PBC Day 6 Interpretation Questions (with answers)

1. Interpret your Western blot data. Your interpretation should include answers to the following questions:

a. Which lanes have bands and which do not?
answers will vary from student to student

b. Is this what you expected?
If bands are observed in the lanes mentioned in the answer to part c below, then the answer should be "yes"—otherwise, "no." Many students' blots also had bands at MW smaller than that of full length Bgal (~112 kDa); these are probably degraded fragments of Bgal, and they should mention these if they saw them.

c. If you don't see any bands, where would you have expected to see them?
Ideally, you would expect to see bands in the following lanes: CL; CL-S; AS-P; DEAE/AF Load; DEAE 0.4M fraction; AF before PD-10, AF after PD-10. Many students will likely see a band(s) in CL-P, as cell lysis was incomplete. These lanes should correspond to fractions that had Bgal activity in their ONPG assay!

d. Suggest reason(s) that your "expected" bands might be missing.
Some possibilities include: forgetting to load a sample/didn't have a sample to load; low protein amounts in sample (below level of detection of Western blot); poor transfer; or experimental errors (forgot to add NBT/BCIP, etc.)

2. Enzyme Kinetics Analysis for **EACH** strain (CSH and HIS461). There is a guide in the manual appendix (page 62-66) that will help you through these calculations. Remember: everything happens **in the reaction tube**, and DON'T FORGET UNITS!!

Use Excel or some comparable program for data analysis and include all relevant charts and graphs:

i. Plot of primary data for EACH strain

Will vary, but watch out for whether they remember to multiple A_{420} by 2 (to get the A420 in the reaction tube).

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ii. Michaelis-Menton plot for EACH strain– Estimate K_M and V_{max}

Will vary, but looks like this:

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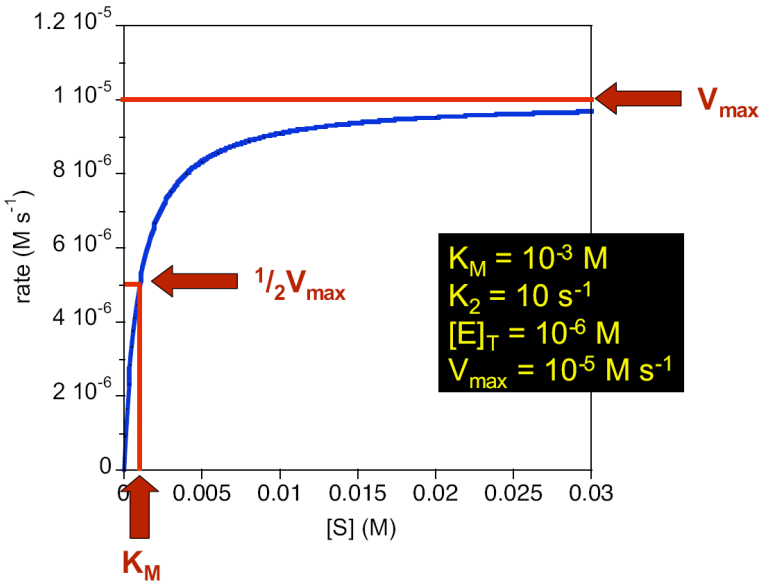
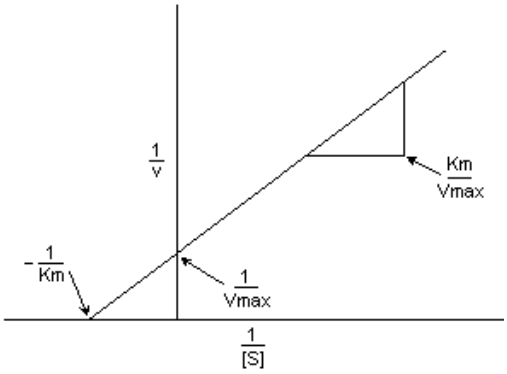


iii. Lineweaver-Burke plot for EACH strain – Calculate K_M and V_{max}

Will vary, but looks like this:



iv. Calculation of k_{cat} for EACH strain.

Varies but the calculation for $[E_t]$ should look like:

1. $\frac{\text{volume of sample added } (\mu L)}{15\ mL} \times \frac{1mL}{1000\ \mu L} \times \text{purity*(mg/mL)} = \text{[protein] in rxn.}$

2. protein conc. in rxn. (mg/mL) x 1 g/1000 mg = g/mL in rxn.
3. g/mL x mol/g (from Bgal MW) = mol/mL in rxn.

***purity comes from Coomassie gel, and should have a value between 0-1)**

$k_{cat} = V_{max}/[E_t]$ and has the units U/mol