Key for Flux Problems-2008

Experiment 5.3

1.

F	[B]	[C]	[D]	[P]
0.2	98.0	97.0	9.5	45.5
0.4	96.0	94.0	9.0	41.0
0.6	94.0	91.0	8.5	36.5
0.8	92.0	88.0	8.0	32.0
1.0	90.0	85.0	7.5	27.5
1.2	88.0	82.0	7.0	23.0
1.4	86.0	79.0	6.5	18.5
1.6	84.0	76.0	6.0	14.0
1.8	82.0	73.0	5.5	9.5
2.0	80.0	70.0	5.0	5.0

a. Decrease

b. See Question 2 below.

2.

F	k_2[B]	k_{-2}[C]	k_4[D]	k_{-4}[P]
0.2	19.6	19.4	4.8	4.6
0.4	19.2	18.8	4.5	4.1
0.6	18.8	18.2	4.2	3.6
0.8	18.4	17.6	4.0	3.2
1.0	18.0	17.0	3.8	2.8
1.2	17.6	16.4	3.5	2.3
1.4	17.2	15.8	3.2	1.9
1.6	16.8	15.2	3.0	1.4
1.8	16.4	14.6	2.8	1.0
2.0	16.0	14.0	2.5	0.5

a. The concentrations of the intermediates decrease. As flux increases, the reaction rate of B to C decreases but the reaction rate of C to B decreases even more.

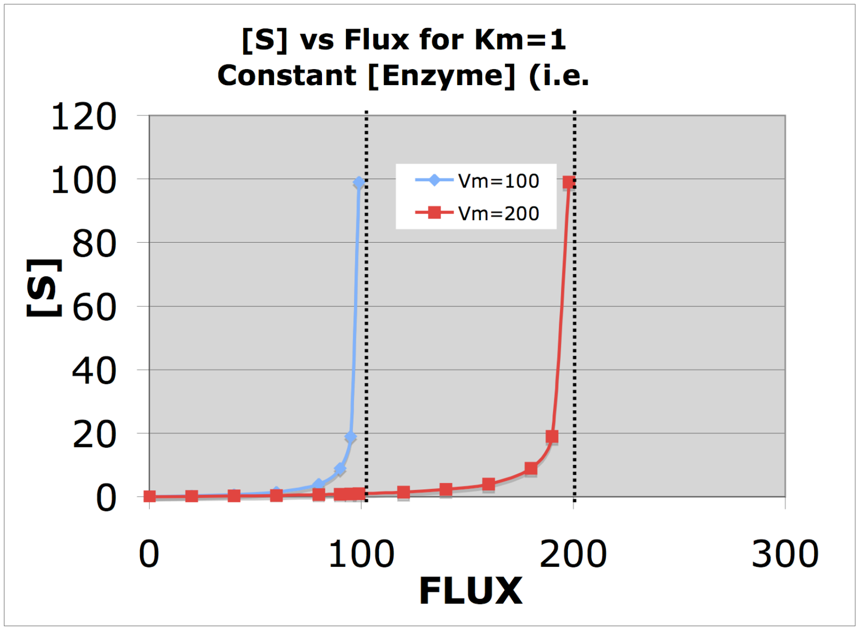
b. The situation is the same as in part a. If the reaction D to C decreases more than the reaction C to D, net conversion of C to D will increase.

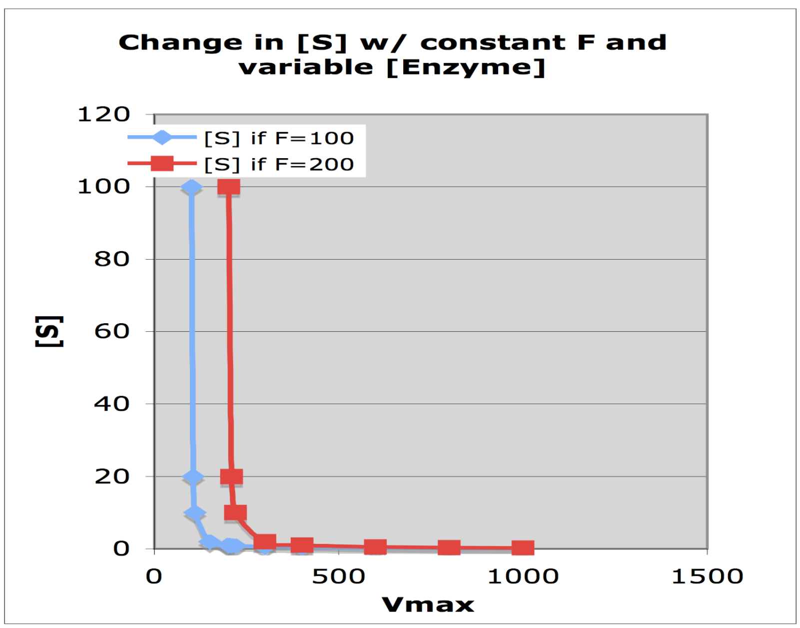
5. $F = k_3$[A]. This is possible in the nonenzymic case because, no matter how small the later rate constants may be, the concentrations of the intermediates can increase until the rate of each step is as great as the initial step. (In this model, no factors that might limit concentrations in the real world, such as solubility, are taken into account.)

6. Set [P] = 0 in your model. Most of the rate constants contribute to this case. Vary the constants to verify that k_3[A] is the maximal possible rate, but that in this case (unlike Question 5) the reverse rate constants affect the rate even though no full reverse reaction is possible.

7. Two generalizations apply here. (1) Changes in the amount of the first catalyst are likely to have the greatest effect because k_1[A] sets the upper limit of flux. (2) Changing the concentration of a catalyst that is present at higher activity than the others is likely to have very little effect, because such catalysts are not significantly limiting.

Experiment 5.4
Q1. As flux approaches Vmax, [S] rises catastrophically to maintain flow.





Q2. Remember Vmax is largely controlled by the amount of enzyme. So just as above when [enzyme] drops to where Vmax is almost the same as flux we again see catastrophic rise in [S]. Notice also how between F=150 to 1000 there is really not much change in [S]

Q3. A. [S] increases by ~ 230% (e.g. 100% x 13.3/5.7)
B. . [S] increases by ~ 20%

Flux =	85	Flux =	95
Vmax =	100	Vmax =	100
Km =	1	Km =	1
[S] =	5.7	[S] =	19

Flux =	85	50%	2X	5X
Vmax =	100	8.505	11.34	28.35
Km =	1	<89.5	<92	<96.6
[S] =	5.67			
	5.666666667			

P1. 85% of Vmax

P2. 35% of Vmax

Flux =	35	50%	2X	5X
Vmax =	100	0.81	1.08	2.7
Km =	1	<45	<52	<73
[S] =	0.54			
	0.54			

The message of these two problems is that an enzyme step at 35% Vmax can absorb much larger changes in flux without getting to dangerous [S] than can a step operating at 85 % Vmax. Notice for example that in Problem 1 only a 13.6% rise in flux leads to a 400% increase in [S]. On the other hand, it takes a 108% rise in flux in a system operating at 35% Vmax to increase [S] by 400%.

P3. There is no real quantitative answer here, it's just a thought problem based on the previous questions. Recall that intermediate steps are at a steady state, often near $\Delta G'=0$, but NOT at equilibrium. Based on Problem 1 and 2 and Question 2 you can see that you can have enormous changes in flux without huge changes in [S], i.e. a steady state. This steady state in turn is enforced by flow from flux controlling points of entry (and to some extent also points of exit) which maintain [S] different from equilibrium. If flux-controlling steps were completely shut off, intermediate steps would quickly move to equilibrium. The [S] that is necessary to maintain a steady state, in turn, is also determined by Vmax (enzyme concentration and intrinsic activity) and Km. These factors can be controlled genetically and by allosteric control.

P5. From the answer to questions above it is clear that if the enzyme present were operating near Vmax a modest increase in flux would lead to big changes in [S] which could have toxicity and could be even more wasteful than synthesizing a little extra enzyme. In order to be responsive to the needs of the organism, metabolic fluxes need to be able to adjust quickly (think fight or flight). The slow reacting organism probably gets eaten!