Biology: Cells and Proteins (Advanced Higher)

The **Mandatory Course key areas** are from the *Course Assessment Specification*. Activities in the **Suggested learning activities** are not mandatory. This offers examples of suggested activities, from which you could select a range of suitable activities. It is not expected that all will be covered. Centres may also devise their own learning activities. **Exemplification of key areas** is not mandatory. It provides an outline of the level of demand and detail of the key areas.

In the **Suggested learning activities**, there are references to the use of case studies. These should be seen as a suggested approach to teaching and learning and not confused with the use of case study as a method of Course assessment. These case studies should make learning active, challenging and enjoyable and identify for the learner the Course content and skills that will be developed. Case studies should be developed in such a way that learners have the opportunity to select activities, where appropriate, and present the opportunity to pursue further study. Case studies need not necessarily be restricted to one Unit but could include biology drawn from different Units.

Mandatory Course key areas	Suggested learning activities	Exemplification of key areas
1 Laboratory techniques for biologists (a) Health and safety	Standard laboratory rules and familiarity with risk assessment.	Chemicals or organisms can be intrinsically hazardous. Their use may involve risks to people, to other organisms or to the environment. The use of control measures, including personal protective equipment as a last resort, to reduce risk.
(b) Liquids and solutions The use of: linear and log dilution series; standard curve for determination of an unknown; buffers to control pH; colorimeter to quantify concentration.	Use of cylinders, pipettes, burettes, autopipettors and syringes. Practice measuring and making solutions and using buffers before embarking upon important experimentation. Use a colorimeter or spectrophotometer	pH can be measured using a meter or an indicator.

	to calibrate a known solution and determine an unknown using eg Bradford reagent.	
(c) Separation techniques Centrifugation to separate pellet and supernatant of differing density. Paper, thin layer and affinity chromatography for amino acids and proteins. Protein electrophoresis uses current flowing through a buffer to separate proteins. Size and charge are factors affecting protein migration in a gel. Proteins can be separated using pH; at their iso-electric point they have an overall neutral charge and precipitate out of solution.	Use protein electrophoresis to identify different muscle proteins. Determine the iso-electric point of a soluble protein such as casein.	
(d) Antibody techniques Detection and identification of specific proteins. Immunoassay techniques use antibodies linked to reporter enzymes to cause a colour change in the presence of a specific antigen. Fluorescent labelled antibodies in protein blotting and immunohistochemical staining of tissue. Monoclonal antibodies are produced using hybridomas formed from the fusion of a B lymphocyte with a myeloma cell using polyethylene glycol (PEG).	Use of monoclonal antibodies in the diagnosis and detection of disease. Use the ELISA technique to identify the presence of specific antigens.	

(e) Microscopy Use of bright field to examine whole organisms, parts of organisms or thin sections of dissected tissue. Fluorescence microscopy allows particular protein structures to be visualised.	Refresh skills in the use of microscopes and making slides. Discuss the ethics of dissection in an educational context.	
(f) Aseptic technique and cell culture Use of inoculum, explants or cells. Use of: haemocytometers to estimate total cell counts; vital staining to estimate viable cell counts. Complex media containing growth factors from serum for animal cell lines. Lifetime of primary cell lines and cancer cell lines in culture. Use of growth regulators in plant tissue culture.	Sterilisation of containers, equipment and materials. Disinfection of working area. Culture bacterial, yeast and algal cells using aseptic technique. Use a haemocytometer to make an estimate of cell count.	Culture media contain requirements of the cells.
2 Proteins (a) Proteomics The proteome is the entire set of proteins expressed by a genome. The proteome is larger than the number of genes due to alternative RNA splicing and post-translational modification. Not all genes are expressed as proteins in a particular cell.		
(b) Protein structure, binding and conformational change (i) Amino acid sequence determines protein structure	Use amino acid chromatography to distinguish between different amino acids.	Individual names or structures of amino acids are not required.

Proteins are polymers of amino acid monomers. Amino acids link by peptide bonds to form polypeptides. The primary structure is the sequence in which the amino acids are synthesised into the polypeptide. Hydrogen bonding along the backbone of the protein strand results in regions of secondary structure — alpha helices, parallel or anti-parallel beta sheets, or turns. Structure of amino acids including main classes of R groups based on functional group: basic (positively charged); acidic (negatively charged); polar; hydrophobic. The polypeptide folds into a tertiary structure; this conformation is caused by bonding, such as interactions of the R groups in hydrophobic regions, ionic bonds, van der Waals interactions (including hydrogen bonds) and disulfide bridges. Prosthetic group is a non-protein unit tightly bound to a protein necessary for its function. Quaternary structure exists in proteins with several connected polypeptide subunits. Interactions of the R groups can be influenced by temperature and pH.	Use protein electrophoresis to identify different muscle proteins. Determine the iso-electric point of a protein and explain the result using understanding of protein structure. Molecular modelling, eg computer aided drug design. Primary structure comparisons of enzymes from different evolutionary backgrounds — alcohol dehydrogenase from different organisms. Post-translational modification and activity in trypsinogen and trypsin.	
(ii) Hydrophobic and hydrophilic interactions influence the location of cellular proteins.	Look at history of evidence-based models	

The R groups at the surface of a protein determine its location within a cell. Hydrophilic R groups will predominate at the surface of a soluble protein found in the cytoplasm. In these proteins, hydrophobic R groups may cluster at the centre to form a globular structure. The fluid mosaic model of membrane structure. Regions of hydrophobic R groups allow strong hydrophobic interactions that hold integral proteins within the phospholipid bilayer. Some integral proteins are transmembrane, for example channels, transporters and many receptors. Peripheral proteins have fewer hydrophobic R groups interacting with the phospholipids.	of membrane structure as an example of refinement of scientific ideas.	
(iii) Binding to ligands A ligand is a substance that can bind to a protein. R groups not involved in protein folding can allow binding to these other molecules. Binding sites will have complementary shape and chemistry to the ligand. DNA binds to a number of proteins. Positively charged histone proteins bind to the negatively charged sugar–phosphate backbone of DNA in eukaryotes; the DNA is wrapped around histones to form		

nucleosomes packing the DNA in chromosomes. Other proteins have binding sites that are specific to particular sequences of double stranded DNA and when bound to can either stimulate or inhibit initiation of transcription.		
(iv) Ligand binding changes the conformation of a protein As a ligand binds to a protein binding site or a substrate binds to an enzyme's active site, the conformation of the protein changes. This change in conformation causes a functional change in the protein. Induced fit in enzymes occurs when the correct substrate starts to bind resulting in a temporary change in shape of the active site increasing the binding and interaction with the substrate. Activation energy; binding of modulators at secondary binding sites in allosteric enzymes; positive and negative modulators. The conformation of the enzyme changes and this alters the affinity of the active site for the substrate. Some proteins with quaternary structure show cooperativity in which changes in binding at one subunit alter the affinity of the remaining subunits. Cooperativity in the binding and release of oxygen in	Enzyme kinetic studies measure turnover rate and affinity. Importance of measuring the initial rate of reaction in enzyme kinetics studies. The impact of inhibitors on enzyme kinetics. Analyse haemoglobin dissociation curves.	In enzymes, specificity between the active site and substrate is related to induced fit. The chemical environment produced lowers the activation energy required for the reaction. Once catalysis takes place, the original enzyme conformation is resumed and products are released from the active site. Positive modulators increase the enzyme affinity whereas negative modulators reduce the enzyme's affinity for the substrate.

haemoglobin and the influence of temperature and pH.		
(v) Reversible binding of phosphate and control of conformation The addition or removal of phosphate from particular R groups can be used to cause reversible conformational changes in proteins. This is a common form of post-translational modification. In this way the activity of many cellular proteins such as enzymes and receptors are regulated. Kinase is often responsible for phosphorylation of other proteins and phosphatase catalyses dephosphorylation. Some proteins (ATPases) use ATP for their phosphorylation. Myosin has heads that act as cross bridges as they bind to actin. When ATP binds to myosin, the myosin head detaches from actin, swings forwards and rebinds. The rebinding releases the ADP and a phosphate ion drags the myosin along the actin filament.	Muscle contraction experiment using ATP. An opportunity to focus on experimental design associated with pilot studies, measurement accuracy, sample size and replication.	
3 Membrane proteins (a) Movement of molecules across membranes The phospholipid bilayer as a barrier to ions and most uncharged polar molecules. Some small molecules such as oxygen and carbon dioxide pass through. Specific transmembrane proteins, which		

act as channels or transporters, control ion concentrations and concentration gradients. To perform specialised functions, different cell types and different cell compartments have different channel and transporter proteins. Passage of molecules through channel proteins is passive, eg aquaporin. Some channel proteins are gated and change conformation to allow or prevent diffusion, eg sodium channels, potassium channels. 'Gated' channels can be controlled by signal molecules (ligand-gated channels) or changes in ion concentrations (voltage-gated channels). Transporter proteins change conformation to transport molecules across a membrane. Transport can be facilitated, eg glucose symport or active eg Na/KATPase. Conformational change in active transport requires energy from hydrolysis of ATP.	CFTR, mutation and cystic fibrosis.	
(b) Signal transduction Some cell surface receptor proteins convert an extracellular chemical signal to a specific intracellular response through a signal transduction pathway. This may result in the activation of an enzyme or G protein, a change in uptake or secretion of		

molecules, rearrangement of the cytoskeleton or activation of proteins that regulate gene transcription.		
(c) Ion transport pumps and generation of ion gradients The sodium potassium pump transports ions against a steep concentration gradient using energy directly from ATP. The transporter protein has high affinity for sodium ions inside the cell; binding occurs; phosphorylation by ATP; conformation changes; affinity for ions changes; sodium ions released outside of the cell, potassium ions bind outside the cell; dephosphorylation; conformation changes; potassium ions taken into cell; affinity returns to start. Functions of Na/KATPase include the following examples: maintaining the osmotic balance in animal cells; generation of the ion gradient for glucose symport in small intestine; generation and long-term maintenance of ion gradient for resting potential in neurons; generation of ion gradient in kidney tubules.		The maintenance of ion gradients by Na/KATPase accounts for a significant part of basal metabolic rate (up to 25% in humans).
(d) Ion channels and nerve transmission Nerve transmission is a wave of depolarisation of the resting potential of a	Daphnia heart rate investigation. The action of chemical agonists can be	

neuron. This can be stimulated when an appropriate signal molecule, such as a neurotransmitter, triggers the opening of ligand-gated ion channels at a synapse. If sufficient ion movement occurs, then voltage-gated ion channels will open and the effect travels along the length of the nerve. Once the wave of depolarisation has passed, these channel proteins close and others open to allow the movement of ions in the opposite direction to restore the resting potential.	assessed. The study can be an opportunity to focus on aspects of experimental design associated with pilot studies, measurement accuracy, sample size and replication. Human reaction time: consider the effects of age or caffeine. Block the design to avoid gender becoming a confounding variable.	
4 Detecting and amplifying an environmental stimulus In archaea, bacteriorhodopsin molecules generate potential differences by absorbing light to pump protons across the membrane. In plants, the light absorbed by photosynthetic pigments drives an electron flow that pumps hydrogen ions across the thylakoid membrane of the chloroplast. In both cases the resulting diffusion of hydrogen ions back across the membrane drives ATP synthase. In animals the light-sensitive molecule retinal is combined with a membrane protein opsin to form the photoreceptors of the eye. A cascade of proteins amplifies the signal.	Investigate vision experimentally. Fish eye dissection.	Photoreceptor system proteins are found across the three domains. Eyes, in which the light sensitive cells are grouped into organised structures for vision, appear to be give an evolutionary advantage; eyes are found in only six animal phyla yet are present in 95% of all animal species.

In rod cells the retinal-opsin complex is called rhodopsin. When stimulated by one photon, a rhodopsin molecule activates hundreds of G-protein molecules, which activate hundreds of molecules of an enzyme. If the enzyme triggers sufficient product formation, a nerve impulse may be generated. A very high degree of amplification results in sensitivities at low light intensities. In cone cells, different forms of opsin combine with retinal to give photoreceptor proteins, each with maximal sensitivity to specific wavelengths (red, green, blue or UV).		
5 Communication within multicellular organisms (a) Coordination Receptor molecules of target cells are proteins with a binding site for a specific signal molecule. Binding changes the conformation of the receptor and this can alter the response of the cell. Different cell types produce specific signals which can only be detected and responded to by cells with the specific receptor. In a multicellular organism different cell types may show a tissue specific response to the same signal.		Multicellular organisms achieve coordination through extracellular signalling molecules, receptors and responses.

(b) Hydrophobic signals and control of transcription Hydrophobic signalling molecules can diffuse through membranes so their receptor molecules can be within the nucleus. Thyroid hormone receptor protein binds to DNA in the absence of thyroxine and inhibits transcription of the gene for Na/KATPase. When thyroxine binds to the receptor protein, conformational change prevents the protein binding to the DNA and transcription of the gene for Na/KATPase can begin raising metabolic rate. The receptor proteins for steroid hormones are transcription factors. Only once the hormone signal has bound to the receptor can the transcription factor bind to gene regulatory sequences of DNA for transcription to occur.	Case study of thyroid disorders. Case study of sex hormone disorders.	Hydrophobic signalling molecules include the thyroid hormone thyroxine and steroid hormones. Hydrophobic signals can directly influence transcription of genes.
(c) Hydrophilic signals and transduction Hydrophilic signalling molecules include peptide hormones and neurotransmitters. Hydrophilic signals require receptor molecules to be at the surface of the cell. Transmembrane receptors change conformation when the ligand binds on the cell surface; the signal molecule does not enter the cell but the signal is transduced across the membrane of the cell.		

Transduced hydrophilic signals often involve cascades of G-proteins or phosphorylation by kinase enzymes. Binding of the peptide hormone insulin to its receptor triggers recruitment of GLUT4 glucose transporter to the cell membrane of fat and muscle cells. Diabetes can be caused by failure to produce insulin (type 1) or loss of receptor function (type 2). Type 2 generally associated with obesity. Exercise also triggers recruitment of GLUT4, so can improve uptake of glucose to fat and muscle cells in subjects with type 2. Binding of peptide hormone ADH to its receptor in collecting duct of kidney triggers recruitment of channel protein aquaporin 2 (AQP2). Aquaporins provide a highly efficient route for water to move across membranes. Recruitment of AQP2 allows control of water balance in terrestrial vertebrates. Failure to produce ADH or insensitivity of its receptor results in diabetes insipidus.	Examine data from glucose tolerance tests. Write a review of data from studies of health and wellbeing, considering the importance of publication of negative results. Find out about health effects associated with type 2 diabetes and the success rate of treatment programmes. Comparative anatomy and physiology of kidneys across different groups of animals.	
6 Protein control of cell division (a) Cell division requires the remodelling of the cell's cytoskeleton The cytoskeleton gives mechanical support and shape to cells. The cytoskeleton consists of different types of proteins	Consider the effects of colchicine and paclitaxel on the cytoskeleton.	

extending throughout the cytoplasm. Microtubules composed of hollow straight rods made of globular proteins called tubulins govern the location and movement of membrane-bound organelles and other cell components. Microtubules are found in all eukaryotic cells and radiate from the centrosome (the microtubule organising centre). Microtubules form the spindle fibres, which are active during cell division.		
(b) The cell cycle An uncontrolled reduction in the rate of the cell cycle may result in degenerative disease. An uncontrolled increase in the rate of the cell cycle may result in tumour formation. The cell cycle consists of interphase and mitosis. Interphase consists of an initial growth phase G1 followed by an S phase where the cell continues to grow and copies its chromosomes and a further G2 growth phase, in preparation for M phase (mitosis and cytokinesis). Mitosis is a dynamic continuum of sequential changes described as prophase, metaphase, anaphase and telophase. Role of spindle fibres in the movement of chromosomes on metaphase plate, separation of sister chromatids and	Stain actively dividing plant meristem tissue and calculate a mitotic index. Examine the role of cell cycle regulators in degenerative diseases such as Alzheimer's and Parkinson's.	The cell cycle regulates the growth and replacement of genetically identical cells throughout the life of the organism.

formation of daughter nuclei. Cytokinesis is the separation of the cytoplasm into daughter cells.		
(c) Control of the cell cycle Progression through the cell cycle is regulated by checkpoints at G1, G2 and metaphase. Checkpoints are critical control points where stop and go ahead signals regulate the cycle. If a go ahead signal is not reached at the G1 checkpoint the cell may switch to a non-dividing state called the G0 phase. As the cell size increases during G1, cyclin proteins accumulate and combine with regulatory proteins called cyclin-dependent kinases (Cdks) and activate them. Active Cdks cause the phosphorylation of proteins that stimulate the cell cycle. If a sufficient threshold of phosphorylation is reached the cell cycle moves on to the next stage. If an insufficient threshold is reached, the cell is held at a checkpoint. The G1 Cdk phosphorylates a transcription factor inhibitor, retinoblastoma (Rb) protein, allowing DNA replication in the S phase. DNA damage triggers the activation of several proteins including p53 that can stimulate DNA repair, arrest the cell cycle or cause cell death.	Use an online simulation of mitotic checkpoint control. Investigate cell cycle mutation in yeast *Shizosaccharomyces pombe*. Research the types of mutations associated with cancer. For example the influence of environmental factors and viruses, the conversion of proto-oncogenes into oncogenes and mutations in tumour suppressing genes.	For many cells the G1 checkpoint is the most important.

(d) Control of apoptosis Programmed cell death (apoptosis) is triggered by cell death signals that activate inactive forms of DNAase and proteinases (caspases) that destroy the cell. Cell death signals may originate outwith the cell (for example from lymphocytes) and bind to a surface receptor protein to activate a protein cascade that produces active caspases. Death signals may also originate within the cell, for example as a result of DNA damage the presence of p53 protein can activate a caspase cascade. In the absence of cell growth factors cells may also initiate apoptosis.	Consider apoptosis in development of tetrapod limbs. Research the challenges in overcoming apoptosis in maintaining animal cell culture lines.	The destruction of cells must be carefully controlled in a multicellular organism.