

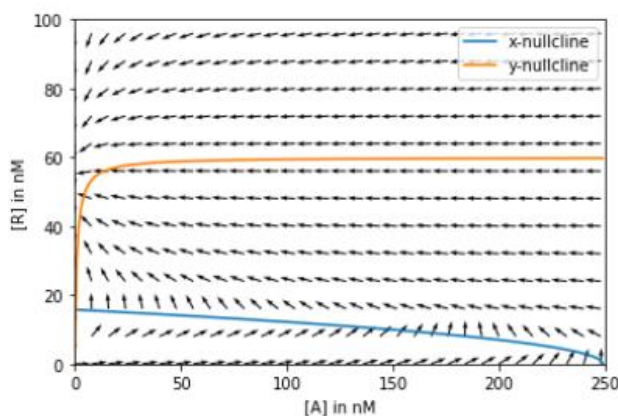
1.

- When the reporter is expressed on the repressilator plasmid but not on a separate high-copy plasmid, oscillations are more regular.
- The competition for shared proteases for the degradation tagged reporter proteins, result in 'retroactivity' effects on oscillations. Removing the interference created very regular oscillations.
- The variance in the three interpeak distances showed that the noisiest phase was when TetR levels were low, so they use titration sponge, which is plasmid with PLtet-binding sites, to soak up TetR molecules away from the TetR-controlled promoter, which raising the threshold effectively and reduced noise.

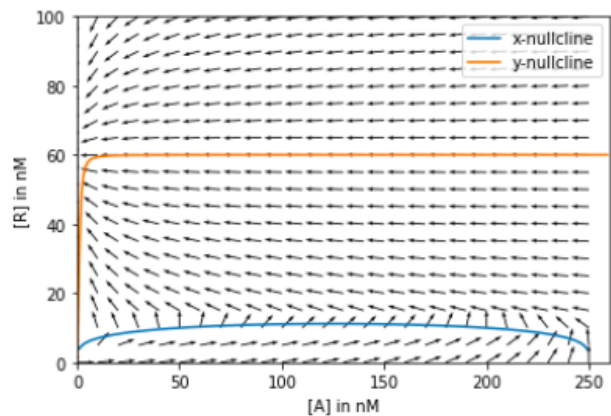
2.

- Both are present the leak of transcription rate of A and R, respectively. The unit is as  $\text{AlphaA, nM} \cdot \text{hrs}^{-1}$ . (What we derive from beginning goff to gon, the Alpha0 was usually separate with the probability. It would be a little confusing when is divided by denominator.)

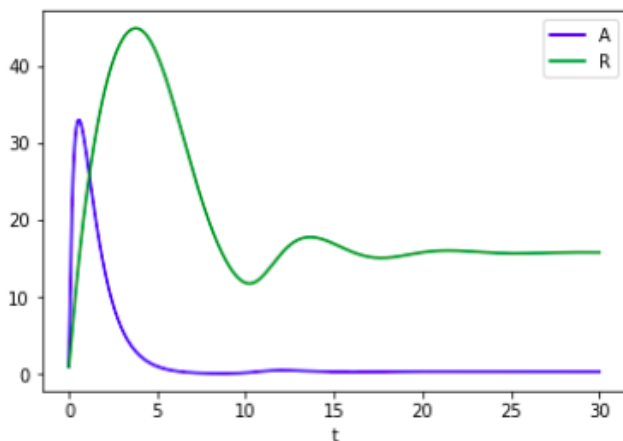
b\_i.



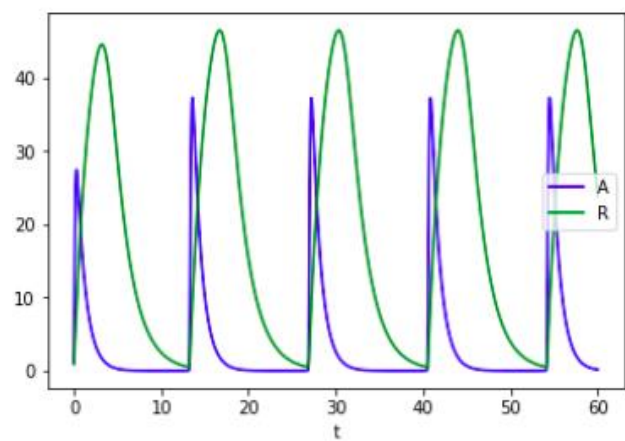
b\_ii.



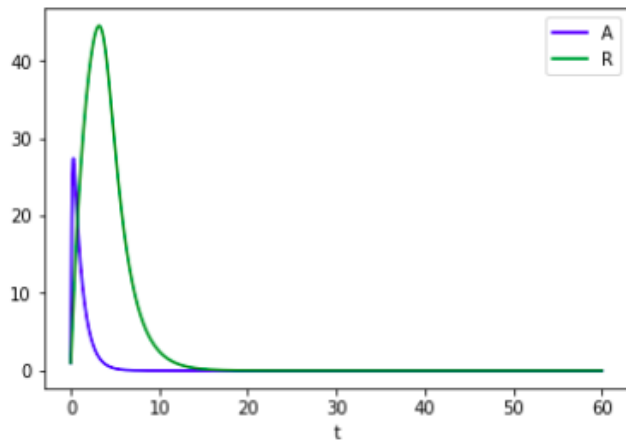
c.(n=1, m=2)



c.(n=2, m=4) sustained



d. (n=2, m=4, alphaA0=0)



d.

When the  $\alpha A_0 = 0$ , the reboost or restart of the signal A is harder or can't be. The oscillation begins again when the signal hit 0, but when there's no leak of transcription of A and A still be degraded, the system absents of A, then there's no next round, in the other words, the whole system shut down.

e. When  $\gamma_A = \gamma_R$ , or  $\gamma_A$  bit smaller than  $\gamma_R$ , result in oscillations (ex:  $\gamma_A = 0.5$ ,  $\gamma_R = 0.5$ ), but the response drops down through t, finally the signal approaching 0. When the  $\gamma_A$  is bit higher than  $\gamma_R$  (ex:  $\gamma_A = 0.51$ ,  $\gamma_R = 0.5$ ), it results in sustained oscillation. However, when  $\gamma_A$  is too big (ex:  $\gamma_A = 3.1622$ ,  $\gamma_R = 0.5$ ), there's no oscillation. The rate setting is pretty critical, it still sustained oscillation when (ex:  $\gamma_A = 3.1621$ ,  $\gamma_R = 0.5$ ). When the  $\gamma_A$  bit higher than  $\gamma_R$ , the frequency of oscillation is highest, the higher the  $\gamma_A$ , the lower the frequency.

3.

$$a. [m]_{ss} = \frac{\alpha_m}{\gamma_m + \frac{k \times C \times \alpha_m}{\gamma_s}}$$

$$b. \gamma_m \ll \frac{k \times C \times \alpha_m}{\gamma_s}$$

$$[m]_{ss} = \frac{\gamma_s}{k \times C}$$

The  $[m]_{ss}$  is independent with its own production rate,  $\alpha_m$ .

$$c. S\left(\frac{\frac{\Delta[m]_{ss}}{[m]_{ss}}}{\frac{\Delta\alpha_m}{\alpha_m}}\right) = \frac{\alpha_m}{[m]_{ss}} \times \frac{d[m]_{ss}}{d\alpha_m} = \frac{\gamma_m}{1 + \gamma_m}$$

When  $\gamma_m = 0$ , sensitivity = 0.

When  $\gamma_m = 1$ , sensitivity = 1/2.

The calculation procedure and plot are showed in picture below: (next page)



$$c) \quad S([M]_{ss}, \alpha_m) = \frac{\Delta [M]_{ss} / [M]_{ss}}{\Delta \alpha_m / \alpha_m} = \frac{\alpha_m}{[M]_{ss}} \cdot \frac{\Delta [M]_{ss}}{\Delta \alpha_m} = \frac{\alpha_m}{[M]_{ss}} \cdot \frac{d[M]_{ss}}{d\alpha_m}$$

$$= \left( \gamma_m + \frac{K \cdot \alpha_m}{\gamma_s} \right) \cdot \frac{d[M]_{ss}}{d\alpha_m} = (\gamma_m + 1) \cdot \frac{d \frac{\alpha_m}{\gamma_m + \frac{K \cdot \alpha_m}{\gamma_s}}}{d\alpha_m}$$

$$\frac{d \cdot \frac{\alpha_m}{\gamma_m + \frac{K \cdot \alpha_m}{\gamma_s}}}{d\alpha_m} = \frac{d}{d\alpha_m} \frac{\alpha_m / \gamma_m}{\left( \frac{K \cdot \alpha_m}{\gamma_s \gamma_m} + 1 \right)}$$

$$= \frac{1}{\gamma_m} \frac{d}{d\alpha_m} \frac{\alpha_m}{\mu \alpha_m + 1}$$

$$= \frac{1}{\gamma_m} \times \frac{1 + \cancel{\mu \alpha_m} - \alpha_m \times \mu}{(1 + \mu \alpha_m)^2}$$

$$= \frac{1}{\gamma_m} \times \frac{1}{\left[ 1 + \left( \frac{K \cdot \alpha_m}{\gamma_s \gamma_m} \right) \right]^2} = \frac{1}{\gamma_m} \times \frac{1}{\left( 1 + \frac{1}{\gamma_m} \right)^2}$$

Assume = 1

$$= \frac{\gamma_m}{(1 + \gamma_m)^2}$$

$$S = (\gamma_m + 1) \cdot \frac{\gamma_m}{(1 + \gamma_m)^2} = \frac{\gamma_m}{1 + \gamma_m}$$