Graphing Kinetics using Prism 5

Prepare a linear regression analysis of the standard curve data, abs vs. the p-nitrophenol product. You should FORCE THE LINE THROUGH ORIGIN! Use the equation of the line to calculate the amount of product made during your kinetic runs. After you convert your data and are ready to graph the kinetics curves use the following steps.

Begin Prism 5 on the network. Choose XY graph…Points only (first option). Choose for Y – “enter and plot a single Y for each point” hit create.

Enter X and Y data values (remembering which values go on which axis).

FOR MICHAELIS/MENTEN:
Analysis button, choose nonlinear regression (found to the right of the linear regression button). Choose and equation…pick “Enzyme Kinetics”…then Michaelis-Menten, then ok button at the bottom. Vmax and Km values are given in the “Results” section. Make sure you use the appropriate units to describe them. Vmax has the same units as the Y axis, Km has the same units as the X axis. Click on the graph button to see the graph. Add a title to make it “pretty” and publication quality. Do this for the uninhibited and both the inhibited reactions.

FOR LINEWEAVER-BURK:
Click back to the original data table. Near the top click “Analyze” (below the linear regression button on the analysis tab). Choose transform, then hit ok at the bottom. From the function list that appears, choose “Pharmacology / biochemistry transformations”, then Lineweaver-Burk (double reciprocal plot). Finally, hit ok. Click on the Lineweaver-Burk graph. This is a linear rearrangement of the Michealis-Menten graph so the data points should be in a fairly straight line rather than the curve. Choose linear regression tab at the top to create a line of best fit through these points. DO NOT FORCE IT THROUGH THE ORIGIN! Add a title to make it “pretty” and publication quality. Do this for the uninhibited and both the inhibited reactions. Click on the results folder and choose data results from linear regression of Lineweaver-Burk. Record values of…Y-intercept when X=0. Remember at this point the value is equal to 1/Vmax. Solve for Vmax. X-intercept when Y=0. Remember at this point, the value is equal to -1/Km. Solve for Km.

FOR WHEAT GERM EXPERIMENT:
Use linear regression to get slope values as instructed in the hand out.

Have Fun!
DR. T.