PART 2 Simplified Model

Although ODEs provide a thorough, precise description of the whole system, they contain too many equations and parameters which would act as a barrier for simulation and further analysis. A simplification of complicated ODEs is necessary. We simplify every single ODE according to certain appropriate assumptions. Finally, we came up with a set of DDE equations.

Assumptions we have made to deduct the original equations involve:

- Relatively faster reactions such as transcription reactions and binding reactions will reach to Quasi-equilibrium.
- Basal expression of protein is so meager that they can be ignored in modeling.
- Two series of Hill Kinetics equations can be estimated through a single Hill Kinetics equation.
- Protein which is translated from mRNA is proportional to corresponding mRNA in a previous time.

Consider gene lasR and luxR which are all constant genes, since AHL has a relatively low concentration compared to other substances, we assume that protein concentrations of PlasR and PluxR are irrelevant to AHLs. Therefore, concentrations of these proteins will reach to constant quantities. Moreover, certain mRNAs will also be constant. That is

$$\frac{\mathrm{dM_{lasR}}}{\mathrm{dt}} = \frac{\mathrm{dM_{luxR}}}{\mathrm{dt}} = \frac{\mathrm{dP_{lasR}}}{\mathrm{dt}} = \frac{\mathrm{dP_{luxR}}}{\mathrm{dt}} = 0$$

We have:

$$M_{\mathrm{lasR}} = \frac{V_{MlasR}}{d_{MlasR}} 、 M_{\mathrm{luxR}} = \frac{V_{MluxR}}{d_{MluxR}}$$

$$\mathrm{P_{lasR}} = \frac{K_{TL1} \times M_{\mathrm{lasR}}}{d_{PlasR}} = \frac{K_{TL1} \times V_{\mathrm{MlasR}}}{d_{PlasR} \times d_{MlasR}}$$

$$\mathrm{P_{luxR}} = \frac{K_{TL4} \times M_{\mathrm{luxR}}}{d_{PluxR}} = \frac{K_{TL4} \times V_{\mathrm{MluxR}}}{d_{PluxR} \times d_{MluxR}}$$

Then focus on compound LA1 and LA2. The binding process is far quicker than other reactions such as translation reactions, thus we assume that the compounds are Quasi-equilibrium:

$$\frac{\mathrm{dLA1}}{\mathrm{dt}} = \frac{\mathrm{dLA2}}{\mathrm{dt}} = 0$$

We have:

$$\mathrm{LA1} = \frac{\mathrm{k_1} \times P_{lasR}}{\mathrm{k_2}} \times A1_{\mathrm{C1}} = \rho_1 \times A1_{\mathrm{C1}}$$

$$\mathrm{LA2} = \frac{\mathrm{k_3} \times P_{luxR}}{\mathrm{k_4}} \times A2_{\mathrm{C2}} = \rho_2 \times A2_{\mathrm{C2}}$$

Take the expressions to equation (6) (15), the functions are transformed to:

$$\frac{dA1_{c1}}{dt} = \gamma(A1_e - A1_{c1})、\frac{dA2_{c2}}{dt} = \gamma(A2_e - A2_{c2})$$

The transformed functions have nothing to do with feedback factors compared to original ones. Knowing that such feedback is essential for our system, we add additional feedback factors k_a、k_b manipulatively.

$$\frac{dA1_{c1}}{dt} = -k_a \times A1_{c1} + \gamma(A1_e - A1_{c1})$$

$$\frac{dA2_{c2}}{dt} = -k_b \times A2_{c2} + \gamma(A2_e - A2_{c2})$$

Then we want to come up a clear expression of $A2_{c1}$、$A1_{c2}$. They are generated by protein PluxI and PlasI, whose translation rate is much slower than the transcription rate. So we assume that certain mRNA is Quasi-equilibrium:

$$\frac{dMLuxI}{dt} = \frac{dMlasI}{dt} = 0$$

We have:

$$M_{luxI} = \left. k_{MluxI} \times [\beta_{MluxI} + (1 - \beta_{MluxI}) \times \frac{LA1^{n1}}{{K_{MI}}^{n1} + LA1^{n1}}] \middle/ d_{MluxI} \right.$$

$$M_{lasI} = \left. k_{MlasI5} \times [\beta_{MlasI} + (1 - \beta_{MlasI}) \times \frac{{K_{MT}}^{n3}}{{K_{M3}}^{n3} + TetR^{n3}}] \middle/ d_{MlasI} \right.$$

By ignoring the basal expression:

$$M_{luxI} = \left. k_{MluxI} \times \frac{LA1^{n1}}{{K_{MI}}^{n1} + LA1^{n1}} \middle/ d_{MluxI} \right.$$

$$M_{lasI} = \left. k_{MlasI} \times \frac{{K_{MT}}^{n3}}{{K_{M3}}^{n3} + TetR^{n3}} \middle/ d_{MlasI} \right.$$

Knowing that protein PtetR is controlled by LA2 through Hill Function, we assume that concentration of mRNA is directly related to LA2:

$$M_{lasI} = \left. \mathrm{k_{MlasI}} \times \frac{\mathrm{K_{M4}}^{\mathrm{n4}}}{\mathrm{K_{M4}}^{\mathrm{n4}} + \mathrm{LA2}^{\mathrm{n4}}} \middle/ d_{MlasI} \right.$$

Parameter KM4 is relevant to both KM1 and KM3, however accurate function depicting the relationship is unknown. We estimate the quantity of KM4 by multiplying KM1 and KM3. Noting LA1 and LA2 have already been expressed, we have:

$$M_{luxI} = \left. \mathrm{k_{MluxI}} \times \frac{\mathrm{A1C1}^{\mathrm{n1}}}{(\mathrm{K_{MI}}/\rho_1)^{\mathrm{n1}} + \mathrm{A1C1}^{\mathrm{n1}}} \middle/ d_{MluxI} \right.$$

$$M_{lasI} = \left. \mathrm{k_{MlasI}} \times \frac{\left(\mathrm{K_{M4}}/\rho_2\right)^{\mathrm{n3}}}{\left(\mathrm{K_{M4}}/\rho_2\right)^{\mathrm{n3}} + \mathrm{A2C2}^{\mathrm{n3}}} \middle/ d_{MlasI} \right.$$

Then we have trouble in expressing protein PluxI and PlasI. Since concentration of protein is the integrals of its mRNA, we assume that it is proportional to concentration of mRNA in a previous time. Thus we have a DDE function:

$$P_{luxI} = \mathrm{k_{TL2}} \times \frac{\mathrm{A1C1}^{\mathrm{n1}}}{(\mathrm{K_{MI}}/\rho_1)^{\mathrm{n1}} + \mathrm{A1C1}(\mathrm{t} - \tau_1)^{\mathrm{n1}}}$$

$$P_{lasI} = \mathrm{k_{TL4}} \times \frac{\left(\mathrm{K_{M4}}/\rho_2\right)^{\mathrm{n3}}}{\left(\mathrm{K_{M4}}/\rho_2\right)^{\mathrm{n3}} + \mathrm{A2C2}(\mathrm{t} - \tau_2)^{\mathrm{n3}}}$$

Equations concerning environmental AHLs remain unchanged.

The original ODEs can be transformed to a much simple DDEs by above deductions.

$$\frac{d\mathrm{A1_{c1}}}{dt} = -k_a \cdot \mathrm{A1_{c1}} + \gamma \cdot (\mathrm{A1_e} - \mathrm{A1_{c1}}) \tag{20}$$

$$\frac{d\mathrm{A2_{c1}}}{dt} = k_{TL2} \cdot \frac{\mathrm{A1_{c1}}(t - \tau_1)^{\mathrm{n1}}}{(\mathrm{K_{M1}}/\rho_1)^{\mathrm{n1}} + \mathrm{A1_{c1}}(t - \tau_1)^{\mathrm{n1}}} + \gamma \cdot (\mathrm{A2_e} - \mathrm{A2_{c1}}) - k_b \cdot \mathrm{A2_{c1}} \tag{21}$$

$$\frac{d\mathrm{A1_{c2}}}{dt} = k_{TL4} \cdot \frac{\left(\mathrm{K_{M4}}/\rho_2\right)^{\mathrm{n2}}}{\left(\mathrm{K_{M4}}/\rho_2\right)^{\mathrm{n2}} + \mathrm{A2_{C2}}(\mathrm{t} - \tau_2)^{\mathrm{n2}}} + \gamma \cdot (\mathrm{A1_e} - \mathrm{A1_{c2}}) - k_a \cdot \mathrm{A1_{c2}} + \mathrm{arab} \cdot (\mathrm{t} > t_1) \cdot (\mathrm{t} < t_2) \tag{22}$$

$$\frac{dA2_{c2}}{dt} = -k_b \cdot A2_{c2} + \gamma \cdot (A2_e - A2_{c2}) \quad (23)$$

$$\frac{dA1_e}{dt} = -\mu A1_e - \gamma \cdot \frac{p \cdot n_{12}}{1 - p \cdot (1 + n_{12})} \cdot (A1_e - A1_{c1}) - \gamma \cdot \frac{p}{1 - p \cdot (1 + n_{12})} \cdot (A1_e - A1_{c2}) \quad (24)$$

$$\frac{dA2_e}{dt} = -\mu A2_e - \gamma \cdot \frac{p \cdot n_{12}}{1 - p \cdot (1 + n_{12})} \cdot (A2_e - A2_{c1}) - \gamma \cdot \frac{p}{1 - p \cdot (1 + n_{12})} \cdot (A2_e - A2_{c2}) \quad (25)$$

Parameters

Some of the parameters are derived from original ones, some of them are created to describe the new equations, and others are set for further testing.

Parameter Name	Value	Description	Reference
n1	2	Parameters of hill equation	Assumption
n2	2	Parameters of hill equation	Assumption
K_{M1}	40nM	Parameters of hill equation	Assumption
K_{M4}	1600nM	Parameters of hill equation	Estimate
ρ_1	434	Calculated as constant	$\frac{k_1}{k_2} \cdot \frac{K_{TL1} \times V_{MlasR}}{d_{PlasR} \times d_{MlasR}}$
ρ_2	1309	Calculated as constant	$\frac{k_3}{k_4} \frac{K_{TL4} \times V_{MluxR}}{d_{PluxR} \times d_{MluxR}}$
k_{TL2}	30 nM/min	Translation rate, connecting with strength of RBS(tunable)	All tunable for test Just estimate as standard
k_{TL4}	30 nM/min	Translation rate, connecting with strength of RBS(tunable)	
γ	2.5min^{-1}	Diffusion rate of AHL through membrane.	published paper
n_{12}	1	Ratio of cell1 volume to cell2 volume	For testing
p	0.2	Ratio of cell2 volume to total volume	For testing
μ	10min^{-1}	Dilution rate of C12 and C6 in environment (tunable)	For testing
k_a	0	Feedback factor	For testing
k_b	0	Feedback factor	For testing
τ_1	30min	Time delay	For testing
τ_2	50min	Time delay	For testing
arab	0 nM/min	Input to change phase	For testing
t_1	0min	Beginning time of arab	For testing
t_2	0min	Ending time of arab	For testing

Table 2 Parameters of DDEs

Results

1) General result

We coded the system in MATLAB. The result shows that every signal AHL is oscillating.

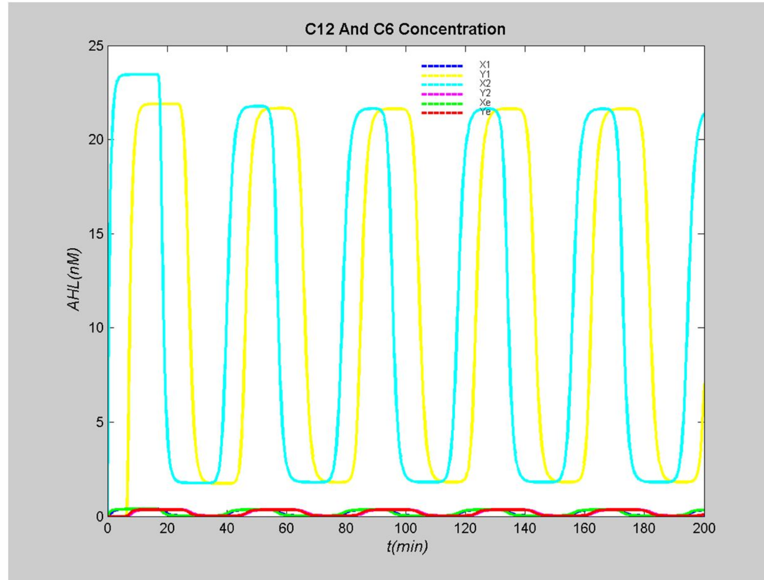

Figure 3 Signal AHL Oscillations

2) Stability analysis

In a nonlinear system, proper value ranges of the parameters are vital for producing a periodic oscillating solution. It's not difficult to find the nonlinear part in our model, thus, we sought to analyze the sensitivity of Hill parameters and thoroughly reveal the internal relationship between Hill parameters and the system's robustness. For simplicity, here we denote $^{K_{M1}}/_{\rho_1}$, $^{K_{M4}}/_{\rho_2}$ and k_{TL2},k_{TL4} by k_{m1},k_{m2} and β_1, β_2 respectively. Without loss of generality, we determined to search the affection to the system's robustness caused by the fluctuation of binary parameter (k_{m2}, β_2). After observing distinct oscillation mode (we choose two types of concentration of AHLs in environment as our observed object) and its phase trajectory, we depicted the bifurcation of our system.

Set basic parameters as follows:

$k_a = 0, k_{\square} = 0, \mu = 10\text{min}^{-1}, \gamma = 2.3\text{min}^{-1}, \beta_1 = 30\text{nm/min}, k_{m1} = 1, n_1 = n_2 = 2, p = 0.2, arab = 20, t_1 = t_2 = 0, \tau_1 = 6, \tau_2 = 10.$

We changed the binary parameter $(\beta_2,\ k_{m2})$ and got the result below.

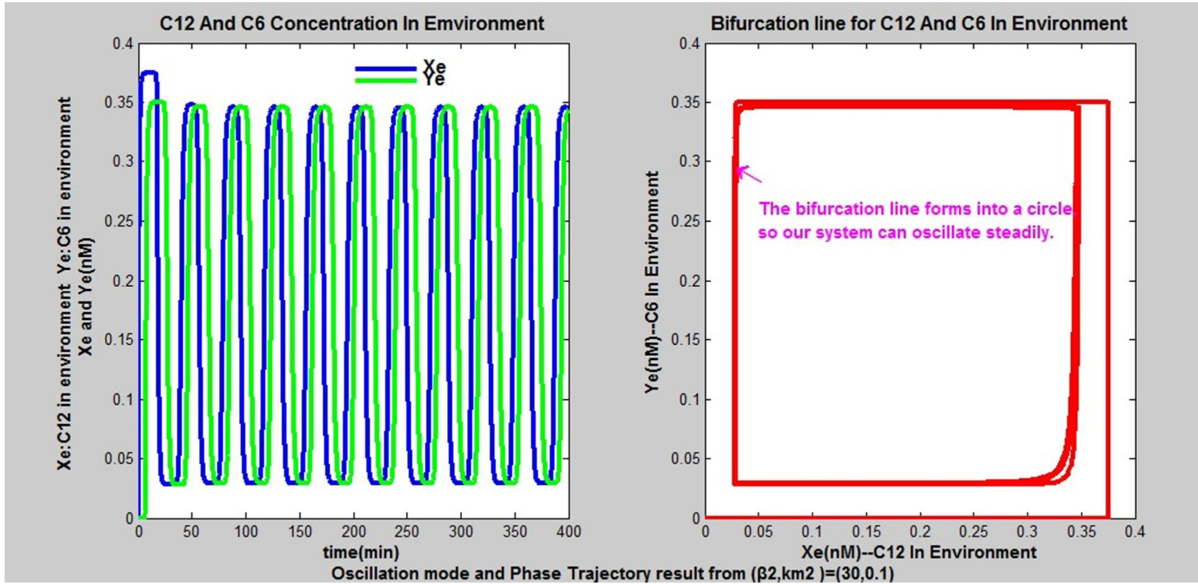

Figure 4 C12 and C6 when $(\boldsymbol{\beta}_2, k_{m2}) = (30, 0.1)$

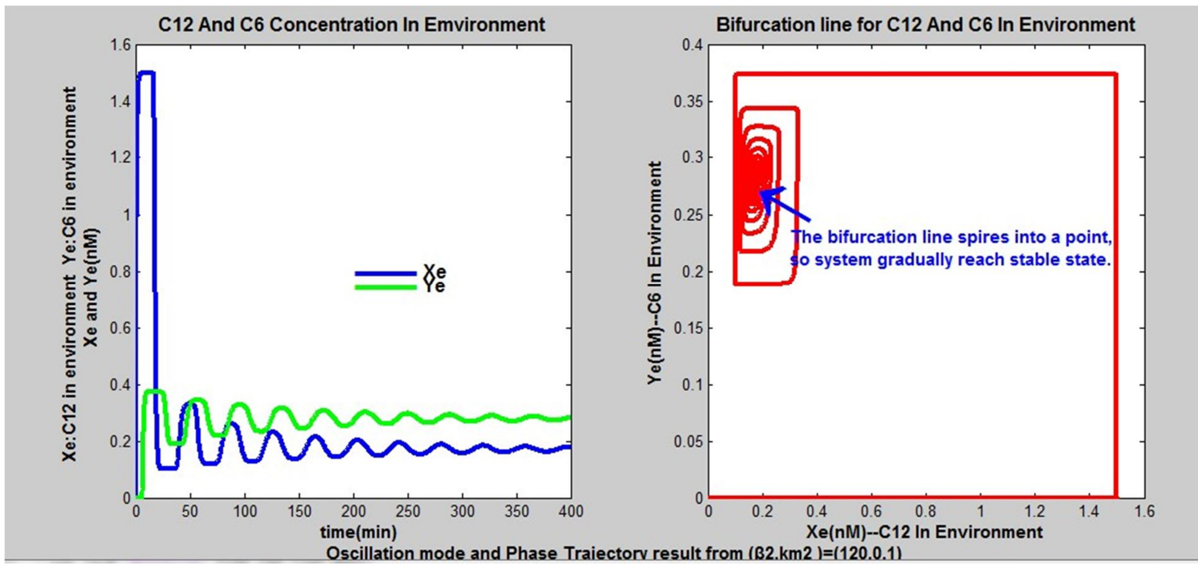

Figure 5 C12 and C6 when $(\boldsymbol{\beta}_2, k_{m2}) = (120, 0.1)$

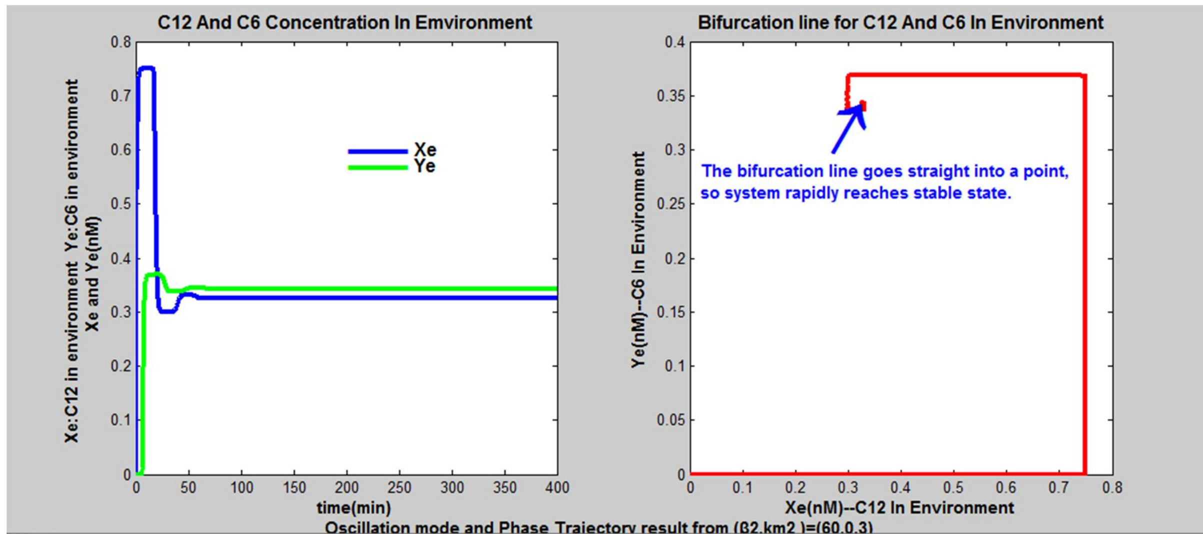

Figure 6 C12 and C6 when $(\beta_2, k_{m2}) = (60, 0.3)$

After simulating the system at different parameters, we recorded several critical points for oscillation and made a table as follows.

k_{m2}	0.05	0.05	0.075	0.075	0.1	0.1	0.125	0.125	0.15	0.15	0.175	0.175
β_2	0.9	900	1.1	575	1.3	170	1.5	145	1.7	105	1.9	84
k_{m2}	0.2	0.2	0.25	0.25	0.3	0.3	0.35	0.35	0.4	0.4	0.5	0.5
β_2	2.1	55	2.6	35	4.2	19	4.8	12	6.9	8	7.6	7.6

Table 3 Critical points (β_2, k_{m2}) for oscillation

Depicting those critical points on an axis, we immediately got the bifurcation line of parameters (β_2, k_{m2}), which indicates the parameters' value range when our system can oscillate stably, being marked in '***bistable***'.

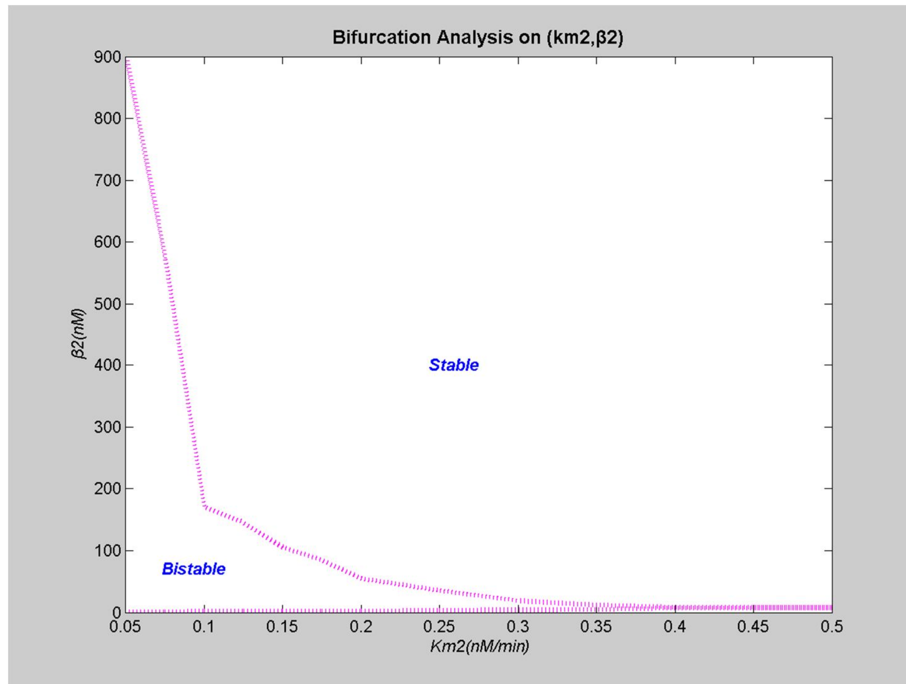

Figure 7 Bifurcation Analysis on (β_2, k_{m2})

3) Proportion of cell volume

In actual vivo experiment, volumes of two separate cells might not be the same. We simulate the system by changing the proportion of two types of cells. What we know from the simulation is that cell proportions only affect the amplitude of signal molecules' oscillation, but no influence to the period or stability of oscillation. The following graphics are drawn under cell proportion 1 and 0.7.

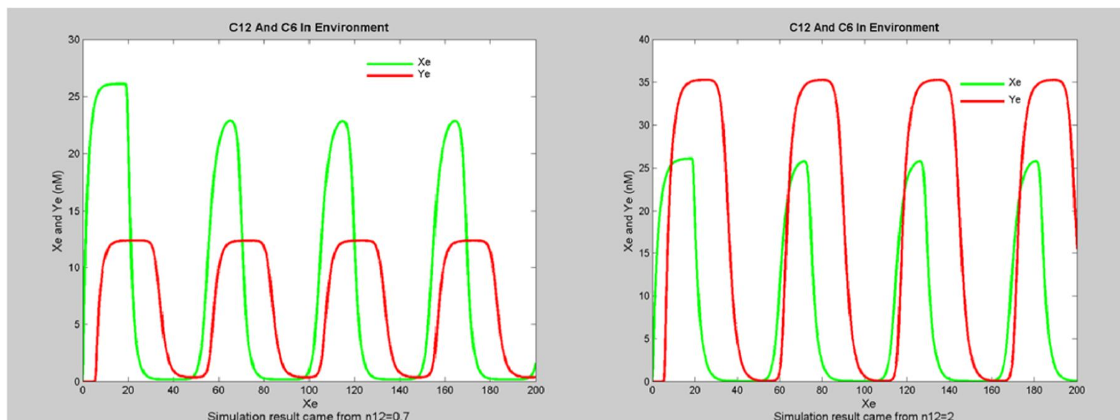

Figure 8 oscillations under different cell proportions

We can find that under different cell proportions, our system can oscillate at different amplitude, and varying in cell proportions may also lead to the change of oscillation period.

4) Period adjustment

As **figure 2** indicates, we expected to adjust oscillation period by adding signal molecule aTc into our system. A small molecule as aTc is, it can easily bind to protein TetR and quickly depletes TetR in cell 2, which results in reduction of TetR net production rate, and indirectly, the protein's inhibition on promoter5 is crippled. Thus, it would take a longer time for our system to reach each threshold, which is equivalent to prolonging the time delay τ_2 in our simplified model. So we can deduce that changing the amount of aTc added into the system in precise model is equivalent to varying the time delay τ_2 in simplified model.

Simulation results under distinct τ_2 are presented as follows.

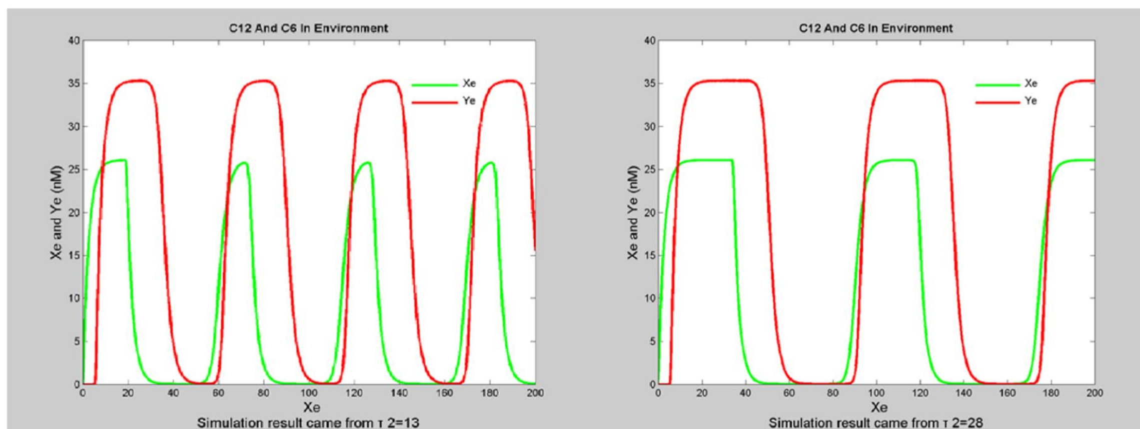

Figure 9 Oscillation cycle's regulation

The result is exactly what we expected, which clearly demonstrates our system can truly be controlled by adding in external signal molecules.

5) Phase adjustment

In cell 2's gene circuit, we designed a promoter induced by arabinose, marked by promoter 6 (see in **figure 2**). Promoter 6 is in a suppressed state until being induced by adding arabinose, and after the inhibition is relieved, signal molecule 3O12HSL will be generated extra. We can also analyze the differential equations describing $\frac{dA1C2}{dt}$, when adding arabinose during period from t_1 to t_2, $\frac{dA1C2}{dt}$ contains an extra item $\text{arab} \cdot (\text{t} > t_1) \cdot (\text{t} < t_2)$. The parameter arab can reflect the rate of adding arabinose nonlinearly. Here we set $\text{arab} = 20$, $t_1 = 80$, $t_2 = 120$ and simulation result is presented as follow.

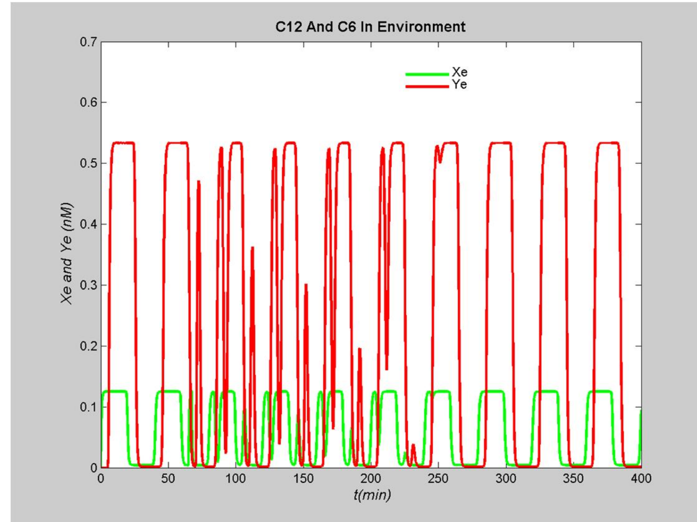

Figure 10 Oscillation phase's regulation