UNIVERSITY OF YORK
BSc Stage 3 Degree Examinations 2017-18

Department:
BIOLOGY

Title of Exam:
Biocatalysis

Time allowed: 2 hours
Total marks available for this paper: 100

This paper has two parts:

Section A: Short Answer / Problem / Experimental Design questions (50 marks)
  ● Answer all questions in the spaces provided on the examination paper

Section B: Essay question (marked out of 100, weighted 50 marks)
  ● Answer one question from question A, question B or question C
  ● Write your answer on the separate paper provided and attach it to the
    back of the question paper using the treasury tag provided
  ● The marks available for each question are indicated on the paper

For marker use only:

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Total as %

DO NOT WRITE ON THIS BOOKLET BEFORE THE EXAM BEGINS
DO NOT TURN OVER THIS PAGE UNTIL INSTRUCTED TO DO SO BY AN INVIGILATOR
SECTION A: Short Answer / Problem / Experimental Design questions

Answer all questions in the spaces provided

Mark total for this section: 50

1. a) What advantages do enzymes offer the chemical industry compared to chemocatalysts? (2 marks)

b) Biosensors comprise a biological recognition component (usually an enzyme or antibody) and a transducer that converts a biological signal into a signal that can be quantified or processed. Describe why enzymes make excellent recognition components and why the involvement of a cofactor like NAD(P) is desirable? (2 marks)

c) You have been asked by HM Customs & Excise to develop an enzyme based biosensor to detect illicit heroin and cocaine; however, there are no suitable enzymes commercially available. Describe a strategy to find a new microbial enzyme that is active towards heroin (diacetylmorphine) and suitable for use in a biosensor. (5 marks)
d) You identify two enzymes, a heroin esterase and a NADP\(^+\)-dependent morphine dehydrogenase. Show on the scheme below how these two enzymes can be coupled to bioluminescence to detect heroin?

(5 marks)

![Diagram showing the coupling of heroin esterase and morphine dehydrogenase to bioluminescence]

e) Why are members of the Old Yellow Enzyme family of flavoenzymes proving to be important biocatalysts?

(3 marks)
f) Why is Old Yellow Enzyme such a well characterized enzyme?
(3 marks)

2. Phenylalanine Dehydrogenase (PheDH) catalyses the interconversion of L-phenylalanine 1 (below), and a ketoacid 2 (structure not shown) with both water and an oxidised nicotinamide cofactor (NAD+) required for the reaction in the oxidative direction.

a) Complete the reaction Scheme above to show the structure of the ketoacid product 2 in the box provided, and identify co-products 3 and 4.
(3 marks)
A representation of the active site of PheDH, in complex with L-phenylalanine is shown below, with selected residues numbered. A table with the genetic code is presented alongside.

<table>
<thead>
<tr>
<th>TTT</th>
<th>TTC</th>
<th>Phe</th>
<th>TCT</th>
<th>TCC</th>
<th>Ser</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTA</td>
<td>TTG</td>
<td>Leu</td>
<td>TCA</td>
<td>TCG</td>
<td>TAC</td>
</tr>
<tr>
<td>CTT</td>
<td>CTC</td>
<td>Leu</td>
<td>CCA</td>
<td>CGG</td>
<td>CAT</td>
</tr>
<tr>
<td>CTT</td>
<td>CTA</td>
<td>Leu</td>
<td>CCA</td>
<td>CGG</td>
<td>CAT</td>
</tr>
<tr>
<td>ATG</td>
<td>AGA</td>
<td>Met</td>
<td>GTT</td>
<td>GCG</td>
<td>Asp</td>
</tr>
<tr>
<td>GTA</td>
<td>GTG</td>
<td>Val</td>
<td>GCT</td>
<td>GCC</td>
<td>GAT</td>
</tr>
<tr>
<td>GTA</td>
<td>GTC</td>
<td>Val</td>
<td>GCT</td>
<td>GCC</td>
<td>GAT</td>
</tr>
<tr>
<td>GTA</td>
<td>GTG</td>
<td>Val</td>
<td>GCT</td>
<td>GCC</td>
<td>GAT</td>
</tr>
<tr>
<td>ATG</td>
<td>TGG</td>
<td>Met</td>
<td>TTA</td>
<td>TGC</td>
<td>Thr</td>
</tr>
<tr>
<td>AAA</td>
<td>AAC</td>
<td>Thr</td>
<td>TAA</td>
<td>TAC</td>
<td>Thr</td>
</tr>
<tr>
<td>AAA</td>
<td>AAG</td>
<td>Lys</td>
<td>TAA</td>
<td>TAC</td>
<td>Thr</td>
</tr>
<tr>
<td>AAA</td>
<td>AAG</td>
<td>Lys</td>
<td>TAA</td>
<td>TAC</td>
<td>Thr</td>
</tr>
<tr>
<td>GAA</td>
<td>GAG</td>
<td>Glu</td>
<td>TAA</td>
<td>TAC</td>
<td>Thr</td>
</tr>
<tr>
<td>GAA</td>
<td>GAG</td>
<td>Glu</td>
<td>TAA</td>
<td>TAC</td>
<td>Thr</td>
</tr>
<tr>
<td>GAA</td>
<td>GAG</td>
<td>Glu</td>
<td>TAA</td>
<td>TAC</td>
<td>Thr</td>
</tr>
</tbody>
</table>

b) The structure suggests that aspartate residue D119 makes an interaction (black dashed lines) with the nitrogen atom of L-Phe. Name this type of interaction, and suggest a mutation that would enable you to study the role of this residue.

(2 marks)
The coding sequence for PheDH is shown below:

```
1   atgagcattgatagcgcgctgaactgggatggcgaaatgaccgtgacccgctttgatgcg
61  atgaccggccgcgcagttgtgattgctcgctggatagcacccagctgggcccggcggg
121 gcacccgcgcgcgcagtataagcaaccctgcgctgaccctgcggtcgcgctgacccgct
181 gcggtcgcgctgaccctgaccctgtgatcgcggtgatcgcgctgacccgctgaccctg
241 gttgaccgctgacccgctgacccgctgacccgctgacccgctgacccgctgacccgct
301 gcggctgacccgctgagctggccggccggccggccggccggccggccggccggccggccgg
361 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
421 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
481 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
541 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
601 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
661 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
721 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
781 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
841 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
901 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
961 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
1021 gcggccggccggccggccggccggccggccggccggccggccggccggccggccggccggcc
```

c) Showing your working, design a forward 5’-3’ primer for a site-directed mutagenesis experiment that would generate your mutation suggested in (b).

(3 marks)

d) Describe an experiment to investigate the role of D119 in the recognition of the amino acid nitrogen atom using the mutant you have designed, and outline the expected effects on your measurements.

(3 marks)
e) The structure suggests that lysine K67 and asparagine N263 interact with the carboxylate of L-Phenylalanine. Focusing on these residues, suggest TWO protein engineering strategies that may enable you to create an enzyme competent for the interconversion of 5 and 6 below.  

f) Describe a mutational strategy for the creation of a PheDH variant competent for the formation of D-Phenylalanine from 2, dependent on random mutagenesis.  

(5 marks)  

(4 marks)
3. What differentiates a hammerhead ribozyme cleaving in *trans* from most other site-specific nucleolytic ribozymes? (2 marks)

4. Mg\(^{2+}\) is important for ribozyme function. Using examples, briefly describe the two roles it can have in ribozymes. (3 marks)

5. a) How are Group I and II self-splicing mechanisms different in the first phosphoryl-transfer reaction step? (2 marks)

   b) What intronic structure is uniquely generated during the Group II self-splicing reaction compared to Group I? (1 mark)

   c) Is this structure always generated in the Group II self-splicing reaction? Briefly explain your answer. (2 marks)
SECTION B: Essay question

Answer one question on the separate paper provided

Remember to write your candidate number at the top of the page and indicate whether you have answered question A, B or C

Mark total for this section: 50

EITHER

A) Discuss the roles flavins play in enzyme catalysis and their importance in a variety of enzyme-based applications.

OR

B) Using the bacterial protease subtilisin as an example, discuss the extent to which structure-informed, site-directed mutagenesis is an appropriate tool for engineering enzymes for altered or improved performance in industrial reactions.

OR

C) Discuss the role of primary, secondary and tertiary structure in natural ribozymes, and compare and contrast with protein-based enzymes.