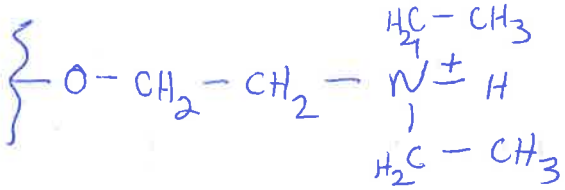
Names: Key (2 x 10 pts)

**Succinate Dehydrogenase Purification (Section 2.1 from posted paper)**

1. TSK column
   a. Identify the type of chromatography:
   Affinity  Size-exclusion  (Anion Exchange)  Cation Exchange

   b. What is the pKa of DEAE? 11.3

   c. Draw the chemical structure of DEAE as it would appear in the buffer listed in the protocol. The hydroxyl functional group is where this molecule is covalently attached to the media that makes it part of the stationary phase.



   d. Describe the chemical basis or theory for how SDH is eluted from the column.

The authors elute the proteins by increasing the ionic strength (salt concentration) with potassium and phosphate.

2. Hydroxyapatite column
   a. Identify the type of chromatography:
   (Affinity)  Size-exclusion  (Anion Exchange)  (Cation Exchange)

   b. What is the purpose or goal of the hydroxyapatite column (e.g., what is going on in this step)?

The SDH does not stick to this column. The authors are attempting to remove proteins that do stick to hydroxyapatite.

3. Sephacryl S-300 column
   a. Identify the type of chromatography:
   Affinity  (Size-exclusion)  Anion Exchange  Cation Exchange

   b. What is the fractionation range for this media (with units)?

10,000 – 1,500,000 g/mole (or Daltons)

10 to 1500 kDa

**Activity Assay Preparation for Lab**

Imagine that you collect the following data for three trials of an SDH activity assay using 10 μL of protein solution:

| Time (seconds) | Trial 1 A600 | Trial 2 A600 | Trial 3 A600 |
|---|---|---|---|
| 30 | 0.95 | 0.94 | 0.98 |
| 45 | 0.85 | 0.86 | 0.89 |
| 60 | 0.8 | 0.85 | 0.85 |
| 75 | 0.75 | 0.78 | 0.81 |
| 90 | 0.7 | 0.75 | 0.78 |
| 105 | 0.69 | 0.72 | 0.75 |
| 120 | 0.65 | 0.65 | 0.68 |
| 135 | 0.55 | 0.62 | 0.65 |
| 150 | 0.54 | 0.55 | 0.63 |
| 165 | 0.5 | 0.48 | 0.59 |

4. Determine the milliUnits (mU) of the protein solution with an error estimate using jackknife resampling. Make sure to round your answer.

8.7 (± 0.4) mU

**Protein Concentration Determination**

You will test the protein concentration for two dilutions of each of three stages for the purification process (six samples total) in addition to the six standard BSA solutions suggested by the protocol (12 total samples to test). You will be performing test tube assays at 37 °C.

5. What volume of Reagent A will you need?

6. What volume of Reagent B will you need?

7. Fill in the following table to prepare to make solutions.

| Tube # | BSA (ug) | Volume BSA Stock (1 mg/mL) | Stage 1 | Stage 2 | Stage 3 | Volume Stage 1 | Volume Stage 2 | Volume Stage 3 | Volume of buffer to make 0.1 mL of solution |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 μL | - | - | - | - | - | - | 100 μL |
| 2 | 20 | 20 μL | - | - | - | - | - | - | 80 μL |
| 3 | 40 | 40 μL | - | - | - | - | - | - | 60 μL |
| 4 | 60 | 60 μL | - | - | - | - | - | - | 40 μL |
| 5 | 80 | 80 μL | - | - | - | - | - | - | 20 μL |
| 6 | 100 | 100 μL | - | - | - | - | - | - | 0 μL |
| 7 | - | - | original | - | - | 100 uL | - | - | 0 μL |
| 8 | - | - | 1/10th | - | - | 10 μL | - | - | 90 μL |
| 9 | - | - | - | original | - | - | 100 uL | - | 0 μL |
| 10 | - | - | - | 1/10th | - | - | 10 μL | - | 90 μL |
| 11 | - | - | - | - | original | - | - | 100 uL | 0 μL |
| 12 | - | - | - | - | 1/10th | - | - | 10 μL | 90 μL |