Question 1:
By studying a recently discovered species of bacteria, you find that the enzyme Zeta catalyzes the conversion of A to B. You purify Zeta and perform enzyme kinetics experiments, measuring **Vmax** to be **60 µM/min**, **KM** to be **15 µM**, and **kcat** to be **10^6/sec**.
Unfortunately, you lose part of your notes before transcribing them to your notebook, so you **don't know** how much purified Zeta and [A] you added to the different tubes. Try to reconstruct the conditions of your original experiment, detailing your reasoning.

(a) In one of your tubes, you measured the production of **24 µM of B per minute**. What was the concentration of [A] you added? **[1 pt]**

(b) What was the concentration of Zeta you added? **[1 pt]**

(c) You discover that your reaction contained **1.5 µM** of a known competitive inhibitor of Zeta with a **Ki** of **3 µM**. Now, knowing this, calculate the **KM** for the reaction in the absence of the inhibitor. **[2 pts]**

Question 2:
Alcohol dehydrogenases (ADHs) are enzymes that catalyze the oxidation of **primary** and **secondary alcohols** to **toxic aldehydes** and **ketones**, respectively. These are then converted to **less toxic acetate byproducts** by the enzyme **Acetaldehyde Dehydrogenase (ALDH)**.

(a) In cases of methanol poisoning, **intravenous ethanol infusion** is used as treatment. Explain the principle behind this treatment. **[1 pt]**

(b) The **KM** of the **liver ADH isozyme αβ1** for ethanol and methanol is **1 mM** and **15 mM**, respectively. Calculate the kinetic parameters for the **methanol-to-formaldehyde** reaction catalyzed by **liver ADH isozyme αβ1** in the presence of **1 mM ethanol**. **[2 pts]**

(c) A researcher purifies a **new ADH isoform** and finds that it catalyzes the conversion of **0.30 g of ethanol** (**molecular weight = 46 g/mol**) per minute at **37 °C** at **Vmax**. What is the turnover number (**kcat**) of ADH (in **min^{-1}**)? Assume the **molecular weight of ADH** is **81,000 g/mol**. **[2 pts]**

(d) **Alcohol flush reaction** is a condition in which a person develops **flushing** or **blotches** associated with **erythema** on the **face, neck, shoulders, ears**, and, in some cases, the **entire body** after consuming alcohol. Approximately **30–50% of East Asians** carry the **ALDH2*2 allele** on **chromosome 12**, which is associated with **alcohol flush reaction**. **ALDH2*2** bears a **lysine-for-glutamate substitution** at

position **487** within the **cofactor binding site**. By analyzing the reactions depicted in **Figure 3**, derive a **possible mechanism** by which **ALDH2*2** contributes to alcohol flush reaction. **[2 pts]**

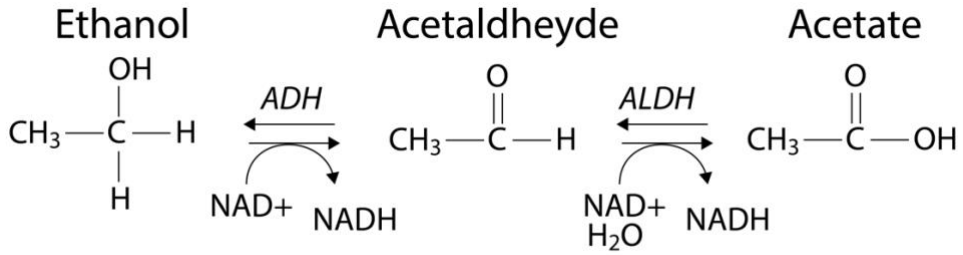

Fig. 3