Stochastic simulation of noise in the genetic regulatory network of bioluminescence in *Vibrio fischeri*

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Abstract

In this study, the stochastic noise within the genetic regulatory network of bioluminescence in *Vibrio fischeri* was analyzed using deterministic and stochastic simulations in a computer program called Dizzy. Two models of the genetic circuit were used in this study to perform the analysis. The deterministic simulations were performed on the models to investigate the steady state of the system. Results for the Zhou model were inconclusive as the steady state could not be established. In contrast, the steady state of the system for the Cox model was established. Analysis of the system continued with this model using stochastic simulations to replicate the noise within the gene circuit. Discrepancies between the stochastic and deterministic simulations posed a problem for analysis of the noise. Although the noise was not analyzed, this investigation provides a foundation for further study.

Introduction

Vibrio fischeri is a bacterium that belongs to the genus *Vibrio* whose species are found typically in saltwater. A few infamous species of this genus are pathogenic to humans such as *Vibrio cholerae*, which causes cholera. Although not pathogenic to humans, *Vibrio fischeri* has many fascinating features and has been much studied. This marine species is found in all oceans

of the world though predominantly in symbiosis with other marine life. An example of this symbiosis is found in the defense mechanism of the bobtail squid. Within the mantle of the squid lies the light organ that *Vibrio fischeri* inhabits. The squid provides the bacteria with nutrition and in return they produce light termed bioluminescence. The light produced by the bacteria allows the silhouette of the squid to be hidden when seen from below by matching the amount of light that hits the mantle, which is the outer covering of the squid's body. The bioluminescence of *Vibrio fischeri* is of particular interest because it involves the phenomenon of quorum sensing which is the ability of the bacteria to communicate with each other over distances and is the method the bacteria use to function within the light organ of the squid. The various biochemical processes and the genetic circuit involved constitute the genetic regulatory network.

The basis of this genetic system is a section of DNA known as the lux operon. The lux operon houses all the genes that are involved in the initiation and cessation of the bioluminescence. An important product of the lux operon is the signaling molecule known as Autoinducer (AI). AI is the language of *Vibrio fischeri*'s quorum-sensing mechanism and how the symbiotic relationship works in the bobtail squid. Within the light organ of the squid, the bacteria build up as well as the concentration of the AI. Once the concentration of AI is sufficient, the biochemical pathways that lead to bioluminescence will open. This shows that quorum sensing is a cell-density dependent process [4]. Thus, the bacteria that are a distance away can regulate the production of light produced by other bacteria.

All the biochemical pathways involved in light production involve both transcription of various proteins as well the regulation of transcription of many genes. AI together with another product of the lux operon LuxR form the complex that binds to the site on the DNA that activates the lux operon and transcription of its genes. This leads to production of the proteins necessary

for bioluminescence. Since these biochemical processes have reactions that involve species with low copy numbers, there is an element of randomness inherent to the system, which therefore cannot be modeled accurately using deterministic methods. A process that has an element of randomness is known as a stochastic process [1]. This element of stochasticity present in the genetic regulatory network of bioluminescence in *Vibrio fischeri* is the object of our investigation.

In our study, we are looking first at the model of the genetic system of bioluminescence. The model consists of the reaction species, the biochemical reactions, and the rates of these reactions. We are using a computer simulator that will look at the kinetics of the system. The simulator known as Dizzy can model the kinetics of system both deterministically and stochastically. Using the simulator, we are able to look at the equilibrium state of the system and determine the relationships that exist between the various species in the biochemical processes. Once the relationships are adequately understood, we will move on to stochastically modeling the system using the stochastic simulators of the Dizzy program [3]. The stochastic simulation is based upon Gillespie's algorithm, which was developed in order to more accurately describe the behavior of some stochastic processes. We will therefore be able to look at the noise due to random fluctuations that exist within different biochemical pathways. In particular, we would like to model the noise within the pathway that involves the production of the protein Lux I from AI. In past studies, the noise of the quorum-sensing mechanism has been investigated. In this study, our goal is to observe the noise present in a single bacterium and compare it with actual experimental results involving its bioluminescence.

Many reasons exist for performing a study such as this. Since the physical bases of many biological processes are not known, this study will further elucidate some of the features of a

system such as this one. It will also confirm the accuracy of the current model for the bioluminescence system and possibly suggest some modification. This study also has some human benefits as well because it will also further unveil the mysteries of the remarkable quorum-sensing mechanism that the bacteria utilize for bioluminescence. If more is known about bacterial communication, perhaps this knowledge can be used to develop more precise antibiotics for bacteria that use communication for virulence.

Model Setup within Dizzy

I began my study by setting up the models of the genetic regulatory network for bioluminescence in *Vibrio fischeri* within a computer program called Dizzy which simulates the kinetic behavior of a system. This windows based program was written by Stephen Ramsey and is publicly available on the web at *magnet.systemsbiology.net/software/Dizzy/*.The systems simulated by dizzy are described by chemical kinetic models that have several components which include the various species of biomolecules, the reactions that describe the various biochemical pathways, and the reaction rate parameters, which describe the speed that a particular reaction proceeds. These models are easily implemented in the Dizzy environment. Similar to a word processor, the components of the model can be typed within the program. The only difference is that Dizzy has certain syntactic rules that must be followed [3].

Dizzy simulations

Once the model has been implemented in Dizzy, the system described by the model is then ready to be simulated. Two general types of simulators exist in Dizzy: stochastic and

deterministic. These simulators differ fundamentally from each other because the method each uses to analyze the kinetics is based on a different view of the nature of the biological system.

One such view is that the system can be described by a coupled set of ordinary differential reaction-rate equations. Thus, if the state of the system is known at a certain point in time, then the state of the system at a later period of time can be predicted. This is the idea behind the method used by the deterministic simulations in Dizzy.

In contrast, the stochastic simulators in Dizzy are based on the idea that due to low copy numbers of the species involved in the biochemical processes, random fluctuations exist in the species numbers, and therefore the state of the system cannot be exactly replicated by deterministic ordinary differential equations. This simulator is based on the ideas proposed by Daniel Gillespie to develop a more accurate method to model certain biological processes that include inherent stochastic effects [1]. The method he developed is called the Gillespie algorithm.

The Gillespie algorithm lies at the heart of the stochastic simulator and is based on trying to solve the so-called chemical master equation that describes the stochastic biological system exactly. The general scheme behind Gillespie's algorithm can be summarized in a few steps. First, the algorithm initializes the number of molecules within the system, the reaction parameters, and the random number generator. Next, the random number generator picks a number that determines the reaction in the model to occur as well as the time interval it occurs over. After the reaction occurs, the time interval is updated based on the result in the previous step, and the molecule count is updated according to the reaction that occurred. Lastly, this process iterates itself until the reactants are zero or the simulation time is exceeded [2].

The results of Dizzy's simulations can be displayed in either a graphical form that shows the species numbers as functions of time or it can be given in the form of a table that can be imported to an Excel spreadsheet for analysis.

Zhou model

The Zhou model was the first model that I analyzed in the study. Details for this model are shown in Figures A.1 and A.2 in Appendix A. I began my analysis of the genetic regulatory network using the Zhou model by first trying to establish the equilibrium state of the system. The equilibrium or steady state occurs when all the species involved in the biochemical pathways stop changing in number. The various simulators in Dizzy allow for this type of kinetic analysis. I used a deterministic simulator to determine the equilibrium of the system. Although not as accurate at modeling the behavior of the system and its component species as the stochastic simulator, the deterministic simulator allows one to get a general idea of when the system comes to equilibrium. The stochastic simulator is more computationally expensive and therefore takes a much longer time to run, so using the deterministic simulation allows for a much quicker analysis. Once the equilibrium is established, the stochastic simulator can be run with initial species obtained from the deterministic simulation to get a more accurate look at the steady state by showing the random fluctuations or noise around the deterministic equilibrium.

I ran the simulation for 25000 minutes, with the results shown in Figure 1.

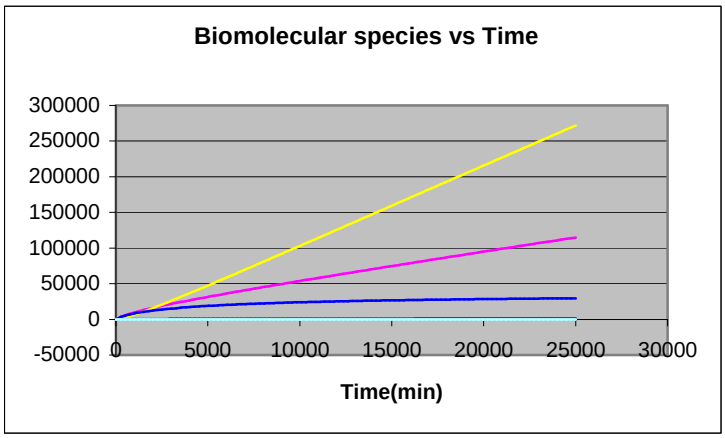

Figure 1. The result of the deterministic simulation for each of the species in the Zhou model where the multi-colored lines represent the different species.

I ran the simulation in several different ways by varying the simulation time and initial species. Each trial gave results similar to the first.

These results present a problem. Our initial goal was to establish the equilibrium state of the system but these results show that there is always a component not at equilibrium. I then began to investigate the origin of the problem by first rechecking the code for the model in Dizzy. I found that there were no errors in the code, so I began to experiment with the model to see if the equilibrium of the system was affected by the initial species. The result after many trials was that it did make changes to the results in such a way that the species that were out of equilibrium were different. After many efforts to investigate the problem failed and equilibrium for the system using the Zhou model could not established, I decided due to time constraints on the study to move on to the investigation of the system using the Cox model.

Cox model

The Cox model is the second model I used in my investigation of the genetic regulatory network. Details for this model are shown in Figure A.3 in Appendix A. I began my analysis

with this model the same way I did with the Zhou model; I wanted to establish the equilibrium state of the system. Although the models are slightly different, the procedure for determining the steady state of the system was the same. Since some of the reactions were different in this model, I tried to see if the system could reach equilibrium without those other reactions in order to reduce the complexity of the model. Therefore, I removed the DNA looping reactions that were present in this model. The results of the simulations showed that the system was able to come to equilibrium.

The next step was to reproduce the equilibrium graph of LuxI versus AI that is present within the Cox paper. This was done by obtaining the equilibrium values of LuxI and AI and having each pair represent a point on the graph. The various points were obtained by varying the AI species by changing the reaction rate of the reaction that provided the system with more AI. The results of this analysis are shown in Figure 2.

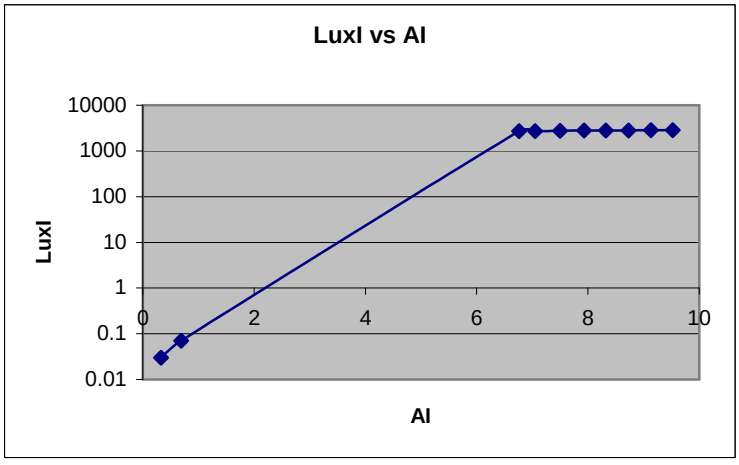

Figure 2. Equilibrium relation between concentration of LuxI and AI. There is a large gap between the first two points and the next several points.

These results show that a problem exists in the relationship between LuxI and AI; there is a large gap in the graph, which is an unexpected result and does not match the equilibrium graph in the Cox paper. I began to investigate the problem by changing various components of the

model to see if it would have an effect. I also rechecked my code for the model and did not immediately find any errors. I later found that I put one of the bimolecular reactions into the model as a single-molecular reaction. I fixed this problem and ran the simulation again which allowed me to reconstruct the LuxI vs. AI equilibrium graph from the Cox paper [4]. This result is depicted in Figure 3.

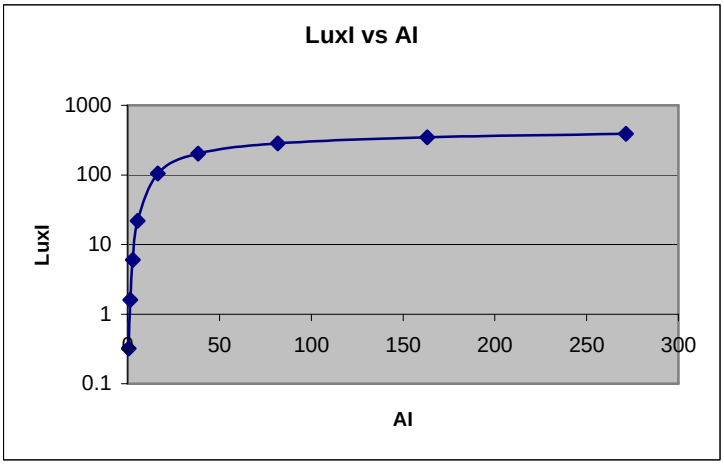

Figure 3. Equilibrium graph of LuxI vs AI with the model corrected.

After obtaining these results, I began the next part of my analysis, which was to look at the noise present at equilibrium in the biochemical pathways of the system. To do this I ran the deterministic simulator to obtain the equilibrium values of each of the species in the system at different AI values. After obtaining the deterministic results, I then ran the stochastic simulations with initial species values that were the same as the deterministic equilibrium species values. This allowed me to analysis the noise around the deterministic equilibrium by starting the stochastic simulations at equilibrium.

The first results that I obtained were those for the equilibrium conditions when AI=1. The comparison between the deterministic and stochastic simulations for AI and LuxI are shown in Figures 4 and 5, respectively.

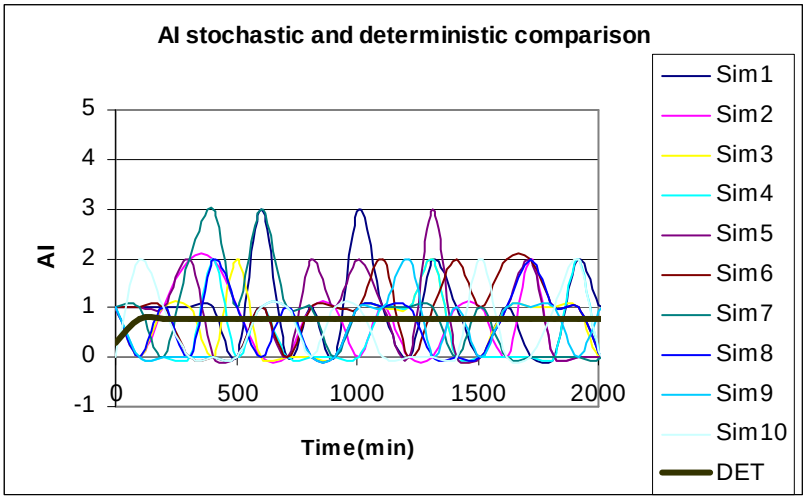

Figure 4. AI is approaching equilibrium at one for the deterministic simulation while the stochastic simulations, indicated by the multi-colored lines, oscillate about that line and average to about 0.90.

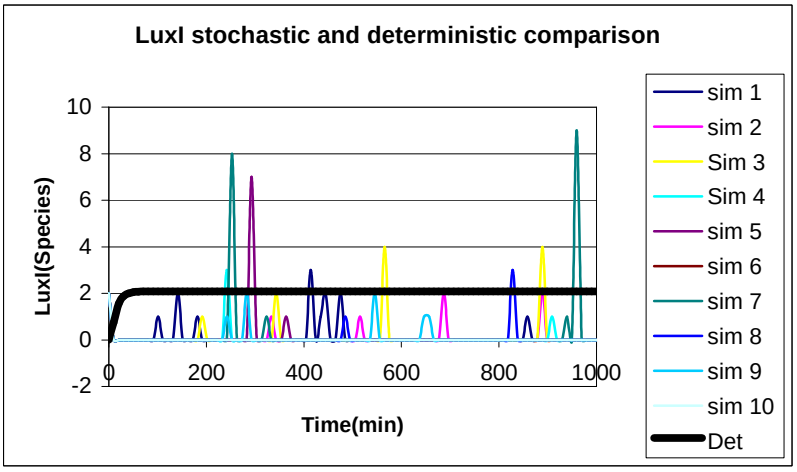

Figure 5. A comparison of the deterministic and stochastic values for LuxI. The deterministic value approaches equilibrium at 2, while the stochastic values (indicated by multi-colored lines) fluctuate with short-lived peaks.

These results present a problem because the various species in the deterministic and stochastic simulations are supposed to agree with each other in such a way that the average fluctuations in the stochastic simulations are about the same as the deterministic values. This discrepancy exists for all other AI values.

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Conclusion

Although the noise within the genetic regulatory was not analyzed for an individual cell and compared with experiment, this study provides a foundation upon which further progress can be made. Once the origin of the discrepancy is found, continuation of the analysis can be made. Also, the noise analysis could lead to the ability to constrain the model parameters thus refining the model. Continuation of this study as well as similar studies is important because if we can better understand the physical basis of the communication mechanism between bacteria, perhaps new, more accurate antibiotics can be developed to intercept the communication between bacteria that use it for organization within the host during virulence.

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Appendix A.

Zhou model

Fast reactions	Slow reactions
$AI + AI \rightleftharpoons^{k_1}_{k_{-1}} AI_2$	$DNA \xrightarrow{k_m} mRNA_{LuxI} + mRNA_{LuxR} + DNA$
$LuxR + LuxR \rightleftharpoons^{k_2}_{k_{-2}} LuxR_2$	$ALD \xrightarrow{\alpha k_m} mRNA_{LuxI} + mRNA_{LuxR} + ALD$
$AI_2 + LuxR_2 \rightleftharpoons^{k_3}_{k_{-3}} AL$	$mRNA_{LuxI} \xrightarrow{k_{pi}} LuxI + mRNA_{LuxI}$
$AL + DNA \rightleftharpoons^{k_4}_{k_{-4}} ALD$	$mRNA_{LuxR} \xrightarrow{k_{pr}} LuxR + mRNA_{LuxR}$
	$LuxI \xrightarrow{k_a} AI$; $AI \xrightarrow{e_a} \emptyset$
	$LuxI \xrightarrow{e_i} \emptyset$; $LuxR \xrightarrow{e_r} \emptyset$
	$mRNA_{LuxI} \xrightarrow{e_{mi}} \emptyset$; $mRNA_{LuxR} \xrightarrow{e_{mr}} \emptyset$

Figure A.1 Biochemical reactions present within Zhou model [5].

Zhou model reaction rate parameters	
k1	0.8/(nM.min)
k-1	8.6/min
k2	0.8/(nM.min)
k-2	8.6/min
k3	6.00E-05
k-3	0.75/min
k4	0.8/(nM.min)
k-4	8.6/min
km	5.4/min
akm	2.27/min
kpi	6.5/min
kpr	6.5/min
ka	3.0/min
ea	(1/6)E-2/min
ei	(1/6)E-1/min
er	(1/6)E-1/min
emi	1.0/min
emr	1.0/min

Figure A.2 Reaction rate parameters for the reactions in the Zhou model [5].

Cox model

AI + LuxR → RAI	$k_{f1} = 0.1$
RAI → LuxR + AI	$k_{r1} = 2$
2 RAI → RAI2	$k_{f2} = 0.06$
RAI2 → 2 RAI	$k_{r2} = 4$
RAI2 + luxD → luxDcomp	$k_{fD} = 0.01$
luxDcomp → RAI2 + luxD	$k_{rD} = 3$
luxDcomp + ipromR → DNAloop	$k_{f\text{-}loop} = 6$
DNAloop → luxDcomp + ipromR	$k_{r\text{-}loop} = 1$
mRNAR → LuxR + mRNAR	$k_{t1R} = 0.03$
mRNAI → LuxI + mRNAI	$k_{t1I} = 0.03$
mRNAR → *	$\gamma_{mR} = 0.006$
mRNAI → *	$\gamma_{mI} = 0.006$
LuxI → *	$\gamma_{PI} = 0.001$
LuxR → *	$\gamma_{PR} = 0.006$
LuxI → LuxI + AI	$k_{AI} = 0.017$
AI → *	$k_{out} = 7.1$
AIsource → AI + AIsource	k_{in} = variable
luxbox + RAI2 + promI + promR → ipromI + ipromR	$k_{f3} = 0.1$
ipromI + ipromR → luxbox + RAI2 + promI + promR	$k_{r3} = 5$
promR → promR + mRNAR	$k_{crpR} = 0.01$
promI → promI + mRNAI	$k_{basII} = 0.000015$

Figure A.3 Biochemical reactions and reaction rate parameters for the Cox model [4].

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