Name: _____ Exam 2

References to published material in this exam are from:

Shukla. S., Bafna, K., Gullet, C., Myles, D.A.A., Agarwal, P.K., and Cuneo, M.J. (2018). Differential Substrate Recognition by Maltose Binding Proteins Influenced by Structure and Dynamics. *Biochemistry* **57:** 5864-5876.

1. In the final step of the protein purification, the authors dialyze their protein in 20 mM Tris (pH 7.5) and 40 mM NaCl. Imagine that you want to prepare 2 L of this dialysis buffer from jars of solid (powder) stock. [Show your work]

    a. How many grams of NaCl will you add to the 2 L volumetric flask?

    Tris has a pKa of 8.08 and comes in two forms:

    Tris HCl (MW: 157.60 g $mole^{-1}$)
    Tris base (MW: 121.14 g $mole^{-1}$)

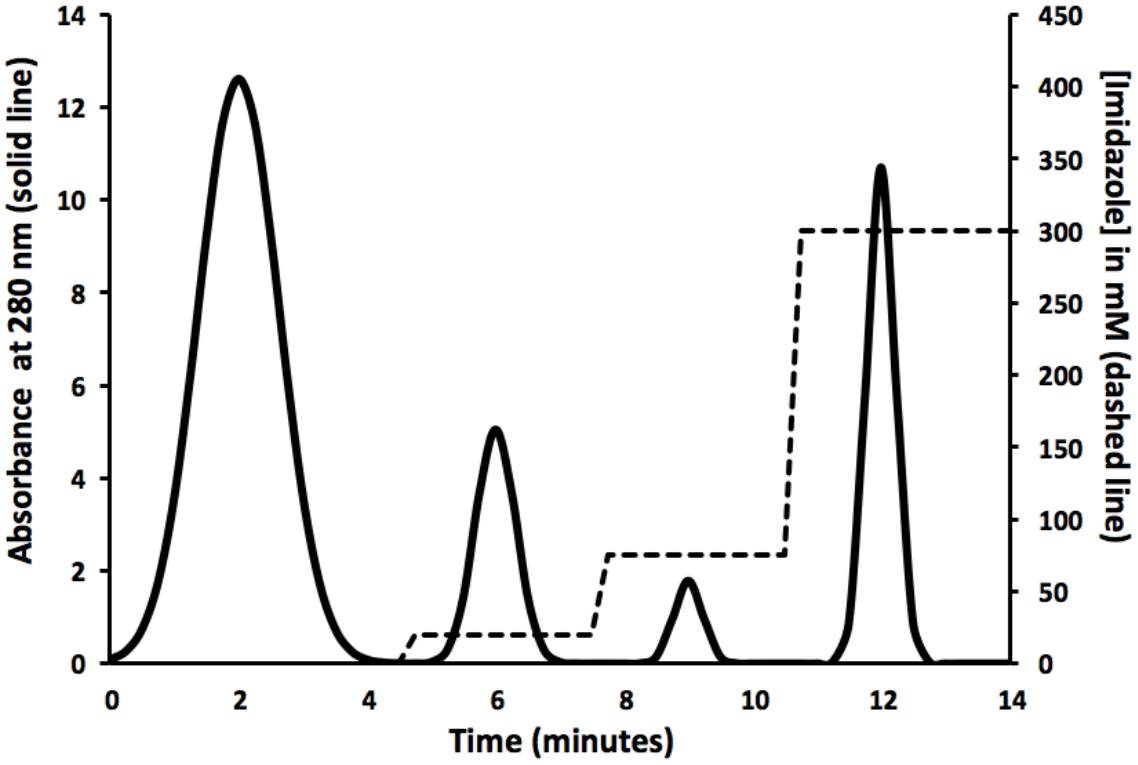
    b. How many grams of Tris HCl will you add to the 2 L volumetric flask?

    c. How many grams of Tris base will you add to the 2 L volumetric flask?

2. Protein Purification
"Briefly, the hexahistidine-tagged tmMBP isoforms were cloned in a pET21a vector and expressed in BL21 RIL *E. coli* cells. The apo form of the proteins was produced using Enfor's minimal medium to avoid binding of endogenous carbohydrates from the medium. The substrate-bound form of the proteins was grown in Terrific Broth. Both media were incubated at 37 C and induced using 1 mM IPTG. The induced cells were harvested and lysed using sonication before an affinity purification step (HiTrap Chelating HP column). The column was washed with affinity buffer A (20 nM imidazole and 300 mM NaCl) and subsequently with affinity buffer B (75 mM imidazole and 150 mM NaCl), and the washes were collected. Finally, elution buffer (300 mM imidazole and 150 mM NaCl) was used to elute the bound protein from the affinity column."

   a. Describe the first step in purifying tmMBP1 after lysing the cells using sonication before an affinity purification step.

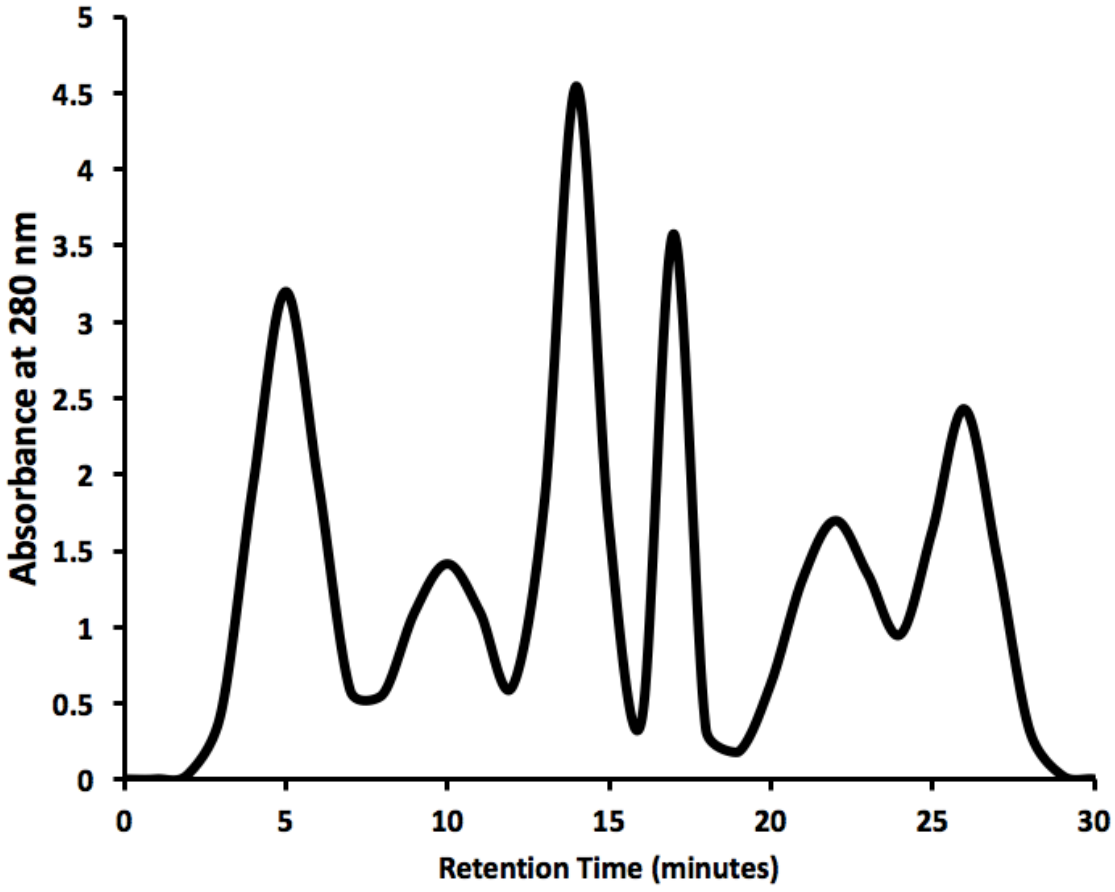
   b. Imagine that the graph below depicts the result of the HiTrap Chelating HP column. Label the peak that contains tmMBP1.



Imagine that the tmMBP1 containing fraction from the previous HiTrap Chelating HP column contains the following additional *E. coli* proteins:

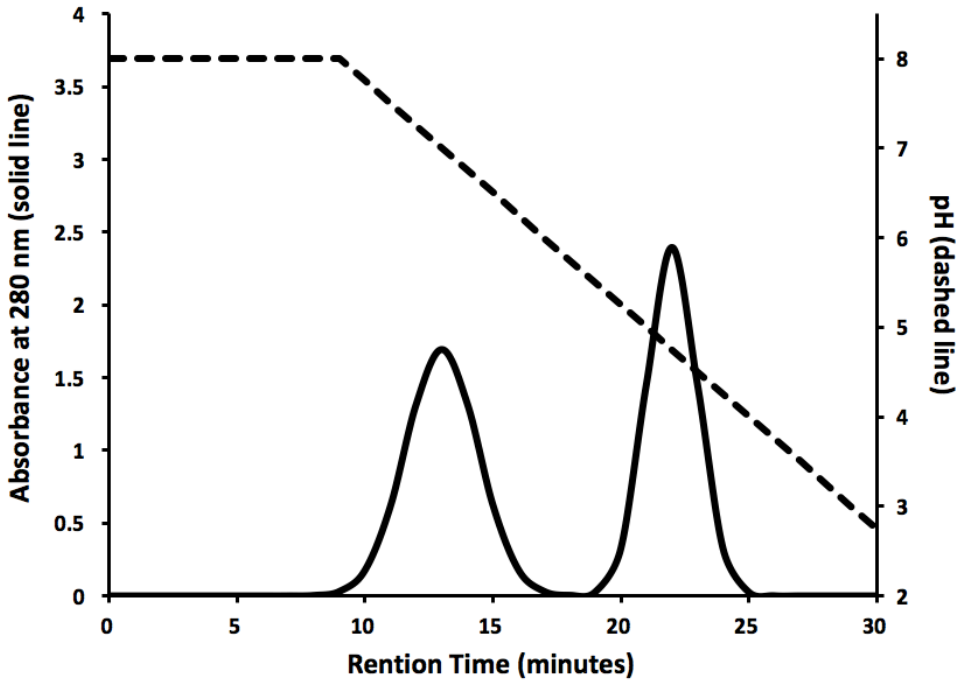
Protein	Length (amino acids)	Molecular Weight (Daltons)	pI
tmMBP1	374	41298	4.75
MetE	753	84674	5.61
RlmM	366	41905	7.04
Rs2	241	26744	6.62
Rs6	135	15704	4.94
RsmA	273	30420	7.83
TorD	199	22473	4.76

c. "Eluted samples were subsequently loaded onto an S75 26/60 gel filtration column (GE Healthcare Life Sciences)." Imagine the graph below depicts the results of the S75 26/60 gel filtration column. Label where each protein eluted from the column.

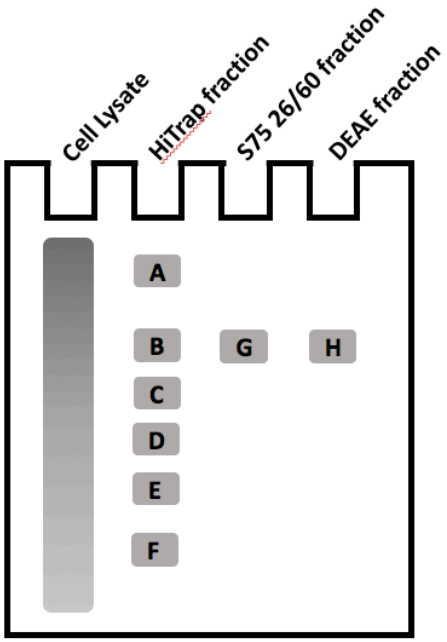


You decide to add a step of ion exchange chromatography to the purification of tmMBP1. You choose a DEAE anion exchange column and dialyze the fraction containing tmMBP1 from the previous gel filtration column into a Tris buffer at pH 8.

d. Imagine the graph below depicts the results of the DEAE column. Label where each applicable protein eluted from the column.



e. "The protein fractions were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis,...". Fill in the table below with the appropriate letter to indicate the protein position on the gel. Add "N/A" for not applicable when a protein does not appear.



Protein	HiTrap fraction	S75 26/60 fraction	DEAE fraction
tmMBP1			
MetE			
RlmM			
Rs2			
Rs6			
RsmA			
TorD			

f. On the above image of the gel, indicate were the positive and negative electrodes were located.

g. At each electrode during electrophoresis, gases are produced. Identify the gas produced at each electrode:

    i. Positive:

    ii. Negative:

h. "..., and fractions containing tmMBP1 were pooled and concentrated." Describe a method to concentrate the tmMBP1.