NAME:_____

1. Chantigian *et al.* named the gene encoding the bifunctional ketoisomerase/acetyltransferase *FdtD*. When the genome of *Shewanella denitrificans* was first sequenced and submitted to the GeneBank, this gene was identified as Sden_2659. Chantigian *et al.* molecularly engineered *E. coli* to express this enzyme using PCR technology.

    a. For both the forward and reverse primers, indicate the bases that are part of the *FdtD* gene (either temple or nontemplate strand) from *S denitrificans*.

5′-AAACATATGATTCATAAATTAGCAGATGTTCAATCTCAAAATATTGGTGACAATACCAAAGTCTGGC-3′

5′-AAACTCGAGGTTTTGTCTCATTTGTTTAAAAGTTGAATAATCACGAATGTAATCATCAGAATCATAGTAATGAG-3′

    b. Indicate the sequence recognized by NdeI.

5′-AAACATATGATTCATAAATTAGCAGATGTTCAATCTCAAAATATTGGTGACAATACCAAAGTCTGGC-3′

5′-AAACTCGAGGTTTTGTCTCATTTGTTTAAAAGTTGAATAATCACGAATGTAATCATCAGAATCATAGTAATGAG-3′

    c. Indicate the cut position of NdeI.

5′-AAACATATGATTCATAAATTAGCAGATGTTCAATCTCAAAATATTGGTGACAATACCAAAGTCTGGC-3′

5′-AAACTCGAGGTTTTGTCTCATTTGTTTAAAAGTTGAATAATCACGAATGTAATCATCAGAATCATAGTAATGAG-3′

    d. Indicate the sequence recognized by XhoI.

5′-AAACATATGATTCATAAATTAGCAGATGTTCAATCTCAAAATATTGGTGACAATACCAAAGTCTGGC-3′

5′-AAACTCGAGGTTTTGTCTCATTTGTTTAAAAGTTGAATAATCACGAATGTAATCATCAGAATCATAGTAATGAG-3′

    e. Indicate the cut position of XhoI.

5′-AAACATATGATTCATAAATTAGCAGATGTTCAATCTCAAAATATTGGTGACAATACCAAAGTCTGGC-3′

5′-AAACTCGAGGTTTTGTCTCATTTGTTTAAAAGTTGAATAATCACGAATGTAATCATCAGAATCATAGTAATGAG-3′

2. During the protein purification process for FdtD, Chantigian *et al.* dialyzed the protein against a solution of 10 mM Tris-HCl (pH 8.0) with 200 mM NaCl. Describe how to prepare 3 L of this buffered solution to be used in dialysis starting with jars of solid NaCl (CAS 7647-14-5); Tris basic form (CAS 77-86-1); Tris acidic form (CAS 1185-53-1) and dH_2O. Show all of your work.

3. Chantigian *et al.* grew cells to an optical density of 0.75 and then induced with 1 mM IPTG.

    a. Draw a cartoon for the expected state of the promoter region for the *fdtD* gene before the addition of IPTG.

    b. Draw a cartoon for the expected state of the promoter region for the *fdtD* gene after the addition of IPTG.

    c. Explain in molecular detail what the authors mean by "the cells were induced with 1 mM IPTG and allowed to express protein at 23°C for 24 hours".

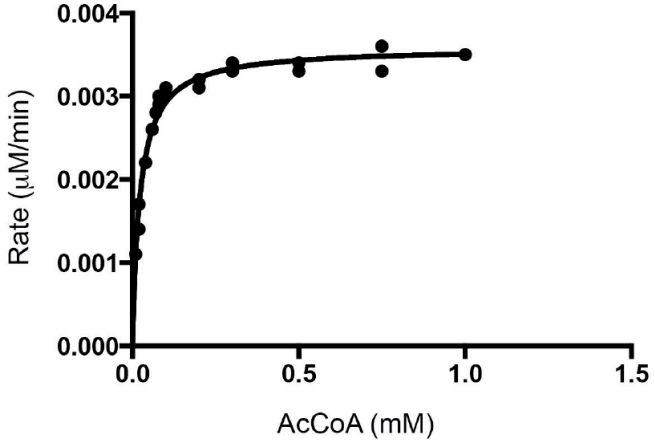
4. Find the first four amino acids in the primary structure of FdtD.
    a. Draw the chemical structure of the peptide of these four amino acids.

    b. Chantigian *et al.* present a Ramachandran plot in Figure S1. Indicate the ϕ and φ bonds on your peptide structure.

    c. Indicate the peptide bonds on your peptide structure.

    d. Why are the peptide bond angles not included on a Ramachandran plot?

5. The authors state that the initial rates were fitted to the standard Michaelis-Menten equation. For the acetyl-CoA substrate, the following data and values were reported:



K_M = 19 (±2) uM
k_{cat} = 4.1 (±0.3) s^{-1}

a. Explain how to quickly assess the validity of the value reported for K_M from the presented data.

b. Explain how to determine k_{cat} from the presented data.

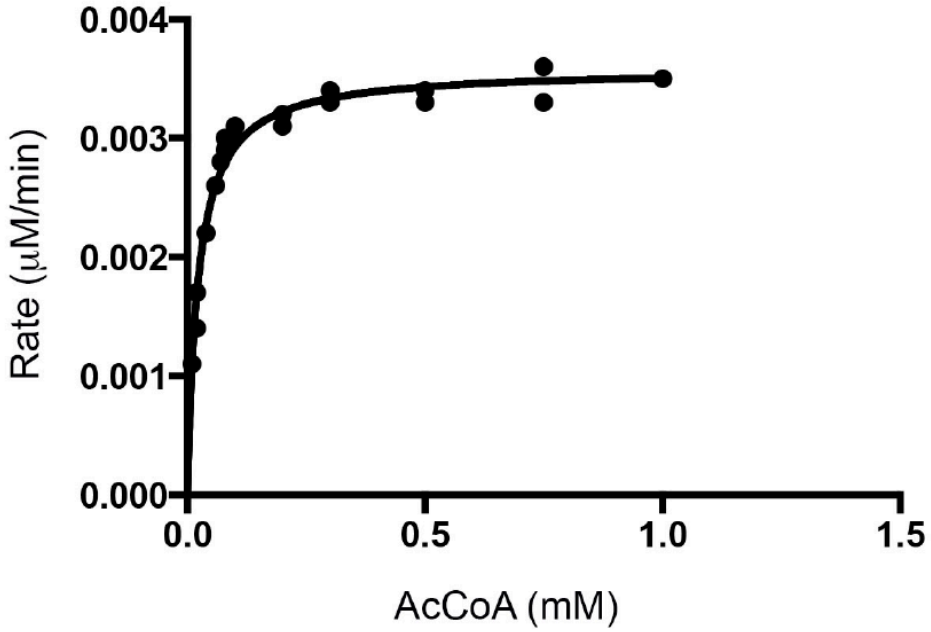
c. What is the molecular weight in grams per mole of the fully functional FdtD enzyme complex? Show your work or site your source.

6. Imagine the authors found a mixed inhibitor of acetyl-CoA for FdtD. The inhibitor has the binding constants: $K_I = 250\ \mu M$ $K_I' = 250\ \mu M$.

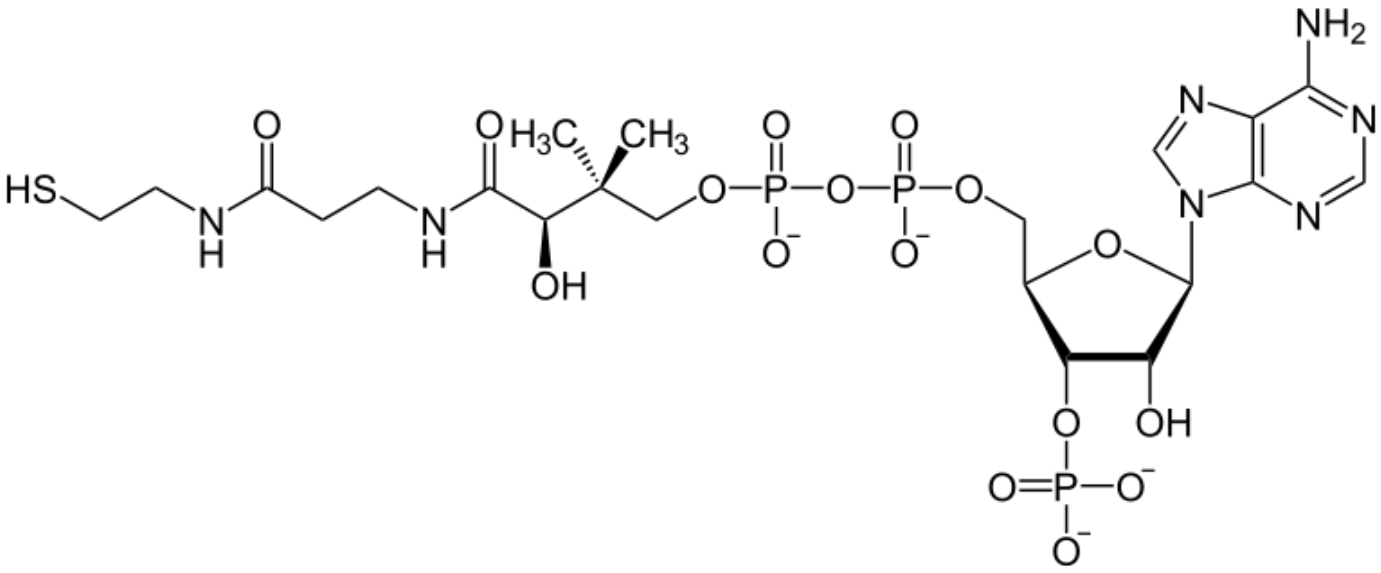
   a. Determine the value of V_{max}^{app} in the presence of 250 uM inhibitor. Show your work and include units.

   b. Determine the value of K_M^{app} in the presence of 250 uM inhibitor. Show your work and include units.

   c. Draw a curve on the following graph to indicate the expected result for initial rates in the presence of 250 uM inhibitor.



7. The chemical structure of CoA cofactor is below. The authors note that several residues are involved in the interaction of FdtD and the CoA cofactor. Indicate the appropriate hydrogen bonds and ionic interaction on the image with a dashed. Involved residues include D82; A107; A125; K83; K131; R145; and T130.



8. Draw “Scheme 6. Possible Catalytic Mechanism for the N-acetyltransferase activity of FdtD” based on the author’s suggestions.