Part I (18 points)

1. (3 points) What is the pH 0.1 M HCl? (a) 0.90, (b) 1.26, (c) 2.26, (d) 3.26
2. (3 points) What is the pH of a 0.05 M solution of acetic acid? (The Ka of acetic acid = 1.8 x 10^-5)
   (a) (pH = 1), (b) (pH = 2), (c) (pH = 3), (d) (pH = 4)
3. (3 points) What is the ratio of [lactate]/[lactic acid] at pH = 5.85? (The pKa of lactic acid = 3.85)
   (a) 100, (b) 0.01, (c) 0.1, (d) 1
4. (3 points) Knowing what we know about the thermodynamics and kinetics of an enzyme, adding an
   enzyme would do the following for a reaction:
   (a) Lower ΔG of the reaction, (b) Lower Ea, (c) Shift Keq, and produce more products, (d) Increase
   Ea and increase reaction rate.

5. (3 points) The following is an enzyme catalyzed reaction:
   1,3-bisphosphoglycerate + ADP ⇄ 3-phosphoglycerate + ATP
   At the start of the reaction, the concentrations of 1,3 bisphosphoglycerate and ADP were 0.1 M each,
   with no products present. However, after the reaction reached equilibrium, the concentration of
   3-phosphoglycerate was found to be 0.0501 M. What is the Keq of the reaction?
   (a) Keq = 1.008; (b) Keq = 0.992; (c) Keq = 0.0499; (d) Keq = -0.0499

6. (3 points) If we know the Gibbs Free Energy of the following two reactions:
   (1) ATP + H2O ⇄ ADP + Pi
       ΔG1 = -33.5 kJ/mol; Keq = 7.485 x 10^5
   (2) 1,3-bisphosphoglycerate + H2O ⇄ Pi + 3-phosphoglycerate
       ΔG2 = -50.2 kJ/mol; Keq = 6.476 x 10^8
   What are the ΔG and Keq for the following reaction?
   (3) 1,3-bisphosphoglycerate + ADP ⇄ 3-phosphoglycerate + ATP

   (a) ΔG = -402 kJ/mol, Keq = 6.483 x 10^8; (b) ΔG = -16.7 kJ/mol, Keq = 865.2; (c) ΔG = -83.7 kJ/mol,
   Keq = 4.84 x 10^14; (d) ΔG = -16.7 kJ/mol, Keq = 6.483 x 10^8.
Part II (12 points)

© Match the statement with answer. Total 12 questions, 1 point for each correct match.

[A] Primitive type of cells such as bacteria, with a single chromosome and no nuclear membrane
[B] The new strand of DNA that is synthesized in short pieces and joined together later
[C] The new strand of DNA that is synthesized continuously during replication
[D] Cell formed by union of sperm and egg which develops into a new individual
[E] Mixing of genetic information from two chromosomes as a result of crossing over
[F] Sequence of mRNA (or corresponding region of DNA) that can be translated to give a protein
[G] Advanced cells of higher organisms that have several chromosomes within a compartment called the nucleus
[H] Process by which information from DNA is converted into its RNA equivalent
[I] The manner in which a long strand of helical DNA is twisted so that it can fit into a cell
[J] Group of three RNA or DNA bases which encodes a single amino acid
[K] Enzyme that makes most of the DNA when chromosomes are replicated
[L] The strand of DNA equivalent in sequence to the messenger RNA, sometimes referred to as the non-template strand, the strand of the DNA which is not read during transcription

① gametes ② quorum sensing ③ supercoiling
② Mendelian ratios ③ luciferase ④ translation
② ribosome ④ global regulation ⑤ recombination
② somatic cell ④ codon ⑥ open reading frame
③ anaphase ④ lagging strand ⑥ coding strand
⑥ DNA polymerase I (Pol I) ⑥ DNA polymerase II (Pol II)
⑤ eukaryotic cell ⑥ anti-Shine-Dalgarano sequence
⑤ Crp protein ⑥ lactose ⑦ DNA polymerase III (Pol III)
③ prokaryotic cell ⑥ meiosis ⑧ zygote
③ complementary base pairing ⑥ complementary sequences ⑨ transport protein
① sigma subunit ⑥ leading strand ⑨ DNA sensor
④ replication ⑥ operon ⑨ nuclear membrane
⑤ primer ⑥ competitive inhibitor ⑨ regulatory region
⑤ deoxyribose ⑥ DNA ligase ⑨ mitosis
⑥ core enzyme ⑥ nucleic acid ⑨ primase
⑥ Transcription ⑥ replication fork ⑨ antiparallel
③ mutation ⑥ antisense RNA ⑨ conjugation bridge
③ supercoiling ⑥ translation ⑨ co-dominance

[A] (  ) [B] (  ) [C] (  ) [D] (  ) [E] (  ) [F] (  )
[G] (  ) [H] (  ) [I] (  ) [J] (  ) [K] (  ) [L] (  )

Part III (70 points)

Please read the questions carefully and refer to the following text. The text was copied from a recent publication. Please be skeptical to the conclusions drawn by the author. When you answer, use ONLY the evidences provided in the text.
Questions:
1. Production of CBP3 was induced by IPTG added to the E. coli cell culture. Notice that the author performed extra steps to purify CBP3. What are the basic features for (a) the cloning vector, and (b) the E. coli host BL21 (DE3)? Please be brief. (10 points)
2. In the result 3.2, the author stated “recombinant CBP3 retained its binding activity to Ca2+ and underwent similar conformational change with native CBP3 upon Ca2+ binding”. Do you agree or disagree? State the evidences if you agree or the skeptical points if you disagree. Please also state your rationale. (10 points)
3. In Fig. 4 the author characterize the structure of CBP3 using CD, then summarized the secondary structures of CBP3 in Table 1. There is probably too much conclusion drawn by this kind of evidence. Can you tell the reason why? (10 points)
4. The molecular weights of CBP3 in Fig. 2 and in Fig. 3 are obviously different relative to the molecular markers. Can you tell the reason why? (10 points)
5. If you are to prove the conformational change induced by Ca2+ binding, what type of experiment you intend to perform? And what is the conclusion you will expect? (10 points)

Text:
1. Basic concept

*Dictostelium discoideum*: A slime mold.
Calmodulin and Troponin C: Two very well-known calcium-binding proteins.
2. Introduction

Calcium ion (Ca^{2+}) regulates wide variety of cellular processes such as cell-cycle progression, differentiation, muscle contraction, signal transduction, nucleotide metabolism, and enzyme activities. These roles of calcium ion must be mediated by a variety of calcium-binding proteins. Calcium-binding proteins can act as buffers storing and releasing Ca^{2+}, or as sensors detecting Ca^{2+} concentration and transducing the signals. These calcium-binding proteins have highly conserved helix-loop-helix structure EF-hand motifs, with a Ca^{2+} ion bound to the inter-helical loop region. In general, a pair of EF-hand helix-loop-helix motifs forms a globular domain. Proteins containing four such motifs will thus have two domains that can be either structurally dependent or independent. Upon Ca^{2+} binding, the sensor proteins undergo conformational change and in turn regulate a large number of target proteins.

*Dictostelium* calcium-binding protein 3 (CBP3) is a small acidic protein, contains four putative EF-hand motifs, and is expressed at the developmental stage. Although several small acidic calcium-binding proteins have been also reported in *Dictostelium*, the structural properties of the calcium-binding proteins have not been reported yet. To characterize structural properties and calcium affinity features of the CBP3 protein, CBP3 and its N- and C-terminal domains were over-expressed in Escherichia coli, respectively, and their conformational properties were characterized.

3. Experimental results
3.1. Expression of recombinant CBP3 and its N- and C-terminal domains

CBP3 is one of the several calcium-binding proteins in *D. discoideum* and contains four EF-hand motifs (residues D19-E30, D58-E69, D102-E113, and D137-E148) (Fig. 1A). We constructed recombinant CBP3, N- and C-terminal domains of CBP3 to
investigate their Ca\textsuperscript{2+}-binding properties and conformational changes induced by Ca\textsuperscript{2+}. CBP3 and its N- and C-terminal domains were cloned into E. coli expression vector pET15b. N- and C-terminal domains of CBP3 consist of residues 1–83 and 84–160, respectively (Fig. 1B) and each domain contains two EF hand motifs. These recombinant proteins were expressed in E. coli strain BL21 (DE3) and the average yields were about 10-25 mg of protein/L of LB medium. The majority of CBP3 proteins was found in the insoluble fraction, inclusion body (Fig. 2A). To solubilize the recombinant CBP3 proteins, inclusion bodies were dissolved in 6 M urea, and purified by nickel-affinity chromatography under denatured condition, and refolded. The N-terminal His-tag leader was removed by thrombin. The cleavage of the His-tag leader and the purity of the protein were assayed by SDS-PAGE. They appeared as single bands on SDS-PAGE under reducing conditions (Fig. 2B).

Fig. 1. (A) Graphical representation of CBP3. Four EF-hand motifs are represented with gray box and numbered with I–IV. The vertical line between EF-hand II and III indicates the cleavage site. (B) Sequences of CBP3 and its N- and C-terminal domains. EF-hand motifs were underlined and italicized in bold. The boundary residues between N- and C-terminal domains were Glutamate (E) and Glutamine (Q). The cleaved site between N- and C-terminal domains was boxed.

Fig. 2. CBP3 expression in E. coli. (A) CBP3 expression profile in E. coli BL21 (DE3) pLysS cells harboring pET-CBP3. E. coli BL21 cells were induced with 1 mM IPTG and prepared at the indicated time after induction. M, molecular size marker (116, 66, 45, 35, 25, 16.4, and 14.4 kDa from upper band); lane 1, cell lysate at 0 h after induction; lane 2, cell lysate at 3 h after induction; lane 3, soluble fraction of cell lysate at 3 h after induction; lane 4, insoluble fraction of cell lysate at 3 h after induction. (B) Purified CBP3 (lane 1), its N- (lane 2) and C-terminal (land 3) domains after the removal of His-tag leader by thrombin. M, molecular size marker (26.6, 17, 14.4, and 6.5 kDa from upper band).

3.2. Calcium induced electrophoretic gel mobility shift of recombinant CBP3

Calcium-binding proteins show different mobility in SDS-PAGE depending on the presence of calcium or EGTA. The refolded recombinant CBP3 protein in SDS-PAGE also migrated slowly in the presence of EGTA (Fig. 3). These results demonstrated that recombinant CBP3 retained its binding activity to Ca\textsuperscript{2+} and underwent similar conformational change with native CBP3 upon Ca\textsuperscript{2+} binding. Besides the CBP3, N- and C-terminal domains also showed a slight mobility shift in gel.
Fig. 3. Calcium-induced gel mobility shift of recombinant CBP3 and its N- and C-terminal domains.

Equal amount of CBP3 with 2 mM CaCl$_2$ (lanes 1, 3, and 5) or 2 mM EGTA (lanes 2, 4, and 6) were loaded on 13% SDS-PAGE. M, molecular size marker (116, 66, 45, 35, 25, 16.4, and 14.4 kDa from upper band); lanes 1 and 2, CBP3; lanes 3 and 4, N-terminal domain; lanes 5 and 6, C-terminal domain.

Fig. 4. Far-UV CD spectra of CBP3 (A) and its N- (B) and C-terminal (C) domains. The far-UV CD spectra were recorded on Jasco J715 spectropolarimeter at ambient temperature. Protein concentration was approximately 8 uM. Each protein was in apo- (dotted line) and 2 mM calcium saturated states (solid line) in 20 mM MOPS buffer, pH 7.2. Measured ellipticities were converted to molar ellipticity, [θ] and the quantification of the contents of secondary structure was carried out with the self-consistent method.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The secondary structure composition of CBP3 and its domains calculated from CD spectra$^b$ in the absence or presence of calcium ion$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CBP3</td>
</tr>
<tr>
<td></td>
<td>Ca$^{2+}$</td>
</tr>
<tr>
<td>α-helix</td>
<td>32.5</td>
</tr>
<tr>
<td>Turs</td>
<td>27.9</td>
</tr>
<tr>
<td>β-sheet and other structure</td>
<td>41.6</td>
</tr>
</tbody>
</table>

$^a$ The secondary structure contents were calculated from Dicoprot [31] program based on the self-consistent method [30].

Text:

A chitinase, Ech30 was purified from yeast. Chitinase degrades chitin, which is the polymeric form
of N-acetyl-D-glucosamine (GlcNAc). Examine the following figures and answer the questions.

6. From Fig. 5 and Fig. 6, can you suggest the specific cutting site for Ech30? (10 points)

7. It seems that each oligosaccharide yields two peaks. Why? (10 points)

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**Fig. 5** Time course of GlcNAc degradation and product formation by Ech30 as analyzed by HPLC on a TSK gel amide 80 column. The top panel shows a standard mixture of GlcNAc oligomers. The other panels show how the reaction proceeds (incubation times are indicated).

**Fig. 6** Time course of product formation upon β-chitin degradation by Ech36. The top panel represents a standard mixture of GlcNAc oligomers.