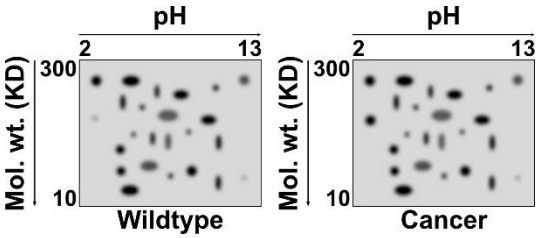
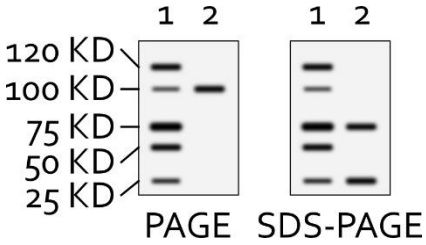
Bio98 W2018 Practice Midterm 2

01. I compare the proteomes of wildtype (WT) and cancer cells from liver, using a 2D gel. My results are shown alongside.



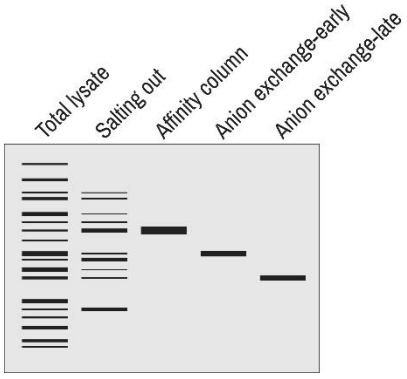
02. I have a sample of the protein *Professorase*, that I run on a native PAGE, and an SDS-PAGE. My data is shown alongside.



03. *Hexokinase* catalyzes the reaction Glucose + ATP → Glucose-6-PO_4 + ADP. I use Glucose on an affinity column to purify *Hexokinase* from my total cell lysate, but am unsuccessful. However, if I first treat the cell lysate with the enzyme *ATPase* (which breaks down ATP to ADP), I am then able to purify the *Hexokinase* using the same Glucose affinity column.

04. I have a mixture of muscle proteins and glycogen. When I heat the solution, the proteins form aggregates, whereas the glycogen does not.

05. I use salt precipitation followed by an affinity column (using the molecule "C") to purify an enzyme. I then ***change the pH*** of the solution and run the affinity-purified sample through an anion exchanger and collect the early and late fractions. I have ***relatively low*** specific activity after the salt precipitation step, and ***very high*** specific activity after the affinity column. However, I see ***no activity*** in either of the fractions after the anion exchanger step. If I mix the early and late fractions from the anion exchange column, I then get **very high specific activity.** Results from a non-denaturing PAGE after each step is shown.



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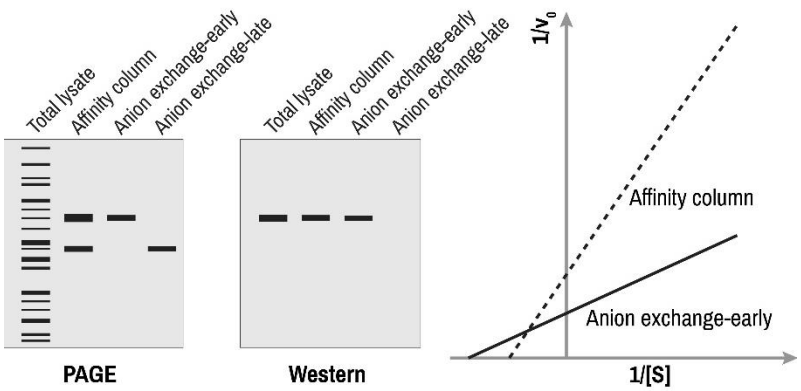
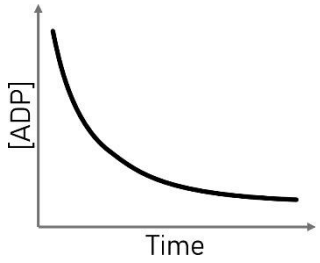
06. I use an affinity column containing glycerol to try and purify a protein, "***X***" from cell lysate. The specific activity of the protein is ***very low*** after this step. So, I run the affinity-purified sample through an anion exchanger. The early fraction has very ***high specific activity,*** whereas the late fraction has ***very low specific activity***. My results from a PAGE and the corresponding Western blot (using an antibody specific to *X*) after each step are shown. Also shown are the enzyme kinetics after the affinity purification, and after the anion exchange column.



07. Consider the following data from the purification of an enzyme that catalyzes the reaction ADP + P_i → ATP. To measure the amount of enzyme present in a sample, I start off with a certain [ADP], add a certain volume of the sample to the ADP, and then measure the [ADP] over time. An example is shown alongside.

Purification step	Total protein (µg)	Activity (Units)
Salt fractionation	1000	20,000
Cation exchanger	500	10,000
Affinity column	75	1,500



08. You take total cell lysate, and use **immunoprecipitation** to purify an enzyme of interest. The purified enzyme has **HIGH** specific activity, and shows a single band (56kD) on an SDS PAGE.
Now, you heat the total cell lysate, and use **immunoprecipitation** to purify the enzyme of interest. The purified enzyme has **LOW** specific activity, and shows two bands (56kD and 122kD) on an SDS PAGE.
You then heat the total cell lysate, allow it to cool down, and use **immunoprecipitation** to purify the enzyme of interest. The purified enzyme has **HIGH** specific activity, and shows one band (56kD) on an SDS PAGE.
Finally, you make total cell lysate from a mutant cell. When you use **immunoprecipitation** to purify the enzyme of interest, the purified enzyme has LOW specific activity, and shows a single band (56kD) on an SDS PAGE. When you heat the cell lysate from the mutant cells and immunoprecipitate the enzyme, the purified enzyme has LOW specific activity, and shows a single band (56kD) on an SDS PAGE.

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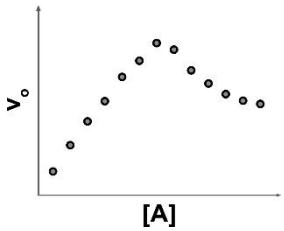
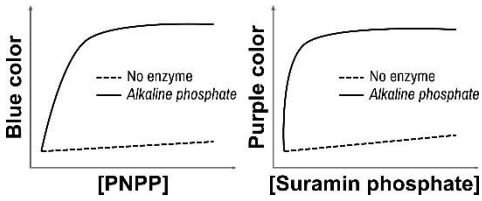
09. The enzyme "**E**" catalyzes the reaction A → B. The v_0 for the reaction at a given [E], but different [A] is shown. Structural data shows that A can bind to E at two different sites.

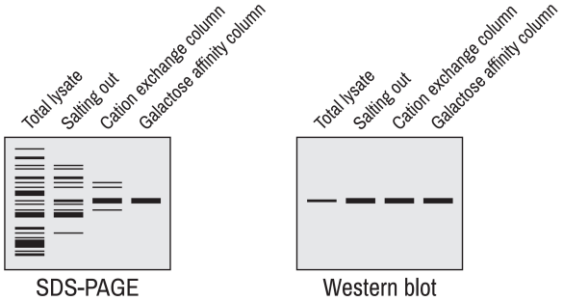


10. *Hexokinase* and *Glucokinase* are isozymes that catalyze the reaction Glc + ATP → Glc-6-PO_4 + ADP. Hexokinase has a K_m of 2.5μM for Glc, whereas Glucokinase has a K_m of 368μM for Glc.

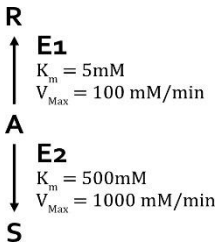
11. PNPP is a yellow substance. When it is hydrolyzed, it is converted to *p*-nitrophenol, which is blue in color. Suramin phosphate is a colorless chemical. When it is hydrolyzed, it forms suramin, a purple substance. I start with different concentrations of either PNPP or suramin phosphate and then measure the amount of blue and purple color produced (respectively) in 10 seconds, either in the absence, or presence of the enzyme *alkaline phosphatase*. My data is shown.



12. You are purifying the enzyme Hexokinase (which converts Glc → Glc-6-PO_4) from rat liver cells. The figure shows your results from the various purification steps. Both the SDS-PAGE results and the corresponding Western blot (using a Hexokinase-specific antibody) is shown. Galactose is a competitive inhibitor of Hexokinase.



13. Consider the hypothetical pathway shown.



14. I have a protein with the sequence shown:

Lys-Lys-Lys-His-Arg-Lys-Ile-Leu-Gly-Phe-Leu-Ile-Ala-Ala-Ala-Leu-Ile-Leu-Leu

This protein forms a micelle in solution.