

# Bioengineering 304: Physiology

October 16, 2008

Homework Assignment #3

Due: 24th Oct 2008

Points awarded for each question are indicated in square brackets. Return assignment with your name clearly indicated at the top of your answer sheet. Return assignments to Fan Lee ([fanlee@u.washington.edu](mailto:fanlee@u.washington.edu))

[25] **Question 1.** The enzyme mechanism for a reversible substrate/product reaction is given below.



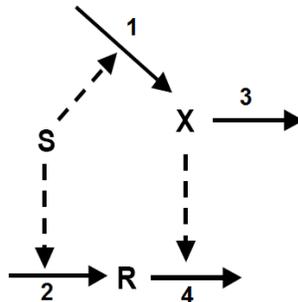
Prove that the Michaelis-Menten rate law for this mechanism is given by the following relation:

$$v = \frac{V_f S/K_S - V_r P/K_P}{1 + S/K_S + P/K_P}$$

Where  $K_S$  and  $K_P$  are some combination of rate constants,  $V_f$  and  $V_r$  are the forward and reverse  $V_{max}$  constants. If the equilibrium constant,  $K_{eq} = P_{eq}/S_{eq}$ , then show that:

$$K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{V_f K_P}{V_r K_S}$$

[35] **Question 2.** The following model shows a signal molecule,  $S$ , regulating two steps, 1 and 2. The signal molecule is under your control. Species  $X$  and  $R$  are part of the model and can change as a result of changes to  $S$  or kinetic constants. Reaction one leads to a molecule  $X$ , which in turn is degraded by reaction 3 or it can act as a regulator of reaction 4. Reaction 2 leads to the formation of a response molecule,  $R$ , which is in turn degraded by reaction 4. The boundary species on the different reactions are not shown and in fact do not enter into these particular model equations. The dotted lines indicate that a particular molecule regulates a reaction and is not consumed in the process.



The rate laws for the different reactions are given as follows:

Reaction 1: Rate =  $k_3 * S$

Reaction 2: Rate =  $k_1 * S$

Reaction 3: Rate =  $k_4 * X$

Reaction 4: Rate =  $k_2 * X * R$

Where  $k_1 = k_2 = 2$  and  $k_3 = k_4 = 1$ .

Based on this model, answer the following questions:

a) Write down the two differential equations that describe this model, one for R and another for X (All stoichiometries are unity). Note that since X regulates reaction 4 it is NOT consumed by reaction 4, X is only consumed by reaction 3. Similarly S is not consumed when it regulates reaction 1 and 2.

b) By running time course simulations investigate what special property this network has. To do this you should run a simulation for a short while until steady state is reached, then increase the concentration of S (say by 25%, try different increases) and investigate the transition of the pathway to a new steady state by observing the effect on R and X. Start simulations with  $X = R = 1$  and  $S = 1.1$ .

c) By setting the two differential equations to zero, solve for the steady state values of X and R in terms of S. Use these equation to confirm the conclusion you give in b)

You may implement your own differential equation solver or use the built-in ODE solvers in Matlab such as ode45.

[40] **Question 3.** The following data was artificially generated by adding **Gaussian noise** to the Michaelis-Menten function,  $S/(K_m + S)$ . That is the first column S is noise free, but the rate data is noisy as if it were generated experimentally. Such data can be used to test new computational methods before being applied to real data.

S	Rate
0	0.004316
0.1	0.278159
0.2	0.587364
0.3	0.476841
0.4	0.721052
0.5	0.654875
0.6	0.754123
0.7	0.761081
0.8	0.829862
0.9	0.853605
1	0.817424
1.1	0.83139
1.2	0.743549
1.3	0.891904
1.4	0.909068
1.5	0.915925
1.6	0.866716
1.7	0.831234
1.8	0.937778
1.9	0.988653
2	0.874342
2.1	0.853545
2.2	0.925618
2.3	0.864231
2.4	0.991577
2.5	0.986454
2.6	0.974255

Your task is to determine the value of the  $K_m$  by fitting the data to a simple Michaelis-Menten function,  $S/(K_m + S)$ .

Using Excel or some other suitable tool such as Matlab, fit the data to the equation,  $S/(K_m + S)$  and use the fit to estimate the  $K_m$  for this system. If you are using Excel, **DO NOT** use the add trend line function (there isn't a suitable function there anyway), instead use the Solver Add-in. You may have to install the Solver Add-in from the Excel options dialog box in Excel 2007 if you can't find it (search online to discover how).

The fitting procedure is as follows. Create four columns, marked, S, Rate Data, Function and Sums of Sqr. In the first and second columns add the data given above. Somewhere on the Excel sheet, designate a cell that has the current value of the  $K_m$ , set it to something like 10, this is your initial guess. In the third column you should compute the value for the Michaelis-Menten function from the S column **and** the current  $K_m$  cell value. Finally, in the fourth column compute the square of the difference between the second and third columns, this is  $(\text{data} - \text{function})^2$ . At the bottom of the fourth column compute the sum of the values in the fourth column. This cell will be the measure of how close the function is to the data, call it the sums of squares or target cell.

Now start the solver package (under tab Data/Analysis, if it is not there, then you need to install it) and enter two cell references, one marked target cell corresponding to the sums of squares and the other marked Changing Cell, corresponding to the Km we will fit. **Make sure** you also select the Min radio button near the top of the solver window, this indicates that you wish to minimize the target cell (ie reduce the distance between the function and the data).

To fit the function (i.e column 3), hit the solve button. You may need to change some options such as the number of iterations to 1000 and the search method to Conjugate (from Newton) in order for the solver to work.

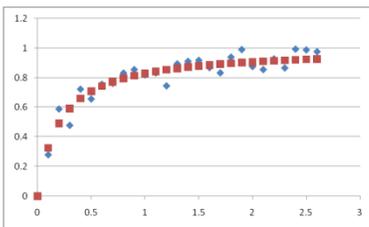
To determine how well your optimization is doing, you should also plot on the same graph, the raw data and the function column. As the fit progresses the function curve should approach the data curve.

### Questions for 3

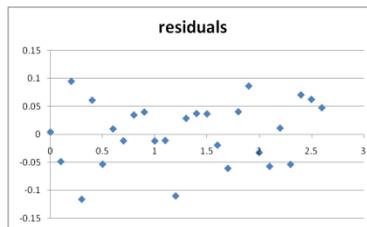
- Explain how the noisy data given above might have been generated.
- In your answer, provide two figures showing the graphs before and after the fit indicating that the fit was successful.
- A procedure that is often carried out after a fitting procedure is to plot the residuals, that is a plot of the data minus the final fit (search online if you are not such what this is). Such a plot can be used to indicate whether there is any bias in your data. In this case the noise in the data was Gaussian so no bias should be present, ie the residuals will be evenly scattered around zero. Provide a plot of the residual to show that the data was not biased.

Example plots:

Fitting



Residuals



- Explain briefly (less than 10 sentences) how curve fitting works, use illustrations if it helps your explanation.

Up to this point, the grade is out of 100 points. However you may obtain additional points to push your grade closer to 100 points if you can answer the following question.

**[15] d)** Because the data we use to fit the function was noisy, it must be the case the fitted Km will not exactly equal the real Km. The only way to obtain the actual Km value would be to measure noise free data which is of course impossible. This means there will always be some error in the fitted Km. The question is, is there any way, perhaps computationally, to estimate that error, for example by quoting a Km plus or minus a standard error, standard deviation or confidence limit. An exact method is not required in your answer but a statement on a general approach to the problem would be satisfactory. Having some measure of confidence in your fitted parameters is important in nonlinear curve fitting.