Modelling of Synthetic Oscillator in Microencapsulation of a Supposed Hypertension Therapy

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1 Introduciton

Hypertension is a worldwide disease causing some severe effects. But up till now, there are not many therapeutic methods besides a wide selection among various antihypertensive drugs. In order to reduce the times the patients taking drugs, we design a genetic oscillator.
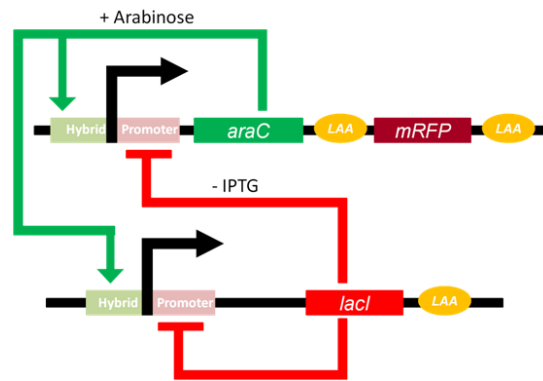

Figure 1: The pathway of genetic oscillator used in this paper.

The enzymes that can induce the expression of propionate is replaced with reporter mRFP. Throughout the whole paper, we chose to study AraC instead of mRFP since they are in the same plasmid and expression rate of both protein is similar. By doing this, we could reduce the number of equations. We put our focus mainly on two aspects:
(1)Oscillation of a single cell.
(2)Oscillation of a group of cells.
To be more specifically, we wanted to find out if this genetic oscillator can oscillate in the first phase, if it is stable against to environment change, if its period can be adjusted, whether a large group of these oscillators can oscillate as well.

2 Delayed Differential Equations

2.1 Establishment of DDEs

The Arabinose Operon and the lac Operon is the core to the functioning of the oscillator. With the presence of Arabinose, dimeric AraC[2]can induce the expression of downstream gene; On the other hand, with minor presence of IPTG, tetrameric LacI[3]may suppress the expression

of downstream gene. For convenience, we assumed that AraC and LacI cannot combined with promoters simultaneously(citation needed). Therefore, we have:

$$D + 2a \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} D_1$$

$$D + 4r \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} D_2$$

where D stands for ratio of promoters which don't combine with any protein among all promoters, a for AraC protein(activator), r for LacI protein(repressor), D_1 for ratio of operons combined with AraC dimer, D_2 for ratio of operons combined with tetrameric LacI, $k_{1,-1}$,k_2 and k_{-2} are reaction rate constants. Assumed that comparing with numbers of operons, that of protein is significantly large enough to ignore whose changes brought by combination of protein and operons. According to law of mass action, we had:

$$\frac{dD}{dt} = -k_1 D a^2 + k_{-1} D_1 - k_2 D r^4 + k_{-2} D_2$$

$$\frac{dD_1}{dt} = k_1 D a^2 - k_{-1} D_1$$

$$\frac{dD_2}{dt} = k_2 D r^4 - k_{-2} D_2$$

With $a, a_0, r, r_0 > 0$, we found that eigenvalues $\lambda < 0$. Thus, when $t \to \infty$ we had $\frac{dD}{dt} = \frac{dD_1}{dt} = \frac{dD_2}{dt} = 0$. We also have $D + D_1 + D_2 = 1$, so we arrive at:

$$D = \frac{k_{-2}}{k_2 r^4 + k_{-2} + \frac{k_1 k_{-2} a^2}{k_{-1}}}$$

$$D_1 = \frac{k_1 a^2}{k_{-1}} D$$

$$D_2 = \frac{k_2 r^4}{k_{-2}} D$$

Denote $a_0 = \frac{k_{-1}}{k_1}$ and $r_0 = \frac{k_{-2}}{k_2}$, we had:

$$D = \frac{1}{1 + \frac{a^2}{a_0} + \frac{r^4}{r_0}}$$

$$D_1 = \frac{a^2}{a_0(1 + \frac{a^2}{a_0} + \frac{r^4}{r_0})}$$

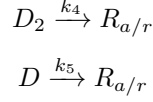$$D_2 = \frac{r^4}{r_0(1 + \frac{a^2}{a_0} + \frac{r^4}{r_0})}$$

What's worth mentioning is that both a_0 and r_0 are constant that are related to IPTG(mM) and Arabinose(%).

$$a_0 = \frac{(6.25 + ara^2)(1 + \frac{iptg^2}{3.24})}{100 ara^2}$$

$$r_0 = \frac{1}{2000000(\frac{0.19}{1 + \frac{iptg}{0.035}^2 + 0.01})}$$
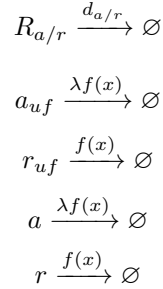
During the transcription process, we had:

$$D_1 \xrightarrow{k_3} R_{a/r}$$

2

$$D_2 \xrightarrow{k_4} R_{a/r}$$

$$D \xrightarrow{k_5} R_{a/r}$$

where $R_{a/r}$ denotes mRNAs of either AraC or LacI. k_3, k_4 and k_5 are transcriptional reaction rates constants. For convenience, we assumed that transcription rate for either AraC or LacI are exactly the same. During the translation and folding processes, we have:

$$R_a \xrightarrow{t_a} a_{uf}$$

$$R_r \xrightarrow{t_r} r_{uf}$$

$$a_{uf} \xrightarrow{k_{fa}} a$$

$$r_{uf} \xrightarrow{k_{fr}} r$$
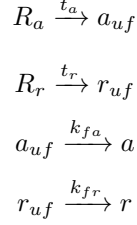
where a_{uf} and r_{uf} are unfolded proteins, t_a and t_r are translational reaction rate constants while k_{fa}, k_{fr} are folding rates constants. In degradation process, we had:

$$R_{a/r} \xrightarrow{d_{a/r}} \varnothing$$

$$a_{uf} \xrightarrow{\lambda f(x)} \varnothing$$

$$r_{uf} \xrightarrow{f(x)} \varnothing$$

$$a \xrightarrow{\lambda f(x)} \varnothing$$

$$r \xrightarrow{f(x)} \varnothing$$

where $d_{a/r}$, $f(x)$ and $\lambda f(x)$ are degradation rate constants.
According to law of mass reaction, we had:

$$\frac{dR_a}{dt} = copy_a(k_3 D_1 + k_4 D_2 + k_5 D) - d_{a/r} R_a$$

$$\frac{dR_r}{dt} = copy_r(k_3 D_1 + k_4 D_2 + k_5 D) - d_{a/r} R_r$$

$$\frac{da_{uf}}{dt} = t_a R_a - k_{fa} a_{uf} - \lambda f(x) a_{uf}$$

$$\frac{dr_{uf}}{dt} = t_r R_r - k_{fr} r_{uf} - f(x) r_{uf}$$

$$\frac{da}{dt} = k_{fa} a_{uf} - \lambda f(x) a$$

$$\frac{dr}{dt} = k_{fr} r_{uf} - f(x) r$$

where $copy_a$ and $copy_r$ are plasmid copies that are transfected into E.coli.
Transcriptional and translational processes of genes take time and consequently, protein that combined to promoters can be seen as those started transcription process before a specific time interval τ. Thus we converted three ODEs into DDEs:

$$D = \frac{1}{1 + \frac{a(t-\tau)^2}{a_0} + \frac{r(t-\tau)^4}{r_0}}$$

$$D_1 = \frac{a(t-\tau)^2}{a_0(1 + \frac{a(t-\tau)^2}{a_0} + \frac{r(t-\tau)^4}{r_0})}$$

3

$$D_2 = \frac{r(t-\tau)^4}{r_0(1 + \frac{a(t-\tau)^2}{a_0} + \frac{r(t-\tau)^4}{r_0})}$$

All values of constants stated above can be found in supplementary data. We solved these DDEs with R language.

We also went one step further. We simulated the situation in which lag τ obeys a specific gaussian distribution, and the lag changes in every certain interval. We hoped by running a random test, we could get closer to real life situation.

2.2 Result
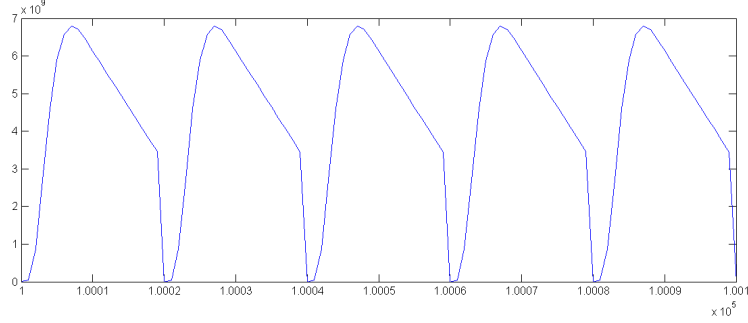
(a) a numerical solve of AraC
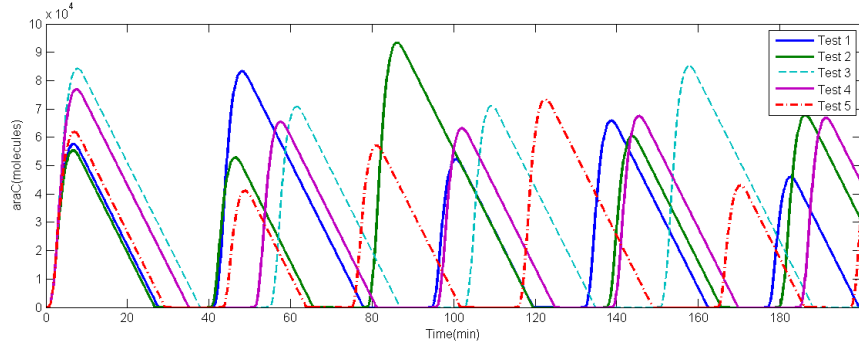
(b) 5 numerical random solves of AraC

Figure 2: (a)A numeric solve of AraC when lag $\tau = 2$, Arabinose concentration is 5%, IPTG concentration is 1mM, time interval is 0.1min.(b)numeric solve of AraC concentration versus time of 5 random test, when Arabinose concentration is 0.7%, IPTG concentration is 10mM, and $\tau \sim (2.0, 0.3^2)$.

The period of this particular solve is 49.0minutes. The numeric solve of DDEs shows that the supposed oscillator is feasible. On the other hand, interval between every adjacent peak is different in a single random test, thus period is calculated by average intervals. Even so, the average period of each random test is different from each other: $T_1 = 43.95min$, $T_2 = 47.65min$, $T_3 = 40.625min$, $T_4 = 39.375min$, $T_5 = 45.975min$. Also, the amplitude of each curve is different.The random solve suggests that extern factors might be introduced to force the period to be the same.

2.3 Stability

Basing on the equations, since applying their Taylor series makes the equations formed in a linear one keeping the topology of the solution to the original equations[4], the chAraCteristic equations can be presented:

$$E(\mu) = \left(\mu + \lambda \frac{\gamma}{(Ce+a)^2}\right)(\mu + d_{a/r})\left(\mu + k_{fa} + \lambda \frac{\gamma}{(Ce+a_{uf})^2}\right) - k_{fa}T_a copy_a(k_3E_1 + k_4E_2 + k_5E_3) = 0$$

4

$$
\begin{cases}
E_1 = \dfrac{\frac{2a}{a_0}e^{-2\mu\tau}\left(1 + \frac{r^4}{r_0}e^{-4\mu\tau}\right)}{\left(1 + \frac{r^4}{r_0}e^{-4\mu\tau} + \frac{a^2}{a_0}e^{-2\mu\tau}\right)^2} \\[4mm]
E_2 = \dfrac{2ae^{-2\mu\tau}\frac{r^2}{r_0}e^{-2\mu\tau}}{\left(1 + \frac{r^4}{r_0}e^{-4\mu\tau} + \frac{a^2}{a_0}e^{-2\mu\tau}\right)^2} \\[4mm]
E_3 = \dfrac{\frac{2a}{a_0}e^{-2\mu\tau}}{\left(1 + \frac{r^4}{r_0}e^{-4\mu\tau} + \frac{a^2}{a_0}e^{-2\mu\tau}\right)^2}
\end{cases}
$$

If there were at least one periodical solution, the equation should have at least one imaginary root.

Denote $\begin{cases} c_1 = \lambda\frac{\gamma}{(Ce+a)^2} \\ c_2 = d_{a/r} \\ c_3 = k_{fa} + \lambda\frac{\gamma}{(Ce+a)^2} \\ c_4 = k_{fa}T_a(k_3 E_1 + k_4 E_2 + k_5 E_3)copy_a \end{cases}$

Namely, $E(\mu) = (\mu + c_1)(\mu + c_2)(\mu + c_3) - c_4 = 0$ has imaginary root(s).

Set $\mu = iy$, $y \in R$ Thus $iy(c_1 c_2 + c_2 c_3 + c_1 c_3 - y^2) - (c_1 + c_2 + c_3)y^2 + c_1 c_2 c_3 = c_4$ is required for imaginary root(s).

Namely, $\begin{cases} y(c_1 c_2 + c_2 c_3 + c_1 c_3 - y^2) = Im(c_4) \\ -(c_1 + c_2 + c_3)y^2 + c_1 c_2 c_3 = Re(c_4) \end{cases}$

Namely $\|y(c_1 c_2 + c_2 c_3 + c_1 c_3 - y^2)\|^2 + \| -(c_1 + c_2 + c_3)y^2 + c_1 c_2 c_3\|^2 = \|c_4\|^2$

Set $x = y^2$

Thus the existence of periodical solution is equal to:

$$
x^3 + (c_1^2 + c_2^2 + c_3^2)x^2 + (c_1^2 c_2^2 + c_2^2 c_3^2 + c_1^2 c_3^2)x + c_1^2 c_2^2 c_3^2 = (k_{fa}T_a copy_a)^2\left(\frac{\left(\frac{2a}{a_0}\right)^2\left(k_3^2 + k_5^2\right)}{\left(1 + \frac{r^4}{r_0} + \frac{a^2}{a_0}\right)^4} + \frac{\left(\frac{2ar^2}{r_0}\right)^2\left(r^4 k_3^2 + k_4^2\right)}{\left(1 + \frac{r^4}{r_0} + \frac{a^2}{a_0}\right)^4}\right)
$$

has at least one positive real root.[5]

Denote p, q, δ as coefficients related to parameters in DDEs. Due to the limited space, these coefficients can be found in supplementary data.

According to the Cardano's Formula, If $q < 0$, $\delta > 0$,then there exists a center of oscillation. In this case, derivative of period with respect to both Arabinose and IPTG are not 0.

If $q = 0$, $p < 0$, then also exists a center of oscillation. In this case, derivative of period with respect to both Arabinose and IPTG are 0.

In fact, in our case - when Arabinose $\in [0, 10]$ and IPTG $\in [0, 10]$ - belongs to the first aspect, which means both Arabinose and IPTG contribute to the period.

In the next subsection, we will discuss the influence of Arabinose, IPTG, lag τ on period of AraC.

2.4 Parameter Range and Sensitivity

To see how different concentration of both IPTG and Arabinose can affect the period of the oscillator and the range of period, we solve the DDEs in various values of IPTG and Arabinose using R language.
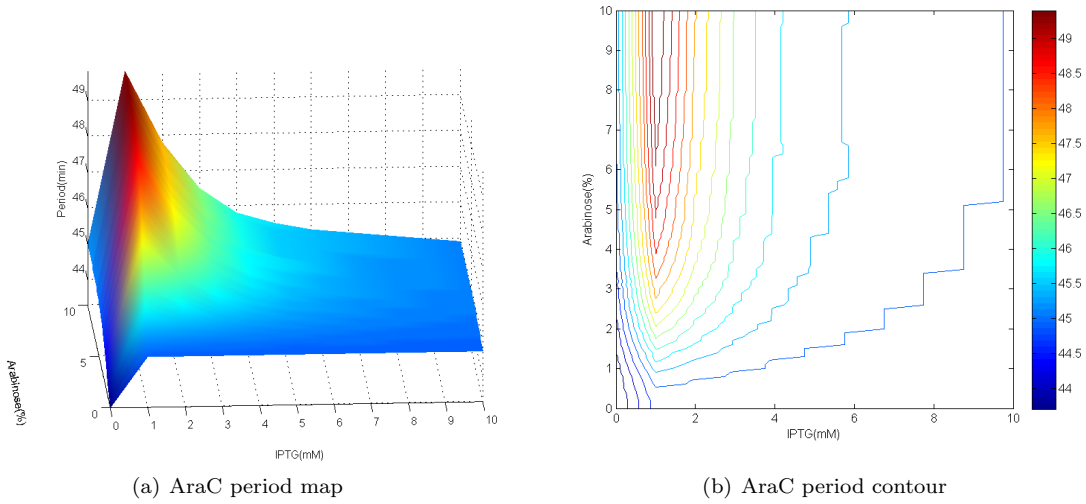
(a) AraC period map   (b) AraC period contour

Figure 3: (a)AraC period map with IPTG from 0mM to 10mM, step is 1mM, Arabinose from 0% to 10%, step is 0.1%. (b)Contour of period concerning Arabinose and IPTG. In both figures, color shows the values of period.

The period map be divide into two areas according to IPTG concentration:'mountain '(0mM∼5mM) and 'plain' (5mM∼10mM). These areas can be more clearly seen in contour. The difference between largest and smallest period is approximately 6 minutes, which is insignificant comparing with the scale of period.In 'mountain' area, when IPTG concentration is fixed, the period increases alongside with Arabinose concentration; on the other hand, when Arabinose concentration is fixed, the period increases at first when IPTG concentration rises, then it decreases when IPTG concentration keep on rising. However, in 'plain' area, the period remain steady against either IPTG or Arabinose change.
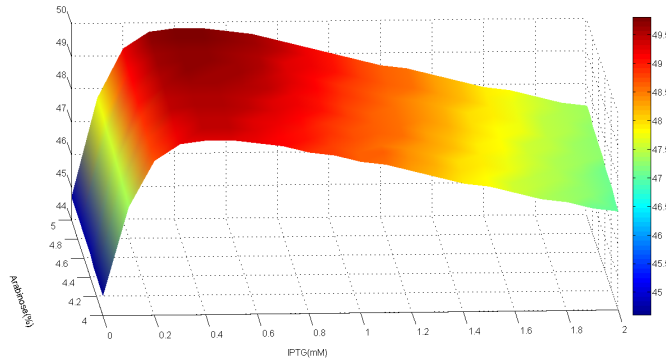We also examined a specific area in a small step size.

Figure 4: AraC period of area whose IPTG is 0∼2mM, step is 0.1mM and Arabinose is 4∼5%, step is 0.1%. The edge is less sharp than the one above.

By examining specific area in a smaller step size, we found that surface of period is actually rather smooth, which suggests that a large step size does not limit its representativeness, since spline interpolation is used in plotting the discrete data and high accuracy is guaranteed by smoothness of the function. Due to the computational expenses and our limited computing power, we set a rather large step. Though coarse, we can still grab the big picture of how IPTG and Arabinose can affect the period of the oscillator.
Then we examined the derivative of AraC's period with respect to both Arabinose and IPTG.
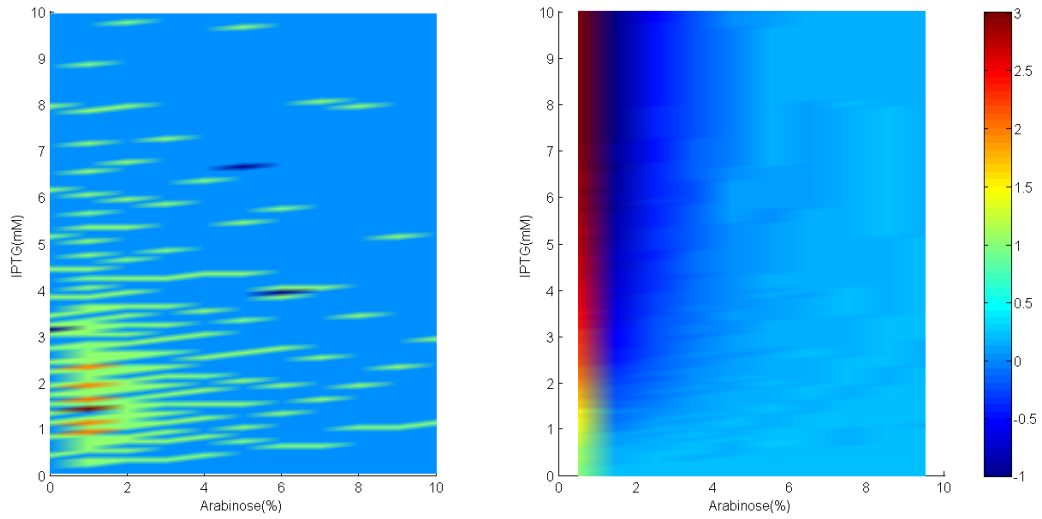
Figure 5: Figure on the left:derivative of AraC's period with respect to Arabinose. Figure on the right:derivative of AraC's period with respect to Arabinose.

These figures further suggest the asymmetry behavior of Arabinose and IPTG in terms of affecting AraC's period.
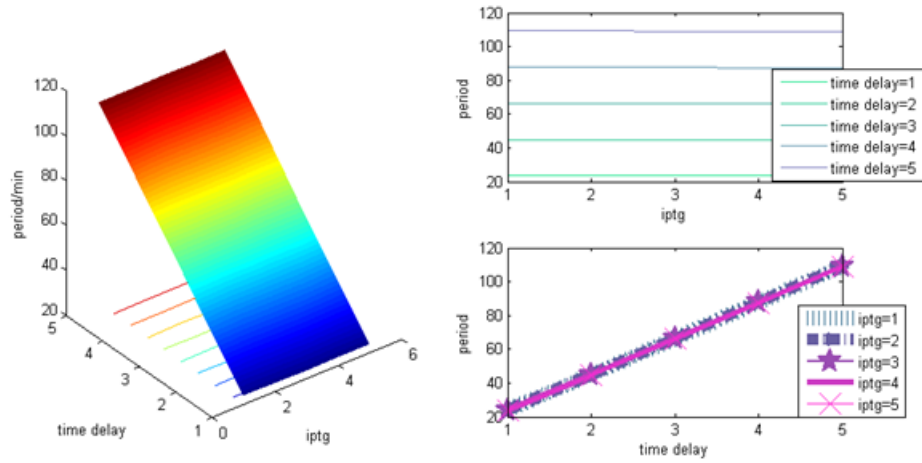Lastly, we dicussed the role of lag τ in the period of AraC.

Figure 6: AraC's period against lag τ.

In these figures, we could clear see that AraC's period increases linearly as lag τ increases. To sum up, the range of period is rather limited while τ is fixed. In other word, it's is rather stable against Arabinose and IPTG changes. On the other hand, we can see that among Arabinose, IPTG and τ, τ has the biggest influence on AraC's period, which should be concerned firstly while adjusting the period of the oscillator.

3 Multi-Cell Oscillation Simulation

3.1 Background

In the last section, we analyzed how a single cell oscillates. When put into practical uses, we should consider real-life limitation and how a cell would behave throughout its life time, in order to see if group would oscillate just like a single cell does.

Prokaryotic fission is binary fission. Under certain circumstances, bacteria reach certain requirement after gaining sufficient nutrition, it would divide into two filial cells. The fission of prokaryotes do not have evident cell phase as eukaryotes do(G1, S, G2, M). For convenience, we assume that all bacteria are in rapid growth.For all cells in rapid growth, they can be assumed to have neither mitosis phase nor interval phase.[6] Throughout their whole life cycle, bacteria are synthesizing fission-related regulatory protein(FtsZ) and polysaccharide concerning synthesis of cell wall.[7] All process stated above are ongoing continuously; in other words, bacteria would synthesize protein regardless of cell fission. In terms of death of cells, according to logistic model of cell population, death rate is linear to population itself. Based on this, we set that a certain amount of cells, which is proportion to squares of population, would be killed due to limited food. Those cells are picked randomly.   We were also interested in lifespan of bacteria. While in rapid grow, the average lifespan of a bacterium is 20 minutes.[6]Without further information, we assumed that the lifespan of bacterium obey a gaussian distribution with $\mu = 20mins$, $\sigma = 1min$.

Moreover, we assumed that the quantity of AraC are linear with plasmid copies. Given a specific environment, the number of initial plasmid copies are a constant[8]. The replication of plasmids can be thought to be completed instantly(within 0.05min)[9]. When cells started to divide, the plasmids are allocated into two filial cells evenly[10]. During the lifespan of a single cell, the number of replicated plasmids obey Poisson distribution.

Lastly, we assumed that when started simulation, all the cell are in the oscillation phase and remain exactly the same oscillation rhythm with each other.   Based on these information, we set several rules for our computer model:

(1)Each round of simulation represent 0.1min.

(2)The lifespan of cells $x \sim (20, 1^2)$.

(3)When reached their lifespan, cells division. Division is completed immediately.

(4)The expression of AraC would not be affected by division, which means the phase of AraC does not change.

(5)Certain amount of cells, which is proportion to square of population, is 'sentenced' to dead in every round. They are picked randomly.

(6)AraC's concentration is proportion to plasmid copies.

(7)When cells are dividing, the plasmid copies would increase by y, and $y \sim Pois(50)$, then split evenly into two filial cells.

(8)When started

3.2 Oral Colon-Specific Drug Delivery System

To simulate real-life situation, we researched for how our genetic oscillator will enter our bodies. As propionate is absorbed in human colon, so the best way to delivery those genetic engineered bacteria into colon. However, considered the possible safety issued brought by bacteria, we sought to solve it by adopting Oral Colon-Specific Drug Delivery System(OCDDS). Such system encapsulate bacteria with semipermeable polymer membrane. Patient take such microencapsulation orally and it will stay in colon for certain days. Due to the semipermeable property, small molecules such as propionate will penetrate the membrane through pores while bacteria are too large to pass through. Bacteria simply stays in microencapsulation and eventually excreted from human body. Such system can prevent bacteria from directly contacting human body and enhance the security[11].

Modified microencapsulation can stay in colon for 70 days[12]. Bacteria concentration in micro encapsulation can reach 10^{10}cfu/mL [13][14] and diameter of microencapsulation can reach $433 \pm 67 \mu m$[15]. For convenience, we assumed that each monocolony originated from a single bacteria. Based on that, the numbers of bacteria are approximately $81713 \sim 208333$[15]. Since numbers of bacteria remain

relatively constant within microencapsulation, the feasibility and stability of supposed therapy are guaranteed.

3.3 Method

We set population of bacteria approximately into 100000 by adjusting death rate, as suggested in previous subsection. We run a 100800 minutes = 70days simulation to see long term outcome of multi cell oscillation system.As for single cell oscillation, we choose the data which is numeric solve of by DDEs stated previous section, whose Arabinose concentration is 0.7%, IPTG concentration is 1mM, and period is 44.8min. This multi cell oscillation simulation is implemented using C++ and compiled through both Visual Studio 2012 and Xcode 4.6.
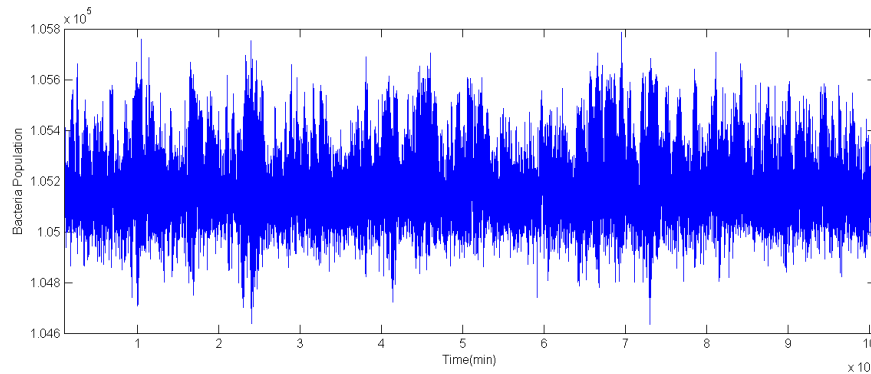
3.4 Result

Figure 7: Population of bacteria against time. Average = 105180, largest difference $\Delta = 5667$

The population of bacteria is fluctuating within a small range $(5667/105180 = 0.0539)$ and generally steady, showing that the logistic model is feasible and this model is successfully simulating the population within microencapsulation.
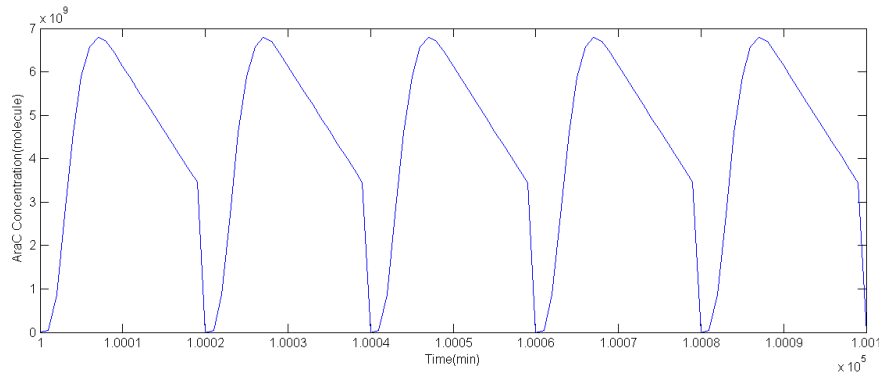

Figure 8: AraC concentration of simulated multi oscillating cells within microencapsulation from 100000 minutes to 100100 minutes since the simulation started.

The multi cells oscillation simulation suggests that even with that amount of cells, the oscillation will still exist just like a single one does. Such result is because of the synchronous of all cells' oscillations throughout the whole process.

9

4 Discussion and Conclusion

Based on the supposed therapy and pathway, we set up a mathematical and a computational model. By setting up and solving DDEs using parameters found in literatures, we found the genetic oscillator is **feasible**. Further, we introduced random factor into DDEs and the result suggest in real-life implementation, **extra work should applied in forcing the period of oscillation into the same**. The supposed genetic oscillator is found to be **stable** mathematically within particular ranges of Arabinose and IPTG, and period of which is **not sensible** to concentration of neither Arabinose nor IPTG but to lag τ. It suggest that when forming the actual therapy, we should mainly focus on how to change τ - the time lag between start of translation and binding to hybrid promoter of regulating protein. Also, a counterpart of **frequency divider** - which is a electronic device - in synthetic biology can be put forward to divide a high frequency biological signal to a lower one. Meanwhile, by running a simulation on computer, we found in a group of cells, the oscillation continue to exist as in a single cell. In real-life application, **extra effort should be put on holding all cells synchronized in oscillating**.

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