INTRODUCTION TO FOCUS AREAS IN BIOINFORMATICS - WS19/20

Project 10: Modeling and Simulation of Boolean Networks

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Abstract

Goal of the project: This week's project was centered on the implementation and simulation of a given set of genes as a boolean regulatory network to analyze the quorum sensing of Vibrio fischeri.

Main result(s) of the project: By analyzing the gene regulation patterns of Vibrio fischeri we noticed that several different states of our boolean network became more prominent than others, meaning the system will behave in specific ways regarding the bacterias's quorum sensing and bioluminescence abilities.

Personal key learnings: Although boolean networks only are able to describe a very simplified version of gene regulation, there are many cases in which they can predict the correct pattern of expressed or suppressed genes.

Estimation of the time: 16 hours

Project evaluation on a scale of 1-5: 4

Word count: 1213

Keywords: boolean networks; gene regulation; vibrio fischeri

Task 1 - Toy model

Implementation and Simulation

We were given a boolean regulatory network consisting of a set of 6 genes which can either have the states 'expressed' or 'not expressed'. These states can be represented by a binary number, i.e. 0 or 1, so by assigning a boolean function for each of the 6 genes to update their values, thus creating the model of the gene network, we are able to implement it. Furthermore, for every state of the model we assign an integer value depending on the current binary representation of the genes, with gene A occupying the least significant bit and gene F occupying the most significant bit. This enables us to analyze the system's behaviour upon simulation.

To perform the simulations we used the Jupyter Notebook in addition to the Docker Machine. Docker provides a variety of modelling and analyzing tool kits such as bioLQM, GINsim and Pint, which we can run using python commands. Running those yields an enumeration of all possible states the system can be in as well as the system's behaviour when set to a specific initial state.

To visualize the results we used GINsim as well as code support from Makeyeva et al. which puts the model in the GINML format for displaying the layout information of the regulatory graphs and networks. As seen in figure 1 we can visualize the activation and inhibition reactions between the nodes of the network, with nodes representing a gene and directed edges representing modulation of one node by another.

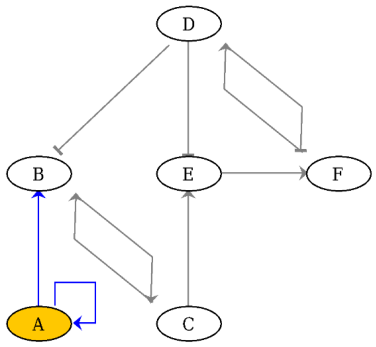

Figure 1 Model graph of a 6 gene regulatory network

Simulations starting from states 1, 4, 21 and 33

We obtained the individual sequences as given in the Figure 2, 3, 4 and 5 respectively. We can see that by having at least one transition between states inside the gene network, the system tends to terminate into a fixpoint after expression of gene D.

Initial State 1

	A	B	C	D	E	F
0	1	0	0	0	0	0

Figure 2 Sequence for state 1; fixpoint with only gene A active

Initial State 4

	A	B	C	D	E	F
0	0	0	1	0	0	0
1	0	0	0	0	1	0
2	0	0	0	0	0	1
3	0	0	0	1	0	0
4	0	0	0	0	0	0

Figure 3 Sequence for state 4; fixpoint after 4 iterations, in which no gene is expressed

Initial State 21

	A	B	C	D	E	F
0	1	0	1	0	1	0
1	1	1	0	0	1	1
2	1	0	1	1	0	1
3	1	0	0	1	0	0
4	1	0	0	0	0	0

Figure 4 Sequence for state 21; fixpoint after 4 iterations, each gene was expressed at least once

Initial State 33

	A	B	C	D	E	F
0	1	0	0	0	0	1
1	1	0	0	1	0	0
2	1	0	0	0	0	0

Figure 5 Sequence for state 33; fixpoint after 2 iterations due to expression of gene D

Attractors and Fixpoints

Although letting the algorithm analyze the behaviour from all 64 possible initial states, we were not able to make the algorithm give us a proper output about the attractors. Therefore we looked for them manually and we found a total of 6 different attractors as seen in figure 6, according to the algorithm each one being a fixpoint. These attractors are at states 0, 1, 5, 19, 27 and 37. As the found attractors denote fixpoints where no transition is possible, the periodic orbit of each attractor has a lentgh of 1. Considering all the state spaces we found that the attractors have a relative coverage of 50 %, 36 % and 9.3 % respectively, the rest having the same coverage with 1.6 %, with their basin sizes indicated in brackets.

The gene regulatory network as seen before in figure 1 highlights the attractor(fixpoint) at state 1 in yellow, where only gene A is expressed. The activation of gene B will never occur because gene C is not expressed, resulting in there never being a transition between states.

Interpretation

As mentioned while analyzing the system's behaviour from specific states, the network flow tends to terminate when gene D is expressed. It is activated by gene F and also suppressing genes B, E and F, meaning it contributes to inhibition of the metabolic process, no matter which genes of the network currently are active. Therefore every attractor needs to have gene D expressed beforehand with the exception of none or only gene A active, which itself is not dependent on any other gene. Since gene D terminates the flow of the network and according to the algorithm every state is leading into a fixpoint it is also the main reason for the period

Attractor	Period	Basin size	<A>	<B>	<C>	<D>	<E>	<F>
1	1	50% (32)	0	0	0	0	0	0
2	1	36% (23)	1	0	0	0	0	0
3	1	9.3% (6)	1	0	1	0	0	0
4	1	1.6% (2)	1	1	0	0	1	0
5	1	1.6% (2)	1	1	0	1	1	0
6	1	1.6% (2)	1	0	1	0	0	1

Figure 6 Attractors with period, basin size and relative coverage

length of the orbit being 1. This can be seen in by comparing the flow in figure 2 and figure 5, in which there is no transition from state 1, but two from state 33, being activation and expression of gene D.

Task 2 - Quorum Sensing Model

Background and description of the model

This hawaiin bobtail squid is a night feeder. However, in the moonlight it casts a shadow which makes it easy to spot for predators, so the squid formed a symbiotic relationship with vibrio fischeri, a bacterium with bioluminescence abilities, to counteract the shadow and in return provides it nutrients. As opposed to the bacteria's low amount in sea water, in high concentrations, like inside the squid's light organ, an auto inducer can form a complex with an operon, starting transcription, producing lux proteins and ultimately emiting blue-green light.

Implementation and Simulation

We were able to a create a boolean regulatory network with a set of 5 genes which also can either have the states 'expressed' or 'not expressed'. Again, these states can be represented by a binary number, i.e. 0 or 1, so by assigning a boolean function for each of the 5 genes to update their values, thus creating the model of the gene network, we are able to implement it. Like in task 1 we worked with the Jupyter Notebook in addition to the Docker Machine to simulate the model.

Visualizing the new system with GINsim (and code support with Makeyeva et al.) then outputs the graph seen in figure 7.

Attractors and Fixpoints

As mentioned in task 1, although letting the algorithm analyze the behaviour from now only 32 possible initial states, we were not able to make the algorithm give us a proper output about the attractors. Therefore we looked for them manually and we found a total of 4 different attractors as seen in figure 8, according to the algorithm each one being a fixpoint.

As the found attractors denote fixpoints where no transition is possible, the periodic orbit of each attractor has a lentgh of 1. Considering with all the state spaces

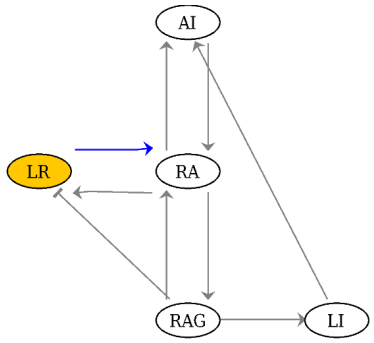

Figure 7 Quorum sensing model of vibrio fischeri

found that the attractors have a relative coverage of 9.3 %, 43.8 % and 34.4 % and 12.5 % repectively, with their basin sizes indicated in brackets.

The gene regulatory network of vibrio fischeri as seen in figure 7 highlights the attractor(fixpoint) in yellow, where only gene LuxR is expressed. Like in the toy model in task 1, a transition will never occur, making this state a fixpoint.

Attractor	Period	Basin size	<LuxR>	<AI>	<LuxR:AI>	<LuxR:AI:Gen>	<LuxI>
1	1	9.37% (3)	0	0	1	0	0
2	1	43.8% (14)	1	0	1	0	1
3	1	34.4% (11)	1	1	1	1	1
4	1	12.5% (4)	0	1	0	1	0

Figure 8 Attractors with period, basin size and relative coverage

Interpretation

We can characterize the 4 different attractors based on the function of the genes. Special genes are namely LuxR:AI:Gen, which when active inhibits the expression of LuxR, therby repressing LuxR:AI and leading to less lux proteins produced and LuxI, which expressed leading to more lux proteins produced therby emitting light. Considering all the basin of attraction we can say that behaviour of the network is entirely dependent on the attractors since about 78% of the total coverage resolves around just two fixpoints.