CW#2 (due May 7th @17:00, 2024)
Instructions:

i. This assignment consists of 50% of the CW marks of BIO312;

ii. You can refer to any materials, but please complete the coursework by yourself.

iii. Please submit your work in Word or PDF format;

iv. Marking: 50 marks for answering Q1-Q2.

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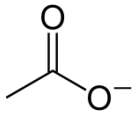
1. Acetyl-CoA is at the core of central metabolism and, in bacteria and plants, can be made from acetate **(1)** and coenzyme A in an ATP-driven reaction catalysed by acetyl-coenzyme A synthetase **(2)**. Protein X is an acetyl-coenzyme A synthetase in *Bacillus subtilis*.



(1)

ATP + acetate + CoA <=> AMP + pyrophosphate + acetyl-CoA **(2)**

When purified protein X was incubated with [1-^{14}C]acetyl-CoA in the presence or absence of a smaller additional protein from *B. subtilis* protein termed AcuA. Autoradiography using a Phosphor Imager indicated that radioactivity was incorporated into the protein X when AcuA was present (**Figure 1**).

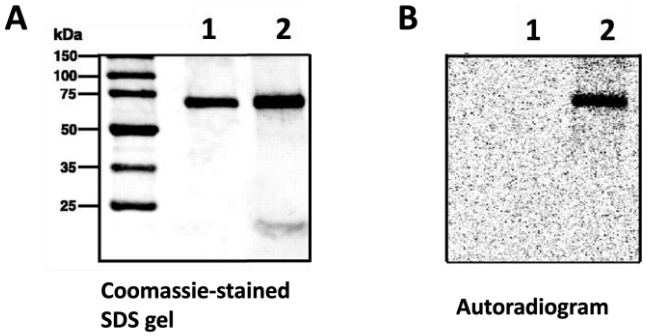


Figure 1. Results of incubating AcsA proteins with radioactive [1-^{14}C]acetyl-CoA. A. Lane 1, *Protein X*; lane 2, *Protein X mixed with* AcuA; B. Autoradiogram of the left panel.

(a) Please analyse the results. **[5 marks]**
This Autoradiogram shows that radioactivity is incorporated into Protein X only when AcuA is present. More detail, after the small protein AcuA is added to the experimental group (lane 2), the original isotope [1-^{14}C] of acetyl-CoA is transferred to protein X through acetate (1) due to the reverse reaction of (2). Then shows the difference visualization result in Autoradiogram (Fig 1).

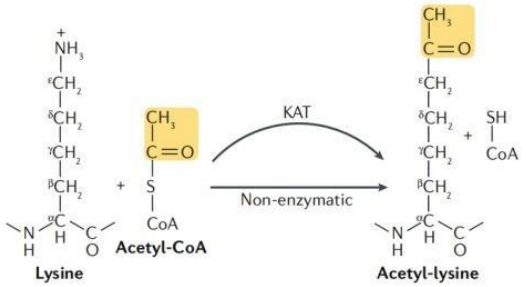
(b) Briefly describe what technique can be used to confirm the results. **[5 marks]**
Mass spectrometry (MS) can be used to confirm the results. Specifically, we can employ matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) or electrospray ionization mass spectrometry (ESI-MS).

(c) Previous MS studies found that a single peptide of protein X, with the sequence SGKIMR and a mass of 690.5 Da, was affected when the protein X was incubated with AcuA. Which

amino acid residue in the peptide is likely to be affected? Draw a diagram of the process. **[10 marks]**

Serine(87.03203)-Glycine(57.02146)-Lysine(128.09496)-Isoleucine(113.08406)-Methionine(131.04049)-Arginine(156.10111)

Since lysine (K) residues are known for acetylation, it's probable that the lysine residue in SGKIMR is affected. The process is shown below:



(d) When protein X was incubated with acetate, ATP and CoA, it produced AMP with a specific activity of 3.3 ± 0.41 μmol of AMP released per min per mg of protein. However, when it was incubated under the same conditions but additionally with AcuA, there was no detectable activity. What implications do these results have for the *in vivo* regulation of the AcsA acetyl-coenzyme A synthetase? **[5 marks]**

In the normal reaction of the experiment, protein X binds acetate to a CoA and requires an ATP to provide energy for catalysis, and then releases the product with AMP, pyrophosphate and acetyl-CoA; But when AcuA is added, it stops the reaction of protein X between the two steps of binding acetate and using ATP, so there is no AMP was produced.

(e) Diagram the process to monitor the level of protein X in *Bacillus subtilis*. **[10 marks]**

From the reaction (2) we can get the relationship between each component:

protein X ↑ + acetyl-CoA ↑ → acetate ↓

The darker the colour of lane2 displayed in the Autoradiogram, the higher the level of proteinX. Alternatively, the lower the content of acetate in the solution, the higher the level of proteinX.

2. Figure 2 below is the thermal shift profile of a wild type DNA polymerase and its two mutants: lysine to aspartate (K to D) and lysine to glutamate (K to E). Based on the diagram below, answer these questions:

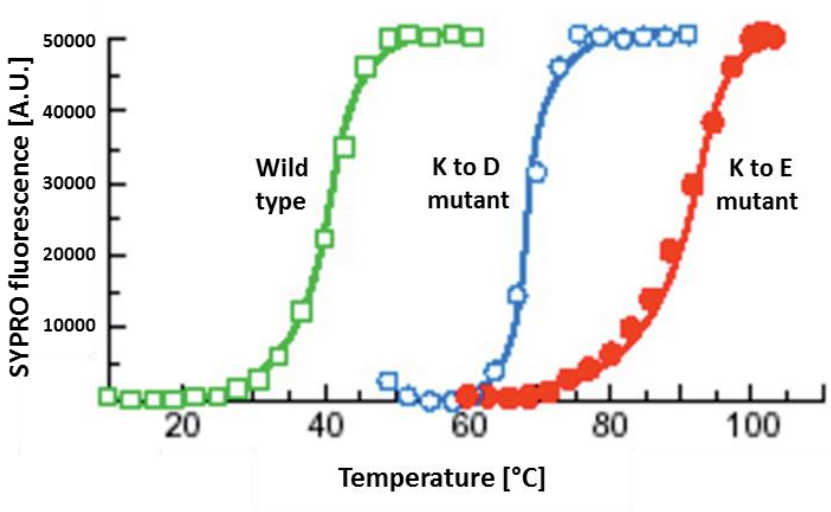


Figure 2

(a) What is the impact of the mutations on the polymerase? **[5 marks]**

Both mutant K to D and K to E showed increased thermal stability relative to Wild type, their difference being that mutant K to E showed greater thermal stability than mutant K to D above high temperatures (higher than 60 ℃).

(b) You are aiming to develop the enzyme for use in polymerase chain reaction (PCR). Which enzyme is most likely to survive twenty repeated PCR cycles? Explain your answer. **[5 marks]**

PCR involves temperature cycling, including high-temperature denaturation steps. Enzymes that remain stable at higher temperatures are more likely to survive repeated PCR cycles. Thus, the **K to E Mutant** is the best candidate for surviving twenty repeated PCR cycles because it denatures at a higher temperature, ensuring its functionality during the cycling process.

(c) The circular dichroism (CD) results of the wild type and the K->E mutant are shown in Figure 3 below. Please analyze the CD results. **[5 marks]**

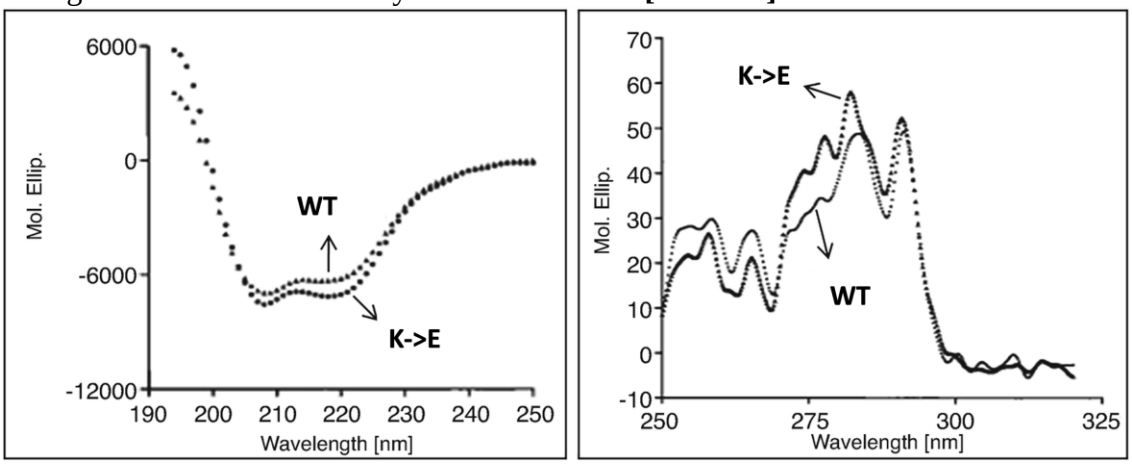


Figure 3

- From the plot on the left (Far-UV wavelength), we can see that mutant K to D shows greater fluctuations in its CD spectrum than Wild type, so mutant K to D has more alpha spirals than Wild type.
- From the plot on the right, we can find out this is the CD spectrum of the protein in near-UV. In this wavelength range, the data for Wild type and K to E mutants had similar patterns, but with different intensities and peaks. This shows that there are significant differences in the secondary structure and aromatic amino acid environment between the two groups at some different wavelengths. To be more specifically, some local regions of the K to E mutant may have undergone conformational changes, resulting in a larger signal displayed in the CD spectrum in the near-ultraviolet region.