HWA CHONG INSTITUTION
JC2 Preliminary Examinations
Higher 3

CANDIDATE NAME		**CT GROUP**	**14S ___**
CENTRE NUMBER		**INDEX NUMBER**	

PROTEOMICS

9815 / 01

Paper 1

Additional Materials: Writing Paper

List of Amino Acids

18 September 2015

2 hours 30 minutes

INSTRUCTIONS TO CANDIDATES

Write your **name**, **CT group**, **Centre number** and **index number** in the spaces provided at the top of this cover page and every sheet of writing paper used.

SECTION A

This section contains **five** questions. Answer **all** the questions.

SECTION B

This section contains **four** questions. Answer **three** out of **four** questions.

SECTION C

Answer the question.

BEGIN EACH QUESTION ON A FRESH SHEET OF WRITING PAPER. A **NIL RETURN** is required.

INFORMATION FOR CANDIDATES

The number of marks is given in brackets [ ] at the end of each question or part question.

The use of an approved scientific calculator is expected, where appropriate.

You are reminded of the need for clear presentation in your answers.

For Examiners' Use	
1	/ 8
2	/ 8
3	/ 12
4	/ 12
5	/ 10
Section A	/ 50
6	/ 10
7	/ 10
8	/ 10
9	/ 10
Section B	/ 30
10	/ 20
Total	/ 100

Section A

Answer all the questions in this section.

QUESTION 1

Rheumatoid arthritis (RA) is an autoimmune disease that primarily affects joints. In its chronic inflammation phase, IL-6 binds to IL-6 receptor (IL-6R) on the immune cell surface, leading to excessive signalling and symptoms of the disease.

(a) Explain the role of signal peptide in IL-6 receptor. [2]

Antibodies against the IL-6R can be used as drugs to treat RA. The binding of antibodies to IL-6R leads the endocytosis and subsequent lysosomal degradation of both IL-6R and its bound antibodies, attenuating IL-6 signalling.

Tocilizumab is a modified antibody against the IL-6R to treat RA. It could rapidly dissociate from IL-6R within the acidic environment of the endosome (pH 6.0) while maintaining its binding affinity to IL-6R in plasma (pH 7.4). Therefore, only IL-6R is degraded, but Tocilizumab can be freed to bind another IL-6R molecule in plasma**.**

(b) Describe the change in binding forces between IL-6R and Tocilizumab at pH 6.0 and pH 7.4. [3]

(c) Suggest the region(s) and identity of amino acid(s) that were modified in Tocilizumab to enable its recycling for repeated actions in plasma. [2]

(d) Suggest one benefit of engineering pH dependency into the interactions of therapeutic antibodies with their targets. [1]

QUESTION 2

A protein can be made up of more than one subunit. Protein complexes are formed by interactions between many proteins.

(a) Describe one protein-protein interaction between:

(i) the subunits of a named protein [2]

(ii) named proteins in a complex [2]

(b) Explain the significance of pyruvate dehydrogenase as a protein complex. [4]

QUESTION 3

Myogenin is a basic helix-loop-helix (HLH) transcription factor involved in the differentiation of muscle cells. Myogenin is kept non-functional via:

- binding of myogenin to Id, another HLH protein
- phosphorylation of myogenin's DNA–binding domain

(a) Suggest how the modifications act to keep myogenin non-functional. [2]

(b) Explain the use of an *in vivo* system to identify the interaction partners of myogenin. [4]

(c) Name an interaction partner of myogenin that cannot be identified by the system described in **(b)**. [1]

Fig. 3.1 shows the Western blot profile of an experiment in which myoblasts were cultured in a differentiation medium (DM) for six days.

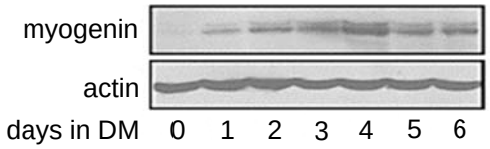


Fig. 3.1

(d) Describe how proteins from muscle cell samples can be prepared for SDS-PAGE. [3]

(e) Discuss why actin was included in the Western blot analysis. [2]

QUESTION 4

IRF-3 is a transcription factor that has been characterised to contain several functional domains including a nuclear export signal, a DNA-binding domain, a C-terminal IRF association domain and several regulatory phosphorylation sites.

(a) Explain how mass spectrometry can be used to study the phosphorylation of IRF-3. You may sketch a graph to illustrate your answer. *(Hint: Enzyme alkaline phosphatase catalyses dephosphorylation. HPO_3 = 80 Da)* [4]

(b) Describe how co-immunoprecipitation (co-IP) can be used to detect the interaction partner(s) of IRF-3 and suggest an advantage of such an *in vitro* system. [3]

(c) The location of a protein may also suggest its functions. It was hypothesised that phosphorylation of IRF-3 leads to its dimerisation with IRF-7, and subsequent nuclear transport.

(i) Suggest an experiment with a proper control to verify this hypothesis. [4]

(ii) Suggest a potential problem in the approach you have suggested. [1]

QUESTION 5

Succinate dehydrogenase is a member of the dehydrogenase protein family and it is the only enzyme that participates in both the Krebs cycle and the electron transport chain.

(a) Explain what is meant by a *protein family*. [2]

Succinate dehydrogenase complex subunit A (SDHA) is a subunit of succinate dehydrogenase.

(b) Explain how FAD^+ facilitates the function of SDHA. [2]

(c) Describe the structure of SDHA and the function of its FAD domain. [4]

SDHA is a substrate for SIRT3, which is a deacetylase. SDHA has 13 acetylation sites through which interaction with SIRT3 is achieved.

(d) Explain how SDHA can be a substrate of SIRT3. [2]

Section B

Answer 3 out of the 4 questions in this section.

QUESTION 6

(a) Describe how ultracentrifugation can be used to determine the molecular weight of haemoglobin. [3]

(b) Explain how the number of haemoglobin subunits can be determined. [3]

(c) Distinguish between velocity sedimentation and equilibrium sedimentation. [4]

QUESTION 7

(a) Discuss the importance of hydrogen bonds in proteins other than those found in protein structures. [3]

(b) Explain how unique amino acid sequences in proteins are specified by genes. [3]

(c) Outline the principle of nuclear magnetic resonance (NMR) and x-ray crystallography in protein structure determination. [4]

QUESTION 8

(a) Discuss the mechanism of enzyme action. [4]

(b) Explain how protein's conformation is caused by side-chain chemical groups using the following examples:

(i) catalytic triad [3]

(ii) hydrophobic and hydrophilic cleft [3]

QUESTION 9

Haemagglutinin (HA) has been studied greatly due to its importance in the reproductive cycle of the influenza virus. Other than its role in binding to receptors on target cells during adsorption, HA also mediates the entry of the influenza viral genome into the cytoplasm by a membrane-fusion event.

(a) Discuss the structural changes of HA in facilitating the release of the influenza viral genome into the cytoplasm. [6]

(b) Explain the necessity for annual vaccine updates and the challenges behind developing vaccines. [4]

Section C

Answer the question in this section.

QUESTION 10

In 1998, Andrew Fire and Craig Mello reported the use of RNA interference (RNAi) as a potent means of gene silencing in *Caenorhabditis elegans*, a soil-dwelling roundworm. RNAi is a useful tool used in the study of developmental biology, where signalling between cells induces cell-fate changes and organ formation.

(a) Suggest the features that make *C. elegans* a model organism in the study of the genetics of development. [4]

(b) Describe the mechanism of RNAi. [6]

Multipurpose minitransposon (mTn) technology is another powerful tool used for genetic studies.

(c) Discuss the advantages and disadvantages of using mTn over RNAi in genetics studies. [4]

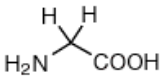
(d) Explain how bioinformatics can analyse disrupted gene sequences generated by mTn technology in a large scale mutant screen. [4]

(e) Suggest how you would verify if the mTn or RNAi has worked. [2]

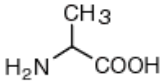
APPENDIX

LIST OF AMINO ACIDS

Small

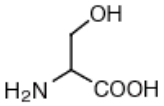


Glycine (Gly, G)
MW: 57.05

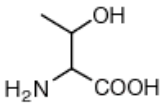


Alanine (Ala, A)
MW: 71.09

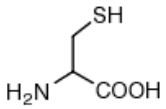
Nucleophilic



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

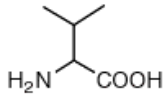


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

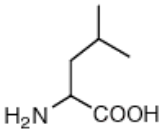


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

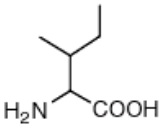
Hydrophobic



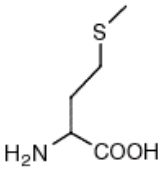
Valine (Val, V)
MW: 99.14



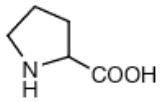
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

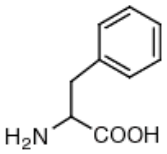


Methionine (Met, M)
MW: 131.19

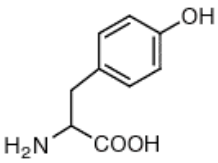


Proline (Pro, P)
MW: 97.12

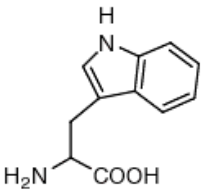
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

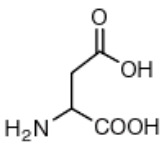


Tyrosine (Tyr, Y)
MW: 163.18

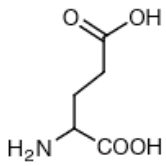


Tryptophan (Trp, W)
MW: 186.21

Acidic

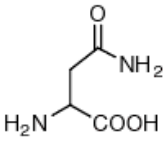


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

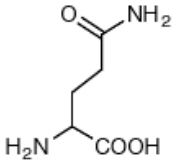


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

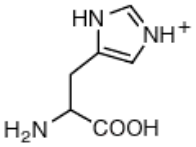


Asparagine (Asn, N)
MW: 114.11

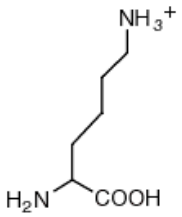


Glutamine (Gln, Q)
MW: 128.14

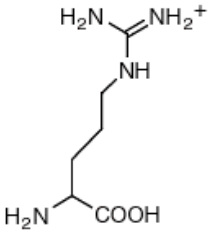
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

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