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1 Question 1 Part A

Starting by writing the equation for the rate of PFK:

$$\hat{r} = r * v$$

Where v for one activator or 35AMP is as follows. The numerator is for cases that lead to activity:

$$v = \frac{W_1 + W_2 * f_2}{1 + W_1 + W_2 * f_2}$$

r is given as follows. All values are given in the problem statement:

$$r = k_{cat} * E_1 * \frac{F6P}{K_{F6P} + F6P} * \frac{ATP}{K_{ATP} + ATP}$$

Now the two extreme cases where there is no 35AMP and when there is a large amount of 35AMP will be examined to find $W1$ and $W2$. The $\hat{r}$ values are from the given data. For 35AMP=0 and thus $f_2 = 0$:

$$\hat{r} = 3.003\mu M/hr = (0.4s^{-1})(3600)(0.12\mu M)\frac{0.1}{0.1 + 0.11}\frac{2.3}{0.42 + 2.3} * \frac{W_1}{1 + W_1}$$

$$W_1 = 0.045$$

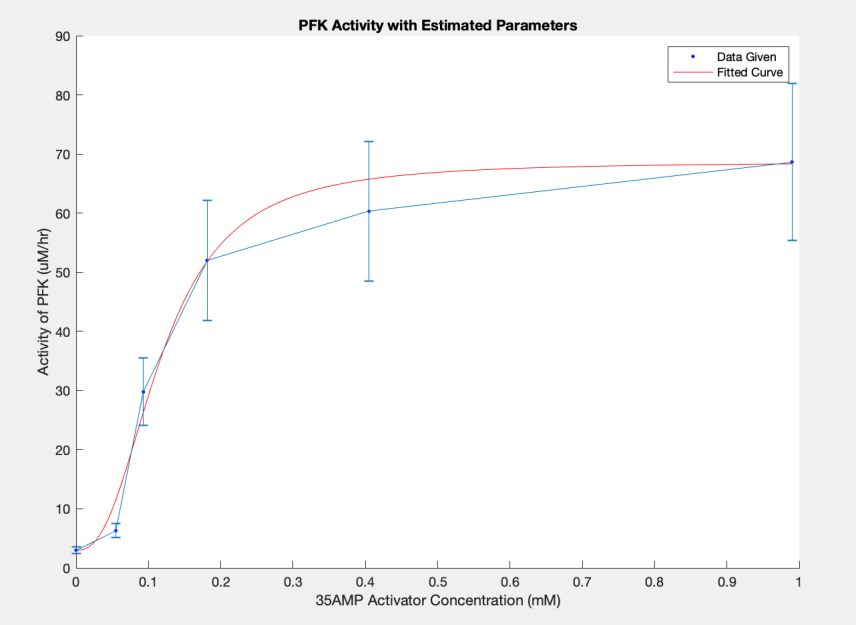
For 35AMP=large or 0.99, f_2 is approximated as 1 where the allosteric sites are all bound by activators:

$$\hat{r} = 68.653\mu M/hr = (0.4s^{-1})(3600)(0.12\mu M)\frac{0.1}{0.1 + 0.11}\frac{2.3}{0.42 + 2.3} * \frac{W_1 + W_2}{1 + W_1 + W_2}$$

$$\hat{r} = 68.653\mu M/hr = (0.4s^{-1})(3600)(0.12\mu M)\frac{0.1}{0.1 + 0.11}\frac{2.3}{0.42 + 2.3} * \frac{0.045 + W_2}{1 + 0.045 + W_2}$$

$$W_2 = 74.03$$

2 Question 1 Part B and C



The above plot was created using CHEME5440Final_1.m. It simply fits the data to the type of curve described in the problem statement with a varying K and n value. The function ReactionRate.m has the curve described in it. The .m file also prints the K and n values found as $K = 0.65731mM$ and $n = 2.4897$. The proposed model formulation does seem to mostly describe the data as it falls within the error bars of the data except at very early activator concentration. With more than 6 data points, the curve could probably be fit better, although it does capture the step-like nature of activating the PFK activity present in the data (and expected of such simple networks). It should also be noted that the curve is sort of "forced" to level out by the last data point because this was assumed to be the point where all the allosteric sites were bound ($f_2 = 1$). More data at higher 35AMP values would certify that this indeed is the saturation point of the activator.

3 Question 2 Part A

1. u and v are the repressors
2. α is the lumped effective rate of synthesis for a repressor
3. n is the cooperativity of repression
4. The second term in each equation is the degradation/dilution rate for each repressor and if considered to be first order is dependent on the concentration of repressor making the constant equal to 1.

4 Question 2 Part B

Starting from the given equations and setting them equal to 0 for the nullclines:

$$\frac{du}{dt} = \frac{\alpha}{1+v^n} - u = f(u,v) = 0$$

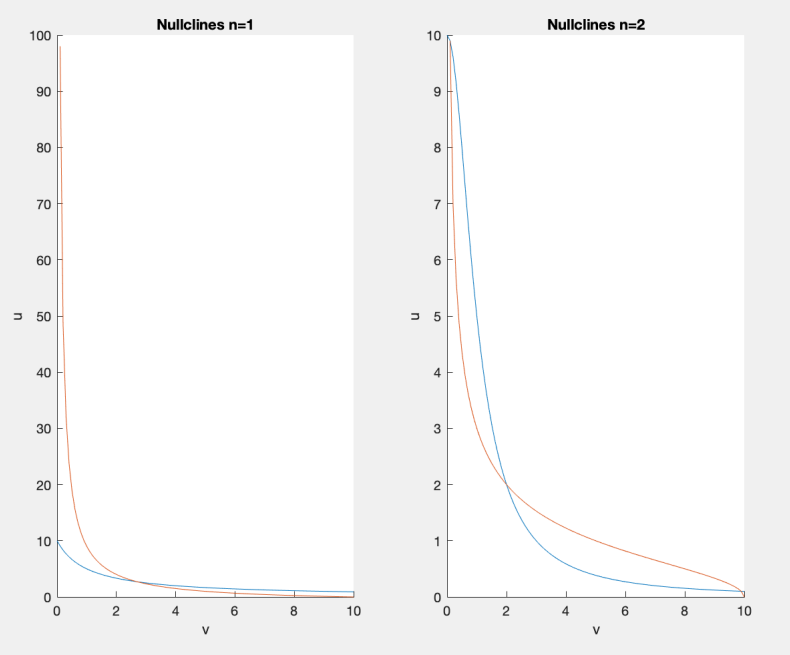
$$\frac{dv}{dt} = \frac{\alpha}{1+u^n} - v = g(u,v) = 0$$

Reveals:

$$u = \frac{\alpha}{1+v^n}$$

$$v = \frac{\alpha}{1+u^n}$$

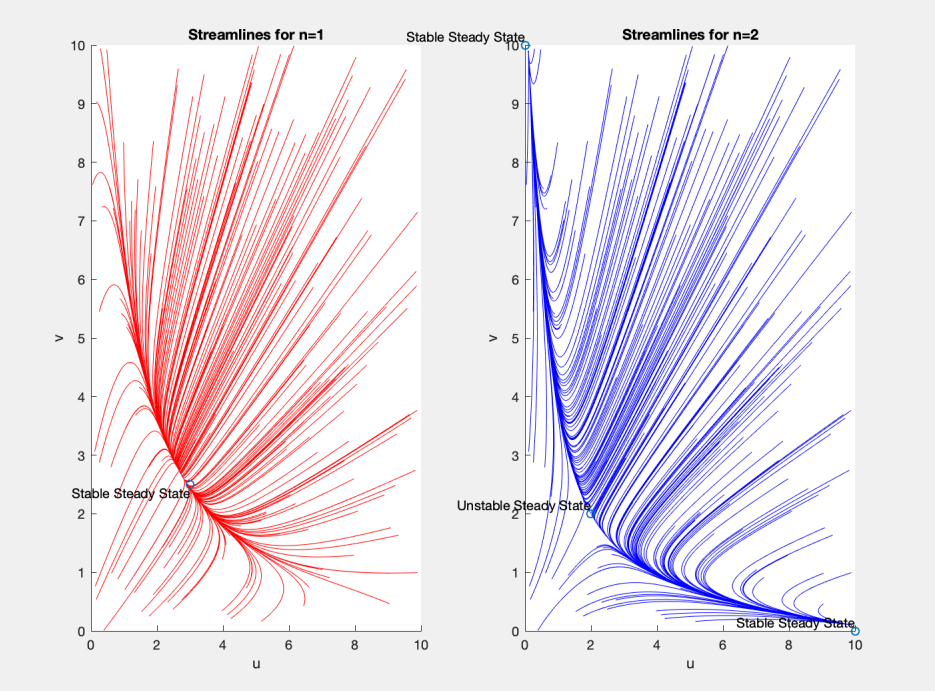
Plotting these gives the following plot. This was done with CHEME5440Final_2b.m



From these nullclines plots, it seems that there is 1 solution when n=1 and 3 solutions when n=2. A higher degree of cooperativity leads to more solutions or steady states. Increasing the degree of cooperativity increases the effect of the inhibitor making the production of the inhibited protein more step-like. This allows the lines to cross over each other more often.

5 Question 2 Part C

The vector with components $f(u, v)$ and $g(u, v)$ indicates the direction in which the state of the network is heading (which steady state it is heading towards). This is relevant as to what amounts of u and v are changing and whether or not the system is heading towards the steady state it was just perturbed off of. The plot below (created in CHEME5440Final_2c.m) has the steady states and their assessments with random starting points for each streamline. With higher cooperativity, the opportunity for a toggle switch arises where two stable steady states arise where there is either all u or all v. Also with higher cooperativity, there is an unstable steady state in the middle that is not returned to when perturbed away from (the center steady state).



6 Question 2 Part D

To build the Jacobian start by taking the derivatives of f and g:

$$J = \begin{pmatrix} f_u & f_v \\ g_u & g_v \end{pmatrix}$$

$$J = \begin{pmatrix} -1 & \frac{-\alpha n v^{n-1}}{(1+v^n)^2} \\ \frac{-\alpha n u^{n-1}}{(1+u^n)^2} & -1 \end{pmatrix}$$

Now the stability criterion is that λ_1 and λ_2 are less than 0.

$$\lambda_{1,2} = \frac{tr(J) \pm \sqrt{tr(J)^2 - 4 * det(J)}}{2}$$

Now:

$$tr(J) = -2$$

$$det(J) = 1 - \frac{\alpha^2 n^2 v^{n-1} u^{n-1}}{(1+v^n)^2(1+u^n)^2}$$

So:

$$\lambda_{1,2} = \frac{-2 \pm \sqrt{\frac{4\alpha^2 n^2 v^{n-1} u^{n-1}}{(1+v^n)^2(1+u^n)^2}}}{2}$$

$$\lambda_{1,2} = -1 \pm \sqrt{\frac{\alpha^2 n^2 v^{n-1} u^{n-1}}{(1+v^n)^2(1+u^n)^2}}$$

$$\lambda_{1,2} = -1 \pm \frac{\alpha n v^{\frac{n-1}{2}} u^{\frac{n-1}{2}}}{(1+v^n)(1+u^n)}$$

Now the u and v values are those at steady state u_s and v_s. The stability criterion is as follows where the real parts must be less than zero:

$$Re(-1 \pm \frac{\alpha n v_s^{\frac{n-1}{2}} u_s^{\frac{n-1}{2}}}{(1+v_s^n)(1+u_s^n)}) < 0$$

Analyzing the steady state where $u_s = v_s$:

$$Re(-1 \pm \frac{\alpha n v_s^{n-1}}{v_s^{2n} + 2v_s^n + 1}) < 0$$

From this equation, when $n = 1$ the value of both eigenvalues is less than zero because the value of the second term will always have an absolute value less than 1 and thus a stable steady state (unless α increases to a very large value or the rate of synthesis gets really fast. This could cause an unstable steady state by increasing the value of the second term). As n increases, one can see that the second term increases above one as long as alpha is also large enough. The factor of n in the numerator being greater than 1 increases this chance. If this term increases above one, then an eigenvalue is no longer negative and there is an unstable steady state. If the rate of synthesis is lower, than the steady state stays stable. In summary, a higher rate of synthesis and higher n value lead to an unstable steady state at the center steady state.

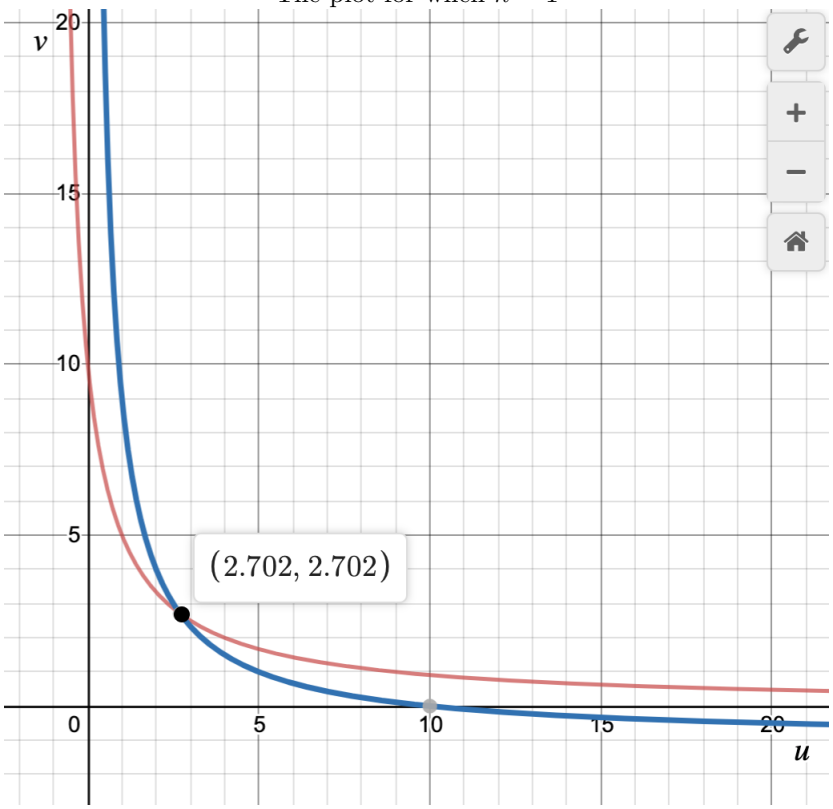
7 Question 2 Part E

The steady state values of u_s and v_s for the center steady state occur when $u_s = v_s$. Therefore plotting the following equations (at steady state) will reveal their value:

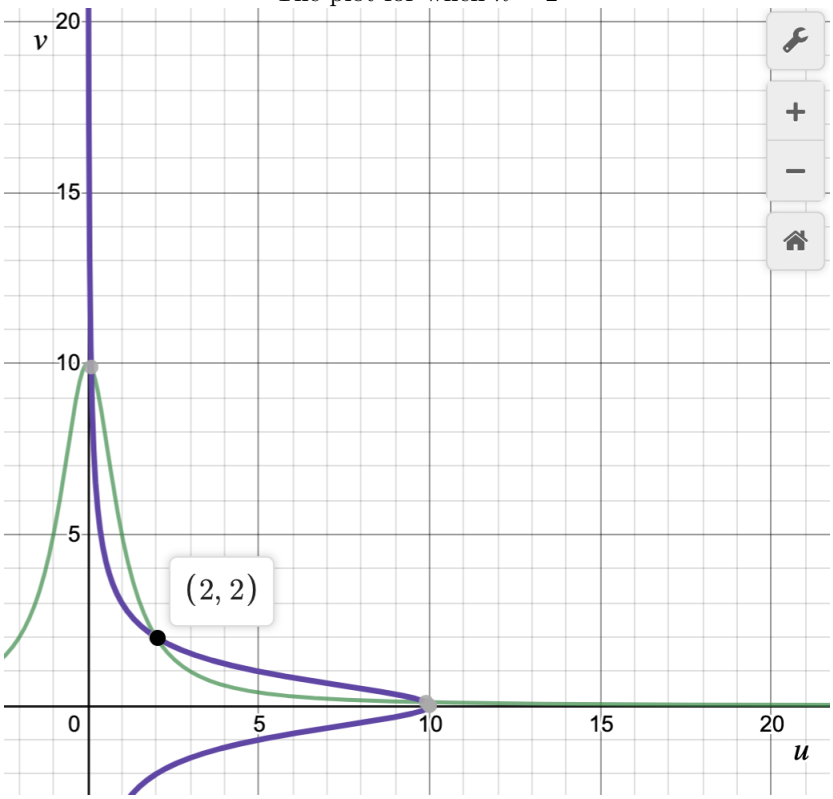
$$u = \frac{\alpha}{1+v^n}$$
$$v = \frac{\alpha}{1+u^n}$$

They were plotting using the online graphing calculator Desmos for ease.

The plot for when $n = 1$



The plot for when $n = 2$



Therefore $u_s = v_s = 2.702$ for $n = 1$ and $u_s = v_s = 2$ for $n = 2$. Now solving the eigen value equation:
For n=1

$$\lambda_{1,2} = -1 \pm \frac{\alpha n {v_s}^{\frac{n-1}{2}} {u_s}^{\frac{n-1}{2}}}{(1+v_s^n)(1+u_s^n)}$$

$$\lambda_{1,2} = -1 \pm \frac{10 * 1}{(1+2.702)(1+2.702)}$$

$$\lambda_1 = -1.73 \ and \ \lambda_2 = -.27$$

For n=2

$$\lambda_{1,2} = -1 \pm \frac{10 * 2 * 2^{\frac{1}{2}} 2^{\frac{1}{2}}}{(1+2^2)(1+2^2)}$$

$$\lambda_1 = 0.6 \ and \ \lambda_2 = -2.6$$

We can see from these eigen values that increasing the cooperativity from 1 to 2 makes the center steady state unstable (has a positive eigenvalue). This means that the steady state for equal values of u and v is unstable and the system moves away from it if perturbed. This makes sense for the toggle switch nature of the system because the system actually moves to the stable steady states where there is either all u or all v. This also means that having more step like or sensitive synthesis of the repressors leads to the possibility of a toggle.

8 Question 2 Part F-1

Since we are assuming fast equilibrium, this means we can assume equation (3)-(5) are at steady state when considering the timescale of equation (6). Therefore:

$$k_f L R_i = k_r R_i^*$$

$$k_f^{ND} N_i D_j = k_r^{ND} N_i^*$$

$$k_D R_i^* = \gamma_D D_i$$

Now plugging into the equation (6) for $\frac{dR_i}{dt}$:

$$\frac{dR_i}{dt} = \frac{\beta^n}{K^n + N_i^{*n}} - \gamma_R R_i$$

$$\frac{dR_i}{dt} = \frac{\beta^n}{K^n + (\frac{k_f^{ND} N_i D_j}{k_r^{ND}})^n} - \gamma_R R_i$$

$$\frac{dR_i}{dt} = \frac{\beta^n}{K^n + (\frac{k_f^{ND} N_i k_D R_j^*}{k_r^{ND} \gamma_D})^n} - \gamma_R R_i$$

$$\frac{dR_i}{dt} = \frac{\beta^n}{K^n + (\frac{k_f^{ND} N_i k_D k_f L R_j}{k_r^{ND} \gamma_D k_r})^n} - \gamma_R R_i$$

Now this is a function with only R_i and R_j as variables.

$$\frac{dR_1}{dt} = f(R_1, R_2) = \frac{\beta^n}{K^n + (\frac{k_f^{ND} N_1 k_D k_f L R_2}{k_r^{ND} \gamma_D k_r})^n} - \gamma_R R_1$$

$$\frac{dR_2}{dt} = g(R_1, R_2) = \frac{\beta^n}{K^n + (\frac{k_f^{ND} N_2 k_D k_f L R_1}{k_r^{ND} \gamma_D k_r})^n} - \gamma_R R_2$$

9 Question 2 Part F-2

Plugging in the dimensional variables given results in the following (deriving just the R_1 term as the R_2 term follows the same method):

$$\gamma_R \frac{duK}{d\tau} = \frac{\beta^n}{K^n + (\frac{k_f^{ND} N_1 k_D k_f L K v}{k_r^{ND} \gamma_D k_r})^n} - \gamma_R u K$$

$$\frac{du}{d\tau} = \frac{\beta^n / K \gamma_R}{K^n + (\frac{k_f^{ND} N_1 k_D k_f L K v}{k_r^{ND} \gamma_D k_r})^n} - u$$

$$\frac{du}{d\tau} = \frac{\beta^n / K^{n+1} \gamma_R}{1 + (\frac{k_f^{ND} N_1 k_D k_f L v}{k_r^{ND} \gamma_D k_r})^n} - u$$

$$\frac{dv}{d\tau} = \frac{\beta^n / K^{n+1} \gamma_R}{1 + (\frac{k_f^{ND} N_2 k_D k_f L u}{k_r^{ND} \gamma_D k_r})^n} - v$$

These equations have the same form as the Collins toggle switch besides constants in the α synthesis term and the inhibition term of the denominator. Remembering the stability criterion in the center steady state:

$$Re(-1 \pm \frac{\alpha n v_s^{n-1}}{v_s^{2n} + 2v_s^n + 1}) < 0$$

Now by analogy the stability criterion for the Notch-Delta system is as follows. If you calculate the Jacobian it comes out to the same result:

$$Re(-1 \pm \frac{AnV^{n-1}}{V^{2n} + 2V^n + 1}) < 0$$

Where $A = \beta^n / K^{n+1} \gamma_R$ and the center steady state value v_s is within $V = \frac{k_f^{ND} N_1 k_D k_f L v_s}{k_r^{ND} \gamma_D k_r}$ (if analyzing the u case). Now, by analogy to Part D, one can see that increasing values that lead to an increased rate of synthesis

A will lead to a unstable center steady state. This means that increasing β or decreasing K and γ_R lead to the instability. This makes sense because β has something to do with the synthesis of the receptor, γ_R has to do with the degradation of the repressor (decreases effective synthesis), and a lower K means a lower saturation constant and thus steeper step like function (meaning an instability at a center steady state as shown in earlier parts of the question). Now, larger V values lead to smaller second terms in the stability criterion and thus stability. To make V larger, L or ligand can be increased. Also k_f^{ND}, N_i, k_D, and k_f can be increased while k_r^{ND},γ_D, and k_r increasing will lead to instability. So in summary, to drive the system to instability, V must decrease. To do so, ligand L can be decreased as well as k_f^{ND}, N_i, k_D, and k_f while conversely k_r^{ND},γ_D, and k_r can be increased. It makes sense that decreasing the ligand will drive the system towards instability because then less receptors are activated to then up-regulate Delta. The whole system is driven by increasing ligand to change the concentration of Notch or Delta on the surfaces of the cells and eventually decide the fate of the cell. If this ligand concentration is lower, the whole system can relax and assume fates rather than constantly signaling and creating new activated receptors. The other terms that when decreased lead to instability also have something to do with creating Delta (or the R_i^* that activates it) while the ones that when increased lead to instability have to do with depleting Delta. So driving the system to not create/activate Delta (or the R_i^* that activates it) leads to instability. This makes sense because when the communication between the cells at Notch-Delta goes away, the cells can assume a fate as one has reached the point where it has more Notch than Delta and is moving towards the stable steady state.