Modelling of Kinetics

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Abstract

A mathematical investigation into the behaviour of a genetic circuit can give predictions into the behaviour the circuit. Each reaction within a genetic circuit can be modelled as a first order kinetic law. Here we analyse the proposed circuit replicating the behaviour of a biological clock for the UQ-Australia iGEM 2011 entry. Open-source software CellDesigner [1] was used to perform the simulation of the kinetic laws over time. Although it was expected that there would be some oscillation behaviour, our simulations have shown that there would be no oscillations. This implies that even if the circuit was successfully constructed we should not expect it to show oscillatory behaviour, but could be a reflection on the limited range of simulations. However, if the physical genetic circuit did display oscillatory behaviour, then literature values taken would then need to be revised for the model. Further analysis of the circuit could give greater insight into the behaviour of the circuit and potentially biological clocks in general.

1 Introduction

We wish to model the circuit to be used for the UQ-Australia iGEM Team for the 2011 competition. The task would be to see if the proposed circuit would produce a steady oscillation. If such an oscillation is predicted, then the characteristics, such as frequency and regularity would be explored. If the circuit does not produce an oscillation, this could be furthered by taking another model or taking another circuit design which would produce an oscillation. By performing the modelling, we hope to gain an idea of the shape of the expression levels of the circuit. The model could then be confirmed experimentally.

2 Theory

We shall consider a system as described in Figure 1. Plac/ara, pBAD and glnAp2 are promoters which produce araC, glG and lacI proteins respectively. The araC gives a positive feedback loop back to the Plac/ara and drives the promtoer pBAD. glnG drives glnAP2. LacI then inhibits Plac/ara thus creating the oscillating behaviour.

From literature, we find that this system can be represented as a network as shown in Figure 2.

All of the nodes (vertices or bubbles) represent an entity such as a protein, promoter, mRNA or degraded states. Connecting the nodes are arrows, which represent reactions. For all reactions, we can write a kinetic law to describe that reaction.

We can then simulate this circuit, which is just a collection of vertices and reactions, over a desired time frame. CellDesigner has a graphical interface which allows us to play with the parameters of the reactions as well as the initial concentration of the nodes.

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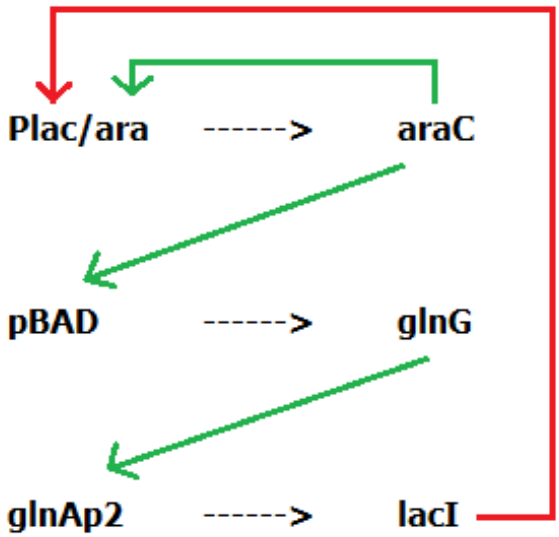

Figure 1: The circuit considered for the UQ-Australia iGEM Team 2011. promoters Plac/ara, pBAD and glnAp2 produce araC, glG and lacI protein respectively. However, these are connected in a set of feedback loops to produce an oscillation.

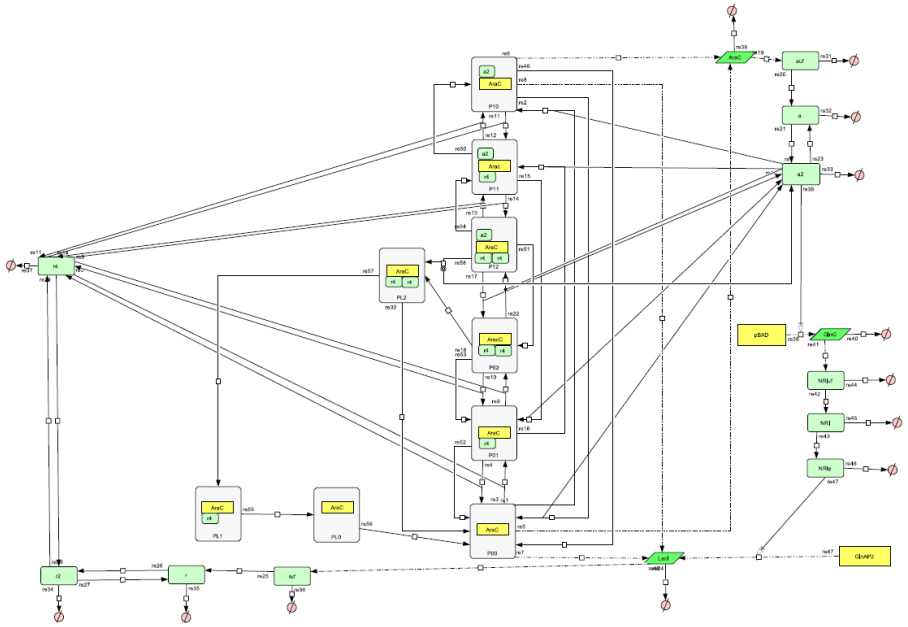

Figure 2: The circuit considered for the UQ-Australia iGEM Team 2011 as drawn out in a network. Note in this diagram, degradation of entities are recorded. Compare with Figure 1. Note that Plac/ara consists of nine components. Note that a larger version of the circuit can be viewed at http://2011.igem.org/wiki/images/f/f3/Circuit-works.pdf.

3 Method

We shall consider our circuit as a system of 24 entities. To handle this, we shall create a 24-element vector containing them. These entities were given an element index for its position in the vector, to be handled by MATLAB. They were also assigned a unique id to be handled when the circuit is handled by CellDesigner. Of course, they also have a name. These are recorded in Table 1.

Table 1: Names of entities to be handled for modelling in MATLAB and CellDesigner

Vector index	Unique ID	Name
1	s6	Plac/ara 00
2	s8	Plac/ara 10
3	s14	Plac/ara 01
4	s15	AraC mRNA
5	s16	LacI mRNA
6	s20	Plac/ara 02
7	s25	Plac/ara 11
8	s30	Plac/ara 12
9	s36	Plac/ara L2
10	s85	Plac/ara L1
11	s88	Plac/ara L0
12	s38	AraC unfolded
13	s39	AraC molecule
14	s40	AraC dimer
15	s56	LacI unfolded
16	s57	LacI molecule
17	s58	LacI dimer
18	s59	LacI tetramer
19	s68	pBAD
20	s70	GlnG
21	s71	NRI unfolded
22	s74	NRI molecule
23	s75	NRI-phosphorylated
24	s82	GlnAP2

With the nodes defines, the reactions need to be defined. Interaction between entities are defined by reactions. There are fifty reactions in all to be listed below. We list the reactions with their products and reactants and the kinetics relating them. These reactions were found from literature [2, 3, 4, 5, 6, 7, 8, 9, 10] These reactions use constants called parameters. These parameters were generally provided with the reaction.

Note that there were 59 reactions. They are not listed here, but can be viewed in the CellDesigner file [11] accompanying this discussion. The parameters are all recorded with the reactions visually linking the entities.

With these defined we can then perform the simulation in CellDesigner[1]. An example of the interface is shown in Figure 3.

The changes in the values were performed to try to produce an oscillation. If an entity appeared to be decreasing too quickly, then the values of the associated parameters and entities were adjusted, increased or decreased, to change the shape. If a concentration appeared to fall below zero, this would be rectified by altering the values of the parameters. This was performed over all of the parameters and reactions.

We define a regular oscillation as a pattern of two reasonably spaced peaks, i.e. two hills of equal height. A more rigorous definition could be taken by considering the Fourier transform, of the expression

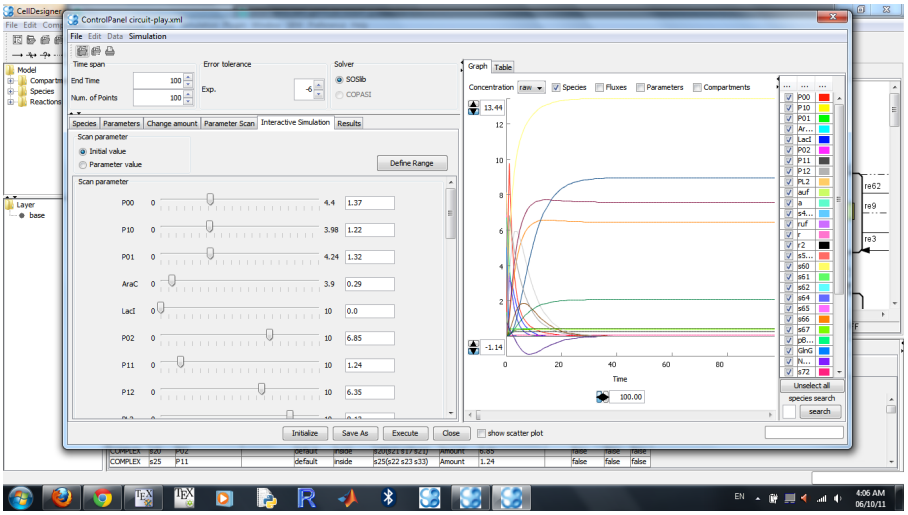

Figure 3: A screenshot of the CellDesigner [1] simulation interface. This allowed us to vary the values of the initial concentrations and parameters with an (almost) instantaneous view of the resulting concentrations over time. Note that this allows us to do our analysis visually.

level. This would provide a plot of the distribution of frequencies (so periods) of the oscillations involved in the plot. This was unnecessary for the data collected in our simulations. Note that there is very little structure in the analysis method. By only changing one variable at a time, as restricted by the interface, there are many points off that contour that are not being run in the simulations.

4 Results

We used the Simulation Control Panel in CellDesigner [1] to look for a set of initial concentrations of entities and values of parameters. The typical plots showing behaviour closest to an oscillation is shown in Figure 4

Note that this plot is for all of the entities in the circuit. Many of them are not of physical interest to us, so they can be unselected when viewing the plots. Note that the behaviour is different when looking at different time domains as can be seen by comparing Figures 4 and 5.

We can see that some of the entities increased and never decreased. These were generally the degraded states. Note that the degraded states were generally recorded as degraded states and assumed to not further interact with the circuit.Note also, that the degraded states do not increase infinitely; they plateau after some time. This could give us further information about the reagent being degraded.

Note also in this diagram that the concentration of an entity falls below zero. This implies that we have a negative concentration of an entity within the circuit.

5 Discussion

From our simulation, we can see that there were no combinations that produced a regular oscillation. This would indicate that this structure of circuit, i.e. the arrangement of reactions within the circuit, would not produce an oscillation.

However, it might not be to the fault of the shape of the circuit. We can see in Figure 4 that it is for some time the concentration of an entity becomes negative. This is physically impossible. It would be reasonable here to assume that the entity was decreasing by too much to an extent that the reactions

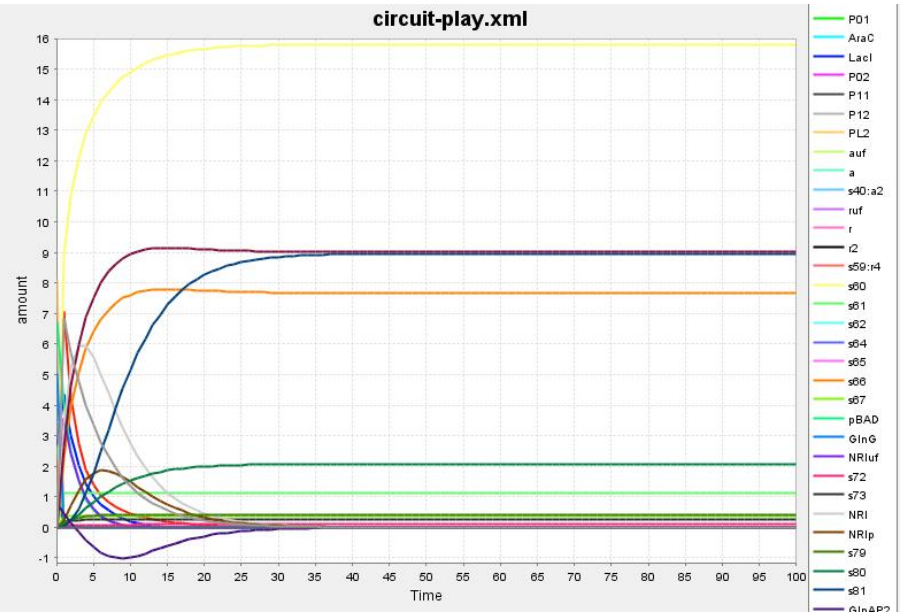

Figure 4: A commonly seen plot of the concentrations of the entities over time produced by the simulations for the UQ-Australia iGEM circuit. Note here that the concentration of a particular entity falls below zero for a certain amount of time. The time domain shown here is the first 100 minutes.

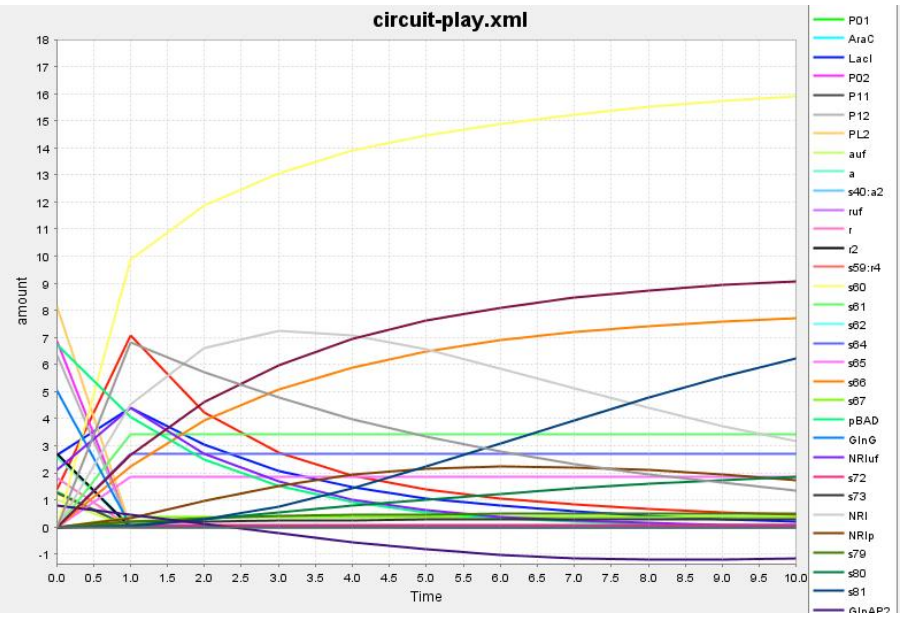

Figure 5: Looking at a smaller domain of time of up to 10 minutes with the CellDesigner [1] simulation of the UQ-Australia iGEM circuit.

may not simultaneously be valid.

Note that the kinetics describing the reactions were taken from literature. Note that different text would reference certain conditions relevant to the situation they are reporting. It is very likely the

kinetic equations used in situations are different to those required in our situation. Most likely, it could be possible that some of the reactions were valid under certain conditions or within a time frame which did not line up with another reaction's conditions.

It is possible that there were too many or not enough equations. It is also possible that there are more interactions within the circuit that should be considered. We could then consider that some of the reactions were insignificant compared to other reactions, thus can be excluded thus simplifying the circuit. For instance, we assumed that the degraded states just degrade and do not further interact with the system. This could be inaccurate.

Note that we could have also considered our kinetic simulations in another program such as MATLAB. This was done. The individual reactions were condensed into twenty-four first order Ordinary Differential Equations (ODEs). These were then solved numerically with hand-coded and precoded algorithms, specifically the fourth order Runge-Kutta methods (ode45) and MATLAB precoded algorithms ode23 and ode113. These algorithms took an unreasonably long time to solve, thus CellDesigner was favoured. Note also, Mathematica would have also been appropriate, but CellDesigner is designed specifically for handling these types of equations.

However, with the MATLAB computations taking unreasonably long times to run and physically impossible concentrations, it would be fair to conclude that the reactions describing the system were not realistic models. Further attempts to model this circuit would involve taking reactions for which we were reasonably certain that all reactions would be reasonably accurate within the domain we are analysing.

Doing the analysis in with other software could result in a simulation over a greater range of values for the parameters and initial conditions. It is very possible that this circuit could produce a regular oscillation in the concentration of one of its entities, but this was not found as a required relation between initial conditions and parameters. There was little structure in choosing the range of values for initial conditions and reaction parameters chosen, aside from a trial and error approach. A further analysis of our circuit could be performed in Mathematica and would hopefully produce simulations over a greater domain. A further investigation into the significance of each reaction with respect to other reactions and parameters could also reveal the key reaction involved, thus making it easier to find the exact set of condition to give an oscillation.

6 Conclusion

We have shown that through our simulations that there would be no regular oscillatory pattern. This indicates that an experimental arrangement as considered for the UQ-Australia iGEM circuit would not produce an regular oscillation of gene expression levels. However, this is limited to the range of values for the parameters and initial conditions taken.

References

[1] A. Funahashi, Y. Matsuoka, A. Jouraku, M. Morohashi, N. Kikuchi, and H. Kitano. CellDesigner 3.5: A Versatile Modeling Tool for Biochemical Networks. *Proceedings of the IEEE*, 96(8):1254–1265, 2008.

[2] J. Stricker, S. Cookson, M. R. Bennett, W.H. Mather, L.S. Tsimring, and J. Hasty. A fast, robust and tunable synthetic gene oscillator. *Nature*, 456:516–19, November 2008.

[3] Peng Jiang and Alexander J. Ninfa. Regulation of Autophosphorylation of Escherichia coli Nitrogen Regulator II by the PII Signal Transduction Protein. *Journal of Bacteriology*, 181(6):1906–11, 1999.

[4] Verena Weiss and Boris Magasanik. Phosphorylation of nitrogen regulator I (NRI) of Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America*, 85(23): 8919–23, 1988.

[5] Brian A. Blaugrund. A Mathematical Model of the bacterial Gene Network Partially Responsible for Regulating Nitrogen Assimilation, 2005.

[6] Alexander J. Ninfa, Lawrence J. Reitzer, and Boris Magasanik. Initiation of transcription at the bacterial glnAp2 promoter by purified E. coli components is facilitated by enhancers. *Cell*, 50(7): 1039–46, 1987.

[7] John R. Sadler and Aaron Novick. The properties of repressor and the kinetics of its action. *Journal of Molecular Biology*, 12(2):305–27, 1965.

[8] Mariette R. Atkinson, Michael A. Savageau, Jesse T. Myers, and Alexander J. Ninfa. Development of Genetic Circuitry Exhibiting Toggle Switch or Oscillatory Behavior in Escherichia coli. *Cell*, 113 (5):597–607, 2003.

[9] Mariette R. Atkinson, Narinporn Pattaramanon, and Alexander J. Ninfa. Governor of the glnAp2 promoter of Escherichia coli. *Molecular Microbiology*, 46(5):1247, 2002.

[10] Zhang Xin, Reeder Thadd, and Schleif Robert. Transcription Activation Parameters at ara pBAD. *Journal of Molecular Biology*, 258:14–24(11), 1996.

[11] Ann Bui and Josh Li. UQ-Australia Circuit in Cell Designer. Available at `http://2011.igem.org/wiki/images/2/2d/UQ-Australia-circuit.zip`, 2011.