CONFIDENTIAL

1-62	60	
63	3	
64	3	
65	9	
66	4	
67	2	
68	6	
69	3	
70	3	
71	9	
72	8	
Total	110	

SEAT NUMBER: .....

SURNAME: .....
(Block letters)

OTHER NAMES: .....
(Block Letters)

SID: .....

FACULTY OF PHARMACY
BACHELOR OF PHARMACY DEGREE
Second Year
DRUG DISCOVERY AND DESIGN A (PHAR 2811)

June 2009 **Time Allowed: 2.5 Hours**

ANSWER **ALL** QUESTIONS

- **Questions 1 - 62** must be answered on the computer sheet provided.
- Use a soft pencil to mark the box corresponding to the most correct answer. Mark alphanumeric characters corresponding to your name and SID, and also write them on the computer sheet.
- Marks will **NOT** be deducted for incorrect answers in the multiple-choice questions.

- **Questions 63 to 72** are to be answered in the spaces provided on pages 23 – 31 of this examination paper**.**
- Non-programmable calculators may be used.

- **All copies of this examination paper are to be returned to the examiner with all pages intact.**
- **None of the examination paper may be removed from the examination room by candidates or supervisors, nor may any portion be copied.**

This examination paper consists of 32 pages, numbered 1-32 inclusive. There are 72 questions, numbered 1- 72 inclusive. Students are asked to check that their booklet is complete, and to indicate that they have done so by signing below.

I have checked this booklet and affirm that it is complete.

SIGNATURE: _____

Students finding an incomplete booklet should obtain a replacement from the examination supervisor immediately.

1. The following amino acid side chains are aromatic:

   A. Phenylalanine (Phe) and Tyrosine (Tyr).
   B. Phenylalanine (Phe), Tryptophan (Trp) and Glycine (Gly).
   C. Tryptophan (Trp), Valine (Val).
   D. Aspartic Acid (Asp), Glutamic Acid (Glu).
   E. Glycine (Gly), Valine (Val) and Isoleucine (Ile).

2. L-alanine and D-alanine

   A. are present in virtually all proteins.
   B. are superimposable isomers of each other.
   C. are enantiomers.
   D. lack an R group.
   E. are aromatic.

3. The zwitter ion terminology refers to the amino acid property of

   A. hydrophobicity.
   B. two charges at the one time.
   C. ionisation of a sidechain.
   D. chirality for all except glycine and proline.
   E. containing two sidechains.

4. A protein retained on an affinity chromatography column is usually eluted off the column by

   A. gradually increasing the salt concentration of the elution buffer.
   B. adding the protein's free ligand.
   C. changing the pH of the elution buffer.
   D. allowing the retained protein to naturally come off the column after the non-specifically bound proteins have first passed through the resin.
   E. achieving good quality packing of the matrix material (stationary phase).

5. Which of the following experimental techniques is useful for determining **high-resolution** 3D-protein structures?

   A. Circular Dichroism (CD) Spectroscopy.
   B. Nuclear Magnetic Resonance (NMR) Spectroscopy.
   C. Powder X-ray crystallography.
   D. Single crystal X-ray crystallography.
   E. Amino Acid Sequence analysis.

6. To determine the **primary** amino-acid sequence in a protein, a useful technique is:

   A. Mass Spectrometry.
   B. Edman degradation.
   C. Molecular Modelling.
   D. Nuclear Magnetic Resonance (NMR) Spectroscopy.
   E. Circular Dichroism (CD) Spectroscopy.

7. An example of a Type 1 receptor is:

   A. Adrenoreceptor.
   B. $GABA_a$ Receptor.
   C. Insulin Receptor.
   D. Steroid Receptor.
   E. Nuclear Receptor.

8. When a drug binds tightly to a target, which of the following is **INCORRECT**?

   A. There is a high degree of structural complimentarity between drug and target.
   B. It is entropically unfavourable for the drug to bind, due to liberation of water molecules from the active site.
   C. The electrostatics of drug and target should be mirror images to increase charge-charge interactions.
   D. The drug isomeric form is important for steric interaction reasons.
   E. The protein must remain flexible to allow the drug access to the active site.

9. A Ramachandran Plot is calculated for a protein in which the only secondary structure represented is α-helices. The Plot can be used to:

   A. indicate sidechain conformations.
   B. determine the backbone conformation of β-strands.
   C. find the range of phi and psi angles for the α-helices.
   D. predict the protein's behaviour in ion exchange chromatography.
   E. determine the prevalence of *cis* and *trans* peptide conformations in the protein.

10. As a check on the scanning procedure record an answer of A to question **10**.

11. Which of the following is **NOT** one of the different interactions involved in substrate binding to an enzyme?

   A. Enzymes chiefly bind substrates by noncovalent interactions.
   B. Enzymes use ionic and hydrogen bonds to bind ligands specifically.
   C. All amino acids in an active site are involved in the chemical reaction that is catalysed.
   D. Enzyme active sites ensure that substrates come together in the correct orientation for chemical reaction to occur.
   E. Changes in the active site may change the type of substrate that is bound but not the chemistry that is catalysed.

12. Which statement about enzyme catalysed reactions is **NOT** true?

   A. Enzymes form complexes with their substrates.
   B. Enzymes lower the activation energy for chemical reactions.
   C. Enzymes change the Ks for chemical reactions.
   D. Many enzymes change shapes slightly when substrate binds.
   E. Reactions occur at the "active site" of enzymes, where a precise 3D orientation of amino acids is an important feature of catalysis.

13. In describing the reaction rate for a chemical reaction, which of the following statements about reaction rate is **NOT** true?

A. Reaction rate is the speed at which the reaction proceeds toward equilibrium.

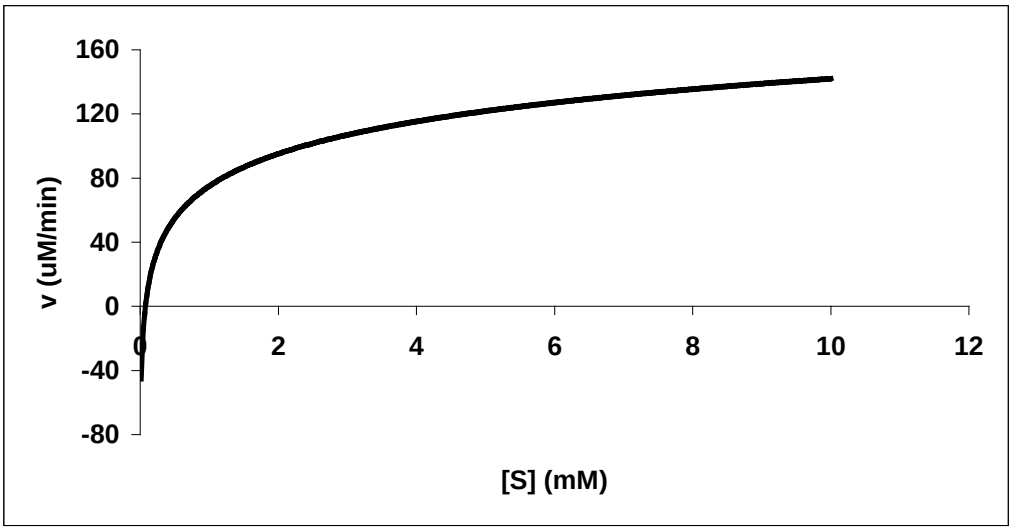
B. Reaction rate is governed by the energy barrier between reactions and products.

C. Enzymes can accelerate the rate of a reaction.

D. Reaction rates are not sensitive to pH.

E. None of these.

The following questions refer to the graph below:



14. Vmax ***estimated*** from the graph above is:

A. 60 uM/min.

B. 75 uM/min.

C. 80 uM/min.

D. 120 uM/min.

E. 150 uM/min.

15. Km ***estimated*** from the graph above is:

A. 1 mM.

B. 2 mM.

C. 4 mM.

D. 5 mM.

E. 10 mM.

16. Which statement is most **CORRECT**?

A. 1 kg of human tissue contains about 1 g ATP.
B. In a healthy cell, the [ATP] is always much less than the [ADP].
C. The total adenine nucleotide pool ([ATP] + [ADP] + [AMP]) in cells is about 50 mM.
D. ATP can be produced in the mitochondria of liver cells and transported in the blood for use by the muscle.
E. At room temperature, a 5 mM solution of ATP will completely hydrolyse into ADP and phosphate within 1 minute.

17. Which statement about fatty acid oxidation is **CORRECT**?

A. Carnitine is a protein embedded in the cell membrane that allows fatty acids to enter from the bloodstream.
B. Fatty acids are covalently attached to Coenzyme A during the FAD/NAD catalysed oxidation reactions.
C. The oxidation reactions involving FAD/NAD occur only in the cytoplasm.
D. Fatty acids attached to Coenzyme A can move freely across the inner mitochondrial membrane.
E. Carnitine is consumed (two carbons at a time) during fatty acid oxidation.

18. Which description of the operation of the Krebs Cycle is **MOST CORRECT**?

A. The cycle goes faster when the electron transport chain is going slowly.
B. The pathway is located in both the cytoplasm and the mitochondria.
C. The cycle reacts fuel molecules with oxygen to produce carbon dioxide.
D. The cycle generates CoA and NADH.
E. Most of the ATP in the cell is made directly by enzymes of the Krebs Cycle by substrate level phosphorylation.

19. Which of the following statements is **INCORRECT**?

A. Electrons can move down the electron transport chain even if proton pumping from the matrix can not occur.
B. Protons are only pumped from the matrix if electrons are passed down the electron transport chain.
C. ATP synthesis at the F_1ATPase requires both ADP and phosphate.
D. Protons will only come in through the F_0F_1ATPase if ATP is simultaneously being made from ADP.
E. Protons can pass freely across the outer mitochondrial membrane.

20. Which statement is **CORRECT**?

A. Two days of continual exposure to a blood glucose concentration of 10 mM will cause a coma.
B. The reaction between proteins and glucose is not an enzyme catalysed process.
C. Glycosylation does not affect the function of proteins.
D. When blood glucose concentration is 5 mM, the rate of glycosylation is negligible.
E. When blood glucose concentration falls below 4 mM insulin is secreted by the beta-cells of the pancreas to compensate.

21. Which statement is **INCORRECT**?

A. It is not practical to measure the glycemic index of meat.
B. The reference food used in glycemic index determinations is normally glucose.
C. The glycemic index is a relative measure of the post-prandial glucose response caused by a particular food.
D. Amylose containing foods have a lower glycemic index than foods which mainly contain amylopectin.
E. Sucrose has a lower glycemic index than amylopectin starch.

22. In white adipose tissue, which process is **NOT** stimulated by insulin?

    A. The rate of glucose uptake.
    B. Pyruvate dehydrogenase activity.
    C. Acetyl-CoA carboxylase activity.
    D. Expression of fatty acyl synthase.
    E. Lipolysis.

23. Which statement best describes the relationship between the pentose phosphate pathway (PPP) and lipogenesis?

    A. The PPP produces the glycerol needed for esterification of newly formed fatty acids.
    B. Lipogenesis provides glycerol 3-phosphate for the PPP.
    C. Lipogenesis uses NADPH produced by the PPP.
    D. The PPP provides ATP to fuel lipogenesis.
    E. The PPP is necessary to provide the carbon dioxide needed to produce malonyl-CoA.

24. Which statement **BEST DESCRIBES** chylomicrons?

    A. Lipoproteins that carry dietary fat to the peripheral tissues.
    B. Discs of phospholipids that mop up loose cholesterol in the blood stream.
    C. Milky droplets formed from the churning of a lipid/salt mixture in the small intestine.
    D. Microscopic droplets excreted by tissues that have too much cholesterol.
    E. An emulsion of fat and protein in the stomach.

25. Which statement regarding the disposal of dietary fat is **CORRECT**?

    A. High Density Lipoprotein (HDL) is formed by the removal of fat from LDL.
    B. Very Low Density Lipoproteins (VLDL) transport dietary fat from the intestine to the liver.
    C. Unsaturated fat goes to the liver, but saturated fat goes to the peripheral tissues.
    D. A high HDL:LDL ratio is positively correlated with heart disease.
    E. Peripheral tissues encounter dietary fat before the liver.

26. What would be the consequences of inhibition of lipolysis during the first few days of starvation?

A. Blood ketone body concentration would rise.
B. Blood glucose concentration would rise.
C. Blood fatty acid concentration would rise.
D. There would be fewer substrates for gluconeogenesis in the liver.
E. Fatty acid oxidation in the muscles would increase.

27. Which statement about ketone bodies is **INCORRECT**?

A. Ketone bodies circulate in the blood stream bound to special carrier proteins.
B. Ketone bodies can be used the peripheral tissues as well as the brain.
C. Ketone bodies can spontaneously decarboxylate to give acetone.
D. Ketone bodies are only formed in the liver.
E. Ketone body oxidation inhibits glucose oxidation.

28. Which of the following hormones/neurotransmitters does **NOT** involve the decarboxylation of a naturally occurring amino acid in its synthesis?

A. Histamine
B. Serotonin
C. GABA
D. Adrenalin
E. Thyroxin

29. Considering the thymidylate synthase reaction, which of the following statements is **INCORRECT**?

The reaction…

A. only occurs in preparation for DNA synthesis
B. is part of the de novo purine synthesis pathway
C. is indirectly inhibited by methotrexate
D. requires folate to supply a methyl group
E. cannot be completed if a F atom replaces the H on C5 of the uracil base

30. Which of the following statements is **INCORRECT**?

A. Introns make up ~90% of most genes
B. Pseudogenes are thought to result from the action of reverse transcriptases
C. Over 90% of the human genome is highly repetitive DNA
D. PolyU-polyA is the fastest sequence to reanneal
E. Repetitive DNA is often found at the centromeres and the teleomers

31. When performing $C_o t$ plot analysis, if a sequence of DNA has a complexity of 100 this means:

A. The 2 DNA strands are 100 bp long
B. The DNA has a unique sequence of 100 bp
C. The 2 complementary strands of the DNA take 100 sec to reanneal
D. The DNA single strand has a $C_0 t$ of 100
E. There are 100 copies of a trinucleotide repeating sequence in the DNA

32. Which of the following statements about trinucleotide repeats (TNRs) is **CORRECT**?

A. TNRs do not have to be transcribed to affect an individual.
B. TNRs producing polyglutamine tracts significantly change the pI of the affected protein.
C. The common CNG sequence destabilises intrastrand loop formation.
D. The TNR tract must be translated above a threshold level to affect an individual.
E. Fragile X syndrome results from a misfolded neuroprotein.

33. RNase P and 28S rRNA both:

A. Act as RNA polymerases.
B. Catalyse the formation of peptide bonds.
C. Contain RNA which has catalytic activity.
D. Have ribonuclease activity.
E. Have reverse transcriptase activity.

34. As a check on the scanning procedure record an answer of A to question **34**.

35. High levels of telomerase activity are typically found in:

A. Normal somatic cells during rapid proliferation.
B. Bacterial cells during transcription.
C. Terminally differentiated somatic cells.
D. Immortal cell lines.
E. Cells infected with a virus.

36. Exposure of DNA to UV light is most likely to result in:

A. the cleavage of backbone phosphodiester bonds
B. the formation of covalent bonds between two adjacent pyrimidines
C. de-purination by cleavage of N-glycosidic bonds
D. the conversion of adenine to its alternative tautomer
E. the conversion of cytosine to uracil

37. A researcher engineers a colony of *E. coli* by removing the trp promoter (including the operator) from the trp operon and replacing it with the lac promoter also including the operator. This would have the effect of:

A. Producing lots of lactose when the cells are fed tryptophan
B. Producing lots of β-galactosidase with low tryptophan
C. Preventing β-galactosidase synthesis only when tryptophan was high
D. Producing lots of tryptophan when [lactose] was high.
E. Degrading lots of tryptophan when the [lactose] is high

Questions 38 to 40 refer to the table below

You have isolated a number of mutations in *E. coli* in the lac operon. The normal (wild type) β-galactosidase protein has a molecular weight of 115 kD. The following information has been provided for each colony. The term "altered" refers to a change from the wild type sequence. The molecular weight of the β-galactosidase produced by each colony was measured using SDS-PAGE. The DNA sequence of lacZ and the amino acid sequence of the gene product, β-galactosidase, were determined and compared to the wild type sequence.

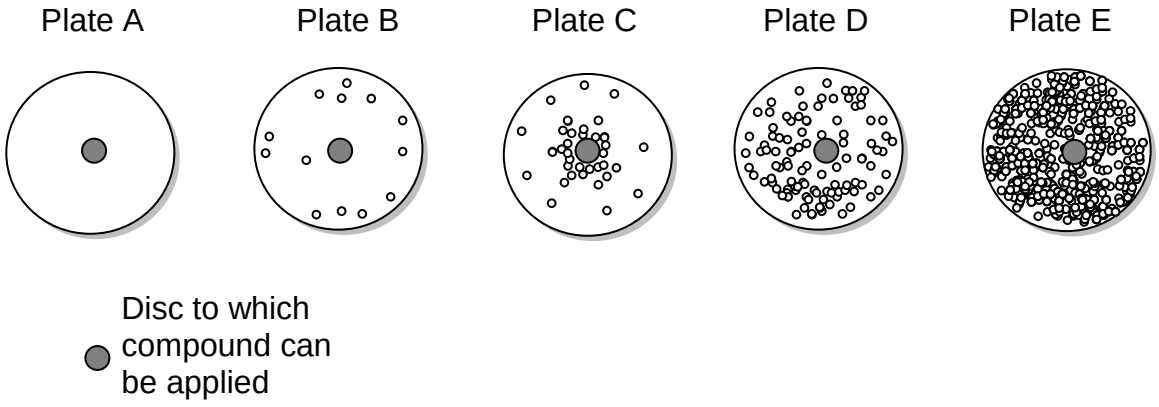
	Colony				
	A	**B**	**C**	**D**	**E**
lacZ gene sequence	altered	altered	altered	unaltered	altered
β-galactosidase molecular weight (kD)	115	5	115	0	115
Amino acid sequence	unaltered	altered	altered	N/A	altered
Activity (% wild type)	100	0	100	0	10

38. Which of the following colonies (A, B, C, D or E) is most likely to result from a frameshift mutation in lacZ?

39. Which of the following colonies (A, B, C, D, or E) displays a mutation which can be explained by the degeneracy of the genetic code?

40. Which of the following colonies (A, B, C, D, or E) is most likely to result from a mutation which disrupts the promoter region?

41. You have set up a number of plates to test a compound's mutagenicity using the Ames test. The figure below shows schematic diagrams of 5 plates. Which plate (A, B, C, D,or E) represents the growth pattern if the medium in the plate contained physiological concentrations of histidine?



42. Which statement about eukaryotic RNA polymerases is **INCORRECT?**

A. RNA Polymerase I transcripts are translated very efficiently
B. RNA Polymerase II promoters often contain a TATA element
C. RNA polymerase II transcripts are the most diverse
D. RNA Polymerase II is the most sensitive to alpha amanitin
E. RNA Polymerase III promoter elements are mostly within the transcribed sequence

43. Normally the eukaryotic C-terminal domain (CTD) RNA polymerase II:

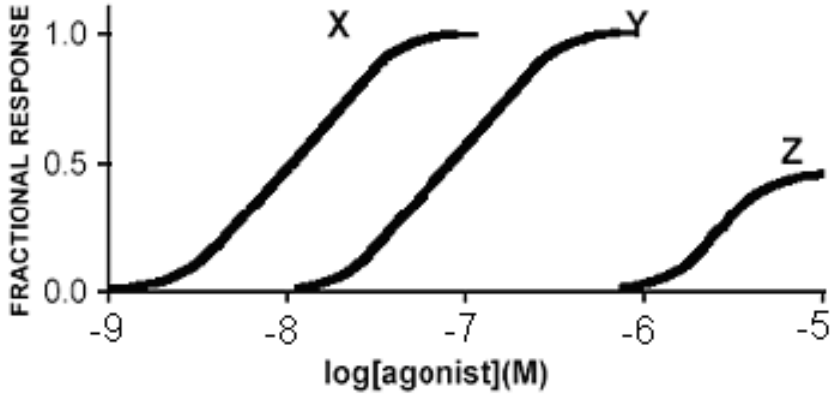
A. adds a ‘cap’ to the 5’ end of mRNA
B. forms part of the spliceosome
C. catalyses polyadenylation at the 3' end of mRNA
D. can be phosphorylated by a transcription factor
E. cleaves mRNA in preparation for splicing

44. PEPCK is up-regulated during starvation. Which of the following statements about this regulation is **INCORRECT**?

During starvation….

A. glucagon binds to cell surface receptors and activates adenylyl cyclase
B. increased cAMP results in increased stability of PEPCK mRNA
C. activated Protein Kinase A phosphorylates a transcription factor, CREB
D. PEPCK is allosterically activated by cAMP
E. glucocorticoids bind to intracellular receptors and activate the transcription of PEPCK

The diagram below is used for the following 1 question (Question 45). The vertical axis is relative biological activity. The curves are labelled X,Y and Z, but they may represent different situations in each question.



45. If curves X,Y and Z represent the dose-response curve for agonist X, Y and Z respectively, the compound Z:

A. has an intrinsic activity the same as X.
B. has a potency half that of agonists X and Y.
C. has a lower intrinsic activity than 1.0 as Z is a partial agonist.
D. answers B and C are correct.
E. answers A and B are correct.

46. An inverse agonist is a ligand that:

A. is an antagonist.
B. requires an allosteric model of drug action to explain its activity.
C. produces a response lower than basal.
D. Answers A and C are correct.
E. Answers B and C are correct.

47. The *specificity* of a drug for its receptor is the:

A. ability of an agonist to produce its maximum response compared to a standard.
B. production of drug effects by interaction with only a single receptor.
C. ability of an agonist to produce the response of a standard agonist.
D. negative log dose of a drug at which the receptor is half maximally occupied.
E. production of a particular drug effect at lower doses than those producing multiple effects.

48. The *occupancy theory* (Clark) of receptor theory of drug action:

A. states that the effects are not directly proportional to the proportion of receptors occupied
B. assumes that full response is not possible from a full agonist at low receptor occupancies.
C. assumes that a maximal response occurs when all the available receptors are occupied.
D. A and C are correct
E. B and C are correct

49. G_s

A. causes the release of calcium ions
B. inhibits adenylyl cyclase
C. activates adenylyl cyclase
D. inhibits phospholipase C-β
E. activates phospholipase C-β

50. Which of the following statements are **CORRECT**? Enzyme-linked receptors

A. are transmembrane proteins with their ligand-binding domain on the outer surface of the plasma membrane
B. are characterised as having a cytosolic domain that either has an intrinsic enzyme activity or associates directly with an enzyme
C. include receptor tyrosine kinases
D. includes the receptors for epidermal growth factor and vascular endothelial growth factor
E. All of the above

51. Which of the following statements are **CORRECT**?

A. GTPase-activating proteins (GAPs) maintain most of the Ras protein (~ 95%) in unstimulated cells in an inactive GDP-bound state
B. Ras inhibits the MAP-kinase serine/threonine phosphorylation pathway
C. GTPase-activating proteins (GAPs) activate Ras by stimulating it to hydrolyze bound GDP
D. Guanine nucleotide exchange factors (GEFs) inhibit Ras
E. Ras is inactive when GTP is bound and active when GDP is bound

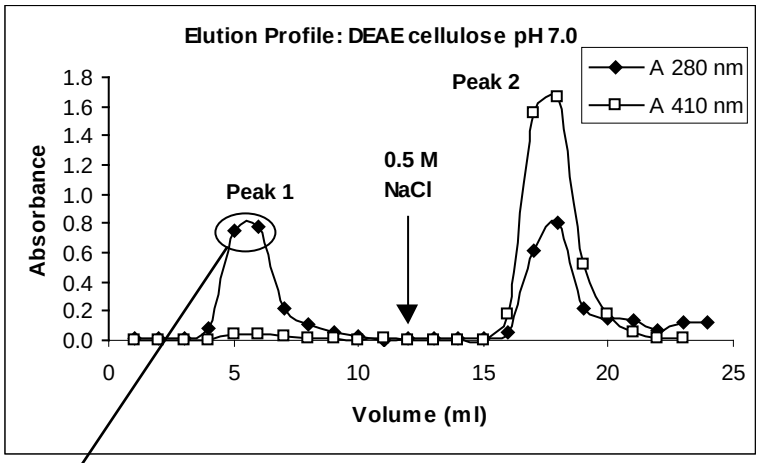
THEORY OF PRACTICAL

Questions 52 to 57 refer to the following information.

You have been given a mixture of 5 proteins (A - E) to separate and identify. You have been told that some of these proteins are coloured, containing a heme prosthetic group, which absorbs strongly at 410 nm. Two of the proteins contain 2 identical subunits (equal molecular weight). You have carried out the following experiments:

Experiment 1.

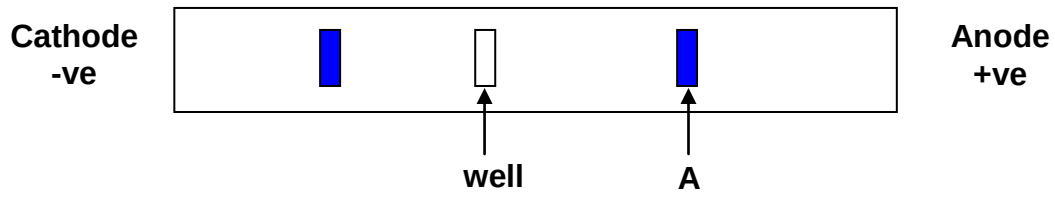
An aliquot of the mixture (A - E) was loaded onto DEAE cellulose at pH 7.0. One mL fractions were collected and the absorbance measured at 410 nm and 280 nm. The first peak eluted contained A and B. A second peak, eluted after the addition of 0.5 M NaCl at pH 7.0 contained two proteins, C, D and E.



15 µL used in Experiment 2

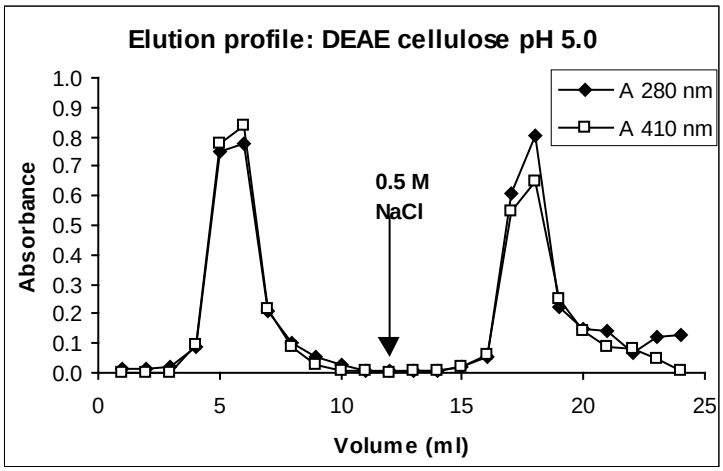
Experiment 2.

A 15 µl sample of Peak 1 from experiment 1 (proteins A&B) was loaded onto a 1.5 % agarose gel, made up in Tris barbitol buffer, pH 8.8. The sample was subjected to electrophoresis at 60 V for 45 min and the gel was then stained with Coomassie blue for 40 min. Destaining followed until the background was clear. The resultant destained gel is shown below.



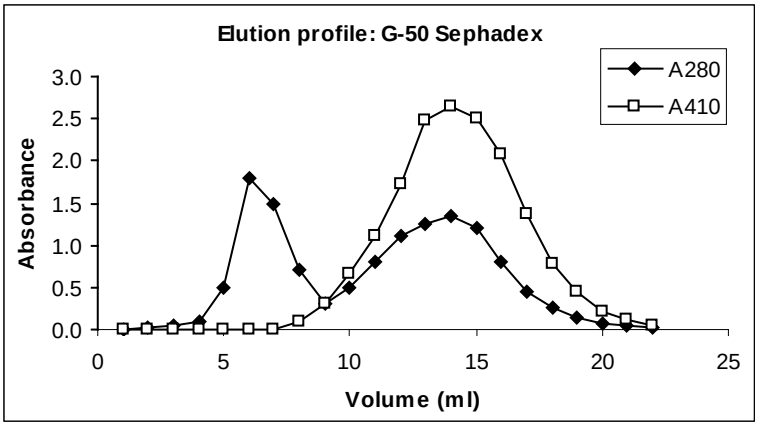
Experiment 3.

Another aliquot of the protein mixture (A-E) was loaded onto DEAE cellulose at pH 5.0 using the same procedure as in experiment 1. The first peak eluted contained three proteins A, B and D. The second peak, C and E, was eluted after increasing the ionic strength of the buffer.



Experiment 4.

Another aliquot of the protein mixture (A-E) was loaded onto a G-50 Sephadex column (cut-off >50 000) equilibrated with 50 mM Tris pH 7.5. One mL fractions were collected after measuring a 30 ml void volume. Two protein peaks were eluted. The first peak contained one protein, A.



Experiment 5.

The original protein mixture was loaded onto an SDS-PAGE at pH 8.8. Three protein bands were observed after Coomassie blue staining:
15 000, 30 000 and 45 000.

52. What is the most likely molecular weight of protein A?

A. 15 000
B. 30 000
C. 45 000
D. 70 000
E. 90 000

53. What is the pI of protein A?

A. 10.5
B. 9.5
C. 8.4
D. 6.5
E. 4

54. One of the proteins in your mixture (A - E) is a histone H2A. Which protein (A - E) is Histone H2A?

A. Protein A
B. Protein B
C. Protein C
D. Protein D
E. Protein E

55. If you loaded the original protein mixture (A-E) onto an ion exchange column containing carboxymethyl cellulose (-ve) at pH 5.0 which proteins would you predict would be eluted **BEFORE** the NaCl wash?

A. All the proteins

B. None of the proteins

C. Proteins A, B & D

D. Protein C and E

E. Protein A and B

56. Which protein (A – E) would have the highest ratio of basic residues (lysine & arginine) relative to acidic residues (glutamate & aspartate)?

A. Protein A

B. Protein B

C. Protein C

D. Protein D

E. Protein E

57. Your mixture contains ovalbumin. This protein is colourless with a pI of 4.7 and a molecular weight of 45 000. Which protein (A – E) is ovalbumin?

A. Protein A

B. Protein B

C. Protein C

D. Protein D

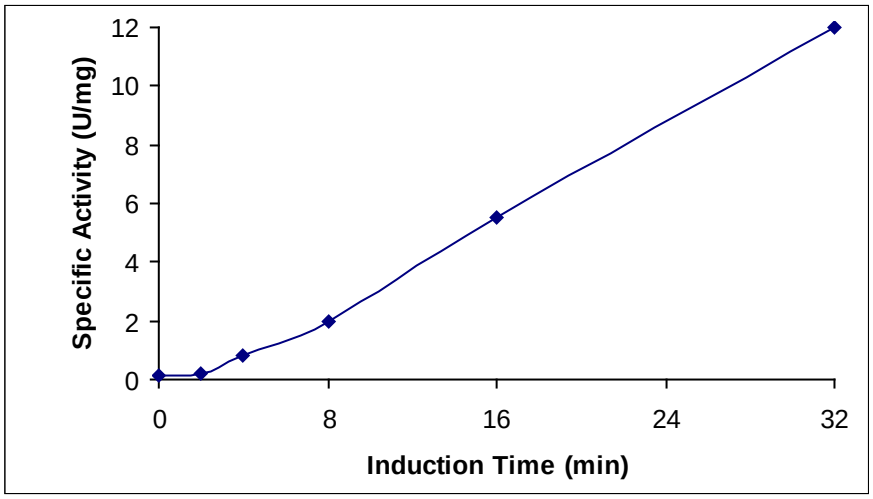
E. Protein E

Questions 58 to 62 refer to the following information.

You wish to investigate the induction of β-galactosidase in a number of strains of *E.coli*. You have been given the following protocol from a researcher's lab notebook:

A wild type colony of *E. coli* was selected and cultured in a medium which did not contain either glucose or lactose as carbon source. After sufficient growth was achieved IPTG was added to the culture to a final concentration of 0.5 mM. This time was denoted as time zero. At 2, 4, 8, 16 and 32 min after the addition of IPTG, 2 mL aliquots of culture were taken and assayed for β-galactosidase activity. The conditions for the assay were as follows:

Samples of culture (up to 50 % of the final volume) were incubated at 28^{o}C in a buffer containing 50 mM phosphate pH 7, 10 mM KCl, 5 mM $MgSO_4$ and 20 mM β-mercaptoethanol. Substrate, *o*-Nitrophenol galactose (*o*-NPG) was added to a final concentration of 2.5 mM. The rate of the reaction was measured over a 10 min time period by monitoring the rate of appearance of the product, *o*-nitrophenol (*o*-NP), spectrophotometrically at 420 nm.



Factor	A	B	C	D	E
Final A_{420}	unchanged	unchanged	**changed**	**change**	**change**
Total amount of ONP in the assay (nmoles)	unchanged	**change**	**change**	**change**	**change**
Rate of ONP production (nmol/min/ml assay)	unchanged	unchanged	unchanged	**change**	**change**
Specific Activity (nmol/min/mg protein)	unchanged	unchanged	unchanged	unchanged	**change**

You are investigating the β-galactosidase assay of culture induced for **8 min** with IPTG. A change is considered significant if it is >±10%. Which of the options (A, B, C, D, E) above would occur if:

58. The induction time was doubled from 8 min to 16 min?

59. The ONPG concentration in the assay was doubled?

60. The assay was scaled up by a factor of 2 (i.e. you added twice the volume of every component in the assay, giving double the assay volume)?

61. The incubation time was increased from 10 to 20 min?

62. The volume of culture added to the assay was halved from 2.0 ml to 1.0 ml? (Water was added to ensure the final assay volume did not alter).

63. (3 marks)

Draw the atomic structure of polypeptides that are in a β-sheet, which hydrogen bonds. The β-sheet can be either parallel or antiparallel, but label which you've shown. You should not require more than 6 amino acids in your figure, but ensure you have the unique hydrogen bonds.

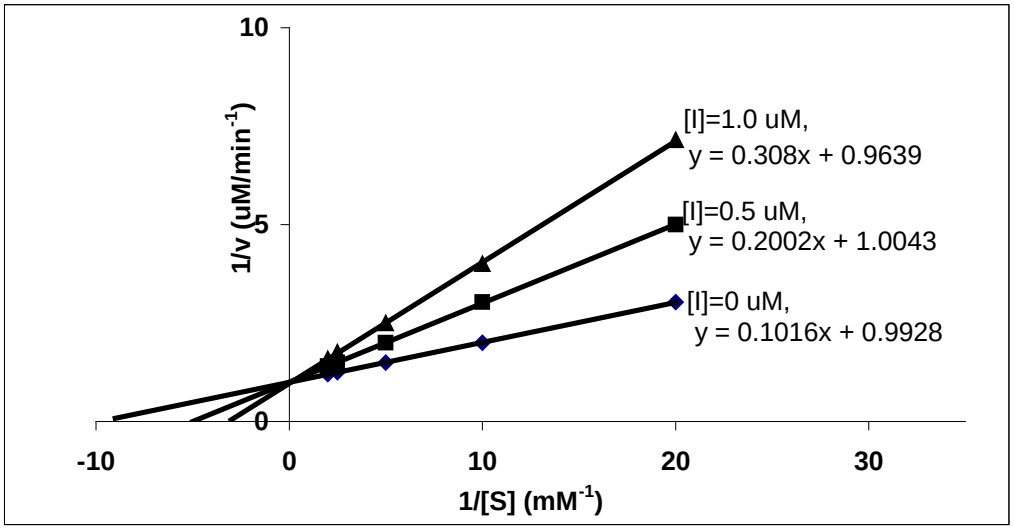
64. (3 marks)

A. What is the pI of a protein? (1 mark)

B. Draw a figure representing the main features of the titration curve of glutamic acid, in which pK1 = 2.19, pKR = 4.25, pK2 = 9.67. (2 marks)

65. (9 marks)

A) **Calculate** Vmax, Km, and Ki from the graph below. Show your working and units (to 4 d.p.). Part marks will be awarded. (7 marks)



Note: $1/v$ = Km/Vmax(1+([I]/Ki)). 1/S + 1/Vmax

B) What is a suicide substrate of an enzyme? (2 marks)

66. (4 marks)

(i) You are advising a pharmaceutical company that is developing uncoupling agents as weight loss drugs. In general terms, how does an uncoupler enable weight loss? (1 mark)

(ii) The company is currently thinking of two approaches 1) B3-agonists 2) weak acids that can cross cell membranes (WACCMs). Give THREE pieces of advice to the company that would enable them to decide which way to proceed. (3 marks)

67. (2 marks)

The relative rate of blood glucose oxidation (glucose uptake through to carbon dioxide) was measured in exercising muscle at various exercise intensities. The rate at rest was set at 1. After 30 minutes of walking, the relative rate was 3, during jogging it was 5 and during competitive running it was 10. What would you expect the relative rate of blood glucose oxidation to be during sprinting? Justify your answer.

68. (6 marks)

A). Describe the spare receptor theory and how it relates to receptor occupancy and drug action. Include aspects of theories prior to the spare receptor theory to assist your explanations. (5 marks).

B). There are phenomenon in which receptors appear the become either more sensitive or less sensitive over time. Can you explain how this might occur? (1 mark)

69. (3 marks)

Outline the molecular mechanism by which bronchospasm can be alleviated by phosphodiesterase inhibitors in asthma.

70. (3 marks).

Breast cancer is one of the most common malignancies. Overexpression of growth factor receptors, particularly the epidermal growth factor receptor (EGFR), has been associated with poor clinical outcome. Please identify TWO current approaches taken toward inhibition of EGFR and outline the molecular basis of their inhibition.

THEORY OF PRACTICAL

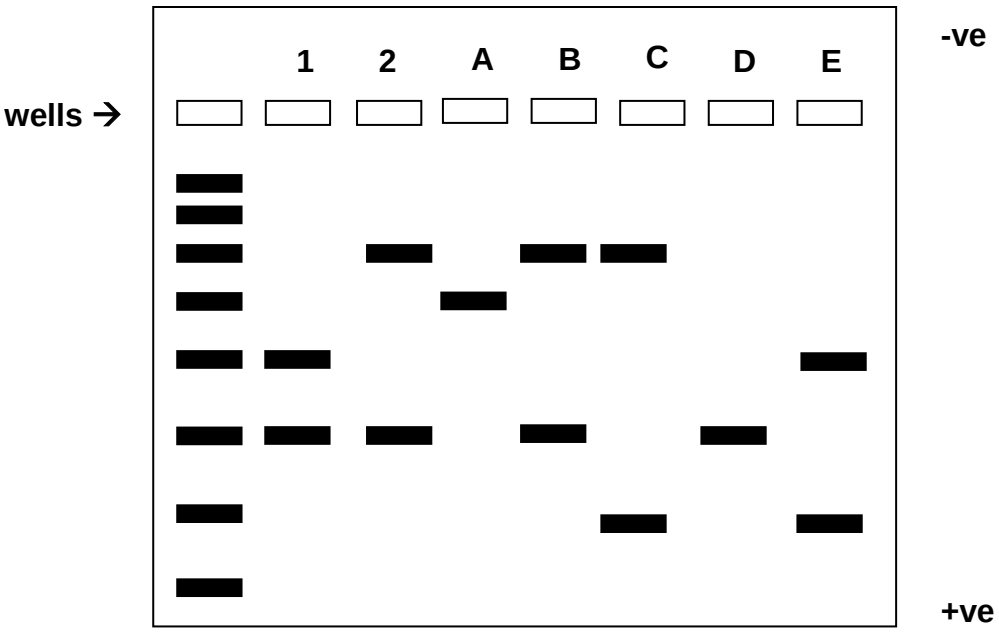
71. (9 marks)

You are performing a genetic profile analysis to determine paternity. You decide to use the polymerase chain reaction (PCR) to investigate a particular variable number tandem repeat (VNTR) called D3S22. This VNTR has a 5' flanking region of 104 base pairs (bp) and a 3' flanking region of 64 bp. The repeats are each 16 bp long, except for the first repeat, which is 12 bp long.

You extract DNA from the child, the mother, and the potential fathers. You prepare separate reactions for each individual by adding 3 μL of the extracted DNA to a mix of PCR buffer, dNTPs, and a mix of forward and reverse primers. You also make a positive and negative control. After adding Taq polymerase as required, you put the reaction mixes into a Thermocycler which you have programmed to perform the following cycle 30 times.

- Stage 1 = 95^{o}C for 1 minute
- Stage 2 = 65^{o}C for 1 minute
- Stage 3 = 72^{o}C for 1 minute

After the cycles are finished, you run a sample from each tube on a 2% agarose gel. The results of this gel are shown below. The sizes of the standards in the left-most lane are 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp



(i) Lane 1 contains the child's sample. Lane 2 contains the mother's sample. Which lane contains the sample from a person who could be the child's father? (1 mark)

(ii) How many repeats are there in the allele represented in lane A? (3 marks)

(iii) The absorbance at 260nm of one of the DNA samples was 0.232. You added 3 μL of this sample to the PCR. Approximately how much DNA was added to the PCR? (2 marks)
Remember that 50 μg/mL DNA has an A260 = 1

(iv) What is happening in the reaction when the temperature in the thermocycler is 65^{o}C? (1 mark)

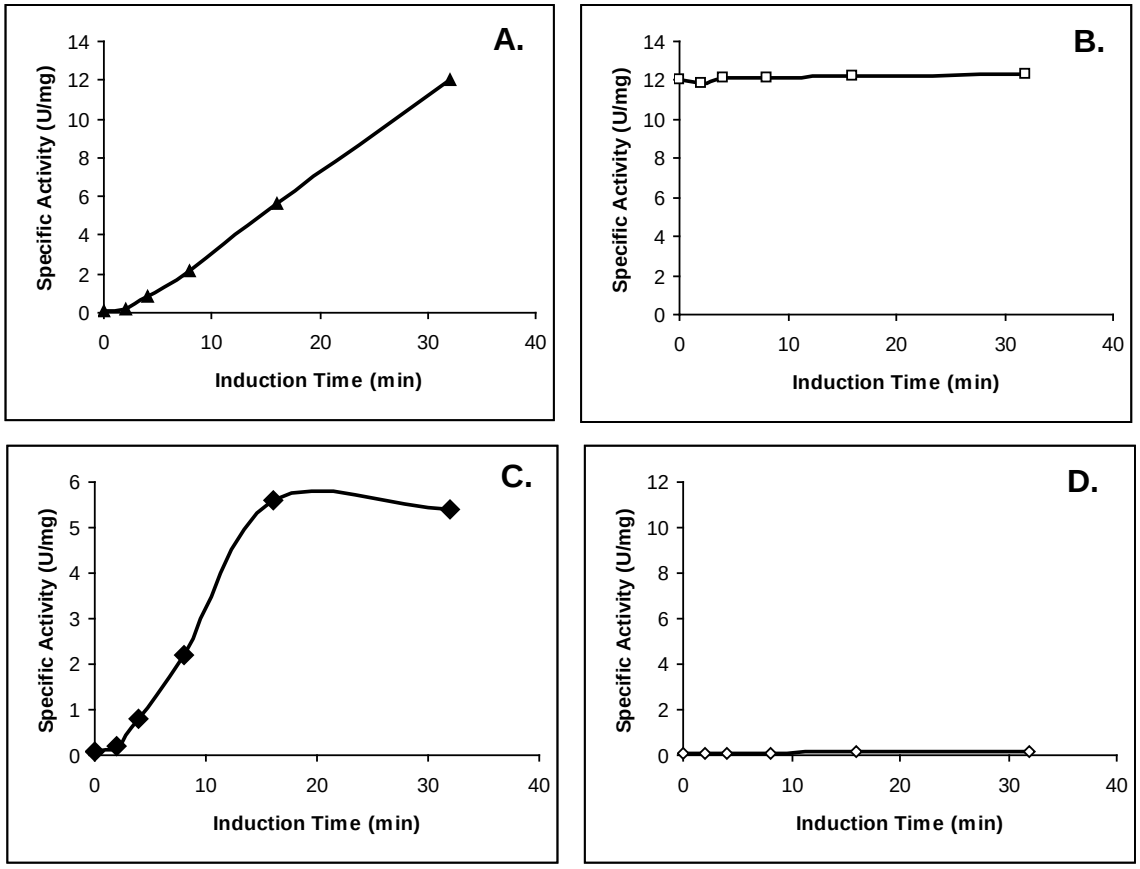
(v) Below is the sequence of the 5'→ 3' strand of D3S22, showing only the regions used to design the primers (the dotted line represents the intervening repeat sequences).

5' ATTCGGACTCGCT----------AACGTGGTATCC 3'.

The forward primer to amplify the intervening sequence would have ATTCG as its first 5 nucleotides. What are the first 5 nucleotides of the reverse primer? (2 marks)

72. (8 marks)

The graphs below (A – D) show the activity of β-galactosidase at various times after adding IPTG. In part (i) draw the lac operon as it would be operating in graph A (see below). Include in your diagram the location of the operator site, the promoter, the CAP binding site, *lacI, lacZ, lacY* and *lacA.*



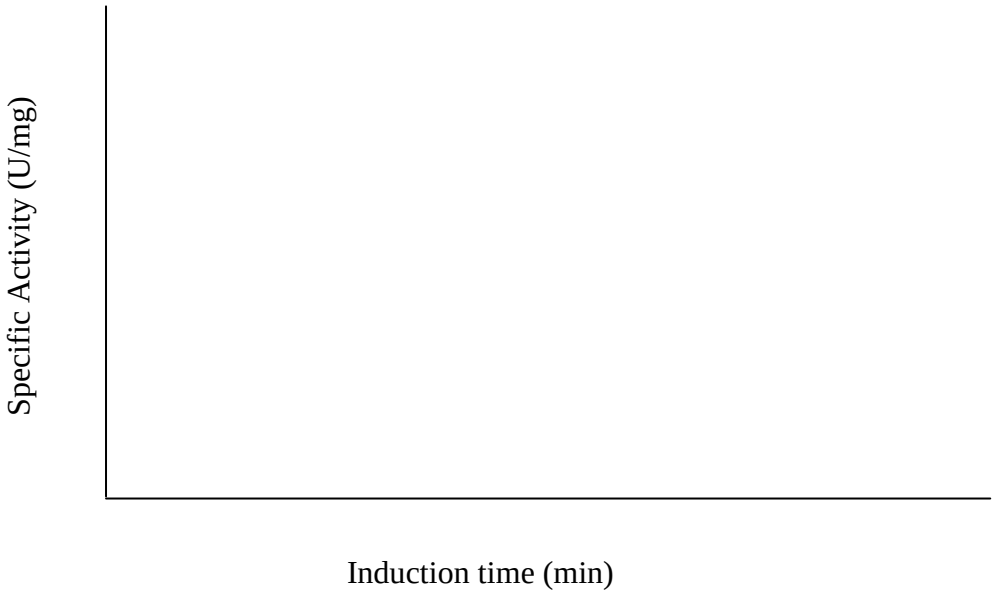
(i) Lac operon as in graph A (3 marks)

(ii) Which graph (A – D) would represent the induction of β-galactosidase in an *E.coli* colony containing a mutation which disrupts the operator binding site? Explain your answer (1 mark)

(iii) Which graph (A – D) would represent the induction of β-galactosidase in an *E.coli* colony containing a mutation which disrupts the repressor (*lacI*)? Explain your answer (1 mark)

(iv) Which graph (A – D) would represent the induction of β-galactosidase in an *E.coli* colony containing a mutation which disrupts the β-galactosidase gene (*lacZ*), resulting in a non-functional protein? Explain your answer (1 mark)

(v) In the space provided below draw the graph you would expect to see if the cAMP binding site on the CAP was disrupted so that cAMP could no longer bind. Consider the induction of β-galactosidase by IPTG in the presence and absence of glucose. (2 marks)



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Rough work space