PROTOCOLS/menu.html introduction to pdf downloads. Some downloadable booklets with a wide range of enzyme-based practical activities http://www.ncbe.reading.ac.uk/NCBE/PROTOCOLS/juice.html NCBE links to downloads for several fruit juice based practicals <http://www-saps.plantsci.cam.ac.uk/worksheets/ssheets/ssheet14.html> interesting experiment using phosphatase, as well as ideas for students to design their own investigations http://www.southernbiological.com/Assets/pdf/Products/Chemicals/TrypsinInfoSheet.pdf trypsin experiment that can be adapted ***Teaching AS Biology Practical Skills*** – practical 5 (part 6) is an enzyme practical. ***Practical Advanced Biology*** has protocols, background information and questions covering several enzyme practicals, as well as numerous ideas for individual planning. ***Comprehensive Practical Biology for A Level*** also has protocols for these investigations. ***Bio Factsheet 43****: Factors affecting enzyme activity*

AO	Learning outcomes	Suggested Teaching activities	Learning resources
C	(e) explain the effects of competitive and non-competitive inhibitors on the rate of enzyme activity;	Only an outline is required here. It is best to concentrate the discussion on reversible inhibitors that act either at the active site (competitive) or elsewhere (non-competitive). Only briefly mention irreversible inhibition (particularly if the students carry out an investigation with an irreversible inhibitor). **Class activities** 1. Investigate the effect of a non-competitive inhibitor (e.g. using copper sulphate solution) on an enzyme-catalysed reaction (e.g. fruit oxidase enzymes and browning of fruit) 2. Become involved in a question and answer / whole class discussion, leading to individual written explanations of the effect of competitive inhibitors (act at active site, reversible, overcome by high substrate concentrations, occupation of active site by inhibitor reduces collisions) and non-competitive inhibitors (act away from active site, may be reversible or irreversible, reduce maximum rate irrespective of substrate concentration, change the shape of the whole enzyme molecule including the active site, so the substrate no longer fits). 3. Explain why, with increasing substrate concentration in the presence of competitive and non-competitive inhibitors, different curves are obtained.	http://www-saps.plantsci.cam.ac.uk/worksheets/scotland/inhibit.htm a practical using Lactozym (ß-galactosidase) involving competitive and non-competitive inhibition by galactose and iodine respectively http://www-saps.plantsci.cam.ac.uk/worksheets/activ/prac2.htm this protocol for an interesting investigation into a non-competitive inhibitor (lead and banana catechol oxidase) can be adapted to avoid the use of lead ethanoate solution by trialling the use of copper sulphate or copper nitrate solution as the inhibitor. Note that authorities have regulations concerning the safe disposal of chemicals that can be hazardous to the environment and it may be preferable to demonstrate this practical. http://www-saps.plantsci.cam.ac.uk/qanda_enzymes.htm check this site for questions and answers regarding different practicals on inhibition To show that an inhibitor is competitive is difficult because students need to make up separate reaction mixtures with different concentrations of the substrate. ***AS and A Level Biology*** (Chapter 3, pp. 47-48) and other textbooks cover this topic thoroughly.

AO	Learning outcomes	Suggested Teaching activities	Learning resources
F	(a) describe the structure of RNA and DNA and explain the importance of base pairing and the different hydrogen bonding between bases;	Begin this topic with a discussion about exactly what DNA does before embarking on the structure. Ask students to recall what they know of protein structure, and then explain that DNA encodes instructions for the sequence in which amino acids are linked together. Consider the requirements for this molecule: • how to carry information (sequence of nucleotides) • expression (transcription and translation) • stability (strong sugar-phosphate backbone, many H bonds) • faithful replication so that the information can be passed on to daughter cells (complementary strands) • ability to provide variation (mutations are the source of evolutionary change). These features can then be linked to the description of DNA structure. The history of the discovery and understanding of DNA makes fascinating reading. You might like to ask students to research this or show them a copy of the original, modest, short article printed in Nature where the structure of DNA (in those days, deoxyribose nucleic acid) was suggested. In teaching DNA structure, remember to discuss the anti-parallel nature (e.g. 3' to 5', 5' to 3') of the two strands of the double helix, one of the stumbling blocks for Watson and Crick. Take care that during teaching confusion is not accidentally caused, e.g. between thymine and thiamine, or between adenine and adenosine - these are frequent errors. A very common wrong answer in examinations is stating that DNA is composed of amino acids. It is a good idea **not** to tell students directly that they will find these things confusing. Once students are confident of DNA structure, move on to RNA structure, giving an outline of the three types of RNA.	http://www.dnaftb.org this deals with many aspects of DNA and genetics. Within the section *Molecules of Genetics* are sections relevant to this Unit http://www.hhmi.org/biointeractive/dna/index.html animations of DNA structure and replication http://accessexcellence.org/AB/GG/ images of RNA and DNA structure http://www.ncbe.reading.ac.uk/ncbe/PROTOCOLS/DNA/extracting.html simple protocols for extracting DNA http://learn.genetics.utah.edu/content/labs/extraction/ a virtual lab and a protocol for extracting DNA http://www.nature.com/nature/dna50/archive.html key papers in the discovery of the structure of DNA are here ***AS and A Level Biology*** (Chapter 5, pp. 64-66) and other textbooks cover this topic thoroughly.

Class activities

1. Label pre-existing diagrams of DNA to show nucleotides, phosphate, deoxyribose, sugar-phosphate backbone, adenine, thymine, cytosine, guanine, hydrogen bonds, base pairing between A and T, and between C and G.
2. Take a diagram of single strand of DNA and add to it appropriate drawings of nucleotides to create a second strand (some students find it easier to turn their page upside down – the antiparallel nature of the double helix).
3. Using cut-outs for nucleotides (using the conventional shapes for bases as in the endorsed text book, so that A naturally complements T and C complements G), build up a small section of double-stranded DNA, showing how base pairing of a 'longer' A with 'shorter' T (and same with G and C) allows the strands to remain parallel. Students can join together their sections to give the idea of a (short!) gene and the class can see that the differences between each group are the equivalent to the different information carried by each gene to code for different proteins.
4. Question and answer / whole class discussion on the relative strength of the bonds that hold the sugar-phosphate backbone together compared to those that hold together the two strands of DNA.
5. Make a summary table of the similarities and differences between DNA and RNA.
6. Make a summary table of correctly matched pairs of pieces of information (e.g. thymine = base only found in DNA, thiamine = vitamin; adenine = base found in DNA and RNA, adenosine = the A in ATP; nucleotide = monomer / building block of DNA and RNA, amino acid = monomer / building block of protein).
7. Consolidate learning of biological molecules by producing a tabular summary of type of macromolecule, components / monomer, name of bond, main function(s), other notes.

AO	Learning outcomes	Suggested Teaching activities	Learning resources
F	(b) explain how DNA replicates semi-conservatively during interphase;	If mitosis has already covered, then begin this topic by reminding students of the necessity for chromosomes to divide before mitosis occurs. Try to ensure that they make connections between mitosis, chromosomes and DNA: each chromosome contains a DNA molecule complexed with (histone) proteins. DNA replication results in two identical DNA molecules, one in each identical chromatid. Animations can be very helpful in aiding understanding of DNA replication. Students should understand the meaning of the term *semi-conservative*. If Class Activity 3 has been carried out towards the end of teaching F(a), use the laid-out models to show how the two strands, by separating, can serve as templates for the addition of complementary nucleotides to give identical molecules. This serves as an introduction to for teaching F(b). Note also that many text book diagrams of replication (possibly for simplicity) show complementary nucleotides being added to each strand in the same direction. It is worth mentioning to students that replication occurs in opposite directions for each strand and possibly discussing the reasons for this later, as extension work. If students have carried out their own research or seen animations of DNA replication. There is no need to go into details of any other possible methods of replication, nor of experiments such as those of Meselsohn and Stahl - though these could form the basis of extension activities or interesting questions to test students' understanding. **Class activity** 1. Use computer simulations and whole class discussion / question and answer to build understanding of DNA replication 2. Use photocopies / jigsaw puzzles / previously made cut-outs of nucleotides DNA diagrams and matching nucleotides to simulate DNA replication	http://www.wiley.com/college/pratt/0471393878/student/animations/dna_replication/index.html explanation and animations of DNA replication – at a higher level so useful for extension work http://highered.mcgraw-hill.com/sites/dl/free/0072437316/120076/bio23.swf animation of DNA replication http://www.accessexcellence.org/AB/GG/dna_replicating.html diagram and notes on semi-conservative replication ***AS and A Level Biology*** (Chapter 5, pp. 66-69) and other textbooks cover this topic thoroughly. ***Bio Factsheet 207:*** *How science works: Meselson and Stahl's classic experiment*

AO	Learning outcomes	Suggested Teaching activities	
F	(c) state that a gene is a sequence of nucleotides as part of a DNA molecule, which codes for a polypeptide and state that a mutation is a change in the sequence that may result in an altered polypeptide; (d) describe the way in which the nucleotide sequence codes for the amino acid sequence in a polypeptide with reference to the nucleotide sequence for HbA (normal) and HbS (sickle cell) alleles of the gene for the β-haemoglobin polypeptide;	It is a good idea to give students an overview of the way in which DNA codes for protein structure, before going into the details of how this process occurs. The important point to get over here is that the sequence of nucleotides in part of a DNA molecule codes for the sequence of amino acids in a protein. Get students to recall what they know about protein structure and function, and remind them how the function of a protein - including enzymes - depends on the sequence of amino acids within it. An error that frequently appears in answers to examination questions on this topic is confusion between nucleotides and amino acids. It is very important to reinforce the correct relationship between nucleotides and DNA / RNA, and between amino acids and protein. A learning methodology called error-free learning shows that when students guess or are given incorrect matches, it is the incorrect matches that they learn, so they must never be given incorrect matches as a learning tool (see also F(a)). **Class activities** 1. Whole class discussion / question and answer to build understanding of the triplet code 2. Use a DNA genetic dictionary to work out, from specific nucleotide base sequences, specific amino acid sequences, including normal and sickle-cell haemoglobin. 3. Make a flow diagram, linear sequential notes or annotated diagram showing that: DNA codes for the amino acid sequence in protein, which is the primary structure; primary structure determines where the protein chain coils and folds (secondary and tertiary structure); secondary and tertiary structure determines the shape; and shape (e.g. of active site, specific channel or receptor site) determines the function.	http://www.sanger.ac.uk/ the human genome project http://www.yourgenome.org/ an excellent site for students and teachers, well worth a visit http://www.kumc.edu/gec/ has links to lots of sites that have information about the human genome project, genetic code and many other related topics ***AS and A Level Biology*** (Chapter 5, pp. 70 & 71) and other textbooks cover this topic thoroughly. For a DNA genetic dictionary, see page 39 of the ***2012 Cambridge International A & AS Level Biology Syllabus, code 9700***.

AO	Learning outcomes	Suggested Teaching activities	Learning resources
F	(e) describe how the information on DNA is used during transcription and translation to construct polypeptides, including the role of messenger RNA (mRNA), transfer RNA (tRNA) and the ribosomes;	It is very important to ensure that students understand the overall sequence of events here, before they get bogged down in the details of transcription and translation. Ensure that they understand the role of mRNA in carrying a copy of the information from DNA to the ribosome, and the role of tRNA in translating this information into the sequence of amino acids that are strung together. Incidentally, trans**C**ription comes before transLation alphabetically as well as in protein synthesis. Animations can be very helpful in describing how translation and transcription take place. **Class activities** 1. Whole class discussion / oral question and answer, animations and reinforcement with written questions to build understanding of the genetic code, the role of mRNA and transcription. 2. Revisit the DNA sequences met in F(c) and (d), plus decode new DNA sequences, with only a mRNA (codon) genetic dictionary, transcribing from DNA to mRNA, and then working out from the dictionary, the sequence of amino acids. 3. As activity 1 but build understanding of translation and the role of tRNA and ribosomes. 4. Use the DNA sequence for the first 6 amino acids in drawing a comprehensive whole page annotated diagram to show transcription and translation – the outlines of cell, nucleus and ribosome (not to scale) can be provided by the teacher.	http://www-class.unl.edu/biochem/gp2/m_biology/animation/gene/gene_a1.html very basic overview to introduce the topic http://www.brookscole.com/chemistry_d/templates/student_resources/shared_resources/animations/protein_synthesis/protein_synthesis.html good information on this topic, with animations at a slightly higher level but still of interest http://www.pbs.org/wgbh/aso/tryit/dna/ the DNA workshop activity on protein synthesis places the student inside the cell. There are also links to other sites on, for example, Crick, Franklin and some relevant applied research ***AS and A Level Biology*** (Chapter 5, pp. 71-75) and other textbooks cover this topic thoroughly. For a mRNA genetic dictionary, see page 38 of the ***2012 Cambridge International A & AS Level Biology Syllabus, code 9700***. ***Bio Factsheet 22****: Protein synthesis I – nucleic acids* ***Bio Factsheet 49****: Protein synthesis II – mechanisms*