HOMEWORK #6 KEY

A. Calculate the specific activity, fold-purification and the % yield for each step as compared to the initial starting material and fill in the values in the appropriate places on the 3 tables.

- Specific Activity: Divide total enzyme activity by total protein.
- Fold Purification: Divide sp. act. by 8.2 (initial sp. act.).
- % Yield: Divide total enzyme activity by starting total activity x 100.

YOUR STEPS	TOTAL RECOVERY		SPECIFIC ACTIVITY	FOLD-PURIFIED	%YIELD
	PROTEIN (mg)	ENZYME (units)			
Initial Starting Material	511	4200	8.2	-	-
40% $(NH_4)_2SO_4$ precipitation	181	3991	**22**	**2.7**	**95**
DEAE flow through, pH 7.0	67.6	3787	**56**	**6.8**	**90**
Isoelectric Focusing (pH 9.7)	3.8	2949	**776**	**95**	**70**

JIM'S STEPS	TOTAL RECOVERY		SPECIFIC ACTIVITY	FOLD-PURIFIED	%YIELD
	PROTEIN (mg)	ENZYME (units)			
Initial Starting Material	714	5880	8.2	-	-
40% $(NH_4)_2SO_4$ precipitation	253	5584	**22**	**2.7**	**95**
DEAE flow through, pH 9.0	5.1	5174	**1014**	**124**	**88**

SUE'S STEPS	TOTAL RECOVERY		SPECIFIC ACTIVITY	FOLD-PURIFIED	%YIELD
	PROTEIN (mg)	ENZYME (units)			
Initial Starting Material	410	3360	8.2	-	-
40% $(NH_4)_2SO_4$ precipitation	146	3192	**22**	**2.7**	**95**
CM, pH 7.0, NaCl elution	1.9	2516	**1324**	**161**	**75**

B. After swallowing your pride you realize your friends have improved upon your method. What factors would you consider in choosing between Sue or Jim's protocol.

- Sue and Jim's protocols both have less steps, a better yield and better fold purification than yours.
- Jim had a slightly better yield than Sue, but Sue had a slightly better fold-purification. Choosing between them would depend on whether quality or quantity were more important.
- Other factors to consider would be whether pH 9 (Jim's) or the NaCl (Sue's) would be detrimental for the subsequent use of the protein.

C. What information in your purification scheme prompted Sue to switch to cation exchange chromatography?

- The isoelectric point of 9.7. Generally do cation exchange below the isoelectric point. Also the failure to bind to an anion exchange column suggested that a cation exchanger might bind the protein.

D. Why did switching the pH from 7.0 to 9.0 result in a better fold-purification at the DEAE chromatography step?

- Raising the pH resulted in more contaminating proteins becoming negatively charged and therefore binding to the DEAE. In other words, proteins with isoelectric points between 7-9 would now absorb to the DEAE column

E. If your protein is approximately 0.1% of the initial starting material, what percentage of the total protein in your last purification step is probably your protein. In other words, how pure is your protein? How could you assess this?

- The approximately 100-fold purification means that approximately 10% of the protein in the final step is your protein.
- To be more quantitative, multiply the initial percentage by the fold-purification (0.1 x 95 = 9.5%)
- Alternatively, approximately 0.5 mg of the starting material is your protein and with a 70% yield approximately 0.35 mg of the 3.8 mg is your protein (0.35/3.8 x 100 = 9.2%).
- the relative purity can be assessed by gel electrophoresis