Designing a Synthetic Noise Tuner Device

Olivia Ho-Shing
May 2010
Biology Department, Davidson College, Davidson, NC 28035

Introduction to Biological Noise

Noise in gene expression refers to the measured level of variation in protein production and behaviour among cells within a genetically identical population. Noise exists in all biological systems as fluctuations in gene expression due to stochasticity in transcription, translation, and molecular coupling processes. Phenotypic variation in genotypically identical populations affords an adaptive benefit for cells to adjust to variations in the external environment without altering the genetic makeup [1]. Furthermore, the cell's allowance of noise in certain pathways lowers the energetic cost of performing the process, while more stringent pathways require more time and energy. While this analog, or graded, response has clear benefits in biological systems, such variation in individual output is at odds with the digital, or binary "on/off", output desired in synthetic systems. Synthetic biologists attempt to minimize the noise level in synthetic devices, or more often, to ignore the effects of noise altogether by averaging noisy output measurements. However, since noise can be beneficial in biological circuits such as the bacteriophage lambda network for environmental sampling and regulatory bistability [2], maintaining or increasing the noise level in a synthetic system could also be beneficial for operating some devices. By accounting for noise in the synthetic design, one may be able to exploit the effects of stochastic behaviour to produce a desirable output. This proposed study aims to design a synthetic circuit that detects and responds to the noise level in a separate synthetic device in order to establish a noise tolerance threshold, or band pass filter, in the cell population, thereby tuning the noise level of the system.

The noise level in a pathway is calculated as the relative deviation from the average measured output. Factors affecting the noise level in a system or synthetic circuit pertain to the random formation and decay of single molecules and multi-component complexes. [1] Most factors displaying stochasticity are involved in the transcription and translation of genes. Because there is a limited amount of transcription factors, polymerases, and other gene

expression machinery within the cellular space, variations in expression rate and protein abundance depend on the timing and kinetics of each protein. This manifestation of noise is known as the finite-number effect – with a smaller number of molecules, such as polymerases and plasmids, affecting protein abundance in a compartment, noise increases. Reversible protein-protein oligomerization, particularly of transcription factors and repressors, reduces noise and increases functional stability in regulatory networks [3]. Noise level is also affected by the degradation rate of proteins in the system. Longer, multistep cascades in synthetic systems have higher noise levels because there are more steps requiring molecular coupling. Negative feedback loops provide a noise-reduction mechanism in downstream processes. Negative feedback can also have a destabilizing effect that may increase noise level if it involves a transcriptional time delay. [1] Alternatively, positive feedback typically amplifies fluctuations and population heterogeneity, increasing the system noise level. Amplification by positive feedback loops can even generate bimodal population distributions [4]. In a toggle switch circuit, positive feedback loops improve the robustness of the network against "leaky" switching [3], which in effect minimizes noise in the final output.

Positive Feedback Loops

Positive feedback loops act as dynamic circuits in both biological and computational systems. Positive feedback, an autocatalytic circuit, underlies bistable or binary responses in both prokaryotes and eukaryotes (1, 5]. In a bistable system, reaching one of the two stable states depends on the system's input parameters. For example, Isaacs and colleagues [4] attempted to tune the fluorescence output of a synthetic circuit by varying the surrounding temperature, thereby destabilizing a repressor protein that controlled a positive feedback loop. They found, however, that noise plays a significant role in the tuning of the positive feedback loop. Stochasticity in the destabilization of the repressor either causes the cell to amplify the amount of GFP by positive feedback, or causes a minimal amount of GFP – "trademark bistability of the positive feedback architecture". [4] Here, the destabilization of the repressor element in the context of a positive feedback loop allows even minute differences in noise level to direct the creation of visually distinct bimodal populations. By utilizing the power of heterogeneity through positive feedback loops, cells can use noise in both biological and synthetic circuits to filter noise. Although noise typically degrades a signal, leading to an

undesirable graded response, noise can also enhance a signal by stochastic resonance. [6] A similar system can be used to construct a noise tuner that creates bimodal populations. One population that operates within the desired noise threshold survives, and the other population outside of the noise threshold dies due to the rapid progression of a fatal feedback loop.

Stochastic resonance is a physical phenomenon manifested in nonlinear systems whereby generally feeble input information, such as a weak signal, can be amplified and optimized by the assistance of noise [7]. While the effects of stochastic resonance are robust, the mechanism simply requires the formation of a threshold, or energetic activation barrier; a weak periodic signal; and a source of noise intrinsic to the system. Because the existing synthetic device that one desires to tune is the source of intrinsic noise, the proposed noise tuner must include the formation of a threshold, and a way to communicate even a weak signal from the existing device. This weak signal can be amplified by the periodic forcing of a positive feedback loop in the circuit. The amplitude of the periodic component depends on the noise strength of the system; thus the noise level of the circuit manipulates the periodic component, the positive feedback loop. Previous applications of the idea of stochastic resonance include simulating neuronal firing and cytoskeleton dynamics, creating bistable ring lasers, and optical trapping. Optical trapping is similar to and used in conjunction with optical band pass filters particularly for single cell sorting [8], suggesting that the proposed noise filter is feasible through a mechanism of stochastic resonance.

Framework Design for Noise Tuner

The proposed noise filter would incorporate an autocatalytic circuit triggered by noise level to induce a bimodal binary response. Cells within the desired noise level can persist, while the cell population outside of the noise threshold triggers the feedback loop to cause death. Thus, the noise level in the output of a synthetic device can be tuned to a desired threshold by killing all other cells. This system could utilize the TetA(C) gene in the positive feedback loop. While low to moderate levels of TetA protein confer tetracycline resistance, over-expression of the gene is detrimental to cell growth and ultimately lethal [9]. The positive feedback loop acting as the periodic force that was initially considered is the Lux system from *Vibrio fischeri*. Conceptually, I propose a circuit model in which noise levels above a threshold defined by the user of the synthetic device cause either the direct induction of a positive feedback loop (Figure

1), or a destabilization event that directs induction of a positive feedback loop (Figure 2), causing the lethal over-expression of TetA. The Lux operon can be used to construct the positive feedback loop. The Lux system contains LuxR, a repressor that when bound by a homoserine lactone autoinducer induces the *pLux* promoter. Induction causes expression of LuxI, the protein that produces the autoinducer to maintain the circuit [10]. The *pLux* promoter can co-regulate LuxI and TetA in order to achieve lethal levels of tetracycline resistance.

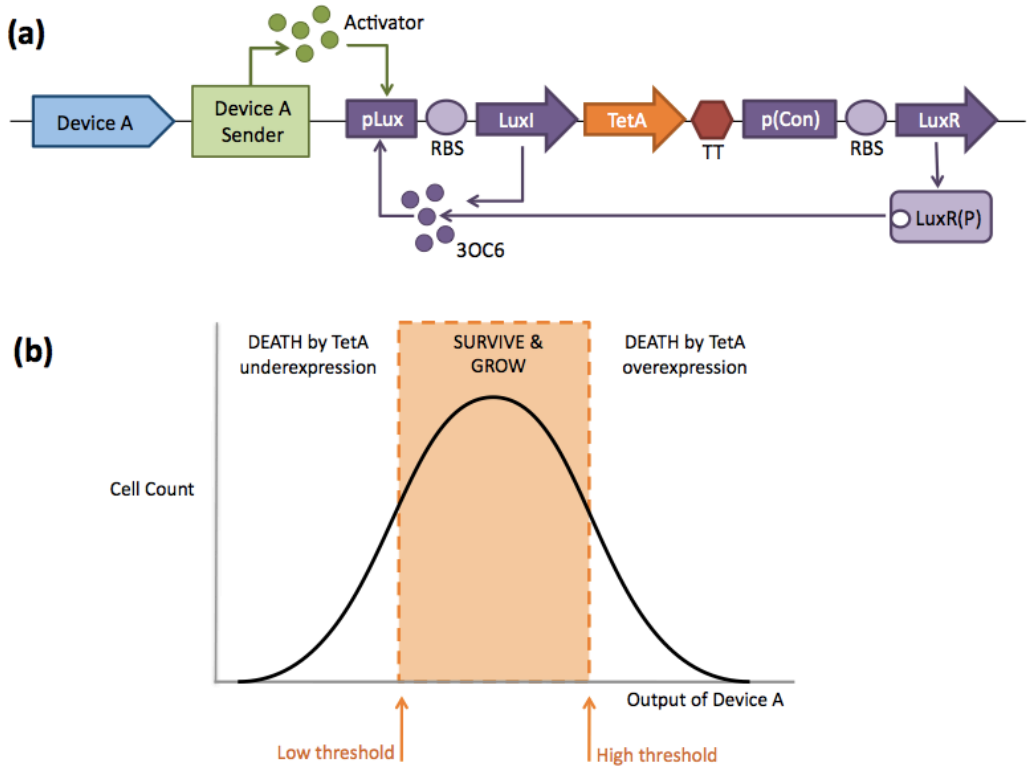

Figure 1. The tentative window design of the noise tuner. *(a)* The design illustrates the Lux system (purple) as the mode for positive feedback in order to express TetA for tetracycline resistance. LuxR is expressed by a constitutive promoter, p(Con), to produce LuxR protein, LuxR(P) that induces the *pLux* promoter when coupled with the autoinducer 3OC6. The existing synthetic device (Device A) to which the noise tuner is connected must have a way to communicate the output level to the noise tuner; this requirement is illustrated in the schematic by expressing a signal sender for Device A, which produces an activator that activates *pLux*. *(b)* The goal of this design is to use tetracycline resistance to select a certain window of cells within the desired noise level; all other cells die, or never grow in the presence of tetracycline. Cells that do not survive would be considered to be a noisy device.

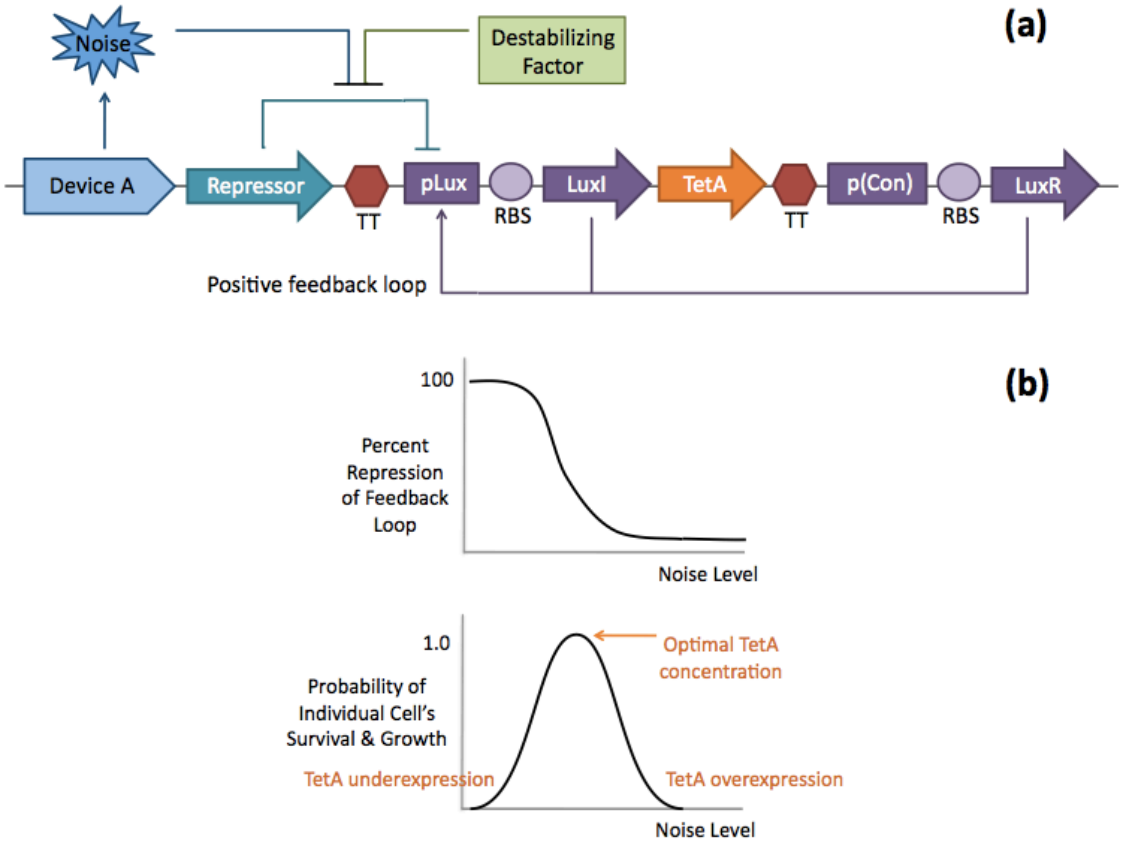

Figure 2. The tentative repressor destabilization design. *(a)* This design utilizes the Lux system (purple) for positive feedback like the window design (scheme simplified). A repressor of the positive feedback loop is expressed with the output of Device A. Noise level of Device A affects the expression level and degree to which the positive feedback loop is suppressed. Another destabilizing factor controlled by the user such as heat affects the destabilization of the repressor. Some destabilization allows the expression of TetA in the feedback loop, granting tetracycline resistance. Over-destabilization causes over-expression of TetA. *(b)* High noise level reduces effective repressing of the feedback loop. Manipulating the destabilizing factor would change the percent repression of the positive feedback loop, allowing the user to tune Device A to the desired noise level. Cells that have tolerable noise level destabilize the repressor enough to produce the optimal concentration of TetA.

Previous studies have attempted to design and construct a bacterial band pass filter in order to select for intermediate levels of gene expression [11, 12]. Sohka and colleagues accomplished this by essentially constructing a low-pass and a high-pass filter in a series (Figure 3B). In their schematic design of the band pass filter (Figure 3A), IPTG induces the *tac*

promoter to express β-lactamase (BLA). If enough BLA is produced, the enzyme hydrolyzes ampicillin, conferring sufficient drug resistance in ampicillin media. Low levels of BLA allow the molecule aM-Pp to build up, which induces the *ampC* promoter. The *ampC* promoter induced expresses GFP and Tet, so that cells can grow in tetracycline media. Thus, viable BLA levels, regulated through an IPTG-inducible promoter, falls within a narrow range such that high concentrations of ampicillin prevent cell growth via inhibition of cell wall synthesis. Cells in regions of low ampicillin are growth arrested due to the protein synthesis inhibitory effects of Tet. Only the cells located in regions of intermediate ampicillin will grow. Increasing the BLA enzyme activity shifts the range of ampicillin concentration required for growth to higher levels, and vice versa.

The crucial difference between this design and the noise filter proposed is that the Sohka band pass filter is not purposed for amplifying noise in order to select for cells within a certain noise range of output. The band pass filter is a good model for studying morphogen gradients that lead to pattern formation [12], and does successfully create two thresholds using drug resistance genes, including tetracycline resistance. However, with the given band pass design, it is very difficult or impossible to narrow the filter to the desired range of gene output. By altering the concentration of ampicillin, it is possible to shift the position of the filter in order to select for a higher or lower expression level. But the size of the window of cell growth, the distance between f_1 and f_2 (Figure 3B), cannot be tuned by the user. Moreover, any adjustment of the window position requires very exact measurement and monitoring of ampicillin, tetracycline, and β-lactamase concentrations. Ideally, the noise filter would be more modular, easily tunable, and self-reliant.

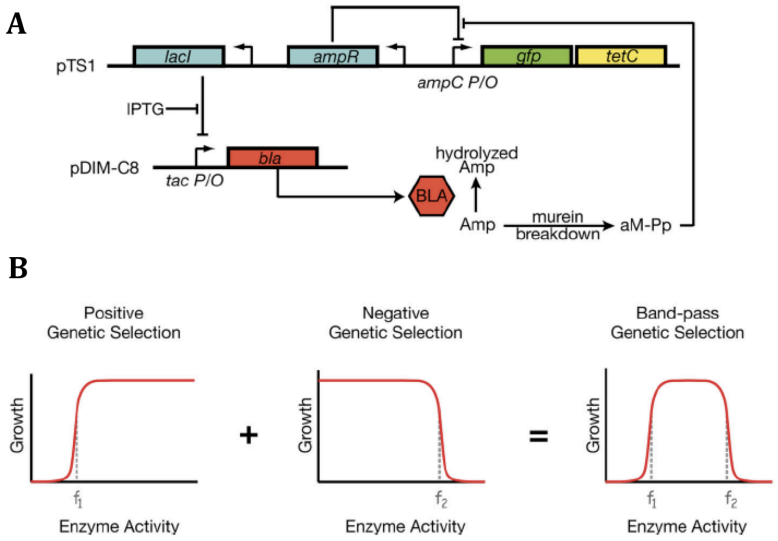

Figure 3. From *Sohka et. al. 2009 (Proc Natl Acad Sci).* (A) Schematic design of the bacterial band pass filter. (B) Schematic representation of how the combination of a positive genetic component and negative genetic component results in the band pass filter.

Design Issues Confronted

The tuning limitations of the Sohka band pass filter raises the first issue confronted in the design of the noise filter: how can we design the circuit to be easily tuned by the user, instead of dictated solely by the biological capacity of tetracycline (or other drug) resistance? One approach to tunability resembles the construct tested by Isaacs and colleagues [4]. This involved a positive feedback loop with a repressor protein whose behaviour they found could be tuned by both altering the temperature of the system, and by the intrinsic noise level of the system. By increasing the temperature, the repressor protein was destabilized, in an attempt to vary the degree of activation of the positive feedback loop. They found, however, that stochasticity in the destabilization of the repressor either causes the cell to amplify the amount of GFP by a positive feedback loop, or causes a minimal amount of GFP, forming bistability in the cell population. For a noise tuner, a similar repressor or other protein could activate the positive feedback loop (Figure 2); theoretically, the noise level of the system could drive the circuit to one stable state or the other, and the system could be tuned by affecting the stabilization of the repressor with a factor such as temperature or some nutrient concentration.

In other words, a noisy device would lead to noisy expression of the repressor; especially if the repressor degrades quickly in the cell, the noise level would significantly affect the positive feedback loop. Having another destabilizing factor like temperature allows the user to make the system even more sensitive to the effects of noise. The primary difficulty in implementing such a system is the initial unpredictability of the effects of destabilizing a protein, and the effects of noise. Determining a fitting protein to use as the destabilized activator could take a large amount of time and testing. Detailed testing of the construct would be needed to verify if and to what extent the noise level could be tuned.

A second approach to tunability could be to make modular tuners that apply different "window sizes", so that the user can select the tuner with the desired window size for desired noise level. Because tetracycline resistance makes cells more sensitive to other drug resistance genes, different window sizes could be constructed by combining drug resistances in a positive feedback loop. One could potentially "slide" the windows higher or lower by changing the plasmid copy number on which the noise tuning circuit is found. Controlling the gene copy number is a proven way to lower the intrinsic noise in gene expression [13]. While this window system is not as tunable as the repressor destabilization system, it could be a simpler circuit to conceptualize and construct.

In either system type, another issue to address in design is how can the noise level be detected so that the noise tuner circuit can be applied to an existing synthetic device? This communication between the two devices, further discussed later, requires the tuner to detect the noise level of the output of the existing circuit; this is at least conceptually very different from detecting the measured output of the circuit. In the window system design, however, one could define the output level above a high threshold or below a low threshold as "noisy" (Figure 1b), making detection in the window design more direct.

Issues Regarding Cell Death or Survival

One of the primary issues in designing the window system is creating the high noise threshold and low noise threshold for the circuit output. The purpose of the thresholds is that all cells generating output above the high threshold or below the low will die, while the cells with output expression in the middle will survive. To accomplish this, a system containing

elements similar to the Sohka band pass filter [11, 12] could be implemented; this would entail an overlapping low-pass filter sub-circuit and high-pass filter sub-circuit so that only cells in the intermediate range can grow or survive. This approach raises the question of how one detects and creates a threshold for noise level within a synthetic circuit versus creating a threshold simply for circuit output. An alternative approach could use elements of previously designed synthetic NOT logic gates. For a NOT logic gate, a low input signal, or low noise threshold, causes a high output. The output here would be some gene product that causes death. Likewise, a high input signal causes a low output in a NOT gate. [14] This portion of the design may or may not be useful for the noise filter schematic. It could be useful if the low output generated is some gene product needed to survive, so that noise inputs at both thresholds will cause death.

The rate of death and rate of variance are another issue to address in designing and testing the construct. While positive feedback loops are useful because of the robust system output and bistability, it is possible that this bistability could be counterproductive in the noise tuner; the positive feedback loop could drive all cells to either the high or low extreme, leaving none in the middle range that will survive. A positive feedback loop could also increase the output and noise level of all individual cells, affecting the position of the window and therefore the population of cells that would die. For this potential problem, the design could incorporate a repressor to attenuate the positive feedback loop at a certain point. Overall, a computational simulation of the effects of a positive feedback loop on output and noise level would be beneficial for predicting the efficacy of the noise tuner design.

It is also unclear how immediately death would occur and how frequently cellular output varies. Because of the numerous factors affecting noise, cells may have a higher noise level during a short period of growth and circuit output, but attenuate that noise level later. Thus, at some point in time, all cells could be within the tolerable noise level but later move outside of that window. Moreover, when a new cell initially develops and begins the synthetic circuit, the measured output will inevitably be lower than the low threshold. The design must account for this so that the noise tuner does not immediately kill all cells before generating any further output. All of these issues with the timing of death or survival affect the gene selected for the mode of killing or survival in the tuner design. One can select a gene so that the user can control when the cells outside of the thresholds will die; for example, antifreeze proteins are

found in vertebrates, plants, fungi and bacteria so that the organism can survive subzero temperatures. By using an antifreeze gene in the noise tuner design, the user can kill noisy cells by subjecting all cells to rapid freezing [15].

Issues Regarding Communication/Signaling

In order for the noise tuner to be both effective and modular, it must be designed to function with different synthetic circuits. There must be some mode of communication between the two circuits such that the noise tuner detects the noise level of the other circuit's output, and a method of positive feedback that will not interfere with the circuit communication. In the initial conceptualization of the tuner design, the Lux system is proposed for the positive feedback mechanism. The Lux system is regulated by the LuxR protein and a homoserine lactone autoinducer such as 3OC6. When the autoinducer binds to the protein LuxR, it activates transcription downstream of the induced *pLux* promoter. The *pLux* promoter is used to express LuxI and other genes downstream. LuxI produces more 3OC6, making an autocatalytic cycle. This system is used in organisms as a powerful quorum sensing mechanism, in which the autoinducer can diffuse outside of the cell to communicate with neighboring cells. [16] Therefore, although the Lux system is well studied and widely used, the diffusion of 3OC6 would cause a synchronized population response instead of the desired fate determination based on the individual cell's noise level. The quorum sensing effect could be reduced by periodically pelletting cells out of media, or flushing 3OC6 from the container using a microfluidic chamber device. Such chambers have previously been built and used to flush out autoinducer and maintain cell density [17], and to measure individual cell fluorescence with single molecule sensitivity [18].

Further issues with using the Lux system, however, arise if the existing circuit to which the noise tuner is added also contains the Lux operon; this would cause cross-communication and perhaps over-amplification due to multiple positive feedback loops. Therefore, alternatives to using the Lux system in the noise tuner design should be explored. A similar system of quorum sensing is the Las system from *Pseudomonas aeruginosa*, which functions the same as the Lux system using a different homoserine lactone autoinducer, 3OC12. According to Waters and Bassler [19], the autoinducer 3OC6 binds specifically with LuxR and 3OC12 is specific to LasR, which would suggest that one could avoid cross-communication between the existing

circuit and the noise tuner feedback loops. However, results from other studies of the Las system [20, 21] including synthetic biology research conducted at Davidson College contradict this claim. Will Deloache and Kin Lau found that in the construction of an XOR logic gate using the Lux system and Las system, 3OC12 activates LuxR as well as LasR [21]. Nonetheless, using any quorum sensing system such as Lux or Las would cause communication between cells, generating a synchronized population response. Therefore, a different mechanism of communicating the existing device with the noise tuner, and a new mechanism of positive feedback or another mode of bimodality are needed for the noise tuner.

Alternative Design Considerations

Other designs that do not incorporate a positive feedback loop can be used to the same effect as the proposed noise tuner if the circuit still elicits bimodality based on noise level. In Kin Lau's research on the construction of a synthetic XOR gate, he demonstrated that the weak inducible promoter *pLux* could be activated for transcription in both the forward and reverse directions. *pLux* is induced in the forward direction when activated by LuxR bound to the autoinducer 3OC6. It is also induced for transcription in the reverse direction, when activated by LuxR in the absence of 3OC6 [22]. Backwards activity has been shown to exist with a stronger inducible promoter *pLac* [23]. The bimodality of promoters can be utilized for the noise tuner if the mode of communication between the existing circuit and tuner is the promoter inducer, such as 3OC6 (Figure 4). Presence of 3OC6 can induce transcription of a viable amount of a gene necessary for growth or survival, namely TetA(C). Again, too much TetA would be lethal to the cell, setting the high noise threshold. Little or no 3OC6 would cause transcription in the reverse direction of a gene causing death. This would set a low noise threshold. This design, however, using the Lux operon still carries the issue of cell-to-cell communication; the design would need to be adapted to avoid quorum sensing.

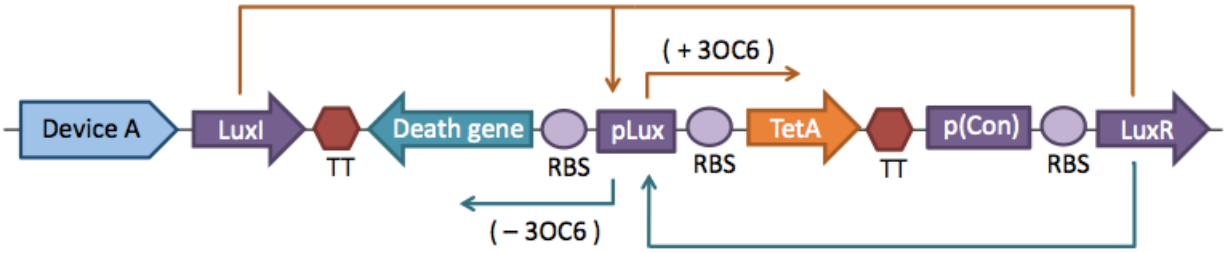

Figure 4. Tentative noise tuner design using forwards and backwards transcription through *pLux*. LuxI expression is connected to the existing device (Device A) output. LuxI produces 3OC6 that complexes with the LuxR protein to induce *pLux* in the forward direction. Moderate expression of TetA affords tetracycline resistance; over-expression causes death. Lack of sufficient 3OC6 causes LuxR to induce backwards transcription, causes cell death.

Another potentially useful component for creating a low threshold, perhaps for making a different window size, is the Cre-Lox system. Cre-Lox recombination is a method of site-specific recombination in which the enzyme Cre recombinase splices out a sequence of DNA flanked by *loxP* sites [24]. Cre expression could be connected to the existing device. By placing a series of stop codons or transcriptional terminators flanked by *loxP* sites directly upstream of a gene needed for the cell's survival, a minimum amount of Cre is needed for cells to live. The benefit of using well characterized parts like Cre-Lox in the noise tuner design is that the design requires less prediction and simulation before testing the construct.

Conclusion

The successful design and construction of a noise-tuning circuit could be applied to numerous existing and future synthetic devices. Principal characteristics to consider in the design are to make the tuner modular, tunable by the user, and representative of the noise level of individual cells instead of representing the level of system output or a population-wide response due to quorum sensing. The design and testing of the noise tuner would benefit from some mathematical simulation of the effects of a positive feedback loop. The benefit of a noise tuner would be more predictable and controllable synthetic biology systems. Discovering how we can control the noise level in biological systems will provide a deeper understanding of noise, its compounding factors, and its effects.

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Works to Consider for Further Study

Breaker RR. (2010). RNA second messengers and riboswitches: relics from the RNA world? Microbe 5: 13–20.

This review may elucidate riboswitches as a possible signaling method that can be used in the noise tuner.

Hale L, Lazos O, Haines A, Thomas C. (2010). An efficient stress-free strategy to displace stable bacterial plasmids. Biotechniques 48: 223–28.

This paper describes a strategy for efficiently removing plasmids instead of lethal gene products and other killing systems.

Hooshangi S, Thiberge S, Weiss R. (2005). Ultrasensitivity and noise propagation in a synthetic transcriptional cascade. Proc Natl Acad Sci USA 102: 3581–86.

Authors discuss noise attenuation and amplification, formation of a low-pass filter, steady-state switching and positive feedback loops.

Levens D, Gupta A. (2010). Molecular biology. Reliable noise. Science 327: 1088–89.

Discusses the bimodal effect of noise particularly on transcription factor regulatory circuits.

Shinar G, Feinberg M. (2010). Structural sources of robustness in biochemical reaction networks. Science 327: 1389–91.

This paper discusses networks that induce robustness in biological networks.

Stricker J, Cookson S, Bennett MR, Mather WH, Tsimring LS, Hasty J. (2008). A fast, robust and tunable synthetic gene oscillator. Nature 456: 516–19.

This paper describes a constructed oscillator using linked positive and negative feedback loops. It discusses the robustness and tunability with a positive feedback loop.

To TL, Maheshri N. (2010) Noise can induce bimodality in positive transcriptional feedback loops without bistability. Science 327: 1142–45.

Authors discuss how noise can generate a system that randomly switches between high and low gene expression.

Acknowledgement

I would like to thank Drs. A. Malcolm Campbell and Laurie Heyer for their thoughts, suggestions, and encouragement.