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Charles Darwin University

Final Examination

Family Name						
Given Name/s						
Student Number						
Teaching Period	Semester 2, 2018					

PHA214 – Biotechnology and Pharmacogenomics	**DURATION**	
	Reading Time:	**10** minutes
	Writing Time:	**120** minutes

INSTRUCTIONS TO CANDIDATES

Answer all questions in Section A on the MCQ answer sheet proved.

Answer all questions in Section B in the booklet provided.

EXAM CONDITIONS

You may begin writing from the commencement of the examination session. The reading time indicated above is provided as a guide only.

This is a CLOSED BOOK examination

Any non-programmable calculator is permitted

No handwritten notes are permitted

No dictionaries are permitted

ADDITIONAL AUTHORISED MATERIALS	**EXAMINATION MATERIALS TO BE SUPPLIED**
No additional printed material is permitted	1 x 16 Page Book 1 x 5-Multiple Choice Answer Sheet 1 x Scrap Paper

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Section B
Short Answer Questions: 11
Total No of Marks for this Section: 75

This section should be answered in the Answer Book provided.

Marks for each question are indicated. Suggested time allocation for Section B: 70 mins

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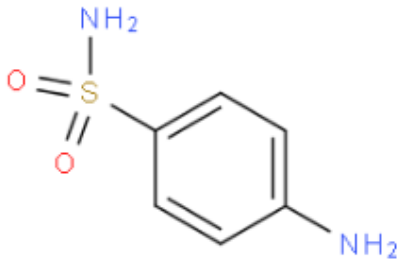
Question 1

Sulfanilamide ($C_6H_8N_2O_2S$) is an antibacterial drug. Its *logP* value is **-0.72**.



(a) Redraw the structure in your answer paper and label all the possible binding interactions that can take place in the binding site between Sulfanilamide molecule and the amino acid residues of theprotein involved.

[3 marks]

(b) Calculate the other properties of sulfanilamide relating to 'Lipinski-Rule of Five'. State whether the molecule is drugable and explain your answer. [Atomic weight of H= 1.01 g/mol, C=12.01 g/mol, N =14.01 g/mol, O =16 g/mol, F =19.00 and S= 32.07]

[4 marks]

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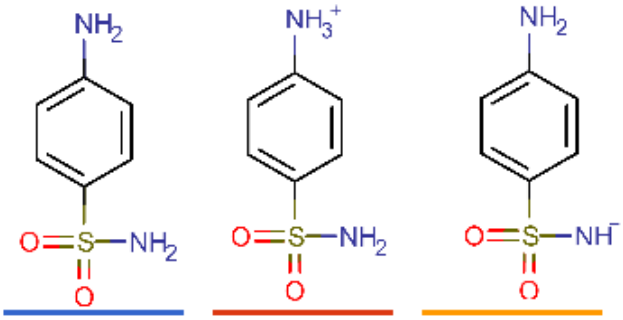
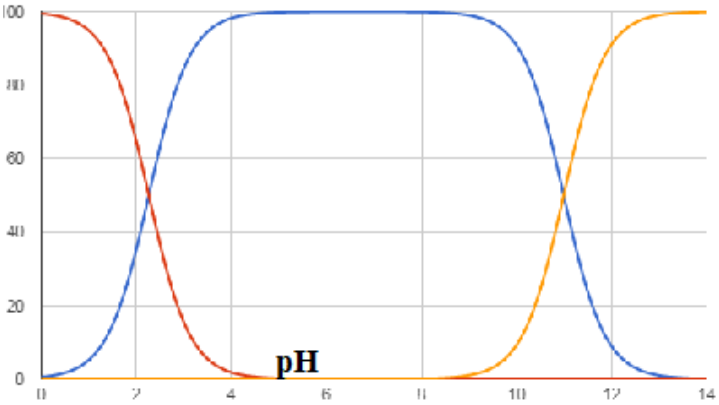
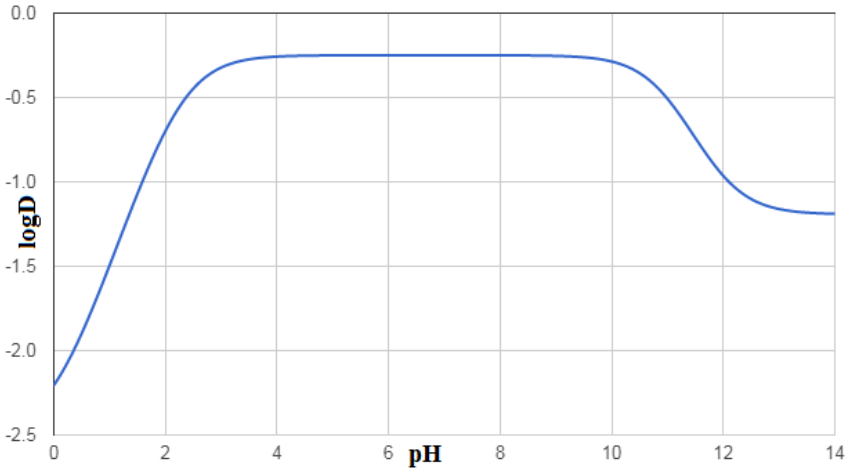
Question 2

(a) The graphs below represents a plot of %concentration versus pH and logD versus pH of sulfanilamide. sulfanilamide's pKa values are 2.27 and 10.99. With reference to the graphs, discuss the nature of the molecule at pH 2.5, 5.5 and 7.4?







[4 marks]

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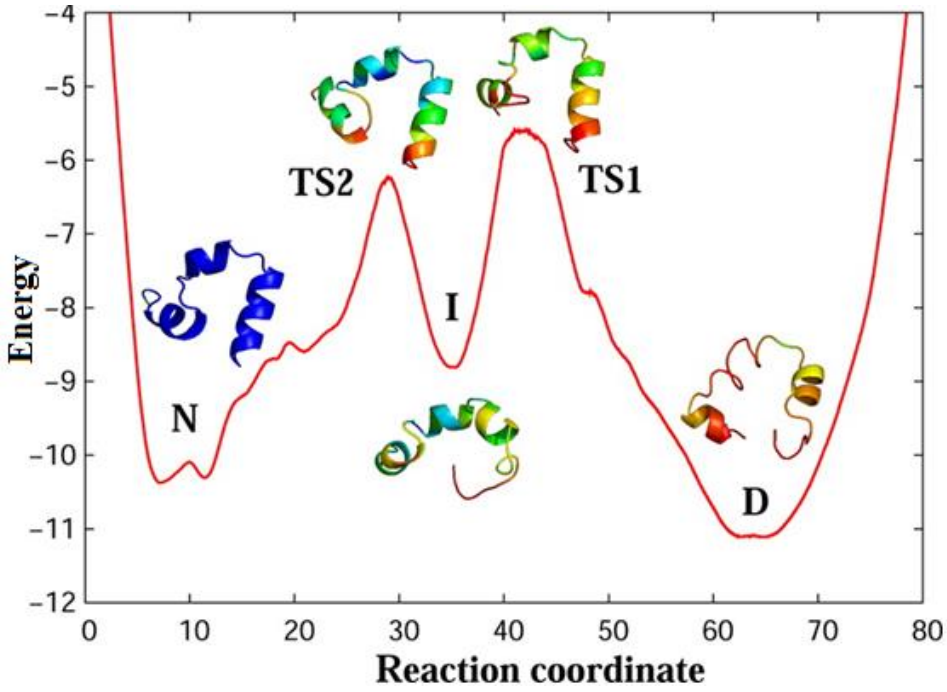
(b) The following figure gives the free-energy landscape diagram of protein folding for villin. The landscape consists of five states: denatured basin (D), first transition state (TS1), intermediate state (I), second transition state (TS2), and native basin (N). Discuss the significance of different states of the protein represented in this diagram.



[3 marks]

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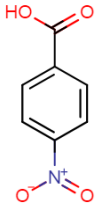
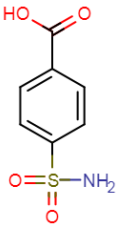
Question 3

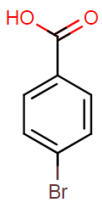
Log P of benzoic acid is 1.89. Using the data provided in the table below, for substituents of benzoic acid answer the questions below.

Substituent	π	σ_m	σ_p
H	0.00	0.00	0.00
Br	0.86	0.39	0.23
Cl	0.71	0.37	0.23
F	0.14	0.34	0.06
I	1.12	0.35	0.18
NO_2	-0.28	0.71	0.78
OH	-0.67	0.12	-0.37
NH_2	-1.23	-0.16	-0.66
SH	0.39	0.25	0.15
SO_2NH_2	-1.82	0.46	0.57

(a) Calculate the log*P* value for

i) 

ii) 

iii) 

[3 marks]

(b) Which of the above 3 compounds will be most hydrophobic? Explain your answer.

[2 marks]

(c) What position of the benzoic acid would you add the -OH group to make the acid stronger? Sketch the structure and explain your answer.

[2 marks]

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Question 4

(a) Thiopurine agents, such as azathiopurine (AZA). Are widely used for the treatment of acute leukaemias, inflammatory bowel diseases and other immunological disorders. AZA is a pro-drug that is rapidly converted to its active metabolites, such as 6-mercaptopurine, 6-thioinosine monophosphate and 6-TGN. Reduced enzymatic activity is associated with increased 6-TGn levels, which may result in direct incorporation of 6-TGN into DNA and cause cytotoxic and immunosuppression.

Using the below table, discuss the data in terms of genotype and drug metabolism.

Factors	TMPT wild type (n = 143)	TMPT heterozygous (n = 6)	Total (n = 149)
Demographics			
Age (yr)	48.6±13.1	48.5±19.5	48.6±13.2
Sex			
Male	47	3	50
Female	96	3	99
Disease			
Behçet disease	111	5	116
Atopic dermatitis	10	0	10
Lichen planus	4	0	4
Chronic eczema	3	1	4
Allergic contact dermatitis	3	0	3
Dermatomyositis	2	0	2
Psoriasis vulgaris	2	0	2
Chronic urticaria	2	0	2
Others	6	0	6
Administration of AZA			
Number of patients	119	4	123
Dosage (mg/d)	62.02 ± 22.68	43.75 ± 12.50	61.42 ± 22.72
Duration (d)	1211 ± 929.66	1074 ± 654.23	1207 ± 924.20

(Marks: 6)

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Question 5

Explain the role of Phase I, Phase II and Phase III proteins in drug metabolism.

(Marks: 3)

Although the phases do not have to be sequential in order, they make sense as a progression from Phase I to III. Explain why the phases are numbered in that sequence in reference to a compound of your choice

(Marks 3)

Question 6

Paracetamol is normally metabolised via conjugation, but has the potential to undergo oxidative metabolism. Explain the inhibition and induction of both the primary and alternative pathways, concentrating on the interaction of paracetamol metabolism with that of ethanol, and explain how incorrect consumption of paracetamol can lead to fatal hepatotoxicity.

(8 marks)

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Question 7

Restriction enzymes are an important tool, with each DNA sequence being unique, producing specific fragment sizes.

(a) Imagine the DNA strand shown in Figure 1 was cut with *Eco*R1 and placed in lane C (Figure 2). Draw the band in lane C as they would appear after electrophoresis, and indicate the size of each of the DNA fragments in base pairs.

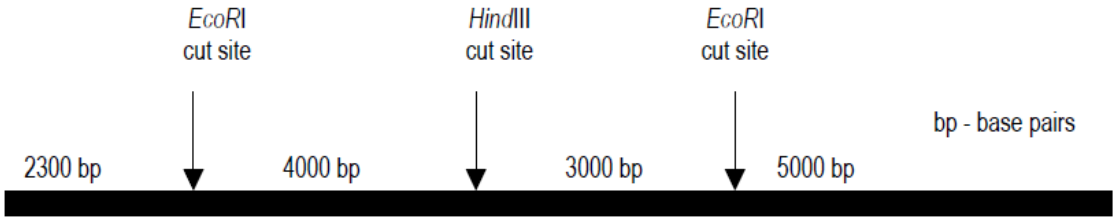

Figure 1: DNA strand with specific *Eco*R1 and *Hind*III sites.

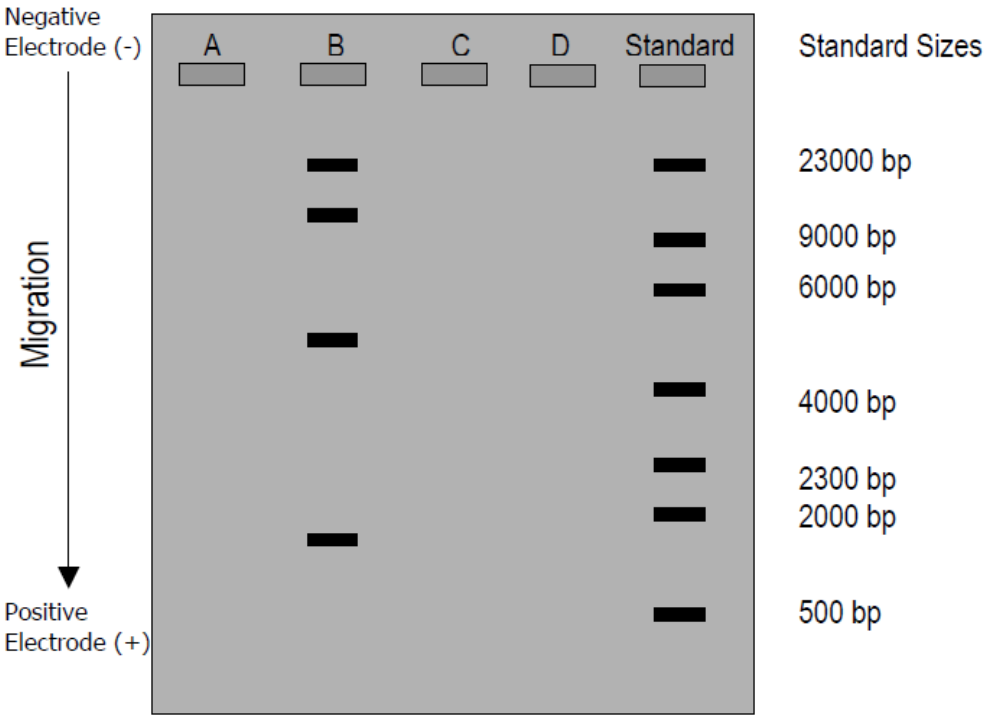

Figure 2: Agrose gel containing DNA fragments separated by electrophoresis.

(b) Explain the process of electrophoresis and how it is used to separate DNA fragments.

(Marks: 9)

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Question 8

A group of 20 recurrence breast cancer patients (taking tamoxifen) were selected for a study, regarding tamoxifen treatment. 19 aged matched women who had already completed 5 years of tamoxifen treatment with no recurrence, were used as controls. (Table 1). Genomic DNA was collected and amplified by PCR to determine if SNPs could influence the efficiency of tamoxifen treatment (Table 2).

Characteristic	Case group (n = 20)	Control group (n = 19)
Median age (years)	45.8	46.47
Menopausal status		
Premenopausal	16 (80%)	15 (78.9%)
Postmenopausal	4 (20%)	4 (21.1%)
Tumour size (cm)		
<2	4 (20%)	3 (15.8%)
2-5	15 (25%)	15 (78.9%)
>5	1 (5%)	1 (5.3%)
Number of positive nodes		
0	10 (50%)	9 (47.4%)
1-3	5 (25%)	5 (26.3%)
>4	5 (25%)	5 (26.3%)
ER status		
Positive	20 (100%)	19 (100%)
Negative	0	0

Table 1: Patient characteristics

SNP	Allele Frequencies	
	Case group (n = 20)	Control group (n = 19)
*CYP2D6*10* (C100T; C- wild type)		
T/T	11 (55%)	1 (5.3%)
C/T	4 (20%)	8 (42.1%)
C/C	5 (25%)	10 (52.6%)
*CYP2D6*4* (G1846A; G – wild type)		
A/A	0	0
G/A	1 (5%)	0
G/G	19 (95%)	19 (100%)

Table 2: Patient genotypes and frequencies

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(a) Using the above data, which of the following SNPs most likely plays a role in tamoxifen treatment?

(Marks: 2)

(b) Using your understanding of drug metabolism, explain why the patients displaying the variant genotype had a shorter disease free survival than the matched controls.

(Marks: 6)

Question 9

DNA fragments for two genes (Bt resistance and GFP) were generated through PCR with the addition of restriction enzymes (*Hind*III) at the 5'- and 3'-ends. The DNA fragments were inserted into a plasmid and transformed into plants so that they were resistant to certain insects (Bt gene) as well as fluoresce (GFP gene).

(a) List the reagents that are necessary for a reaction to occur and explain the role of each reagent.

(Marks: 4)

(b) State the reaction steps and conditions for a general PCR reaction and what is occurring in each step.

(Marks: 3)

(c) Would it have been possible for the plant to be transformed with the Bt gene and not the GFP gene? Explain your answer.

(Marks: 2)

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Question 10

Paul is a 25-year-old schizophrenic who has successfully been treated with clopine for over 12 months. Clopine is metabolised by CYP1A2. With the support and encouragement of his family and friends, he recently started a new job. He regularly smoked two packets of cigarettes a day, but the new job has kept him busy and he now only has a couple of cigarettes a day. During the next couple of weeks, Paul increasingly feels lethargic and fatigued. He assumed this was due to his new job, so started having several cups of coffee (caffeine is a substrate for CYP1A2) a day to try to help 'perk' him up. However, he further deteriorated developed muscle spasms, dizziness, blurred vision and confusion.

a) Explain how the sudden decrease in smoking had effected Paul.

b) What are the potential drug interactions with coffee?

(Marks: 5)

Question 11

A new drug has been developing to treat prostate cancer. This drug is designed to be taken orally every day during the treatment period, and it inhibits the growth of the cancer cell. During the initial clinical trial, it is found that 20% of the studies population has a very low blood concentration of the drug. Briefly outline THREE (3) possible mechanism of genetic variation that can explain this observation.

(Marks: 3)

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