**CHEM 450/550: Advanced Biochemistry**

**Homework #1 (50 points)**

**Due 11/06/18 at the start of class**

**Name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Below are a set of questions based on enzyme kinetics. Please answer all questions completely. Careful with units, and you must completely show your work (calculations, etc.) to receive credit!

1. You are studying an enzyme that recognizes a single substrate. You obtain the following single substrate kinetic data using 200 nM enzyme in all experiments. Please graph your data using a Hanes-Woolf plot and attach your graph to this homework. On your graph, make sure your axes are labeled and both the fit and equation are displayed. Using your graphed data, provide your Km, Vmax, kcat, and catalytic efficiency values. Please show your work. (20 points)

|  |  |
| --- | --- |
| **[Substrate], uM** | **V (uM/s)** |
| 0.5 | 3.5 |
| 1 | 6.2 |
| 2 | 12.5 |
| 4 | 23.5 |
| 10 | 32.2 |
| 17.5 | 36.9 |
| 30 | 41.8 |
| 50 | 44 |

2. You are now studying an enzyme that binds two substrates and converts them to two different products. Below is the data where you varied the concentrations of both your substrates. All underlined values are velocity measurements in units of mM/min. Plot the data below using a Hanes-Woolf primary plot to obtain apparent Km (for both substrates A and B) and Vmax values for all data sets. Next, using Lineweaver Burke secondary plots calculate the true Km (for both substrates A and B), true Vmax, and your turnover number. Your enzyme concentration for all experiments was kept fixed at 5 uM. Please include all plots (with axes labeled and fits shown) with this homework, and please show your work. (25 points)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Substrate A, mM** | **0.46 mM Substrate B** | **0.62 mM Substrate B** | **1.25 mM Substrate B** | **3.08 mM Substrate B** |
| 3 | 0.2 | 0.255 | 0.41 | 0.62 |
| 6 | 0.377 | 0.463 | 0.66 | 0.968 |
| 10 | 0.555 | 0.678 | 0.95 | 1.308 |
| 20 | 0.845 | 1.005 | 1.338 | 1.803 |
| 40 | 1.18 | 1.378 | 1.718 | 2.295 |
| 60 | 1.25 | 1.44 | 1.78 | 2.37 |

3) Finally, do your enzyme examples from problems 1 and 2 obey the limits of diffusional control? Please explain.