Preliminary Designs for Synthetic Chemotactic Oscillators

MIT Summer Synthetic Biology Competition

This document sketches out some of the potential designs for *synthetic chemotactic oscillators*, which are synthetic biological systems which oscillate between various phases of chemotactic and non-chemotactic behavior. Although single-cell synthetic chemotactic oscillators are possible, we hope to build synchronized population level oscillators so that an entire cell population oscillates between chemotactic and non-chemotactic behavior. Ultimately, this could result in an impressive display where a population of cells collectively move up and down a chemical gradient.

The larger system can be decomposed into three primary modules. The *chemotaxis* module provides a hook into controlling the chemotactic behavior of the cell. The *oscillator* module generates a reliable output signal which oscillates between a high and low state. The *cell-to-cell synchronization* module couples the individual oscillator modules across the entire cell population to create synchronized population-level oscillations. Figure 1 illustrates how these three primary modules are interconnected. The oscillator module is connected to the chemotaxis module through a simple pops enable signal. When the chemotaxis module is enabled the cell should be able to respond naturally to chemical gradients, but when the chemotaxis module is disabled the cell should either remain stationary or simply wander randomly. The oscillator module is connected to the cell-to-cell synchronization module through two pops signals. The oscillator module uses one signal to communicate to the cell-to-cell synchronization module which oscillatory phase it is in. Meanwhile, the cell-to-cell synchronization module communicates to the oscillator module which phase the rest of the population is in. These signals are wired directly into the oscillator to bias the oscillator towards the population's phase.

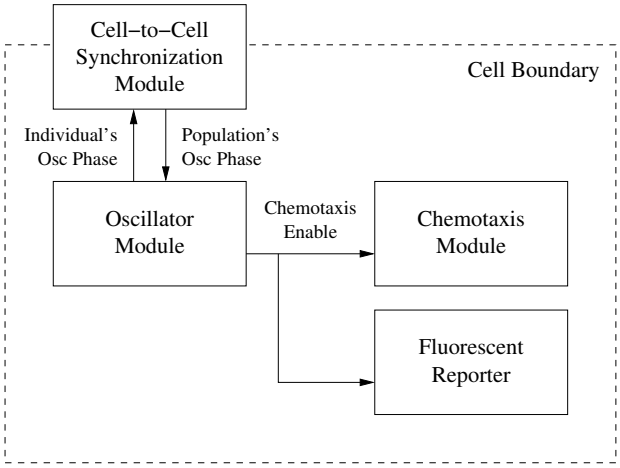

Figure 1: Top-level Schematic of Synthetic Chemotactic Oscillator

We now provide more detail about each of the three primary modules before proposing alternative designs which have a different top-level organization.

1 Chemotaxis Module

The chemotaxis module takes pops in as an input enable signal; when the module is enabled the cell should exhibit natural chemotactic behavior and when the module is disabled, the cell should either be stationary or wander randomly. The current design for the chemotaxis module is basically a protein generator for CheY (see Figure 2). CheY is an integral part of the chemotactic path way [1]. Briefly, *E. coli*'s flagella motor is able to turn both clockwise (CW) and counter clockwise (CCW). By default the motor turns CCW which causes the flagella bundle to wind tightly and push the cell forward. The motor is also able to turn CW which causes the flagella bundle to unwind and results in the cell tumbling randomly. CheY regulates how often the motor switches between CW and CCW rotation; when CheY is phosphorylated it binds to the flagella motor and encourages CW rotation (i.e. tumbling). When CheY is not phosphorylated it does not bind to the flagella motor and the motor turns CCW by default. The phosphorylation of CheY is controlled by transmembrane chemoreceptors: chemoattractants decrease the rate of phosphorylation, while chemorepellents increase the rate of phosphorylation. Or in other words, in the presence of a chemoattractant, the cell is more likely to travel in long straight bursts which encourages the cell to continued towards the attractant. In the presence of a chemorepellent, the cell is more likely to tumble and thus randomly move away from the repellent.

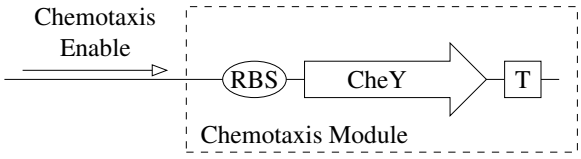

Figure 2: Schematic of Chemotaxis Module

Our chemotaxis module uses CheY expression as a way to transcriptionally turn chemotaxis on and off. Assuming a CheY- cell strain, if pops in is high, then CheY is expressed and the cell exhibits natural chemotactic behavior. If, however, pops in is low, then CheY is not expressed and the cell will not be able to exhibit chemotactic behavior. Instead the cell will mostly like move in random directions with long periods of straight movement.

An alternative design for the chemotaxis module would make use of a mutant form of CheY (termed CheY*) which is constitutively active (i.e. does not need to be phosphorylated to bind to the flagella motor) [2]. This design would probably use a CheY+ strain and the input enable signal would be active low. This means that when pops in is low the chemotaxis module is enabled (CheY* is not expressed and the cell functions normally), and when pops in is high the chemotaxis module is disabled (Chey* is expressed and this synthetically increases the probability of tumbling).

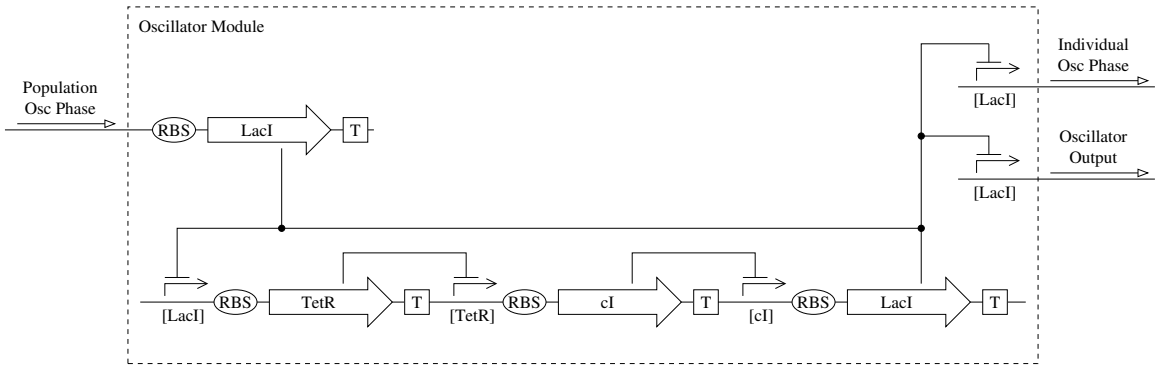

Figure 3: Schematic of Oscillator Module using a Ring Oscillator

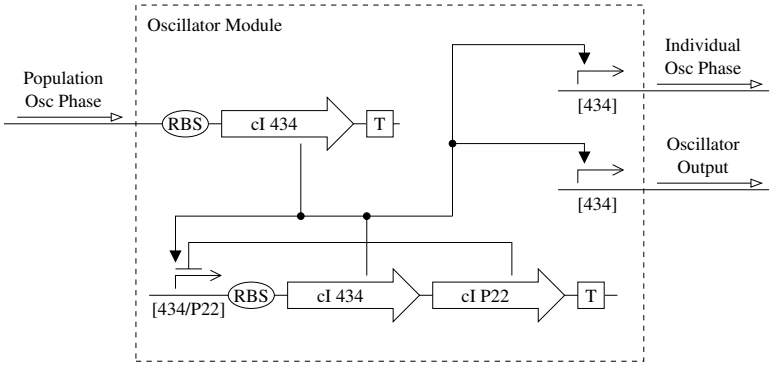

Figure 4: Schematic of Oscillator Module using a Relaxation Oscillator

2 Oscillator Module

The oscillator module is responsible for generating the *oscillator output* pops out signal which oscillates between high pops and low pops in a regular and sustainable way. We have two primary designs for the oscillator module: a ring oscillator and a relaxation oscillator. The *individual osc phase* output signal is similar to the oscillator output signal in that it should be high during one phase of the oscillation and low during the other phase. The *population osc phase* input signal reports what oscillator phase the rest of the population is in. If the oscillator module is designed to be synchronized, then the population osc phase input signal should be used to bias the oscillator towards the corresponding phase.

Figure 3 illustrates a possible design of a ring oscillator based oscillator module. This is the standard design used in Michael Elowitz's repressilator [3]. The oscillator output pops out signal is simply the output of one of the inverters in the ring and the choice shown in the figure is rather arbitrary. The individual osc phase output and population osc phase input are similar to the design proposed by Garcia-Ojalvo et al. [4].

Figure 4 illustrates a possible design for a relaxation based ring oscillator similar to the systems proposed by Hasty et al. [5] and McMillen et al. [6]. This design is basically identical to the one proposed by the California Institute of Technology summer synthetic biology team [7]. The Hasty and McMillen proposals differ from the Cal Tech proposal, since they

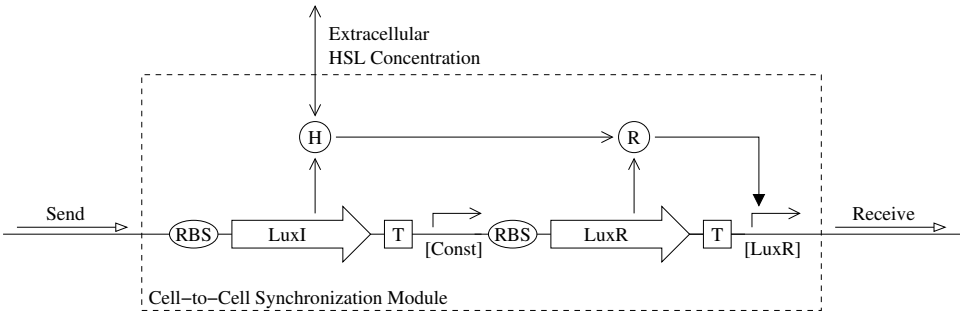

Figure 5: Schematic of Cell-to-Cell Synchronization Module

use an enzymatic decay mechanism to actively degrade the positive feedback and provide the necessary negative feedback loop, while the Cal Tech proposal uses transcriptional repression for negative feedback. Although these are all feasible designs, we are currently focusing more of our effort on the integrated synchronized relaxation oscillator discussed in Section 4.1.

3 Cell-to-Cell Synchronization Module

The cell-to-cell synchronization module is used to synchronize each cell's individual oscillator module with the other oscillators in the population. The hope is to create population level synchronized oscillations. The module assumes that a small molecule is used to communicate between cells. The module has a *send* pops in signal and a *receive* pops out signal. When the send signal is high then the cell-to-cell synchronization module should start sending the inter-cellular small molecule and only stop when the send signal goes low. The receive signal notifies other modules of what the neighboring cells are sending. If the receive signal is high then the neighboring cells are sending inter-cellular small molecules.

The design of this system uses the well studied *Vibrio fischeri* quorum sensing subsystem and it is similar to the designs proposed by Garcia-Ojalvo et al. [4] (which synchronizes ring oscillators) and by McMillen et al [6] (which synchronizes relaxation oscillators). Figure 5 illustrates our planned design for the cell-to-cell synchronization module. Figure 6 is an alternative design which includes an active mechanism (namely the enzyme AiiA) to degrade the small molecule. When the send signal is high the LuxI gene will be expressed producing HSL and enabling the standard cell-to-cell communication mechanism. The X gene will also be expressed which in turn represses the AiiA gene. When the send signal goes low, the expression of LuxI will stop, but the expression of AiiA will start and thus help degrade the HSL left in the cell. This design should have faster response times at the cost of greater design complexity. The choice of which QPI to use is rather arbitrary and depends on what other transcriptional factors are being used in the system as a whole.

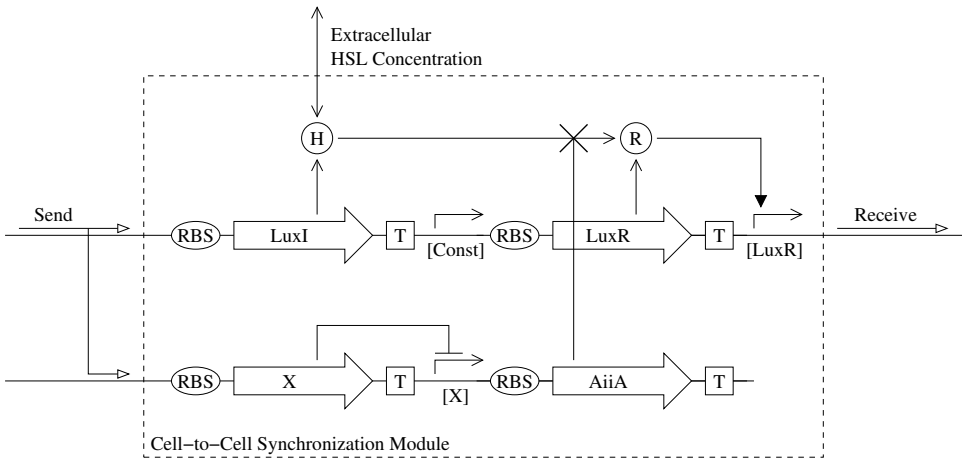

Figure 6: Schematic of Cell-to-Cell Synchronization Module with AiiA

4 Alternative Top-Level Designs

In this section we briefly present three alternative designs which have a different top-level schematic than that presented in Figure 1: an integrated synchronized oscillator approach, a quorum-based chemotactic oscillator, and an unsynchronized chemotactic oscillator.

4.1 Integrated Synchronized Oscillator

We are investigating a novel relaxation oscillator which integrates the oscillator module and the cell-to-cell synchronization module into a single tightly coupled unit. Figure 7 illustrates the design of the integrated synchronized oscillator.

As in previous oscillator module designs, the oscillator output signal is easily obtained from a second LuxR activated promoter. The positive feedback from the LuxR/HSL acti-

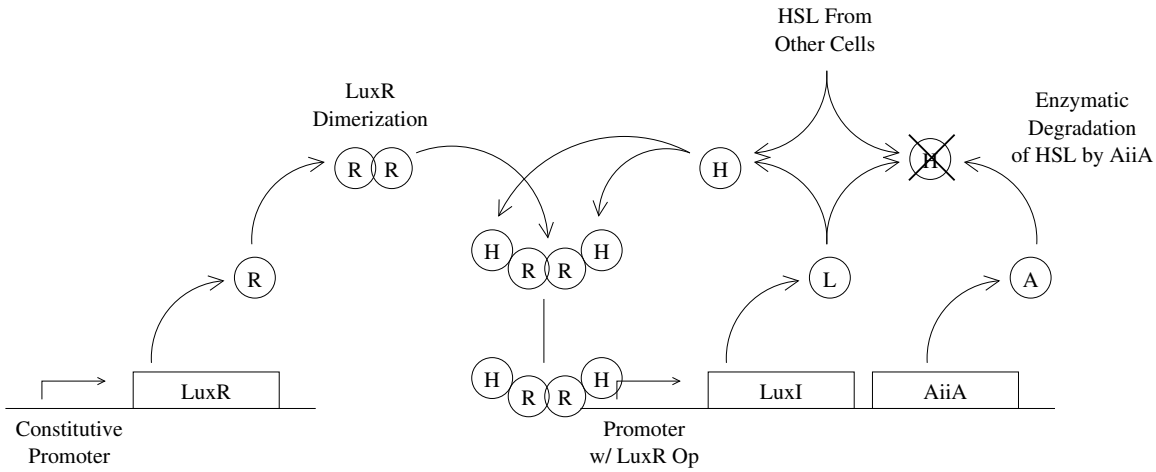

Figure 7: Schematic of Integrated Synchronized Oscillator

vator creates a bistable regime when the degradation of HSL is varied as an input. By self coupling the expression of AiiA (which enzymatically degrades HSL) we create a relaxation oscillator. More details on how this system actually works are in the modeling document.

4.2 Quorum-based Chemotactic Oscillator

All of the designs so far have relied on an internal oscillator module to drive the chemotactic oscillations. A completely different strategy would use the quorum sensing subsystem to create an oscillating system. Figure 8 sketches a top-level design of such a system. The quorum sense module is simply the LuxR promoter upstream of the LuxI gene creating a positive feedback amplifier.

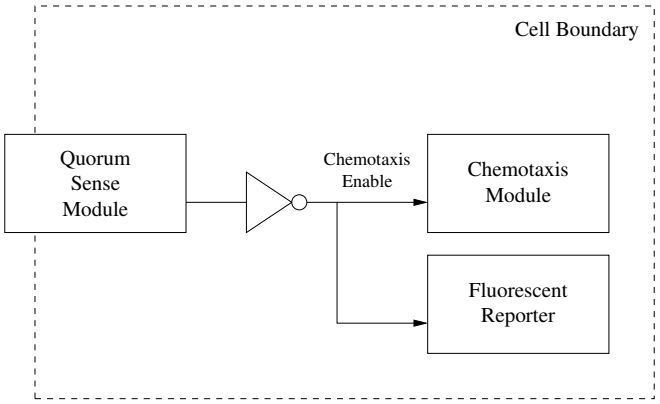

Figure 8: Schematic of Quorum-based Chemotactic Oscillator

This system works as follows. Assume the cell population begins evenly distributed across the environment. Then the quorum sense output will be low and the cells will be exhibiting the natural chemotactic behavior. As the cells start to collect around the chemoattractant the amount of HSL near the cells will increase until a quorum is reached and the output of the quorum sense switches high. At this point the chemotaxis module is disabled and the cells will slowly start to diffuse around the environment. Eventually the levels of HSL will drop and the process will repeat. As in Figure 6 we might integrate AiiA into this system to increase the response time. Note that if the chemotaxis module input is active low then the inverter is not necessary.

4.3 Unsynchronized Chemotactic Oscillator

It is important to remember that synchronized oscillations are not strictly necessary to illustrate the concept of chemotactic oscillations in general. Figure 9 sketches a simple top-level design which would enable individual cells to exhibit chemotactic oscillations.

Observing the oscillations would of course be more difficult, but still possible using the dual fluorescent reporters and assuming that the transcriptional timescale is much slower than the actual chemotactic response. This is a reasonable assumption in liquid chemotaxis buffer, but probably less valid when chemotaxis buffer is mixed with agarose (as in the

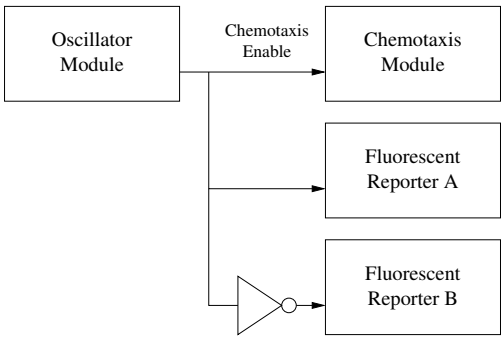

Figure 9: Schematic of Unsynchronized Chemotactic Oscillator

aspartate plug experiments). To observe chemotactic oscillations we take pictures of the cell population at relatively fine grain time scales ($\sim$ 5 min) over a long period. We should see those cells which express fluorescent protein A near the chemoattractant, while those which express fluorescent protein B distributed evenly. When the oscillator module changes phases, the cells will rapidly respond - the cells expressing fluorescent protein A will start to evenly distribute, while the cells expressing fluorescent protein B will start to move towards the chemoattractant. They key point is that the cells will change their chemotactic behavior much faster than the degradation rate of the fluorescent reporters. We should be able to observe these transient effects to verify chemotactic oscillations. Owing to the difficulty of setting up these time courses this alternative is probably best left as a last resort.

References

[1] H. Berg. The rotary motor of bacterial flagella. *Annual Review of Biochemistry*, 72:19–54, 2003.

[2] S. Da Re, T. Tolstykh, P. M. Wolanin, and J. B. Stock. Genetic analysis of response regulator activation in bacterial chemotaxis suggests an intermolecular mechanism. *Protein Science*, 11:2644–2654, 2002.

[3] M. Elowitz and S. Leibler. A synthetic oscillatory network of transcriptional regulators. *Nature*, 403:335–338, 2000.

[4] J. Garcia-Ojalvo, M. Elowitz, and S. Strogatz. Modeling a synthetic multicellular clock: repressilators coupled by quorum sensing. *PNAS*, 101(30):10955–10960, 2004.

[5] J. Hasty, F. Isaacs, M. Dolnik, D. McMillen, and J. Collins. Designer gene networks: Towards fundamental cellular control. *CHAOS*, 11(1):207–220, 2001.

[6] D. McMillen, N. Kopell, J. Hasty, and J. Collins. Synchronizing genetic relaxation oscillators by intercell signaling. *PNAS*, 99(2):679–684, 2002.

[7] Modified barkai-leibler relaxation oscillator. California Institute of Technology synthetic biology team report: http://www.cds.caltech.edu/$\sim$rwald/sbc04/, 2004.