IMPERIAL COLLEGE LONDON

B.Sc. Examination 2016

This paper is also taken for the relevant examination for the Associateship of the Royal College of Science

PROTEINS AND ENZYMES

Thursday 9 June 2016 10.00-13.00

FOR FIRST YEAR STUDENTS IN BIOCHEMISTRY AND BIOTECHNOLOGY

Please use the **MCQ ANSWER SHEET** provided **FOR SECTION A** and a **SEPARATE ANSWER BOOK FOR EACH QUESTION IN SECTION B AND SECTION C**. All parts of a question carry equal weighting unless otherwise specified.

SECTION A
This section consists of 25 compulsory multiple choice questions. Using the answer sheet provided, mark the box or boxes to indicate your answer. Some questions in this section have more than one correct answer. Credit will be given for all correct answers but you will be penalised with a negative mark for incorrect choices. You will not be penalised if you do not select an answer. This section carries 25% of the marks. You should allow approximately 45 minutes to answer this section.

1. **Identify the following amino acid:**
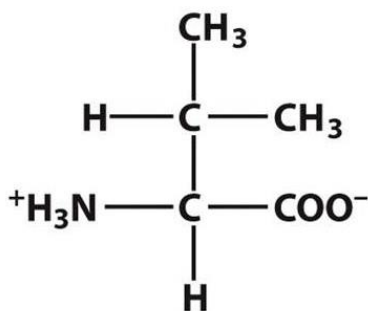
    A) Leucine
    B) Isoleucine
    C) Methionine
    D) Valine
    E) Serine

2. **Given that the rise along an α-helical axis is 5.4 Å, what is the length of a continuous α-helix of 26 amino acids?**
    A) 35 Å
    B) 36 Å
    C) 37 Å
    D) 38 Å
    E) 39 Å

3. **The pK_a values for the ionisation of the amino acid arginine are as follows: pK_1 = 2.2, pK_2 = 9.0, pK_3 = 12.5. Which of the following statements is/are correct?**
   A) At pH 12.5 the overall charge on arginine is +0.5
   B) At pH 4 the overall charge on arginine is -0.5
   C) At pH 8 the overall charge on arginine is +1
   D) The pI of arginine is 10.75
   E) The overall charge on arginine at pH 2.2 is +1.5

4. **Assuming a pK_a of 6.0 for the histidine side chain, what is the charge on the peptide below at (i) pH 6.0 and (ii) pH 1.6**

   R-L-C-D-K-M-H-H-C-I-P-G-A-F-G-D-E-K-C-M-E-V-S-T-A-A-K

   A) (i) +1, (ii) +6
   B) (i) +1, (ii) +7
   C) (i) 0, (ii) +7
   D) (i) 0, (ii) +6
   E) (i) -1, (ii) +6

5. **Western blot analysis of a protein sample requires which of the following?**
   A) Cleavage of the protein
   B) A pure protein sample
   C) A protein-specific antibody
   D) The protein to be stained with Coomassie Blue
   E) The protein to be separated by SDS-PAGE

6. **Trypsin cleavage:**
   A) is sensitive to the presence of Pro residues close to the cleavage site
   B) cuts on the N-terminal side of Lys and Arg residues
   C) is pH dependent
   D) results in the formation of homoserine lactone residues
   E) requires SDS and β-mercaptoethanol

7. **You have been given a pure sample of Protein X. You make a 1:10 dilution of the sample and measure the A_{280} of the sample. The value you obtain is 0.536. Given that the ε_{280} of Protein X is 0.785 ml mg^{-1} cm^{-1} and the molecular mass is 61.1 kDa, what is the molar concentration of protein in your sample assuming a path length of the cuvette of 1 cm?**
   A) 0.112 μM
   B) 1.12 μM
   C) 11.2 μM
   D) 112 μM
   E) 1.12 mM

8. **Which of the following BLAST programs requires a protein sequence as the query sequence?**
   A) blastn
   B) blastx
   C) blastp
   D) tblastx
   E) tblastn

9. An enzyme displays a K_M of 1 mM and V_{max} of 5.1 mmol.s^{-1}. In the presence of 0.35 mM inhibitor, the K_M was measured to be 3 mM and the V_{max} was unchanged. What is the dissociation constant for the inhibitor?
    A) 0.175 mM
    B) 0.70 mM
    C) 2.1 mM
    D) 5.12 mmol s^{-1}
    E) 15 mM

10. The rate constant for a chemical reaction is 8.0×10^{-1} s^{-1} at 6 °C. The activation energy (E_a) is 30 kJmol^{-1}. If the temperature is raised by 5 °C, what is the new rate constant? Assume that the Gas constant, R = 8.31 J mol^{-1} K^{-1}.
    A) 8.0×10^{-1} Jmol^{-1} s^{-1}
    B) 1.0 s^{-1}
    C) 3.0 s
    D) 38 s
    E) 40 s^{-1}

11. Which of the following statements is/are true about suicide inhibitors?
    A) They are competitive inhibitors
    B) They irreversibly modify the active site
    C) They are treated as substrates by the enzyme
    D) They resemble the transition state
    E) They degrade inhibitors bound to the active site

12. What is the catalytic efficiency of an enzyme that has a K_M of 0.36 nM and a turnover number of 200 s^{-1}?
    A) 0.018 nM min
    B) 0.56 μM^{-1} s^{-1}
    C) 2.0 μM s
    D) 72 nM s^{-1}
    E) 200 nM^{-1} min^{-1}

13. In the presence of 0.64 mM uncompetitive inhibitor the V_{max} was reduced by 38% relative to the uninhibited reaction. What is the K_I for the inhibitor?
    A) 0.45 mM
    B) 0.64 mM
    C) 1.04 mM
    D) 3.86 mM
    E) 6.48 mM

14. 'Specific acid' catalysis:
    A) is dependent on the proton concentration
    B) refers to catalysis by a specific protonated amino-acid side chain in an enzyme
    C) is dependent on a specific Brønsted acid
    D) refers to catalysis when pH < 7
    E) refers to catalysis by Brønsted acids not Lewis acids

15. **Which of the following statements is/are true about a catalytically perfect enzyme?**
   A) It is a multienzyme complex
   B) The turnover number is around 10^8-10^9 s^{-1}
   C) It turns over a substrate every time it encounters one
   D) The catalytic efficiency is around 10^8-10^9 M^{-1}s^{-1}
   E) It functions at extremes of temperature and pH

16. **The pK$_a$ of an aspartic acid side chain in pepsin is 4.0. What percentage is protonated at pH 6.6?**
   A) 0.069
   B) 0.25
   C) 0.33
   D) 5.7
   E) 10

17. **The pK$_a$ of a lysine side chain will decrease:**
   A) if the pH of the solution is decreased
   B) if placed in a more hydrophobic environment
   C) if placed close to a metal ion
   D) if placed close to a Glu side chain
   E) if placed close to an Arg side chain

18. **Which of the following intermediates in the Krebs cycle is formed by an oxidoreductase?**
   A) Citrate
   B) Isocitrate
   C) α-ketoglutarate
   D) Succinyl CoA
   E) Malate

19. **What is the common name of the molecule shown below?**
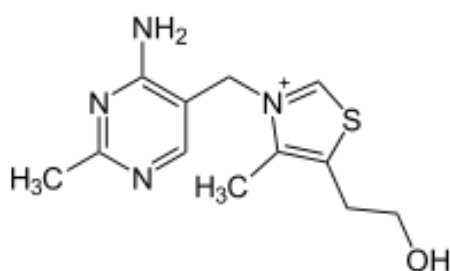
   A) Thiamine
   B) Pyridoxine
   C) Biotin
   D) Niacin
   E) Riboflavin

20. Which of the following molecules can carry 2-carbon units?
- A) CoA
- B) CoQ
- C) FMN
- D) Biotin
- E) Lipoamide

21. Which of the following strategies is/are exploited by elastase during catalysis?
- A) Acid/base catalysis
- B) Metal ion catalysis
- C) Covalent catalysis
- D) Electrostatic catalysis
- E) Preferential binding of the substrate

22. The pyruvate dehydrogenase complex:
- A) is an enzyme of the citric acid cycle
- B) is a membrane-bound respiratory enzyme involved in quinone reduction
- C) contains TPP
- D) contains pyridoxal phosphate
- E) shows substrate channelling

23. Which of the following is/are true about lysozyme?
- A) It is a lyase that catalyses the cleavage of polysaccharide molecules
- B) It has binding sites for 6 sugar residues (subsites A-F)
- C) It cleaves the glycosidic bond linking the sugars in subsite C and subsite D
- D) The sugar in subsite D adopts a half-chair conformation upon binding
- E) The Koshland mechanism proposes the formation of a glycosyl-enzyme intermediate

24. Which of the following is/are true about the Koshland-Némethy-Filmer model of allosteric regulation?
- A) Binding is described in terms of two dissociation constants
- B) The rate of reaction follows Michaelis-Menten kinetics
- C) Binding of ligand induces structural changes in neighbouring subunits
- D) Both negative and positive cooperativity can be explained
- E) It is unable to model the binding of oxygen to haemoglobin

25. Which of the following is/are true about the binding of oxygen to haemoglobin?
- A) Binding occurs at a covalently bound haem
- B) Oxygen is released by a reduction in pH
- C) Binding of oxygen has a Hill coefficient of 4
- D) Haemoglobin is a homotetramer
- E) Haemoglobin and myoglobin are examples of convergent evolution

A TOTAL OF FOUR QUESTIONS MUST BE ANSWERED FROM SECTIONS B AND C, WITH AT LEAST ONE QUESTION ANSWERED FROM SECTION B AND AT LEAST ONE QUESTION ANSWERED FROM SECTION C. Each question is worth 18.75% of the total marks. Candidates should allow about 135 minutes for sections B and C. **USE A SEPARATE ANSWER BOOK FOR EACH QUESTION.**

26. You have been given a mixture of the following untagged native proteins to separate.

Protein	pI	Molecular mass (kDa)
A	5.3	53
B	5.7	48
C	7.4	125
D	8.2	10

    a) With the aid of diagrams, describe how you would obtain as pure a sample of Protein A as possible, explaining your strategy. (40%)
    b) Briefly outline how you will assess how successful your purification has been. (20%)
    c) Which of the other proteins is likely to be the main contaminant of your purified Protein A extract and why? (10%)
    d) Suggest an alternative purification strategy to purify Protein A involving an affinity tag, describing the experimental procedure you would follow. (30%)

27. An unknown protein sequence from Plasmodium falciparum is BLAST searched against the NCBI non-redundant protein database. The unknown sequence has a length of 316 amino acids and shown below are the top two alignments (HSPs) produced by BLAST:

Hypothetical protein [Theileria annulata]
Length: 322

Score	Expect	Method	Identities	Positives	Gaps
318 bits(815)	5e-105	Compositional matrix adjust.	158/311(51%)	216/311(69%)	2/311(0%)

```
Query  7    IVLVGSGMIGGVMATLIVQKNLGDVVLFDIVKNMPHGKALDTSHTNVMAYSNCKVSGSNT  66
            I L+GSG IGG+M  L      L DV   DIV N+  GK+LD  H N +    K  G+N
Sbjct  10   ISLIGSGNIGGIMGYLTQLTELADVNFLDIVPNIGAGKSLDIMHANSIQGKAYKCKGTNN  69

Query  67   YDDLAGADVVIVTAGFTKAPGKSDKEWNRDDLLPLNNKIMIEIGGHIKKNCPNAFIIVVT  126
            Y+D++G+DV IVTAG  KAP KS++EWNRDDL+  N+KI+ ++G +IKK  P AF+IV+T
Sbjct  70   YEDISGSDVCIVTAGLAKAPTKSNEEWNRDDLVGYNSKIIRDVGENIKKYAPEAFVIVIT  129

Query  127  NPVDVMVQLLHQHSGVPKNKIIGLGGVLDTSRLKYYISQKLNVCPRDVNAHIVGAHGNKM  186
            NP+DVMV L+ + +G PKN ++G+GG+LD+SR+  YI++KL V P+ V+   ++GAHG+ M
Sbjct  130  NPMDVMVHLMLKVTGFPKNMVVGMGGLLDSSRMNCYIAEKLGVNPKYVHGSVIGAHGDSM  189

Query  187  VLLKRYITVGGIPLQEFINNKLISDAELEAIFDRTVNTALEIVNLH--ASPYVAPAAAII  244
            + L    TV GIP+ +F+    I+  +++ I +RTV +A EI+ L+    S Y APA A I
Sbjct  190  IPLVSRSTVYGIPILQFVEQGYITMEDIKEIEERTVTSAFEILKLYGSGSSYFAPATAAI  249

Query  245  EMAESYLKDLKKVLICSTLLEGQYGHSDIFGGTPVVLGANGVEQVIELQLNSEEKAKFDE  304
            EMA SYL D K V  CS   LEGQYGH DI+ GTP V+GANGVE+V EL+L  EE+ K+D
Sbjct  250  EMASSYLNDKKCVFPCSCYLEGQYGHRDIYCGTPAVIGANGVEKVFELKLTPEEQDKYDA  309

Query  305  AIAETKRMKAL    315
            +I E KR++AL
Sbjct  310  SIKEIKRLEAL    320
```

L-lactate dehydrogenase [Theileria parva strain Muguga]
Length: 321

Score	Expect	Method	Identities	Positives	Gaps
314 bits(804)	2e-103	Compositional matrix adjust.	151/311(49%)	216/311(69%)	2/311(0%)

```
Query  7    IVLVGSGMIGGVMATLIVQKNLGDVVLFDIVKNMPHGKALDTSHTNVMAYSNCKVSGSNT  66
            I L+GSG IGG+M  L      L D V FDIV N+  GK+LD  H N +     K  G+N
Sbjct  9    ISLIGSGNIGGIMGYLSQLTELADTVFFDIVPNIGAGKSLDIMHANSIQGKAYKCKGTNN  68

Query  67   YDDLAGADVVIVTAGFTKAPGKSDKEWNRDDLLPLNNKIMIEIGGHIKKNCPNAFIIVVT  126
            Y D+AG+DV IVTAG  KAP KS++EWNRDDL+  N KI+ E+   +IKK  P AF+IV+T
Sbjct  69   YKDIAGSDVCIVTAGLAKAPAKSNEEWNRDDLVAFNAKIITEVAENIKKYAPKAFVIVIT  128

Query  127  NPVDVMVQLLHQHSGVPKNKIIGLGGVLDTSRLKYYISQKLNVCPRDVNAHIVGAHGNKM  186
            NP+DVMV L+ + +G  KN ++G+GG+LD+SR+  YI++KL V P+ V+   ++GAHG+ M
Sbjct  129  NPMDVMVHLMLKVTGFSKNMVVGMGGLLDSSRMNCYIAEKLGVNPKYVHGSVIGAHGDSM  188

Query  187  VLLKRYITVGGIPLQEFINNKLISDAELEAIFDRTVNTALEIVNLH--ASPYVAPAAAII  244
            + L    TV GIP+ +F+     ++  +++ I +RT+ +A+EI+ L+    S Y APA A I
Sbjct  189  IPLVSRSTVYGIPILDFVEKGYLTHEDIKEIEERTITSAIEILKLYGSGSSYFAPATAAI  248

Query  245  EMAESYLKDLKKVLICSTLLEGQYGHSDIFGGTPVVLGANGVEQVIELQLNSEEKAKFDE  304
            EMA +YL D K V  CS   LEGQYGH +++ GTP V+GANGVE+V+EL+L   +E+ KF++
Sbjct  249  EMASAYLNDKKSVFPCSCYLEGQYGHKEVYCGTPAVIGANGVEKVLELKLTPQEQQKFND  308

Query  305  AIAETKRMKAL    315
            +I E +R+++L
Sbjct  309  SIKEIRRLESL    319
```

Question continues on next page

a) Based on these two alignments what can be inferred about the query protein sequence and why? (30%)
b) The full sequences of the top 10 alignment hits were extracted from the database and aligned with ClustalO to produce a multiple sequence alignment (MSA). What additional information would the MSA provide that could not be obtained from the BLAST output and why? (30%)
c) A phylogenetic tree was created from the MSA and it is unrooted. What is the difference between a rooted and unrooted tree? (20%)
d) How would you root the unrooted tree? (20%)

28. The initial rate of reaction (v_0) has been measured for an enzyme in the presence and absence of an inhibitor at different substrate (S) concentrations.

[S] (mM)	v_0 (mM/min) no inhibitor	v_0 (mM/min) +0.03µM inhibitor
0.080	10.7	3.56
0.16	14.8	4.92
0.24	16.9	5.65
0.50	20.0	6.67
1.0	21.8	7.27

a) Using a Lineweaver-Burk plot calculate the V_{max} and K_M values for both the uninhibited and inhibited reactions. (50%)
b) What type of inhibition does the inhibitor confer? Explain the mode of action of this inhibitor. (25%)
c) Calculate the dissociation constant for inhibitor binding. (25%)

29.
a) Define what is meant by general acid and general base catalysis. (10%)
b) The enzyme galactose mutarotase from *E. coli* catalyses the conversion of β-D-galactose to α-D-galactose via an acyclic intermediate. Residues His-104 and Glu-309 are thought to be involved in catalysis. Using curly arrows, propose a plausible mechanism, including the structures of the amino-acid side chains. (70%)
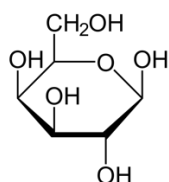
β-D-galactose

c) A second histidine residue, His-175, is also found in the active site. Explain how you would test whether just one or both of the two His residues are required for catalysis. (20%)

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30. Explain with the aid of diagrams the biochemical properties of the peptide bond as well as the key information related to the peptide bond that is represented in a Ramachandran plot. Describe what the different regions of the plot represent and what is meant by the allowed and non-allowed regions.

31. Describe the mode of action and kinetics of competitive (33%), uncompetitive (33%) and mixed inhibition (34%); for each type of inhibition, provide the modified Michaelis-Menten equation (**DO NOT** derive the equations), show a hypothetical Lineweaver-Burk plot (with labels) and discuss which kinetic parameters each inhibitor affects.

32. Define the term 'co-enzyme' and explain **with examples** the roles that **five different co-enzymes of your choice** play in catalysis.

33. Describe how binding of oxygen to haemoglobin is regulated (50%) and, with the aid of oxygen-binding curves, explain the reason why high altitude training is of benefit to athletes competing at sea level (50%).

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