Model Part: Oscillation stability and sensitivity analyses and Oscillation adjustment

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In our project, we are dedicated to design a quorum-sensing oscillator which consists of two types of cells. Cells of the same type can fluctuate synchronously and certain designs were made to adjust the phase and the amplitude of oscillation. These are the things that our modeling part aims to simulate. We built and simplified our simulation system step by step and deepened into further characteristics of the system, which would provide firm evidence proving that our design does work. Here we will introduce our modeling work in four parts.

PART 1 Original Full Model

At our first step, we wanted to describe the system thoroughly without leaving out any seemingly unimportant actions and factors. As a result, the description of the system contains every possible mass actions as well as some hill kinetics, Henri-Michaelis-Menten. We came up a set of ODEs with 19 equations.

Construction of ODE equation

CELL I

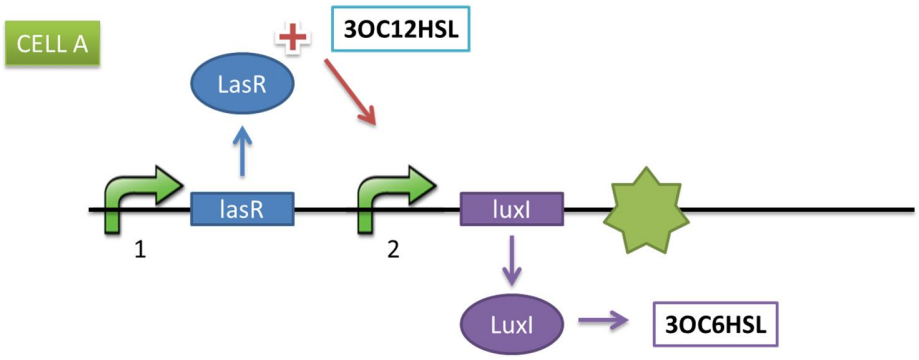

Figure 1 designed circuit of cell I

CELL II

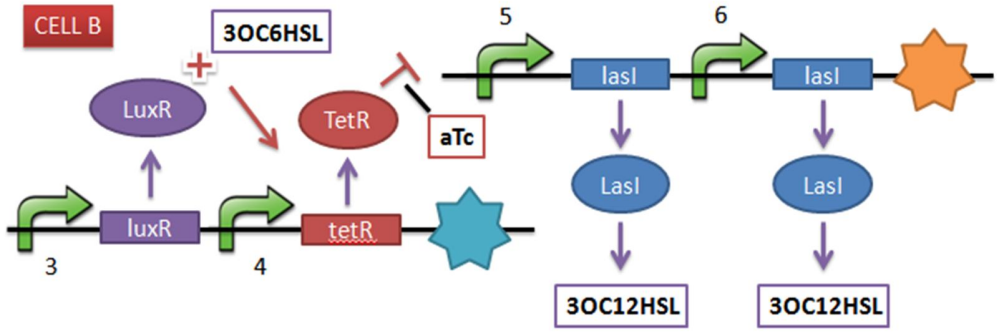

Figure 2 designed circuit of cell II

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Promoter 1 and promoter 2 preceding lasR and luxR genes respectively are constant promoters, which will transcribe and translate into protein PlasR and PluxR. LA1 is the binding association of lasR and 30C12HSL(A2C1) and it can affect the subsequent promoter 2 which can be described by Hill Equation. The same goes to LA2. Gene luxI will be translated into protein PluxI which would generate 30C6HSL(A1C1) through enzymatic reaction. The AHL will diffuse through the membrane to the environment(A1e) and finally enter into Cell 2(A1C2). Protein PtetR which is translated from gene tetR represses promoter 5 which is responsible for transcription of gene lasI. Promoter 6 is constant for translation of protein PlasI. 30C12HSL(A2C2) is generated from Protein PlasI through enzymatic reaction. 30C12HSL in the environment is called A2e which will diffuse to Cell 1. aTc is added manipulatively to change the phase of oscillation by binding the protein PTetR. Therefore, we have these following ODEs:

$$\frac{dM_{lasR}}{dt} = v_{MlasR} - d_{MlasR} \times M_{lasR} \tag{1}$$

$$\frac{dM_{luxI}}{dt} = k_{MluxI} \times [\beta_{MluxI} + (1 - \beta_{MluxI}) \times \frac{LA1^{n1}}{{K_{M1}}^{n1} + LA1^{n1}}] - d_{MluxI} \times M_{luxI} \tag{2}$$

$$\frac{dP_{lasR}}{dt} = k_{TL1} \times M_{lasR} - d_{PlasR} \times P_{lasR} - k1 \times A1_{c1} \times P_{lasR} + k2 \times LA1 \tag{3}$$

$$\frac{dP_{luxI}}{dt} = k_{TL2} \times M_{luxI} - d_{PluxI} \times P_{luxI} \tag{4}$$

$$\frac{dLA1}{dt} = k1 \times A1_{c1} \times P_{lasR} - k2 \times LA1 \tag{5}$$

$$\frac{dA1_{c1}}{dt} = -k1 \times A1_{c1} \times P_{lasR} + k2 \times LA1 + \gamma \cdot (A1_e - A1_{c1}) \tag{6}$$

$$\frac{dA2_{c1}}{dt} = \lambda_1 P_{luxI} + \gamma \cdot (A2_e - A2_{c1}) \tag{7}$$

$$\frac{dM_{luxR}}{dt} = v_{MluxR} - d_{MluxR} \times M_{luxR} \tag{8}$$

$$\frac{dM_{TetR}}{dt} = k_{MTetR} \times [\beta_{MTetR} + (1 - \beta_{MTetR}) \times \frac{LA2^{n2}}{{K_{M2}}^{n2} + LA2^{n2}}] - d_{MTetR} \times M_{TetR} \tag{9}$$

$$\frac{dM_{lasI}}{dt} = k_{MlasI} \times [\beta_{MlasI} + (1 - \beta_{MlasI}) \times \frac{{K_{MT}}^{n3}}{{K_{M3}}^{n3} + TetR^{n3}}] - d_{MlasI} \times M_{lasI} \tag{10}$$

$$\frac{dP_{luxR}}{dt} = k_{TL3} \times M_{luxR} - d_{PluxR} \times P_{luxR} - k3 \times A2_{c2} \times P_{luxR} + k4 \times LA2 \tag{11}$$

$$\frac{dP_{lasI}}{dt} = k_{TL4} \times M_{lasI} - d_{PlasI} \times P_{lasI} \tag{12}$$

$$\frac{dP_{TetR}}{dt} = k_{TL5} \times M_{TetR} - d_{PTetR} \times P_{TetR} - k5 \times P_{TetR} \times aTc + k6 \times TetR^* \tag{13}$$

$$\frac{dLA2}{dt} = k3 \times A2_{c2} \times P_{luxR} - k4 \times LA2 \quad (14)$$

$$\frac{dA2_{c2}}{dt} = -k3 \times A2_{c2} \times P_{luxR} + k4 \times LA2 + \gamma \cdot (A2_e - A2_{c2}) \quad (15)$$

$$\frac{dTetR^*}{dt} = k5 \times P_{TetR} \times aTc - k6 \times TetR^* \quad (16)$$

$$\frac{dA1_{c2}}{dt} = \lambda_2 P_{lasI} + \gamma \cdot (A1_e - A1_{c2}) \quad (17)$$

$$\frac{dA1_e}{dt} = -\gamma \frac{1 - p \cdot (1 + n_{12})}{p \cdot n_{12}} \cdot (A1_e - A1_{c1}) - \gamma \cdot \frac{1 - p \cdot (1 + n_{12})}{p} \cdot (A1_e - A1_{c2}) - \mu A1_e \quad (18)$$

$$\frac{dA2_e}{dt} = -\gamma \frac{1 - p \cdot (1 + n_{12})}{p \cdot n_{12}} \cdot (A2_e - A2_{c1}) - \gamma \cdot \frac{1 - p \cdot (1 + n_{12})}{p} \cdot (A2_e - A2_{c2}) - \mu A2_e \quad (19)$$

Parameters

The parameters are inherent factors determining the behaviors, properties of a system. We selected the quantities thoughtfully from previous iGEM teams and some others were found from published papers.

Parameter Name	Value	Description	Reference
n_1	2	Parameters of hill equation	Assumption
n_2	2	Parameters of hill equation	Assumption
n_3	2	Parameters of hill equation	Assumption
K_{M1}	40nM	Parameters of hill equation	Assumption
K_{M2}	40nM	Parameters of hill equation	Assumption
K_{M3}	40nM	Parameters of hill equation	Assumption
k_{MluxI}	5.25nM/min	Strength decide by R0079	Peking 2009
k_{MTetR}	5.25nM/min	Strength decide by R0062	
k_{MlasI}	5.25nM/min	Strength decide by R0040	
k_{TL1}	42	Translation rate, connecting with strength of RBS(tunable)	All tunable for test Just estimate as standard
k_{TL2}	42	Translation rate, connecting with strength of RBS(tunable)	
k_{TL3}	42	Translation rate, connecting with strength of RBS(tunable)	
k_{TL4}	42	Translation rate, connecting with strength of RBS(tunable)	
k_{TL5}	42	Translation rate, connecting with strength of RBS(tunable)	
v_{MlasR}	5.25nM/min	Transcription rate(tunable)	Peking 2009
v_{MluxR}	5.25nM/min	Transcription rate(tunable)	Peking 2009

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β_{MluxI}	0.01	Basal expression in hill equation	Assumption
β_{MTetR}	0.01	Basal expression in hill equation	Assumption
β_{MlasI}	0.01	Basal expression in hill equation	Assumption
γ	2.5min^{-1}	Diffusion rate of AHL through membrane.	published paper
λ_1	0.06	Generation rate of 30C6HSL	published paper
λ_2	0.06	Generation rate of 30C12HSL	published paper
d_{MlasR}	0.0173min^{-1}	Degradation constant of mRNA	published paper
d_{MluxI}	0.0173min^{-1}	Degradation constant of mRNA	published paper
d_{MluxR}	0.0173min^{-1}	Degradation constant of mRNA	published paper
d_{MTetR}	0.0173min^{-1}	Degradation constant of mRNA	published paper
d_{PluxR}	$2.31 \times 10^{-2}\text{min}^{-1}$	Degradation constant of luxR protein.	2010 MIT
d_{PluxI}	$1.67 \times 10^{-2}\text{min}^{-1}$	Degradation constant of luxI protein.	2010 MIT
d_{PlasI}	0.01min^{-1}	Degradation constant of lasI protein.	2010 MIT
d_{PlasR}	$1.88 \times 10^{-2}\ \text{min}^{-1}$	Degradation constant of lasR protein.	published paper
d_{PtetR}	$1.67 \times 10^{-2}\text{min}^{-1}$	Degradation constant of tetR protein.	assumption
k1	$9.6 \times 10^{-3}\text{nM}^{-1}\text{min}^{-1}$	Rate constant of binding reaction between LasR and 30C12HSL	published paper
k2	$15\ \text{min}^{-1}$	Rate constant of dissociation reaction between LasR and 30C12HSL	published paper
k3	$0.14232\text{nM}^{-1}\text{min}^{-1}$	Rate constant of binding reaction between LuxR and 30C6HSL	2008 KULeuven
k4	60min^{-1}	Rate constant of dissociation reaction between LuxR and 30C6HSL	2008 KULeuven
k5	$0.06\text{nM}^{-1}\text{min}^{-1}$	Rate constant of binding reaction between tetR and aTc	published paper
k6	50min^{-1}	Rate constant of dissociation reaction between tetR and aTc	published paper

p	0.2	Ratio of cell2 volume to total volume	For test
n_{12}	1	Ratio of cell1 volume to cell2 volume	For test
μ	$10min^{-1}$	Dilution rate of C12 and C6 in environment (tunable)	For test

Table 1 Parameters of ODEs

Results

We simulated this system by SIMBIOLOGY, a toolbox embedded in MATLAB. However, unaware of the key parameters to which the system is sensitive, we felt difficult to control or adjust properly, and the simulation result of the system came into a damped oscillation. We ascribed the inability of our model to the fact that the precise descriptions contain too many equations and parameters and we felt obliged to establish a simplified model in place of the precise one for simulation and further analysis.

PART 2 Simplified DDE 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

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

We have:

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

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

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

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:

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

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.

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

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

Then we want to come up a clear expression of $\mathrm{A2_{c1}}$、$\mathrm{A1_{c2}}$. 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:

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

We have:

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

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

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By ignoring the basal expression:

$$M_{luxI} = {k_{MluxI} \times \frac{LA1^{n1}}{K_{MI}{}^{n1} + LA1^{n1}}} \Big/ d_{MluxI}$$

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

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

$$M_{lasI} = {k_{MlasI} \times \frac{K_{M4}{}^{n4}}{K_{M4}{}^{n4} + LA2^{n4}}} \Big/ d_{MlasI}$$

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:

$$M_{luxI} = {k_{MluxI} \times \frac{A1C1^{n1}}{(K_{MI}/\rho_1)^{n1} + A1C1^{n1}}} \Big/ d_{MluxI}$$

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

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:

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

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

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Equations concerning environmental AHLs remain unchanged.

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

$$\frac{dA1_{c1}}{dt} = -k_a \cdot A1_{c1} + \gamma \cdot (A1_e - A1_{c1}) \tag{20}$$

$$\frac{dA2_{c1}}{dt} = k_{TL2} \cdot \frac{A1_{c1}(t-\tau_1)^{n1}}{(K_{M1}/\rho_1)^{n1} + A1_{c1}(t-\tau_1)^{n1}} + \gamma \cdot (A2_e - A2_{c1}) - k_b \cdot A2_{c1} \tag{21}$$

$$\frac{dA1_{c2}}{dt} = k_{TL4} \cdot \frac{(K_{M4}/\rho_2)^{n2}}{(K_{M4}/\rho_2)^{n2} + A2_{C2}(t-\tau_2)^{n2}} + \gamma \cdot (A1_e - A1_{c2}) - k_a \cdot A1_{c2} + arab \cdot (t > t_1) \cdot (t < t_2) \tag{22}$$

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

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

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

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	For testing

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		C6 in environment (tunable)	
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.

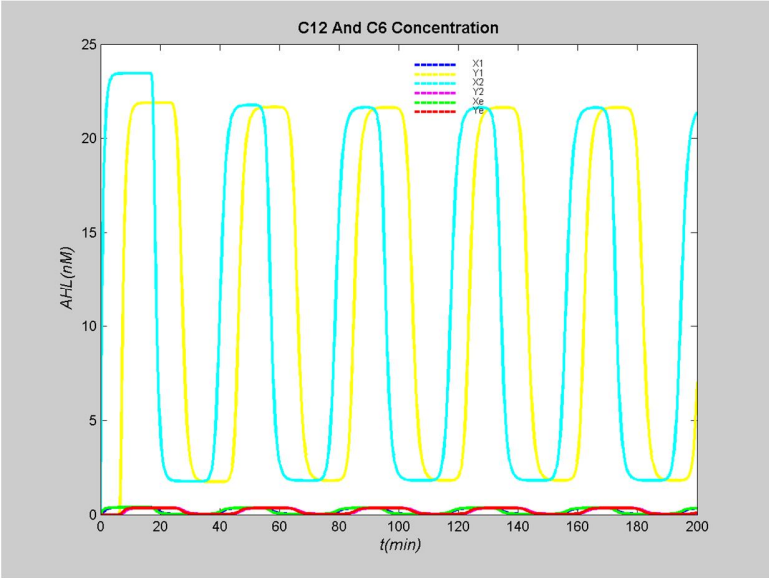

Figure 3 Signal AHL Oscillations

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}/ρ_1, K_{M4}/ρ_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:

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

We changed the binary parameter (β_2， k_{m2}) and got the result below.

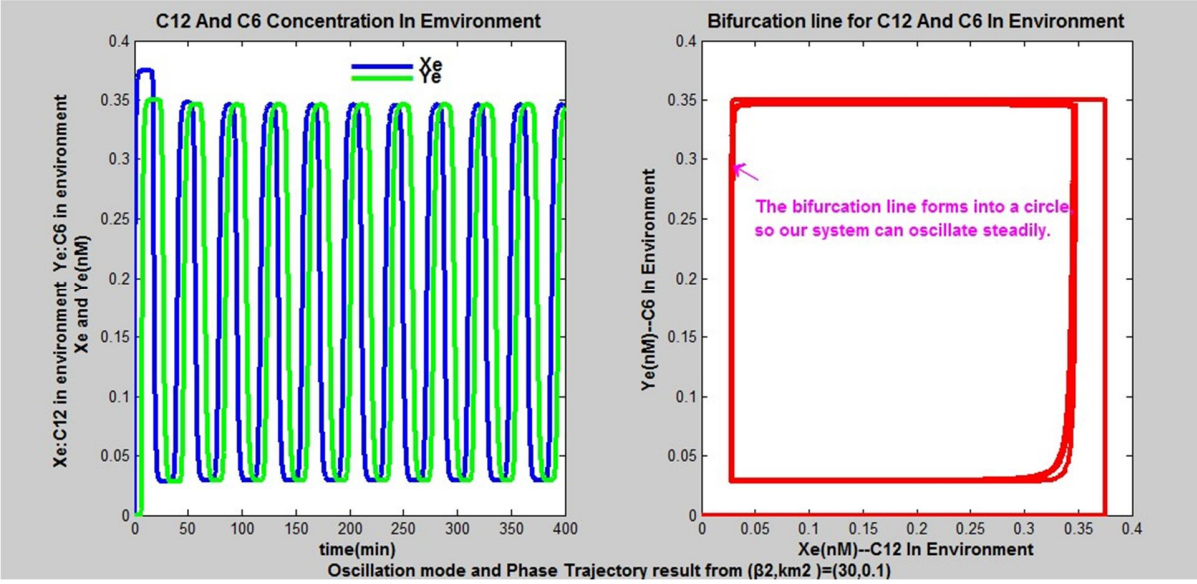

Figure 4 C12 and C6 when $(\beta_2, k_{m2}) = (30, 0.1)$

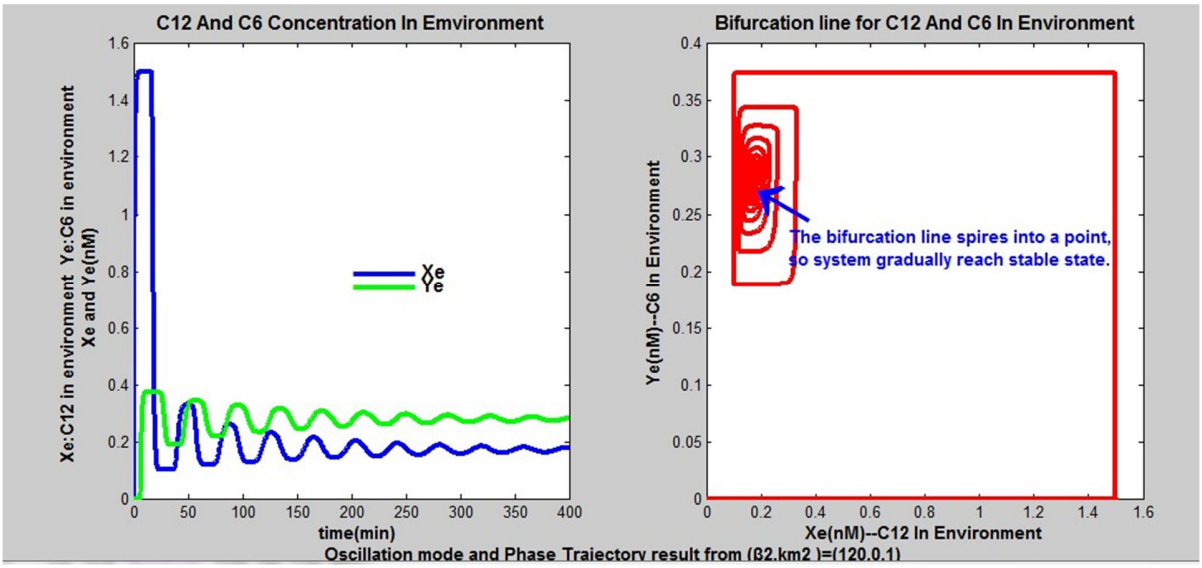

Figure 5 C12 and C6 when $(\beta_2, k_{m2}) = (120, 0.1)$

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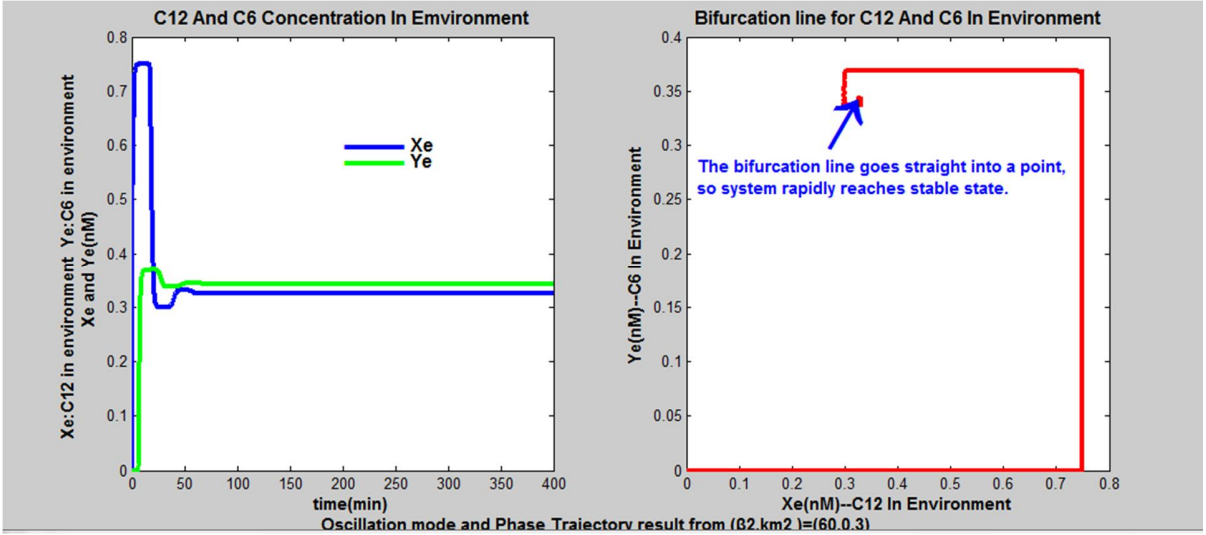

Figure 6 C12 and C6 when $(\beta_2, k_{m2}) = (60, 0.3)$

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***'.

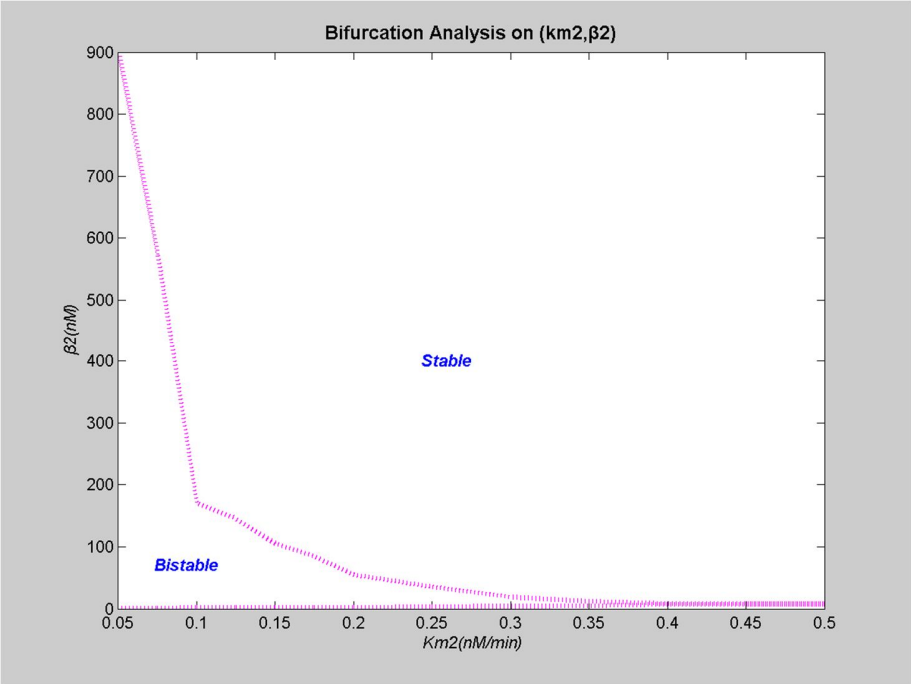

Figure 7 Bifurcation Analysis on (β_2, k_{m2})

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.

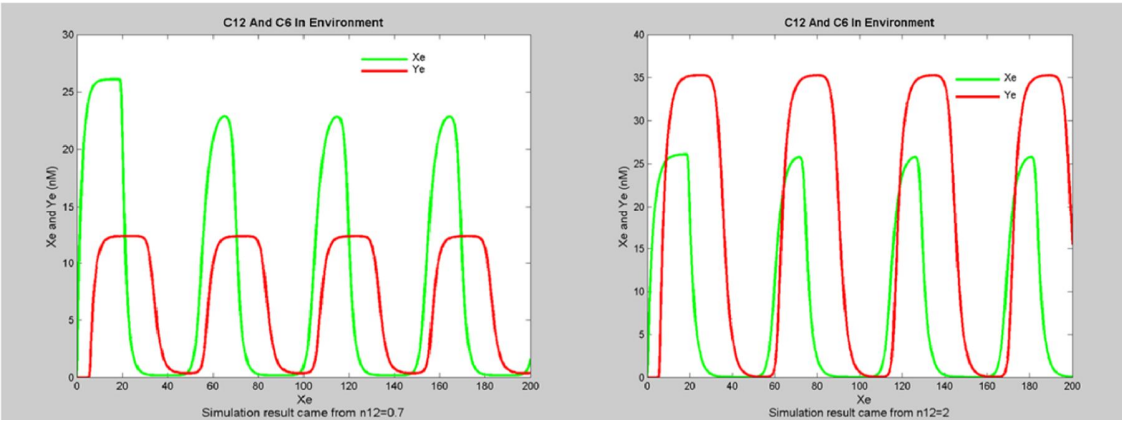

Figure 8 oscillations under different cell proportions

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.

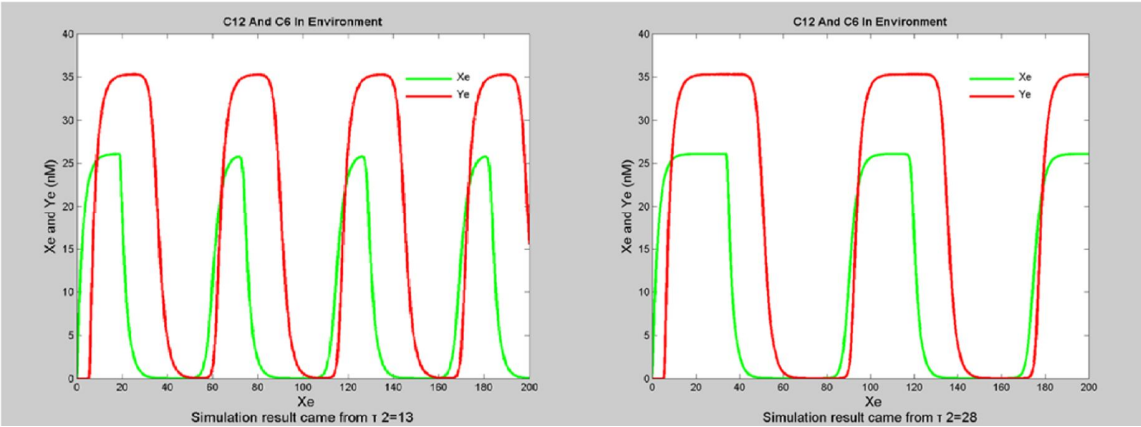

Figure 9 Oscillation cycle's regulation

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 $\mathrm{arab} \cdot (\mathrm{t} > t_1) \cdot (\mathrm{t} < t_2)$. The parameter arab can reflect the rate of adding arabinose nonlinearly. Here we set $\mathrm{arab} = 20$, $t_1 = 80$, $t_2 = 120$ and simulation result is presented as follow.

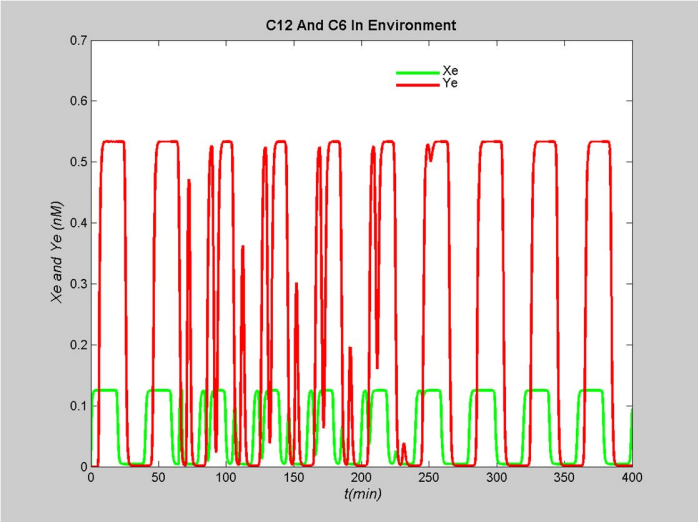

Figure 10 Oscillation phase's regulation

PART 3 Dimensionless Model

In order to make a further analysis on stability of the system, sensitivity of parameters, feedback factors-we manipulate all the arguments and parameters to make them dimensionless. Analysis of this part is crucial since parameters in vivo experiment may be different and even at odds with modeling ones but a proper dimensionless can reveal the mathematical essence of our model.

Considering the Hill equation in the simplification DDEs, $A1_{c1}$ and K_{M1}/ρ_1 should be the same order of magnitude, thus K_{M1}/ρ_1 is a well measurement of quantities of $A1_{c1}$. We have:

$$[A1_{c1}]\sim K_{M1}/\rho_1$$

Similarly,

$$[A2_{C2}]\sim K_{M4}/\rho_2$$

In equation (20) (23), Let:

$$dA1_{c1}/dt = 0\,, dA2_{c2}/dt = 0$$

We have:

$$[A1_e]\sim\frac{(k_a+\gamma)\cdot K_{M1}}{\gamma\cdot\rho_1},\quad [A2_e]\sim\frac{(k_b+\gamma)\cdot K_{M4}}{\gamma\cdot\rho_2}$$

In equation (24) (25), Let:

$$dA1_e/dt = 0\,, dA2_e/dt = 0$$

We have:

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$$[\mathrm{A1_{c2}}]\sim\frac{\mathrm{K_{M1}}}{\rho_1}\cdot\left(1+\frac{\mu}{\gamma}\cdot\frac{1-\mathrm{p}\cdot(1+\mathrm{n_{12}})}{\mathrm{p}}\right)$$

$$[\mathrm{A2_{c1}}]\sim\frac{\mathrm{K_{M4}}}{\rho_2}\cdot\left(1+\frac{\mu}{\gamma}\cdot\frac{1-\mathrm{p}\cdot(1+\mathrm{n_{12}})}{\mathrm{p}\cdot\mathrm{n_{12}}}\right)$$

Define:

$$x_1=\frac{\mathrm{A1_{c1}}}{\mathrm{K_{M1}}/\rho_1},\qquad x_2=\frac{\mathrm{A1_{c2}}}{\mathrm{K_{M1}}/\rho_1}\cdot\frac{1}{1+\frac{\mu}{\gamma}\cdot\frac{1-\mathrm{p}(1+\mathrm{n_{12}})}{\mathrm{p}}},$$

$$y_1=\frac{\mathrm{A2_{c1}}}{\mathrm{K_{M4}}/\rho_2}\cdot\frac{1}{1+\frac{\mu}{\gamma}\cdot\frac{1-\mathrm{p}(1+\mathrm{n_{12}})}{\mathrm{p}\cdot\mathrm{n_{12}}}},\qquad y_2=\frac{\mathrm{A2_{C2}}}{\mathrm{K_{M4}}/\rho_2},$$

$$x_e=\frac{\mathrm{A1_e}}{\mathrm{K_{M1}}/\rho_1}\cdot\frac{k_a+\gamma}{\gamma},\qquad y_e=\frac{\mathrm{A2_e}}{\mathrm{K_{M4}}/\rho_2}\cdot\frac{k_b+\gamma}{\gamma},\qquad t^*=\gamma\cdot t$$

Define:

$$a=\frac{k_a}{\gamma},\qquad b=\frac{k_b}{\gamma},\qquad \mathrm{u}=\frac{\mu}{\gamma},\qquad \mathrm{v}=\frac{p}{1-p(1+\mathrm{n_{12}})},\qquad \mathrm{m}=\frac{\rho_1}{\mathrm{K_{M1}}}\cdot\frac{k_{p2}}{r},\qquad \mathrm{n}=\frac{\rho_2}{\mathrm{K_{M4}}}\cdot\frac{k_{p1}}{r}$$

The dimensionless equations are as follows:

$$\frac{dx_1}{dt^*}=-(a+1)\cdot x_1+(1+a)\cdot x_e \tag{26}$$

$$\frac{dy_2}{dt^*}=-(b+1)\cdot y_2+(1+b)\cdot y_e \tag{27}$$

$$\frac{dx_2}{dt^*}=m\frac{1}{1+u/\mathrm{v}}\cdot\frac{1}{1+y_2(t^*-{\tau_1}^*)^{n2}}+\frac{1}{1+u/\mathrm{v}}\cdot\frac{1}{a+1}x_e-(a+1)x_2 \tag{28}$$

$$\frac{dy_1}{dt^*}=n\frac{1}{1+u/\mathrm{vn_{12}}}\cdot\frac{x_1(t^*-{\tau_2}^*)^{n1}}{1+x_1(t^*-{\tau_2}^*)^{n1}}+\frac{1}{1+u/\mathrm{vn_{12}}}\cdot\frac{1}{b+1}y_e-(b+1)y_1 \tag{29}$$

$$\frac{dx_e}{dt^*}=-(u+(1+\mathrm{n_{12}})\cdot v)\cdot x_e+(1+a)\cdot v\mathrm{n_{12}}\cdot x_1+(1+a)\cdot v\cdot\left(1+\frac{\mathrm{u}}{\mathrm{v}}\right)x_2 \tag{30}$$

$$\frac{dy_e}{dt^*}=-(u+(1+\mathrm{n_{12}})\cdot v)\cdot y_e+(1+b)\cdot v\cdot y_2+(1+b)\cdot v\mathrm{n_{12}}\cdot\left(1+\frac{\mathrm{u}}{\mathrm{vn_{12}}}\right)y_1 \tag{31}$$

Parameters

Parameter Name	Value	Reference
a	0	For testing
b	0	For testing
${\tau_1}^*$	25	For testing
${\tau_2}^*$	70	For testing

u	4	$\frac{\mu}{\gamma}$
v	0.33	$\frac{p}{1-p(1+\mathrm{n}_{12})}$
m	52.08	$\frac{\rho_1}{\mathrm{K}_{\mathrm{M1}}}\cdot\frac{k_{p2}}{r}$
n	39.28	$\frac{\rho_2}{\mathrm{K}_{\mathrm{M4}}}\cdot\frac{k_{p1}}{r}$
n_{12}	1	For testing
n_1	2	Hill constant
n_2	2	Hill constant

Table 4 parameters of dimensionless DDEs

Results

1) Sensitivity analysis

In order to find out the key parameters which will affect stability of the system at most, we need to make a sensitivity analysis on each. At first, we did brief and instinctive analyses on each parameter as follows. $\tau_1{}^*$ And $\tau_2{}^*$ represent time delay in cell 1 and cell 2 respectively, which have been discussed in **part 2**, have little influence on stability of system but intend to affect the oscillation period merely. Parameters a and b refer to feedback factors indirectly, which have not been discussed before, we will see how a and b affect our system later. We have clarified that parameter u is equivalent to μ/γ, thus, u is directly decided by the dilution rate of signal molecules 3OC12HSL and 3OC6HSL in environment and will inevitably influence stability of oscillation. As for m and n, they are inseparably connected to the Hill parameters whose sensitivity have been analyzed in part 2, so we can deduce that m and n are both sensitive parameters to our system.

Here we mainly did sensitivity analyses on parameters m, n and u. Parameters were set fundamentally as **Table 4** shows.

Simulation results reveal that the system can oscillate stably only when $\mathrm{u} < 5.3$(fixed the other two sensitive parameters), $11.9 < \mathrm{m} < 71.3$ and $\mathrm{n} > 34.4$. In other words, to ensure the stability of oscillation, the dilution rate cannot be too high, while the promoter 2 and 4 which affect m and n should be chosen appropriately.

Model Part: Oscillation stability and sensitivity analyses and Oscillation adjustment

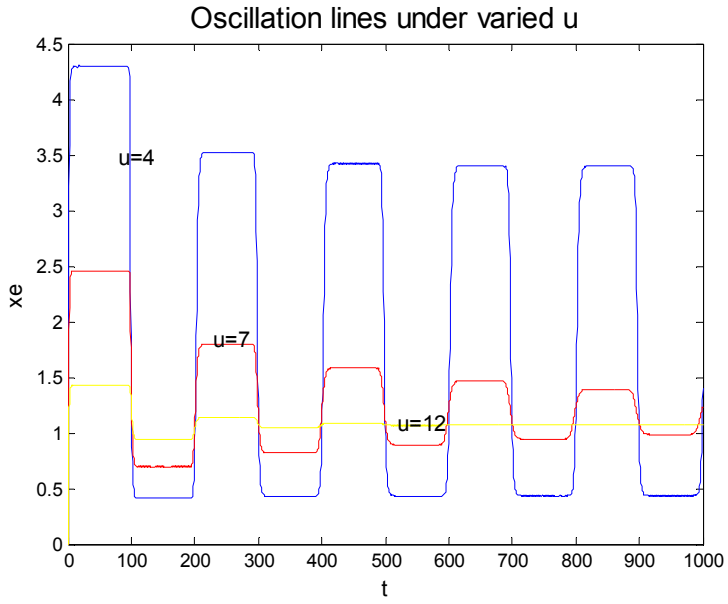

Figure 11 Sensitivity analyses results (On u)

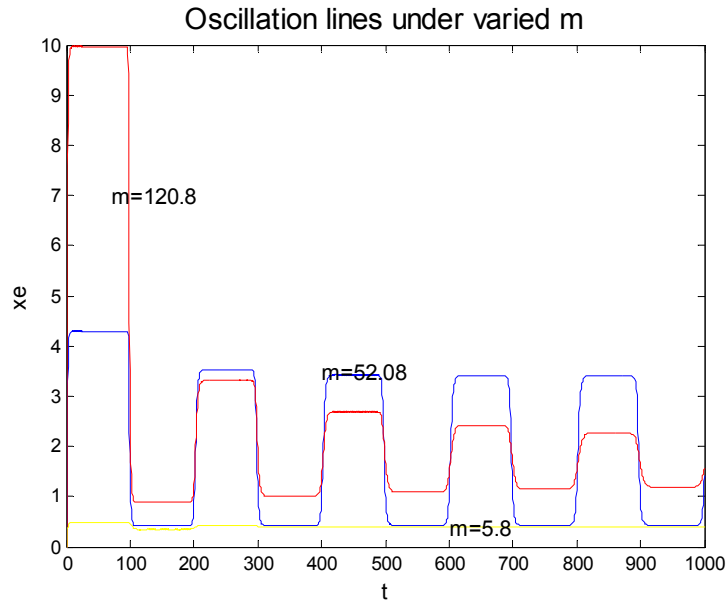

Figure 12 Sensitivity analyses results (On m)

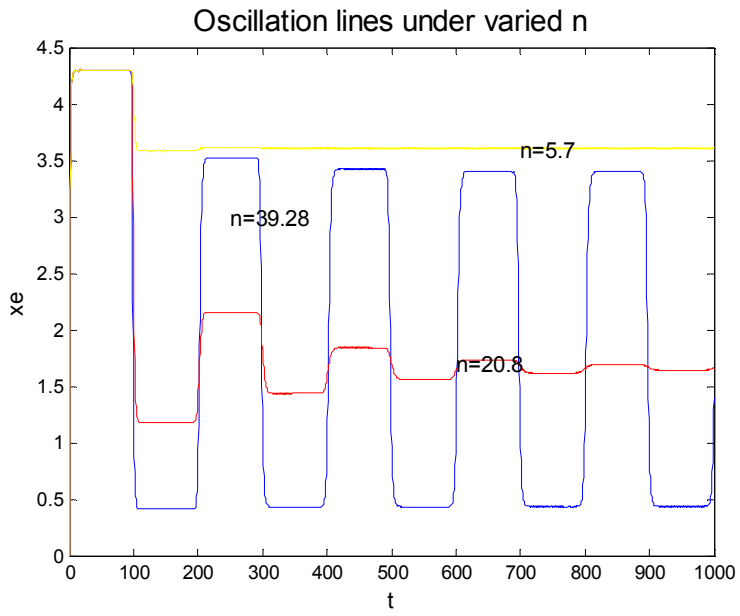

Figure 13 Sensitivity analyses results (On n)

2) Stability analysis

Although we have done sensitivity analyses on some predominant parameters and acquired fabulous results, these analyses were all based on unary composites, holding only a single subject. We are not content with only doing sensitivity analyses, which merely care about single-in-single-out outcomes but not considering binary relation in systematic concept. So we made a bifurcation analysis on binary parameter (u, m) adopting the same method as what we have done in part 2.

u	0.1	0.6	1.1	1.6	2.1	2.6	3.1	3.6	4.1	4.6	5.1	5.6	7	7.8
m	0.03	0.5	1.2	2.1	3.1	4.3	5.6	7.1	8.6	10.2	11.8	14.3	32.1	34.3
u	0.1	0.6	1.1	1.6	2.1	2.6	3.1	3.6	4.1	4.6	5.1	5.6	7	7.8
m	1500	800	370	280	190	150	130	117	101	87	74	54	37.2	34.3

Table 3 critical points (u, m) for oscillation

Depicting those points into an axis, we got the bifurcation line, which indicates the parameters' value range when our system can oscillate stably is in the area marked by '*bistable*' as follows.

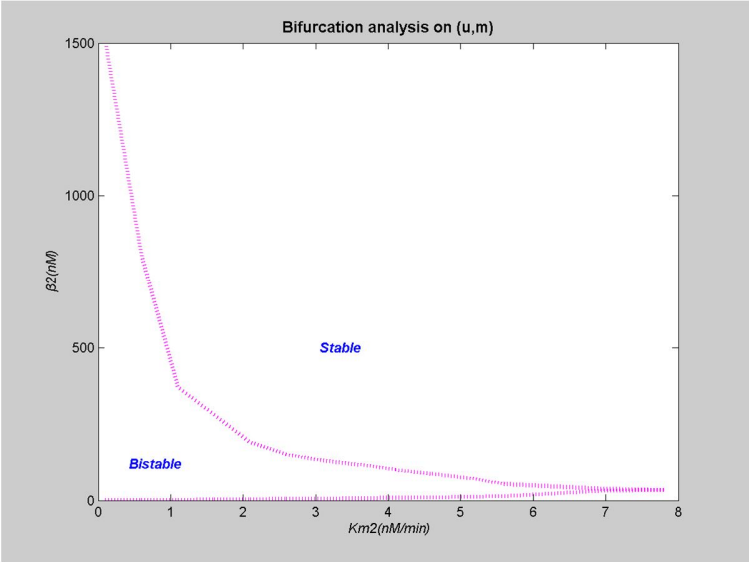

Figure 14 Bifurcation analysis on $(\mathbf{u}, \mathbf{m})$

3) Feedback analysis

By changing parameters a and b, which is equivalent to varying types of feedback introduced, we got simulation results as follows (In order to manifest more clearly, the parameter v was set larger, thus each cell's feedback effect would put greater influence on the whole system).

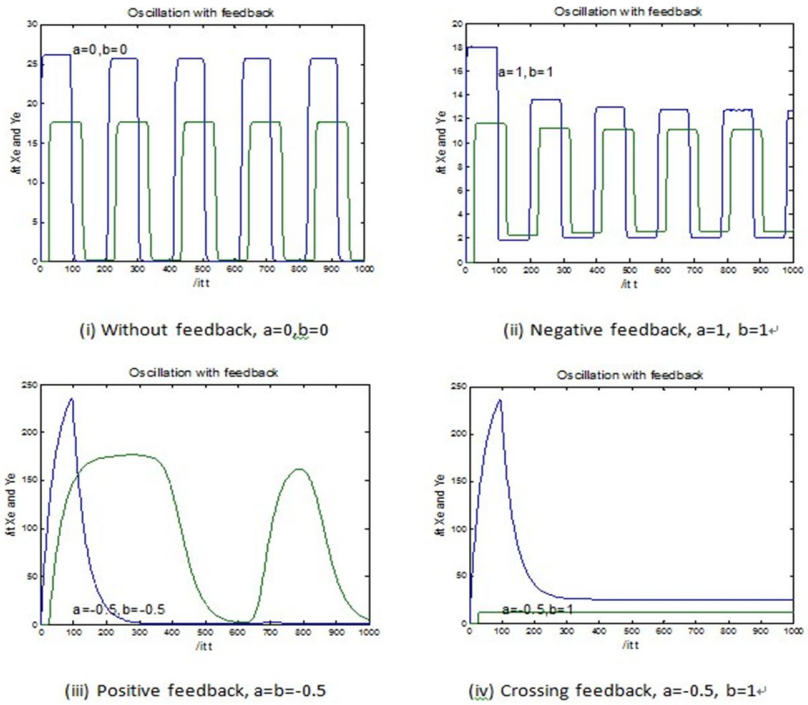

Figure 15 System with feedback

Model Part: Oscillation stability and sensitivity analyses and Oscillation adjustment

The analysis shows that only with a negative feedback mechanism could the overall system be working as an oscillator. When a=b=0, the system contains no artificial negative feedback, but there may be some inherent negative feedback within the system.

PART 4 Quorum-sensing Effect

What we have done insofar is focused on two-cell oscillation. Quorum-sensing oscillator is not simply a matter of expansion in magnitude, but a matter of robustness in allowing difference of each individual cell. Moreover, we test the adjustment of phase and amplitude of oscillation in this part.

As we all know, no two things in this world are the same, so do cells. The major difference of individual cell that we take into considerations is twofold:

- Each cell is distinct at generating AHL.
- The initial amount of AHL can be disproportionally distributed in each cell.

The rate of generation of AHL is closely related to parameter m and n. Therefore, we introduce randomness to both parameters by letting them obey normal distribution. That is:

$$m(i) = \mu 1 + N(0, \sigma 1^2);$$
$$n(i) = \mu 2 + N(0, \sigma 2^2);$$

μ1 and μ2 are the average ability of generating 30C6HSL and 3012CHSL, and normal distribution--$N(0, \sigma^2)$--describes the fluctuations of AHLs in every individual cell. We then expanded our equations from 2 cells to a population of cells. Each cell share a mutual environment in which we assume that AHLs in environment is proportionally distributed.

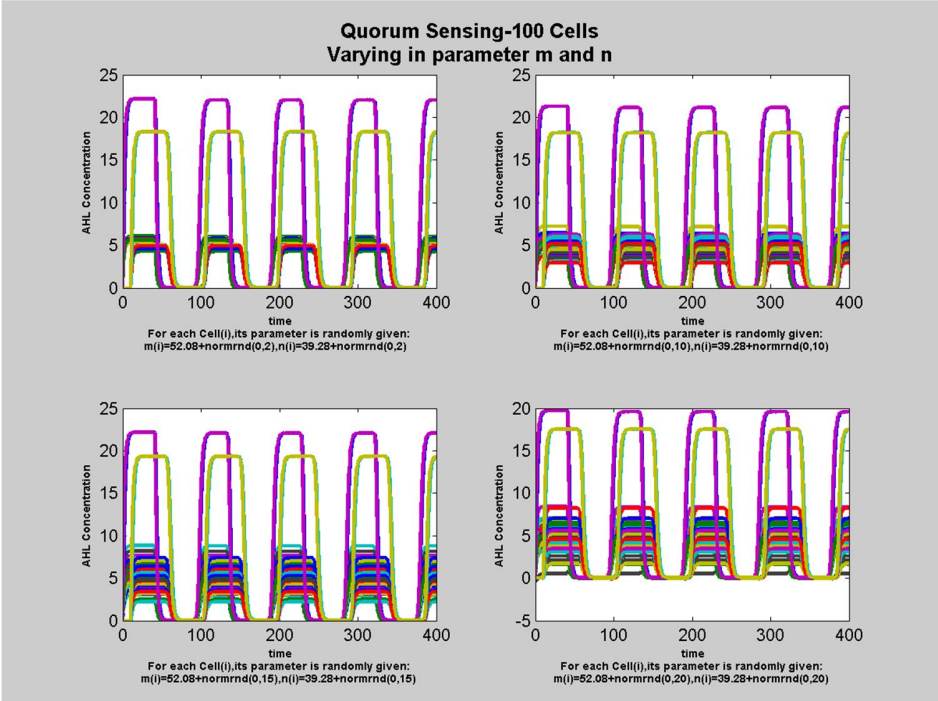

Figure 16 100 Cells Varied in parameter m and n

Model Part: Oscillation stability and sensitivity analyses and Oscillation adjustment

The figures indicate that our system can oscillate synchronically being able to tolerate differences among a population of cells. Furthermore, the figures prove that different ability of generating AHLs of cells have nothing to do with the period and phase of the oscillation. We can also see that the oscillation amplitude of each cell is to a greater extent varied when the Variance of interruption is enlarged.

Moreover, we test whether the oscillation is dependent on initial distribution of AHL by changing the initial amount drastically by letting them follow uniform distribution. That is:

$$\text{Initial(i)} = U(0,20);$$

The results would give evidence to prove that our system can start to oscillate synchronically given variant initial starting numbers.

Based on this distribution restraining the initial AHL concentration in each cell, we simulated out a figure as follows.

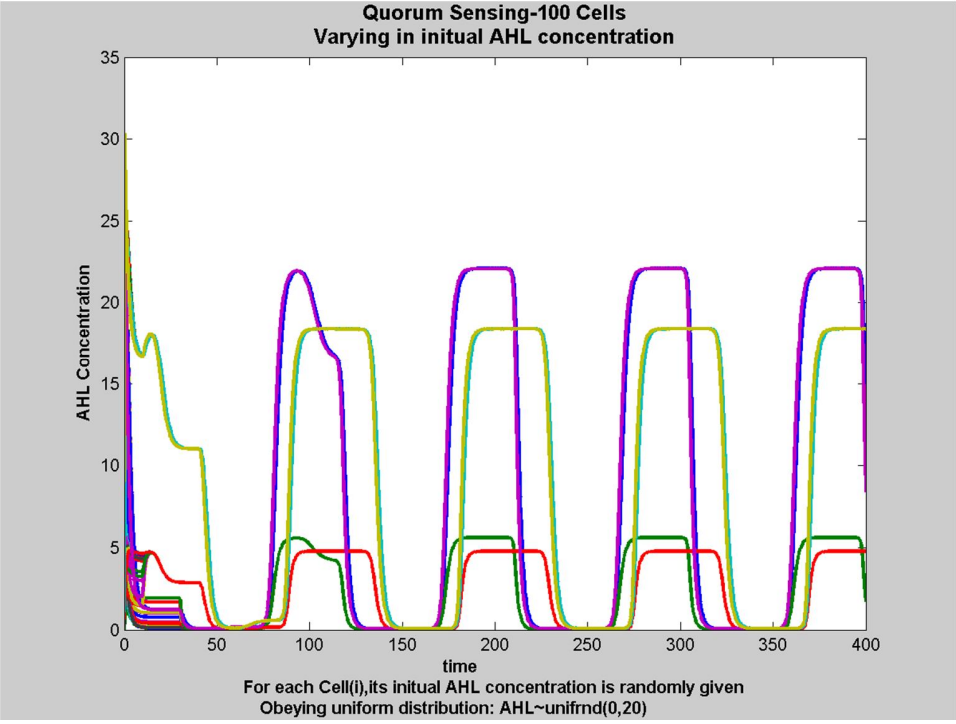

Figure 17 100 Cells Varied in initial AHL concentration

The results demonstratively give evidence proving that our system can start to oscillate synchronically given variant initial starting numbers.

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Model Part: Oscillation stability and sensitivity analyses and Oscillation adjustment

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