Synchronized Chemotactic Oscillators

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Summer Synthetic Biology Competition
Massachusetts Institute of Technology
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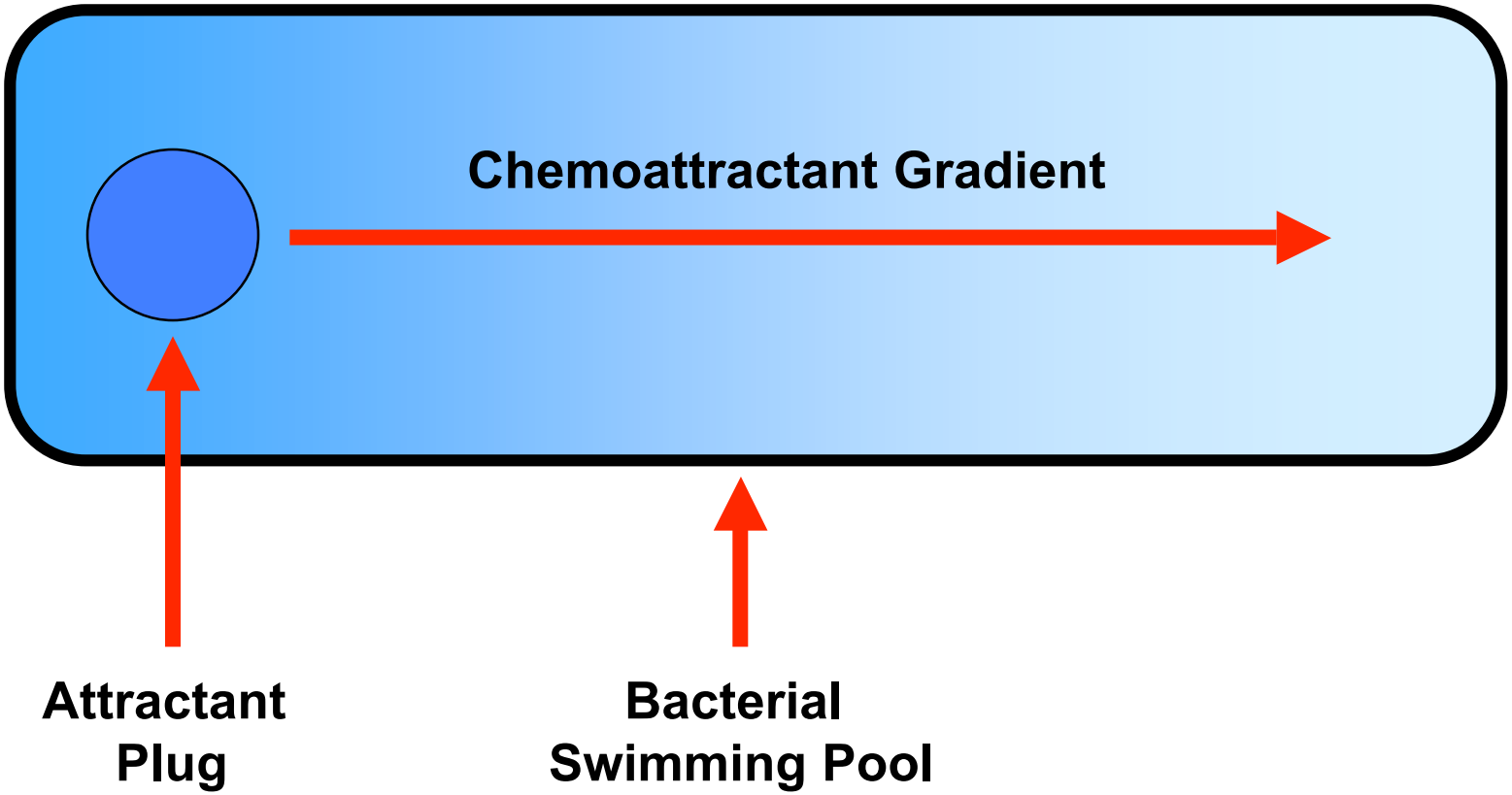
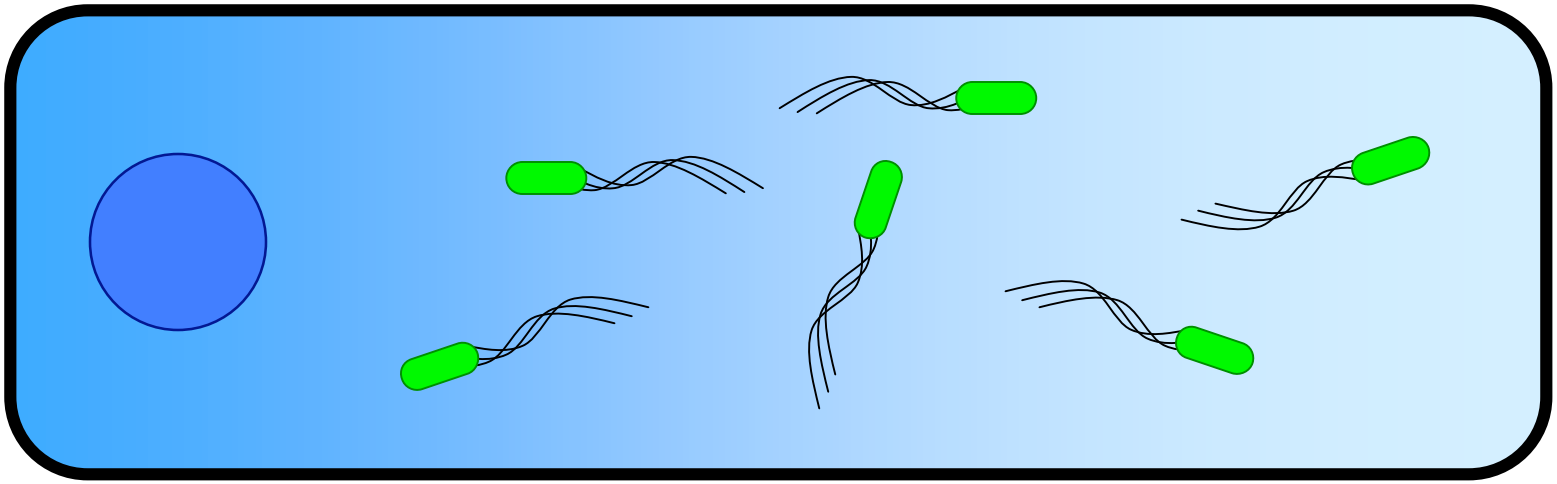
Motivation

- Our goal: an interesting, complex system – something cool. But how to make it happen?
- We focused on implementing **modularity**
  - Breaking biological systems into modular pieces
  - At a low level, this is BioBricks
- Building a modular system allowed efficient division of labor – key for a team this large

A Chemotactic Oscillator

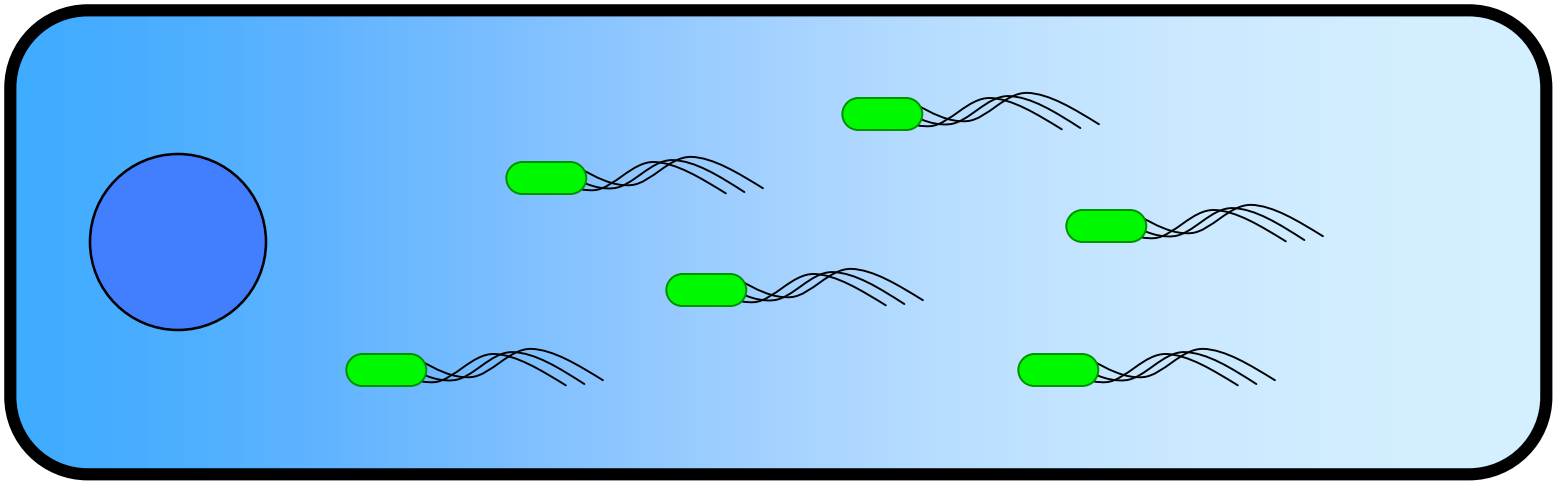


A Chemotactic Oscillator



Bacteria are added to the swimming pool

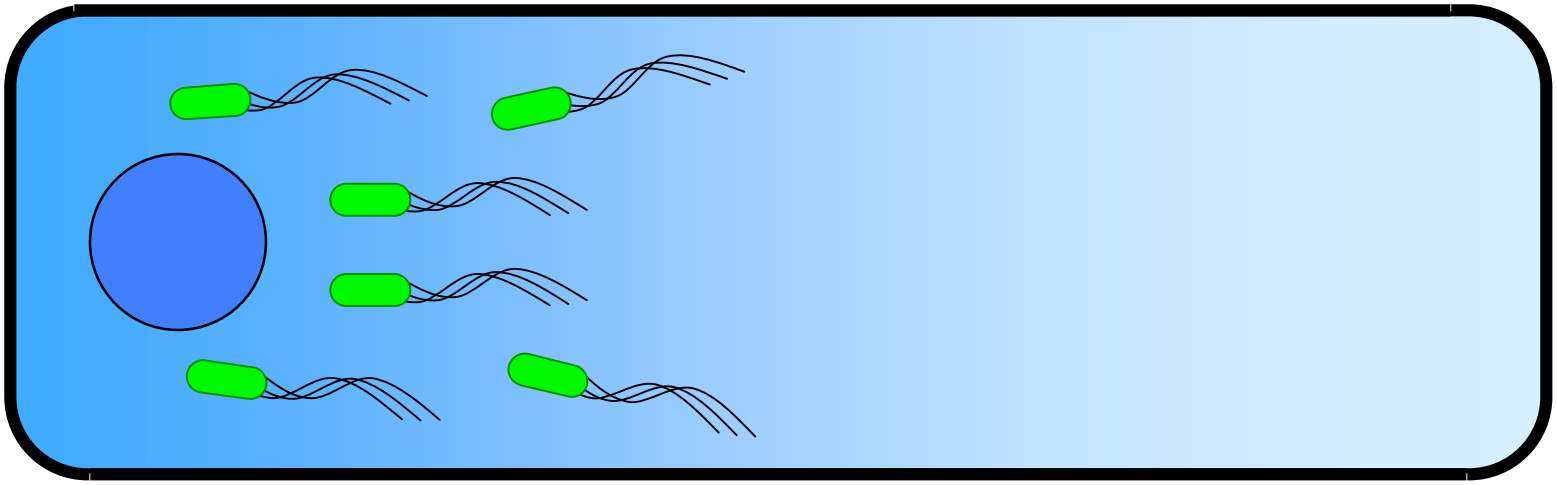
A Chemotactic Oscillator



**Chemotaxis is enabled,
indicated by green bacteria**

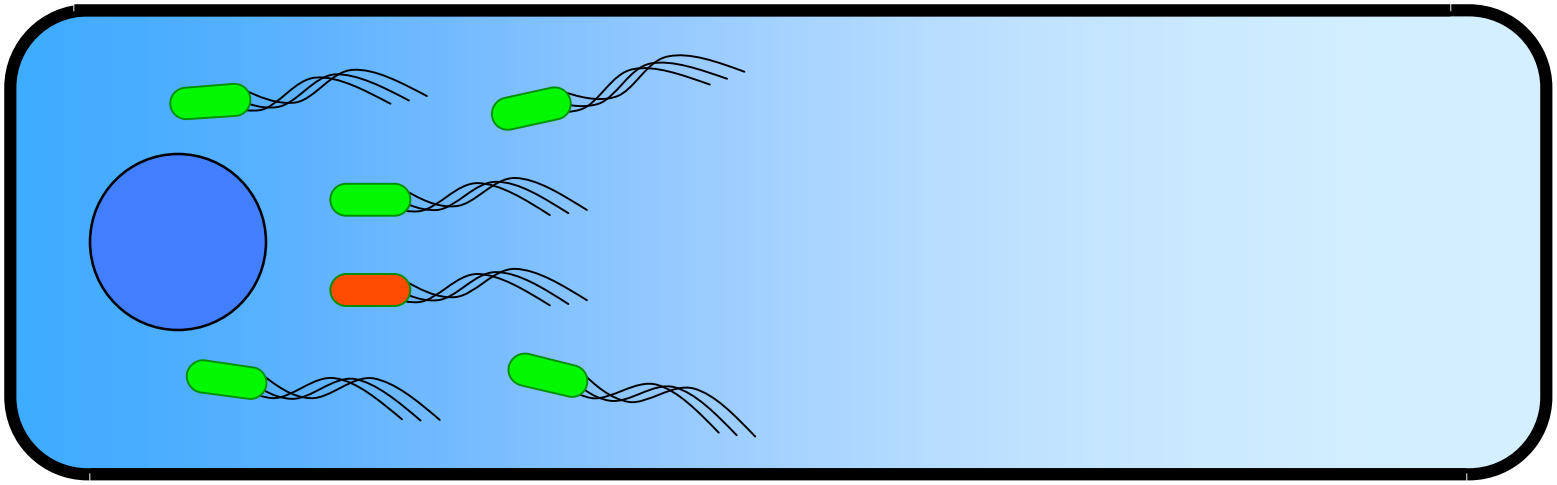
**Bacteria start swimming up the
gradient towards the attractant plug**

A Chemotactic Oscillator



The bacteria congregate around the attractant plug

A Chemotactic Oscillator

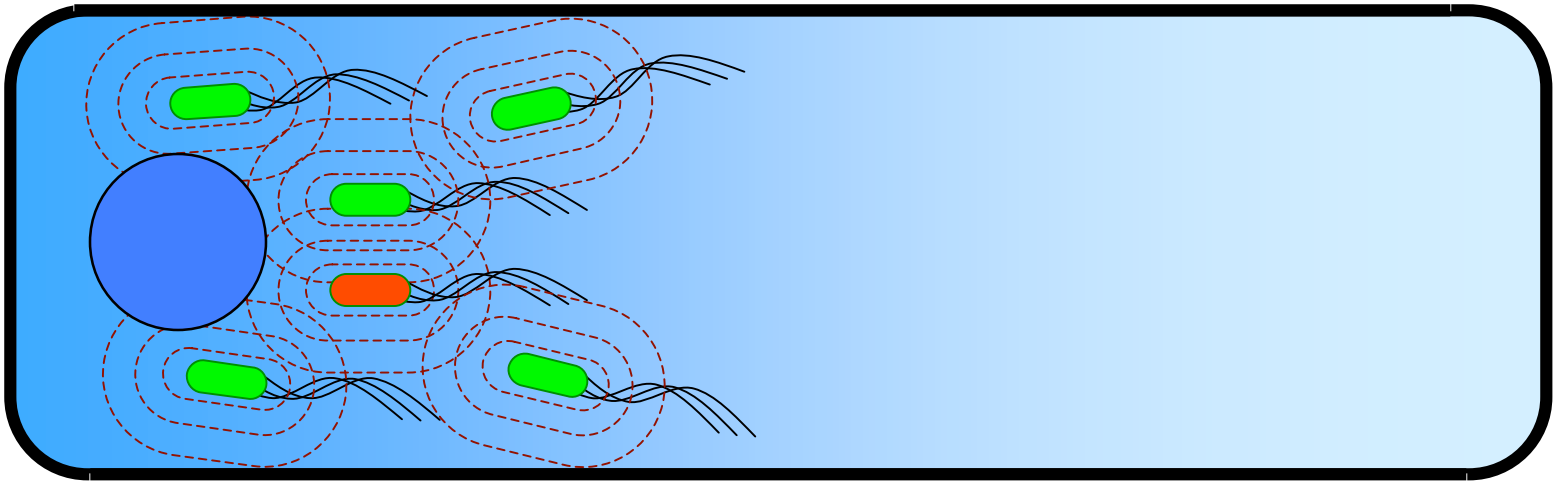


Each bacteria has an internal oscillator, driving switch between:

chemotaxis enabled

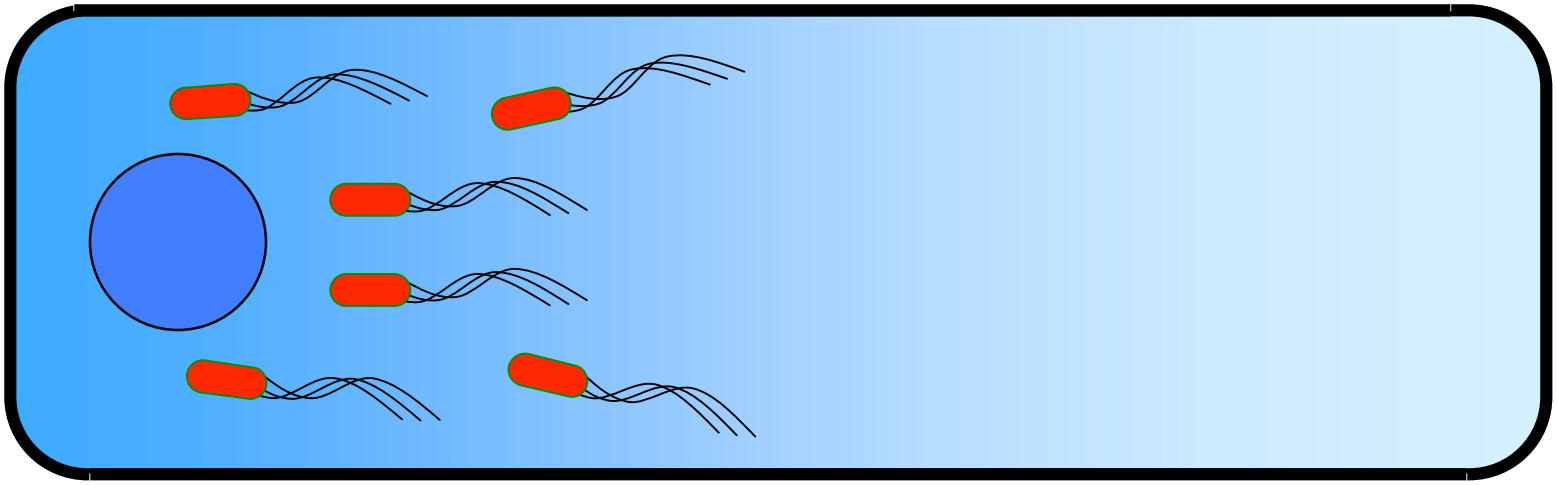
chemotaxis disabled

A Chemotactic Oscillator



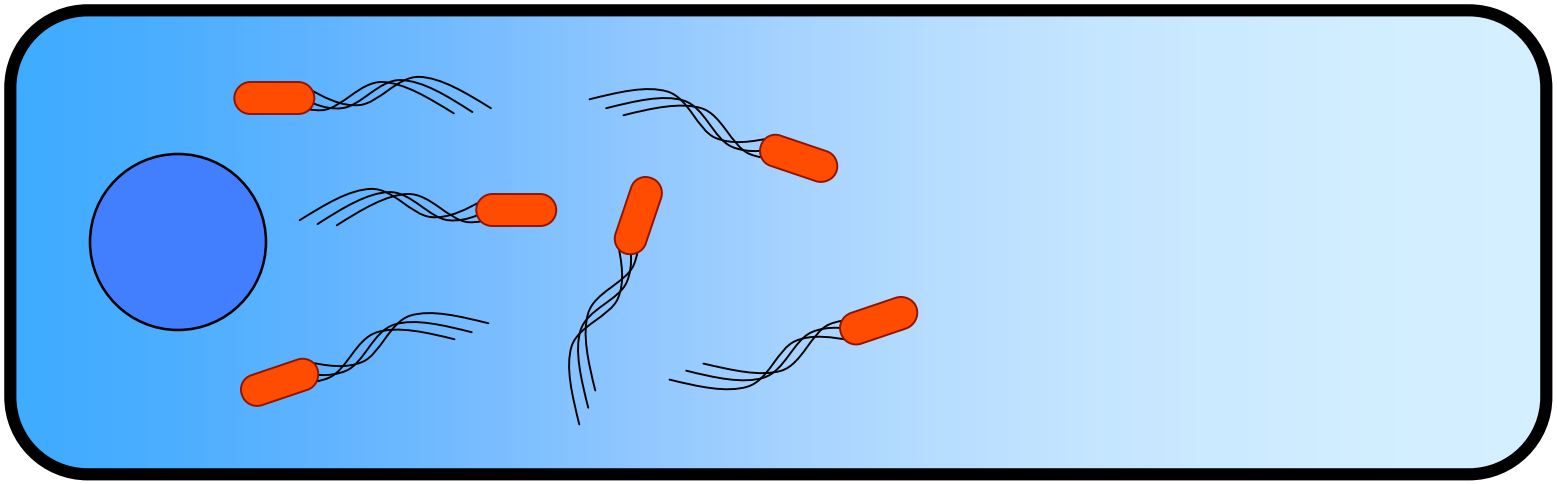
The bacteria communicate their internal oscillator phase with each other using cell-to-cell signaling …

A Chemotactic Oscillator



… enabling the entire population to change state synchronously

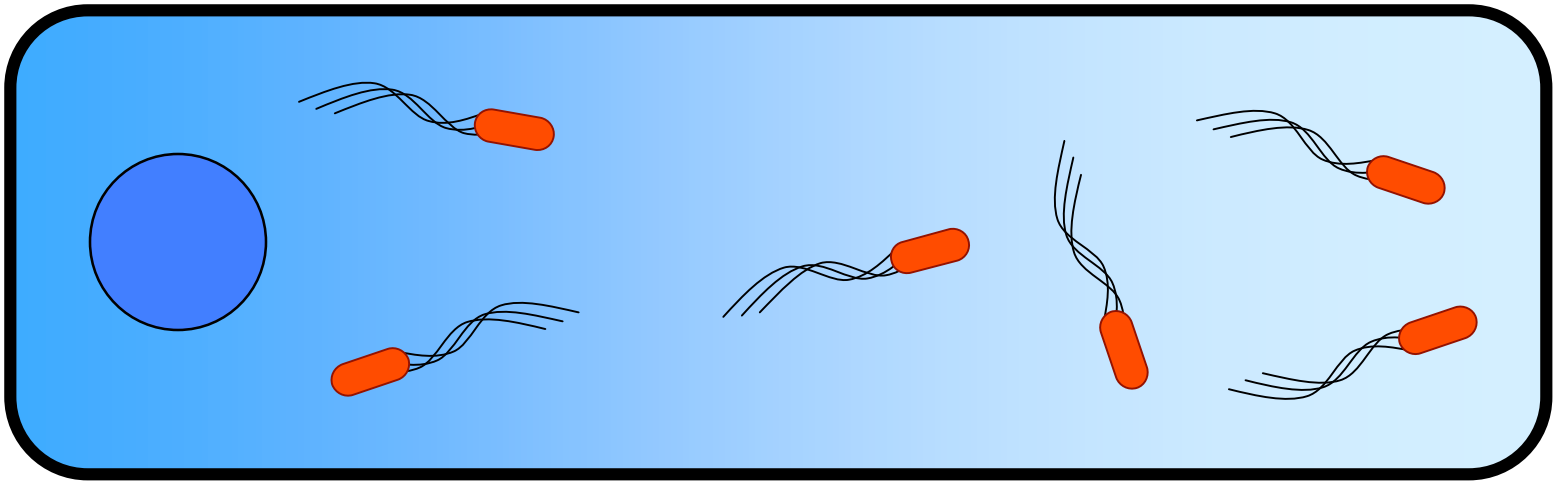
A Chemotactic Oscillator



In red bacteria, chemotaxis is disabled

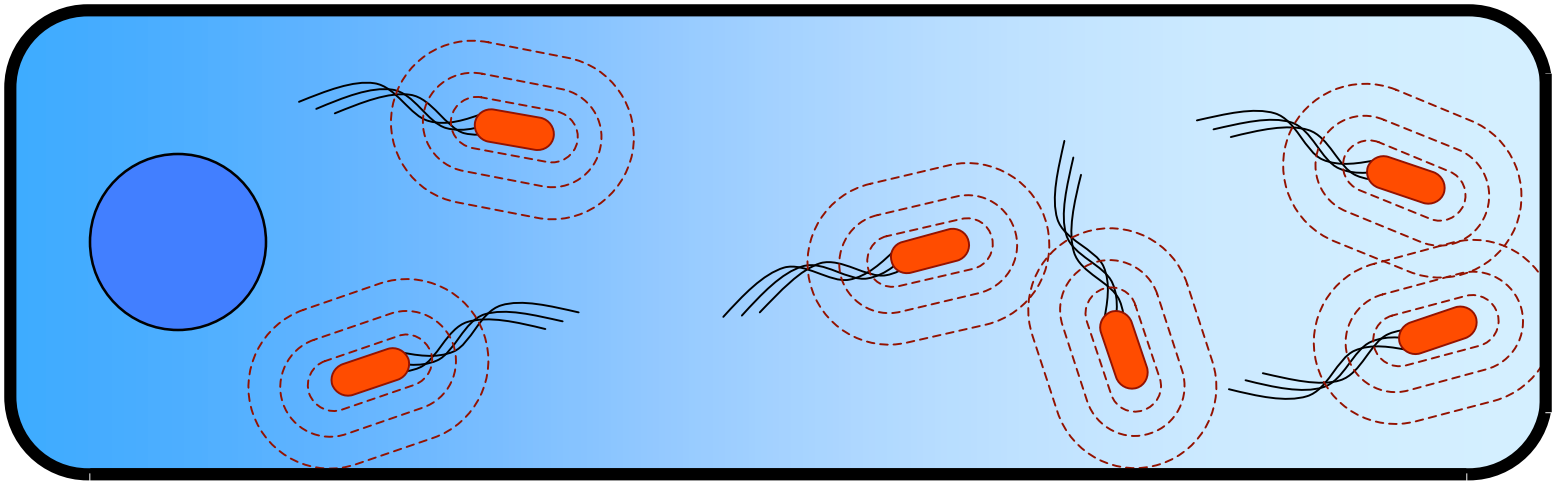
Bacteria start to randomly move away from the attractant plug

A Chemotactic Oscillator



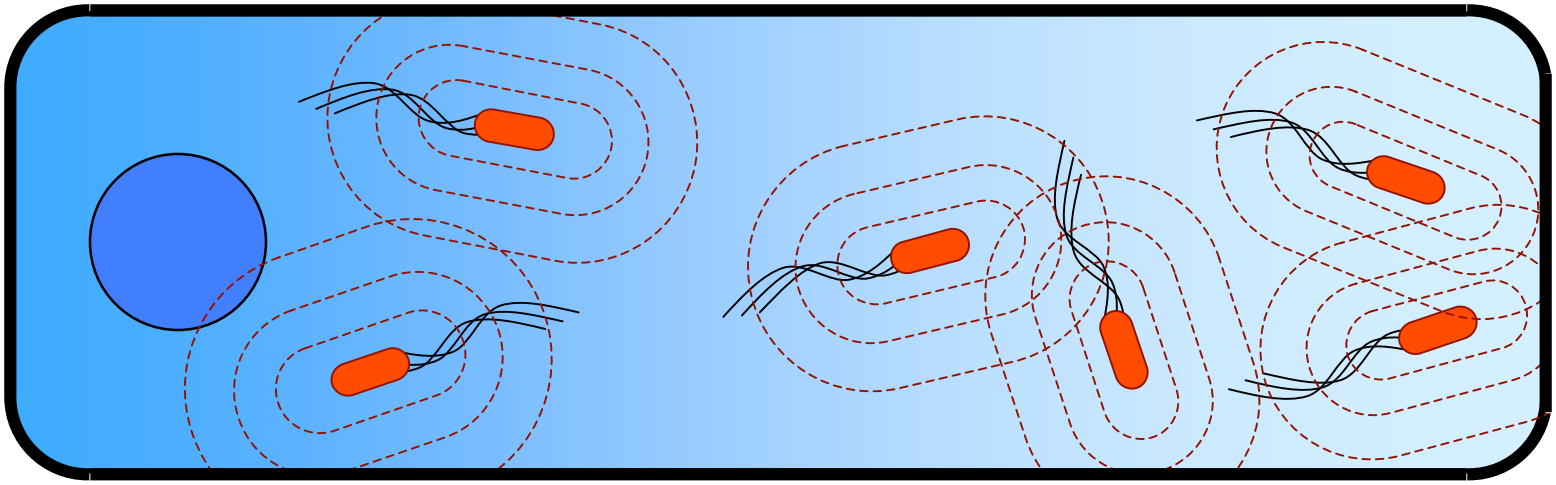
Eventually the bacteria are dispersed around the swimming pool

A Chemotactic Oscillator



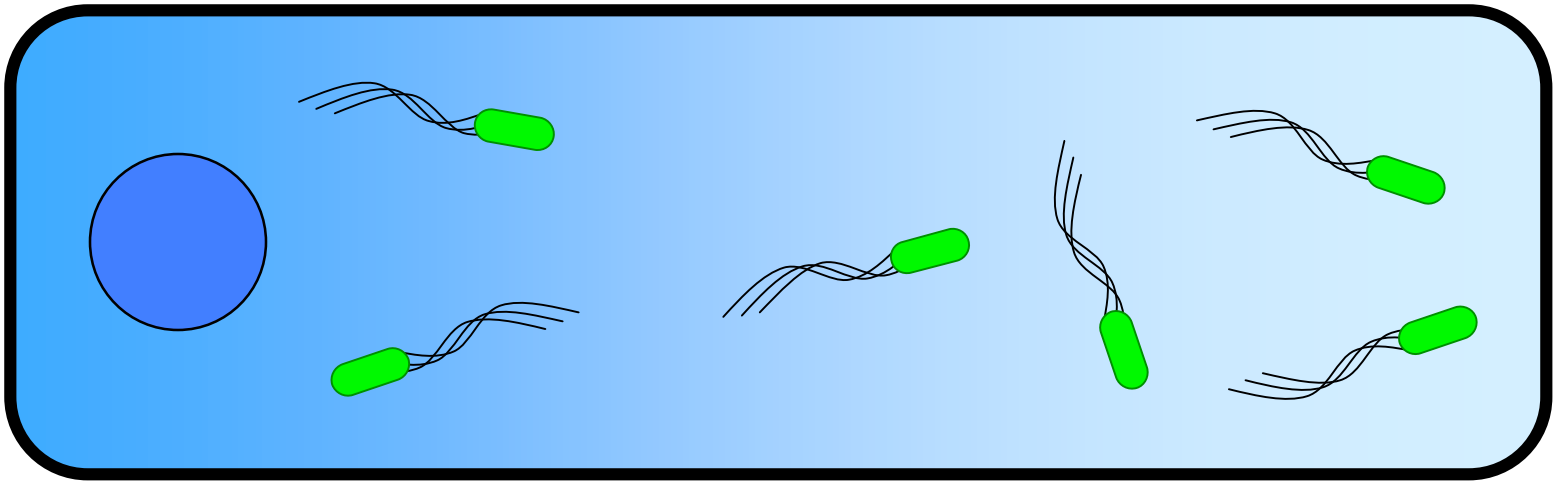
Again the bacteria communicate with each other using cell-to-cell signaling

A Chemotactic Oscillator



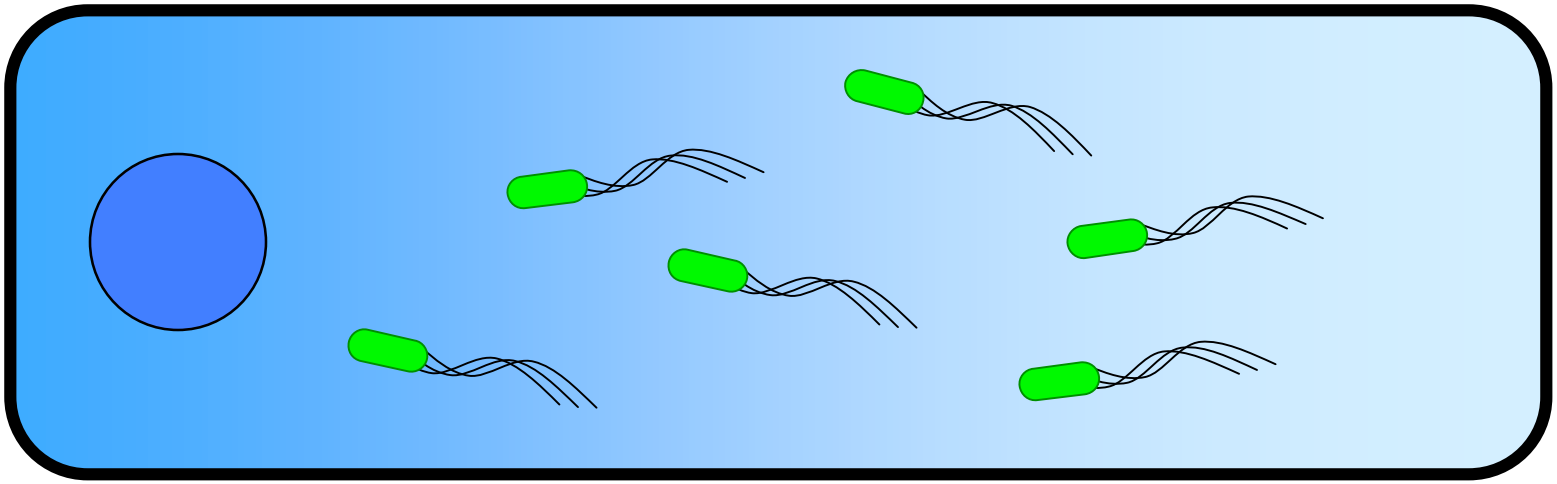
The inter-cellular signaling molecule diffuses throughout out the swimming pool to the entire population

A Chemotactic Oscillator



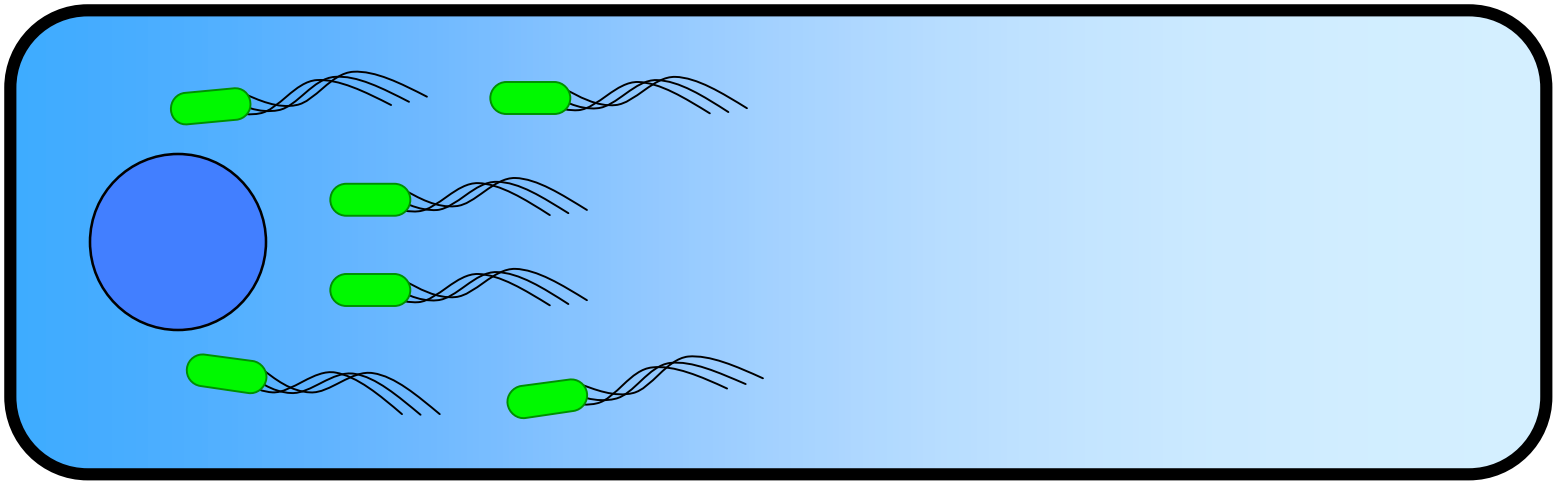
State changes: chemotaxis is enabled

A Chemotactic Oscillator



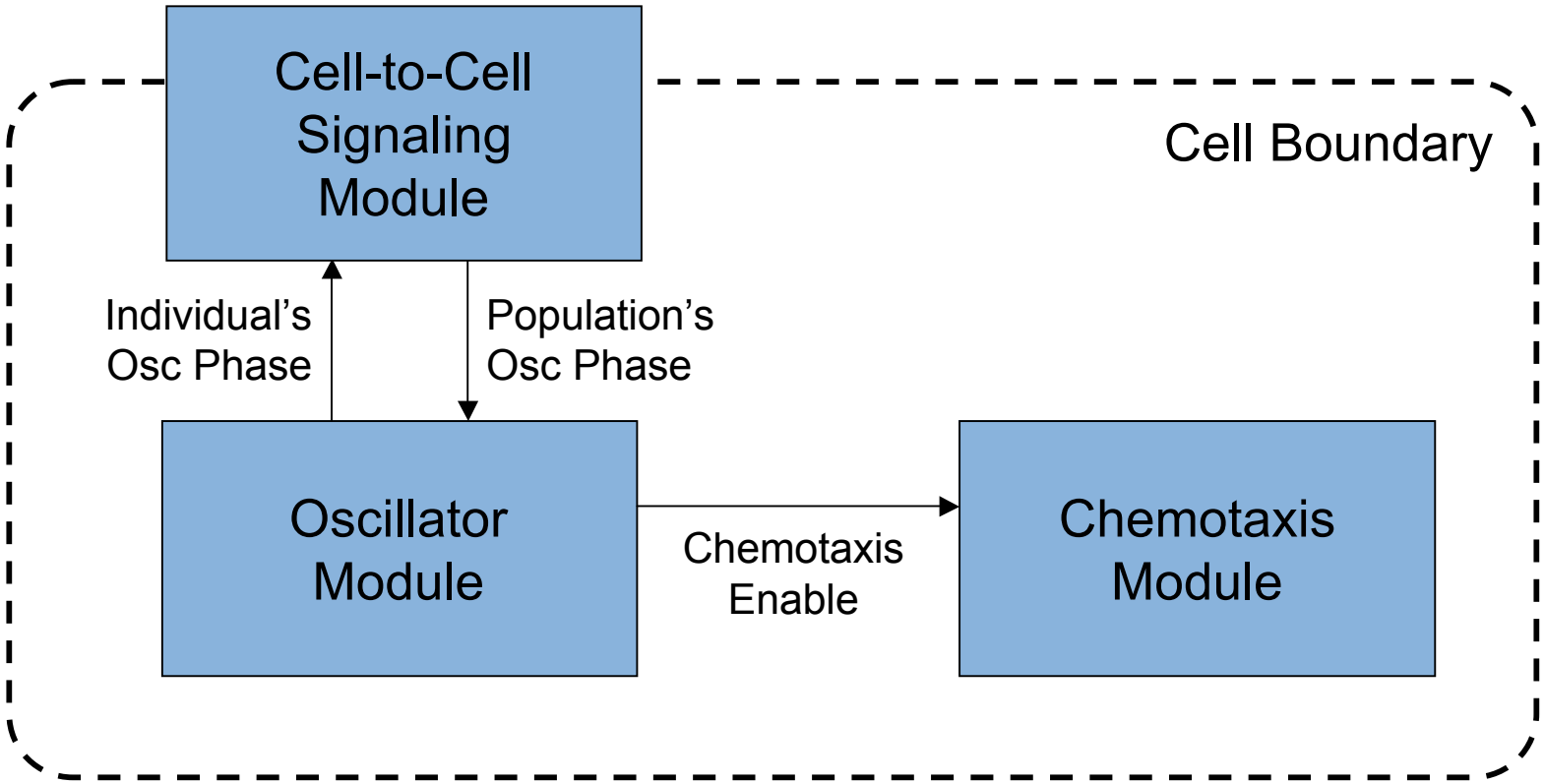
... and onward they swim, doomed to a fate worse than that of Sisyphus

A Chemotactic Oscillator

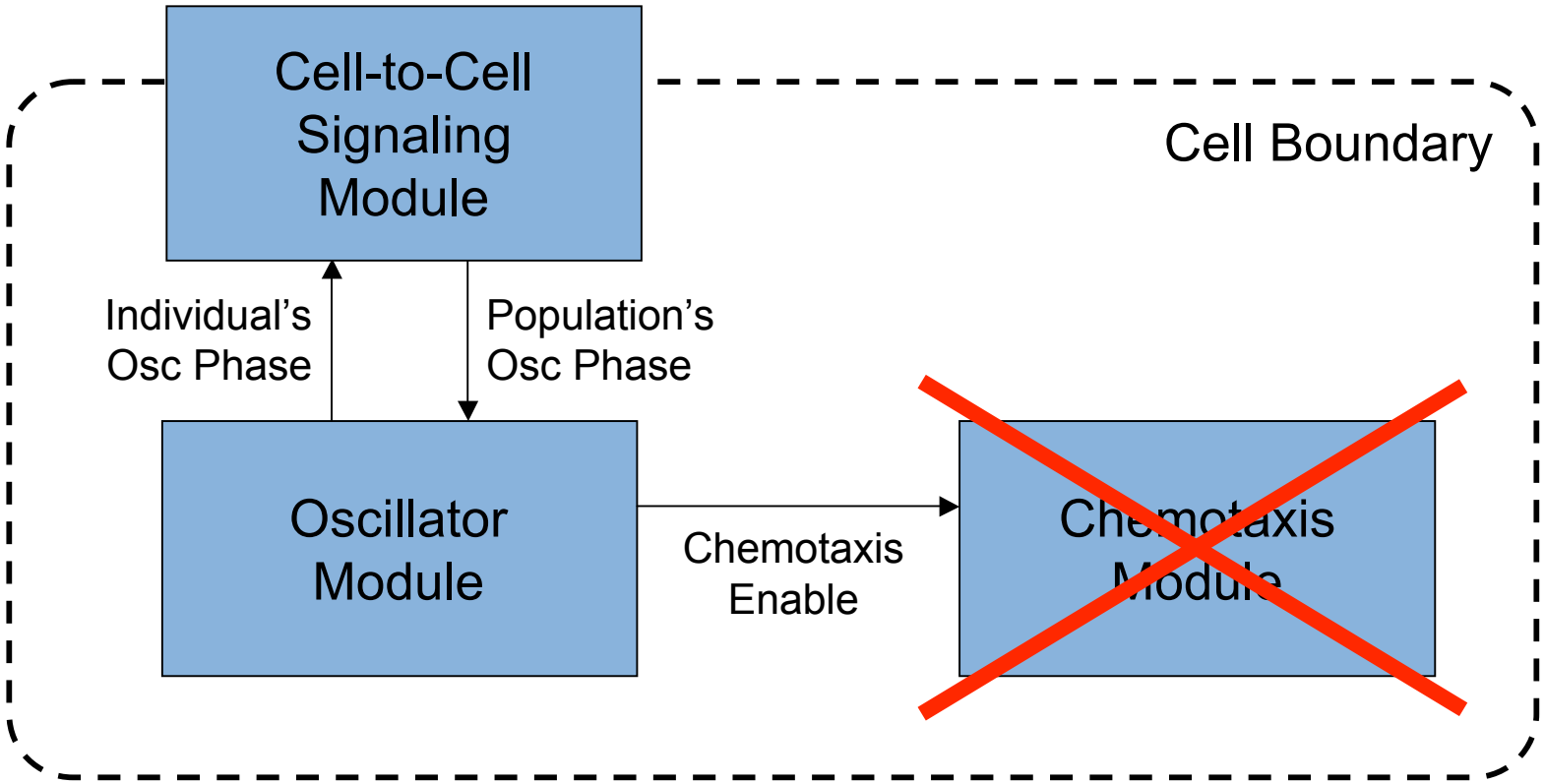


“Futile, this wretched swimming!”

Top-Level System Diagram

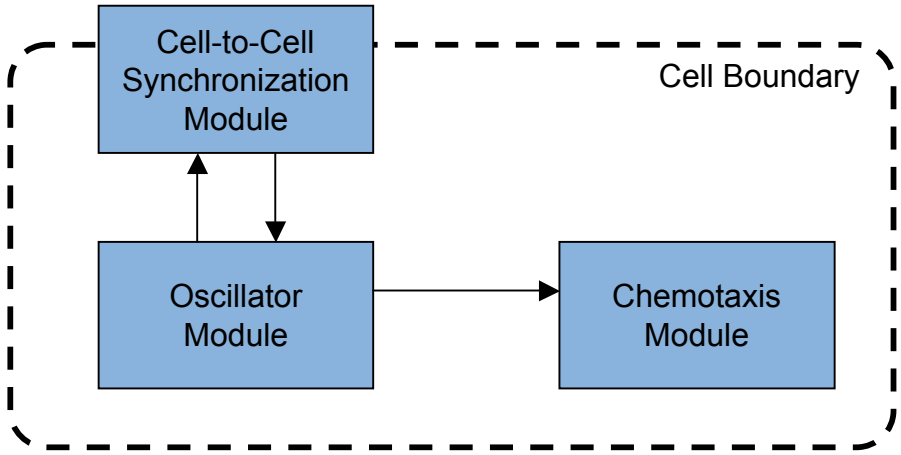


Top-Level System Diagram

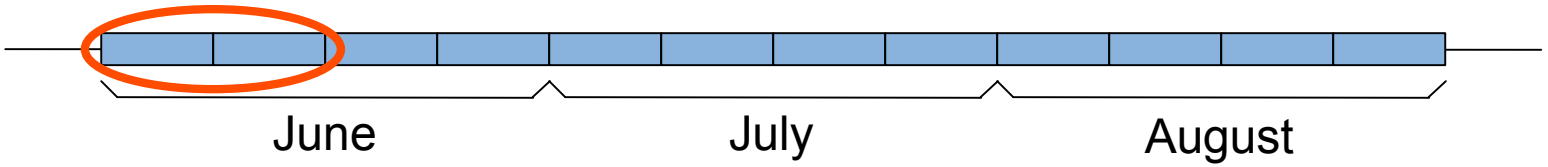


Outline

- Overview
  - Motivation
  - System Description
  - How we got there: Synopsis of Summer Activities
- Module Discussion
  - Cell-to-cell Signaling Module
  - Oscillator Module
  - Chemotaxis Module
  - Module Integration
- Final Remarks



Learning about SynthBio

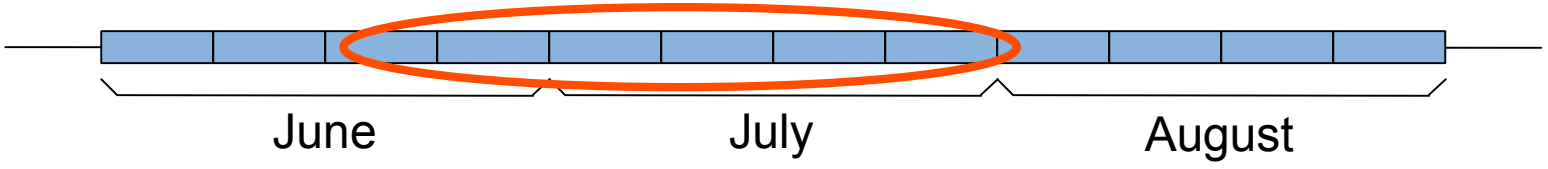


- Preliminary discussion and design work
- Previous Class Experiences at MIT
  - Much design, little implementation

Cool ideas, but we wondered: “Can we do this?”

Had to hit the lab …

Introduction to a Biology Lab



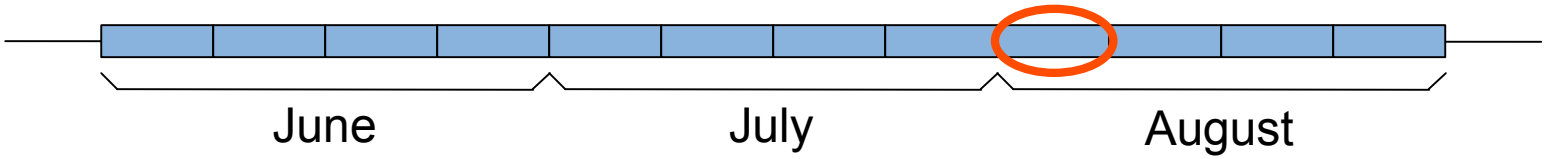
Objective during this period was **parts characterization**

- Achieved useful work on RBS characterization
- Attempted to build sets of linked inverters
- Began work on cell-to-cell signalling

"So...you're saying that was supposed to be refrigerated?"

"Agarose gels, Agar gels...what's the difference?"

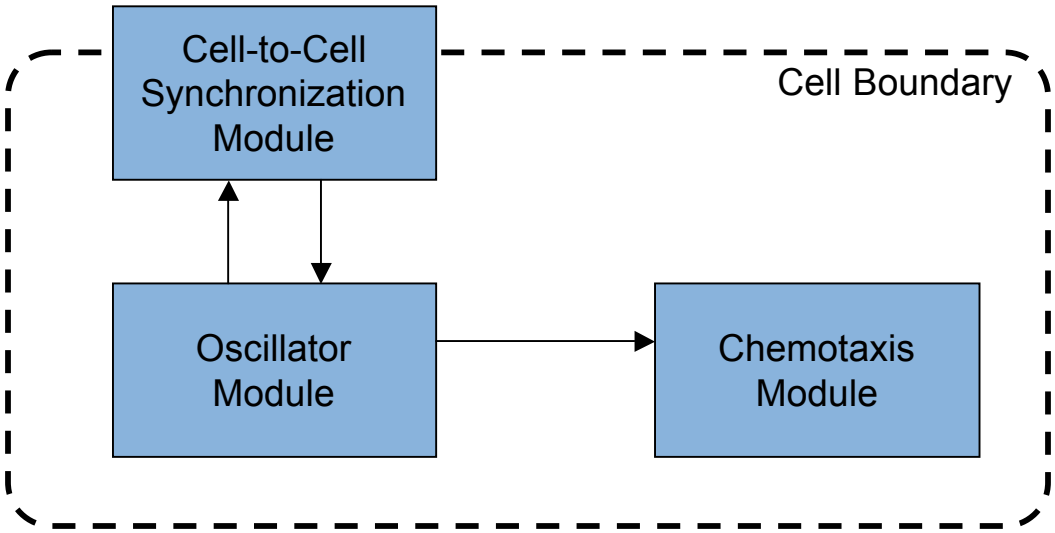
Finalizing Design



Brainstormed several comprehensive and full-system designs

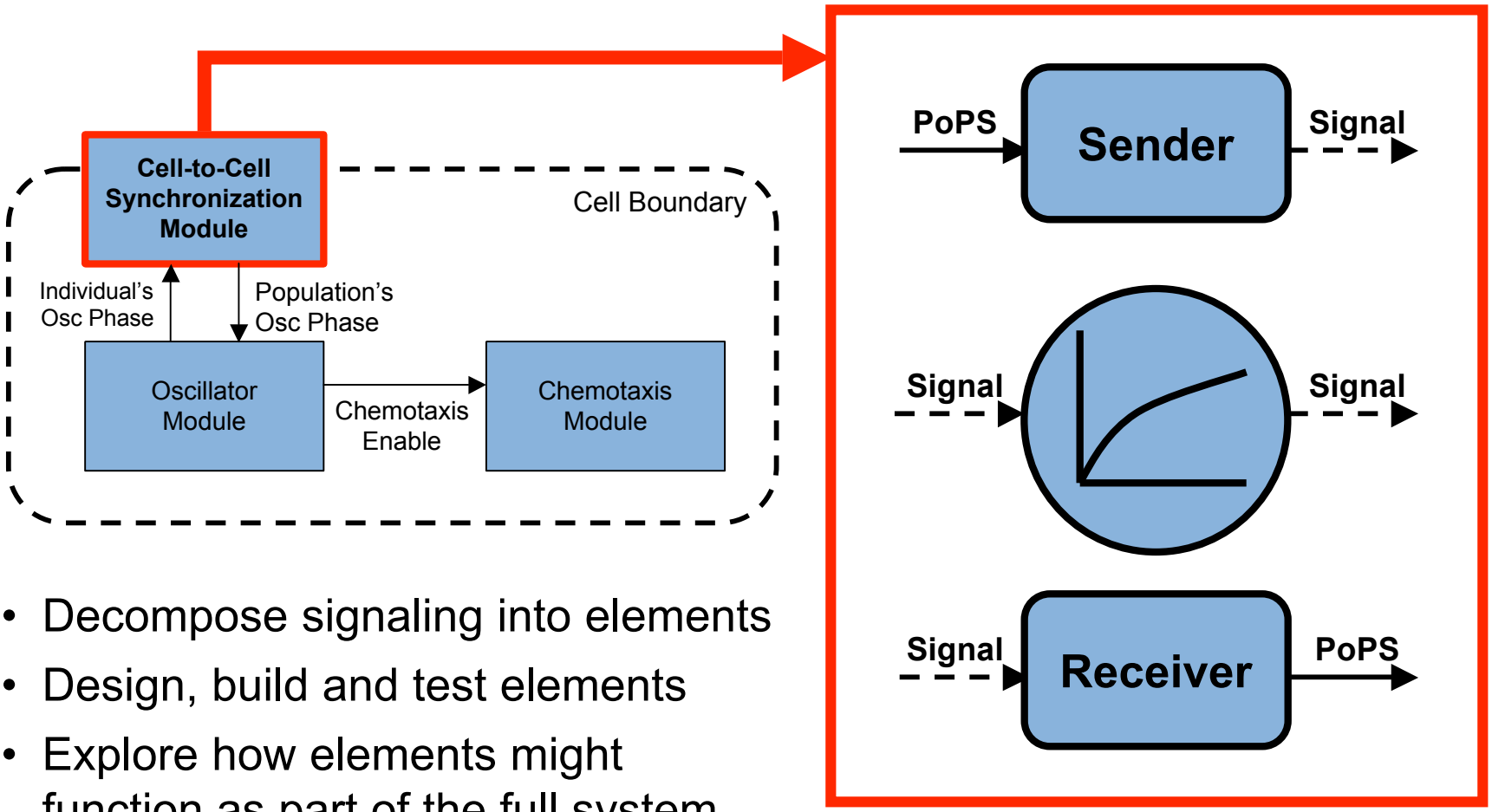
Choosing one design gave us purpose and focus in lab

Thereafter: Making it Happen



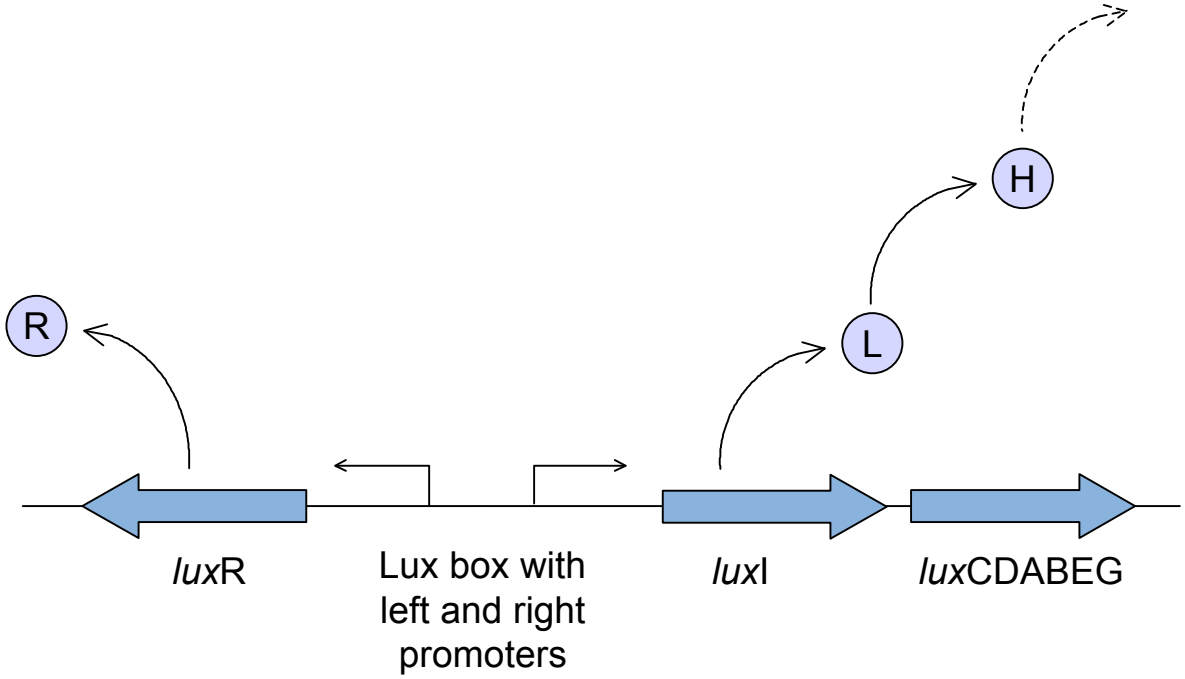
- Final element
  - **Integrating** modules is surely easier said than done
  - How to prepare the **experimental setup** for our work?
- Barry, Jason, Fred, and Vikki will now discuss these topics. Chris will offer final remarks.

Cell-to-Cell Signaling Objectives



- Decompose signaling into elements
- Design, build and test elements
- Explore how elements might function as part of the full system

The Lux System



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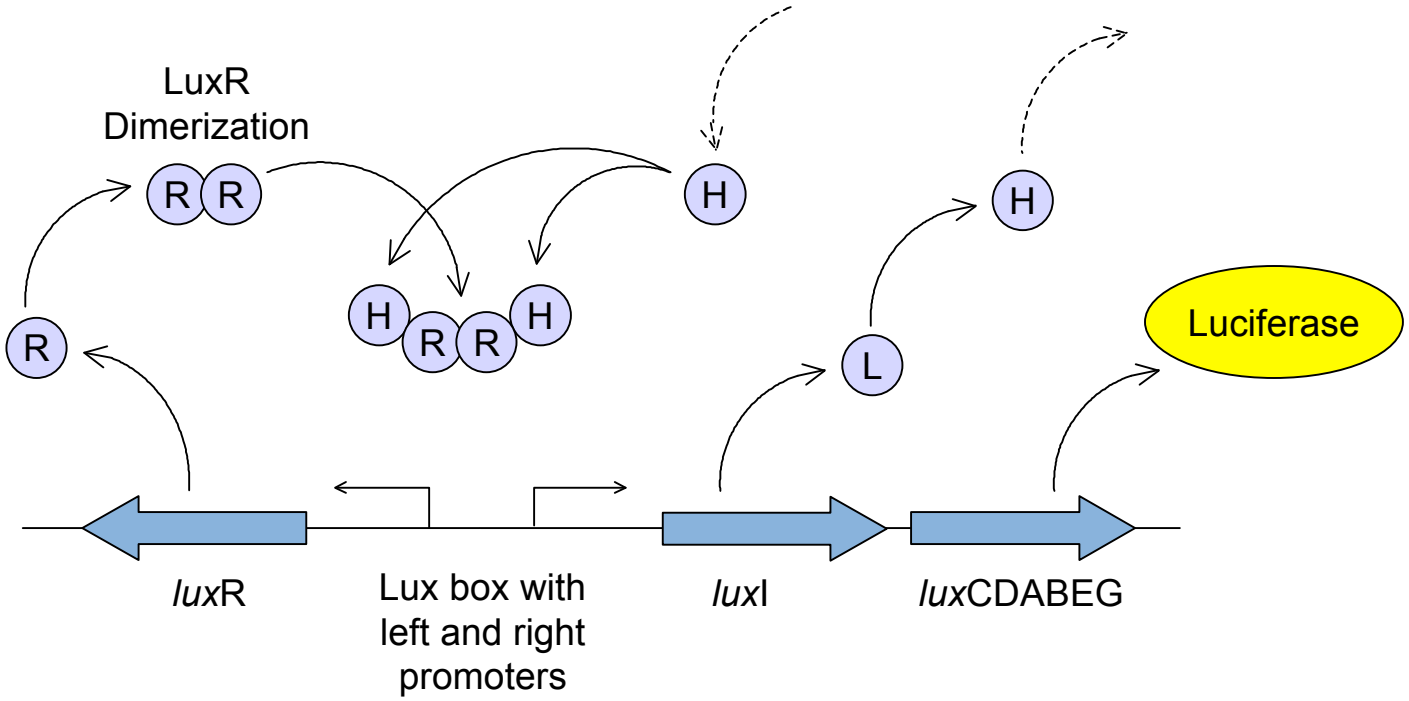
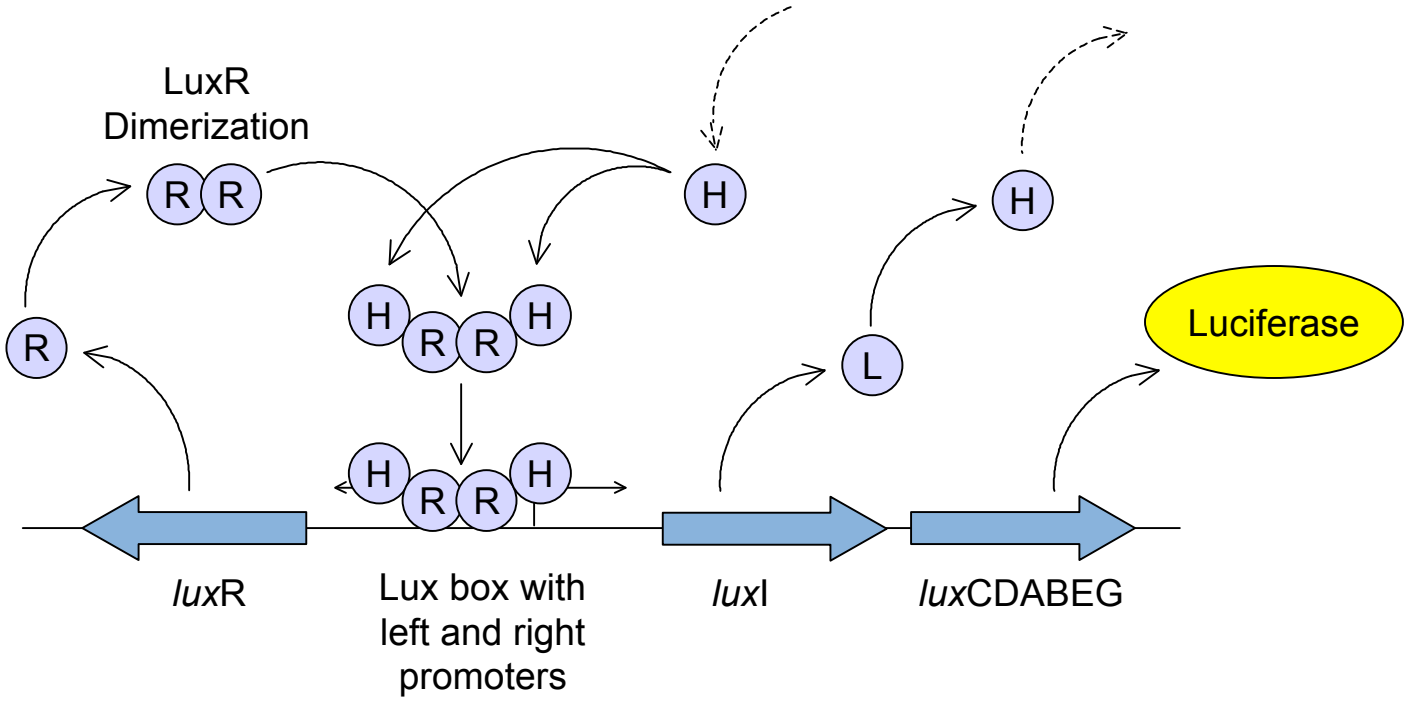
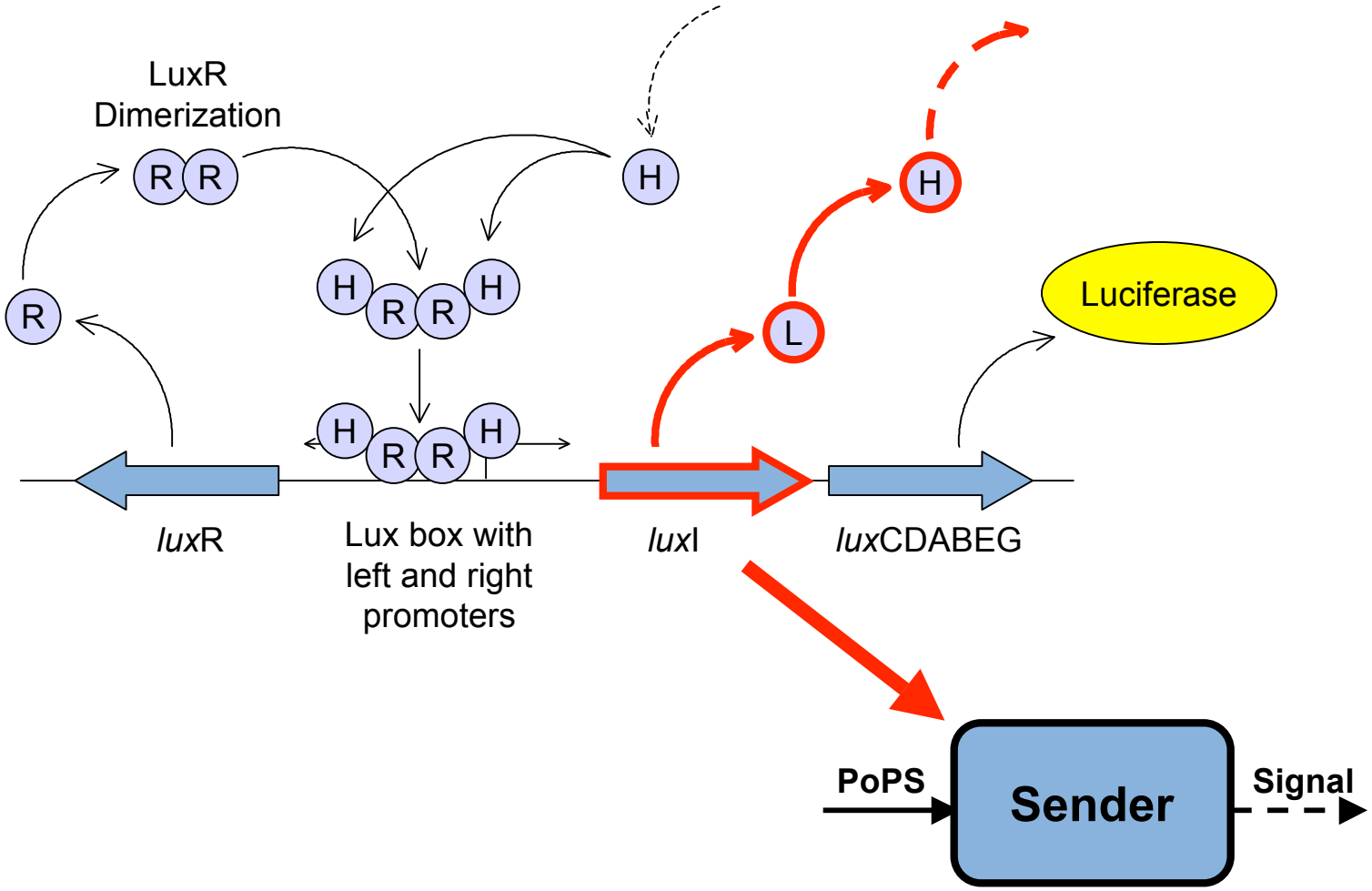


The Lux System



Utilizing Existing Components



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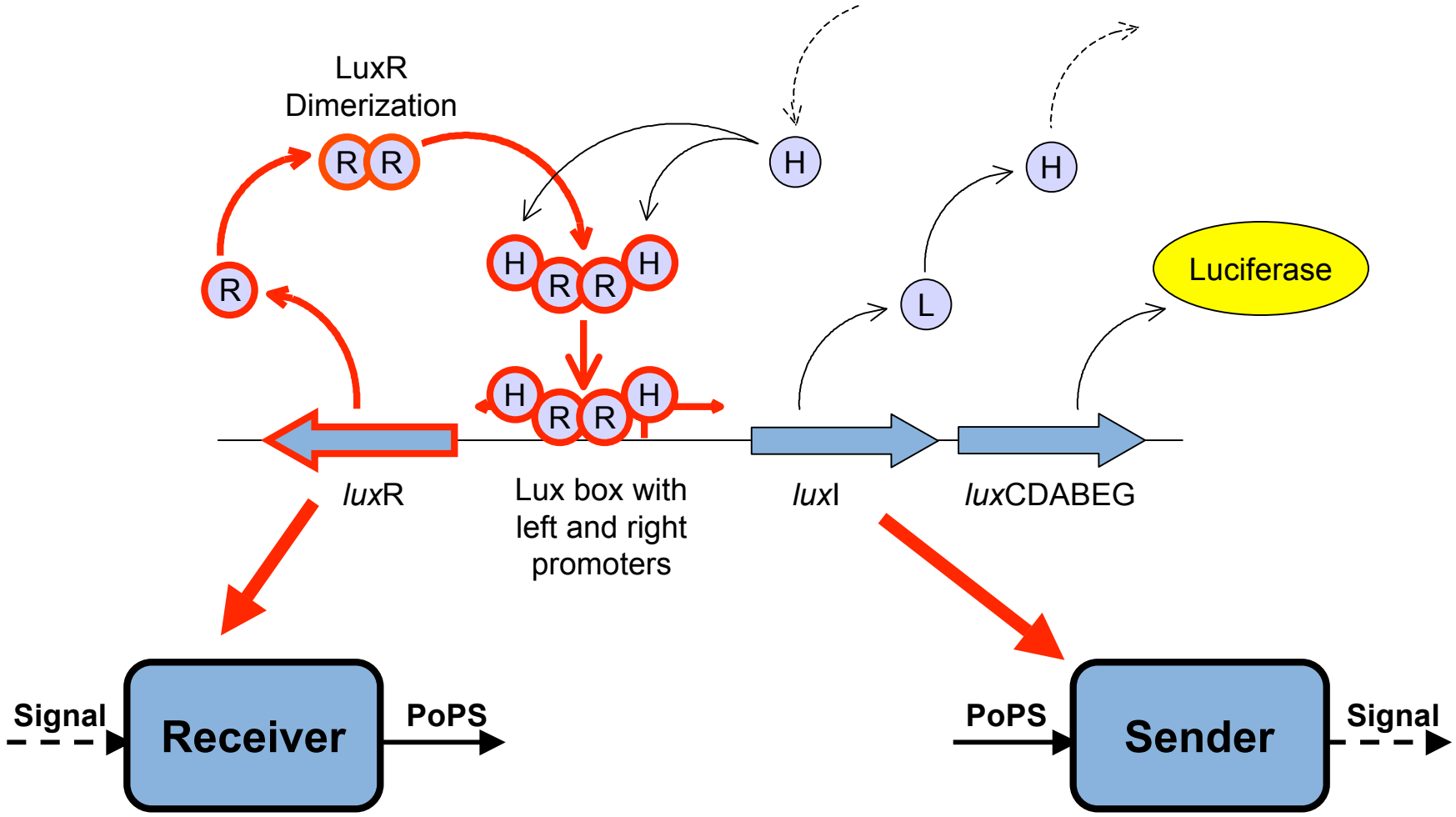
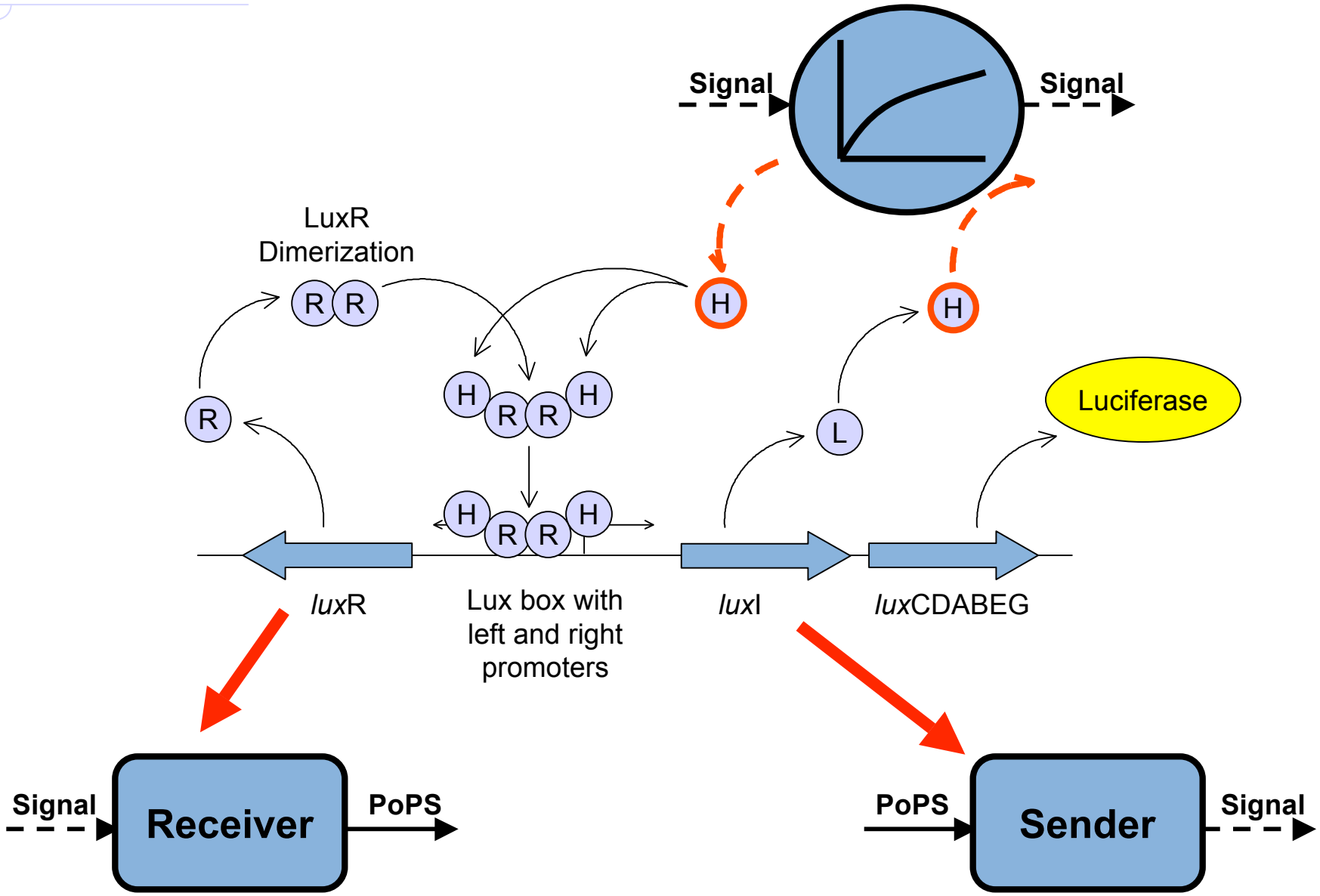
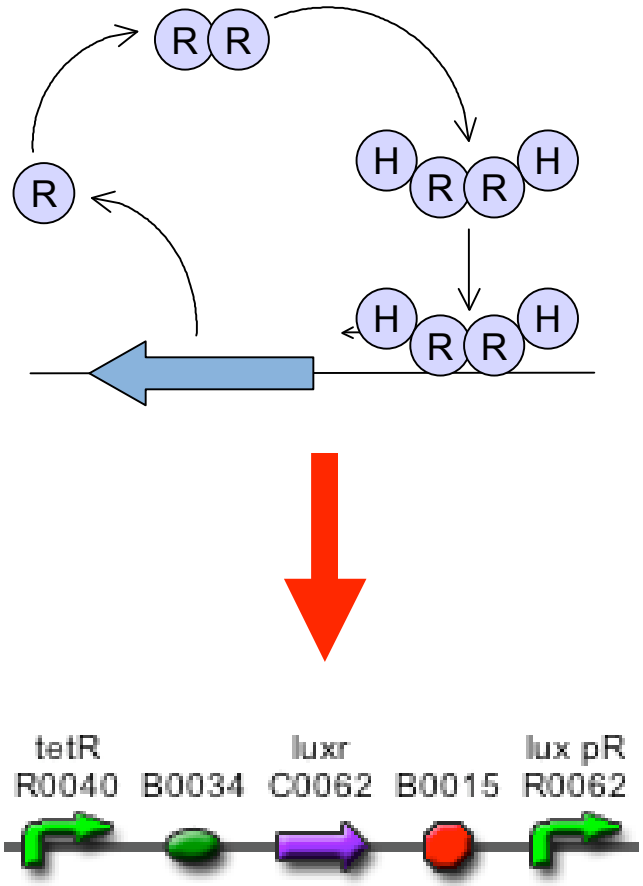


Utilizing Existing Components

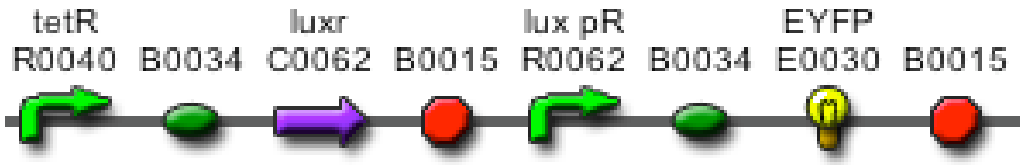


Receiver Design



BBa_I13270

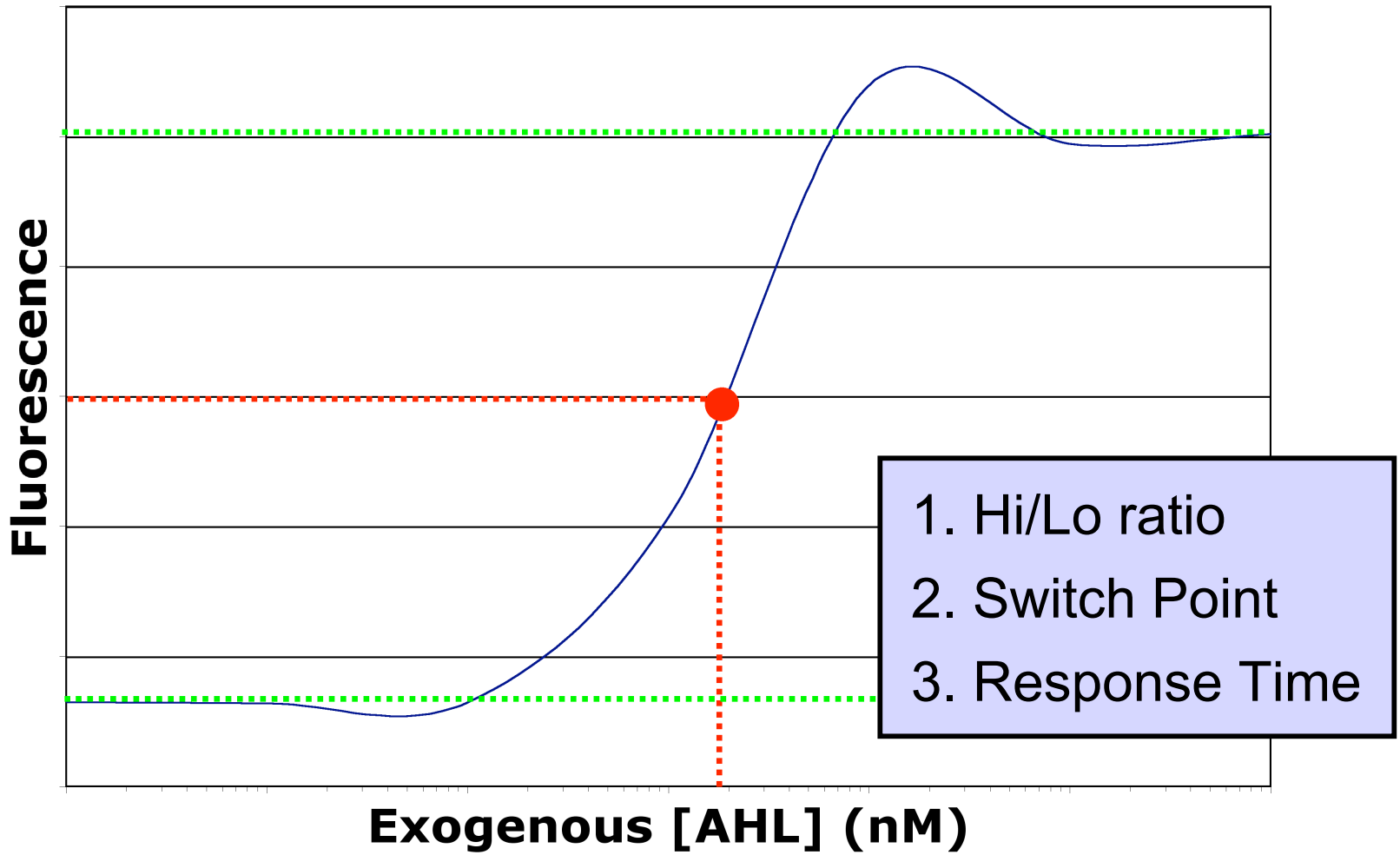
Receiver Building



BBa_I13273

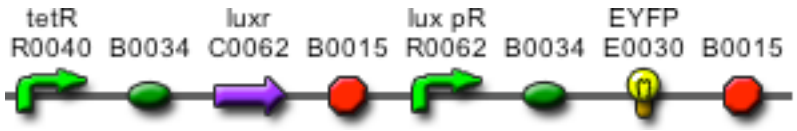
- Varied Upstream Promoter - Ptet, $luxP_L$
- High (100-200) and Low Copy (10-20) Plasmid
- Used YFP Output Device as a PoPS Reporter
- Built in DH5alpha using standardized assembly, Transformed into MC4100 and HCB1103

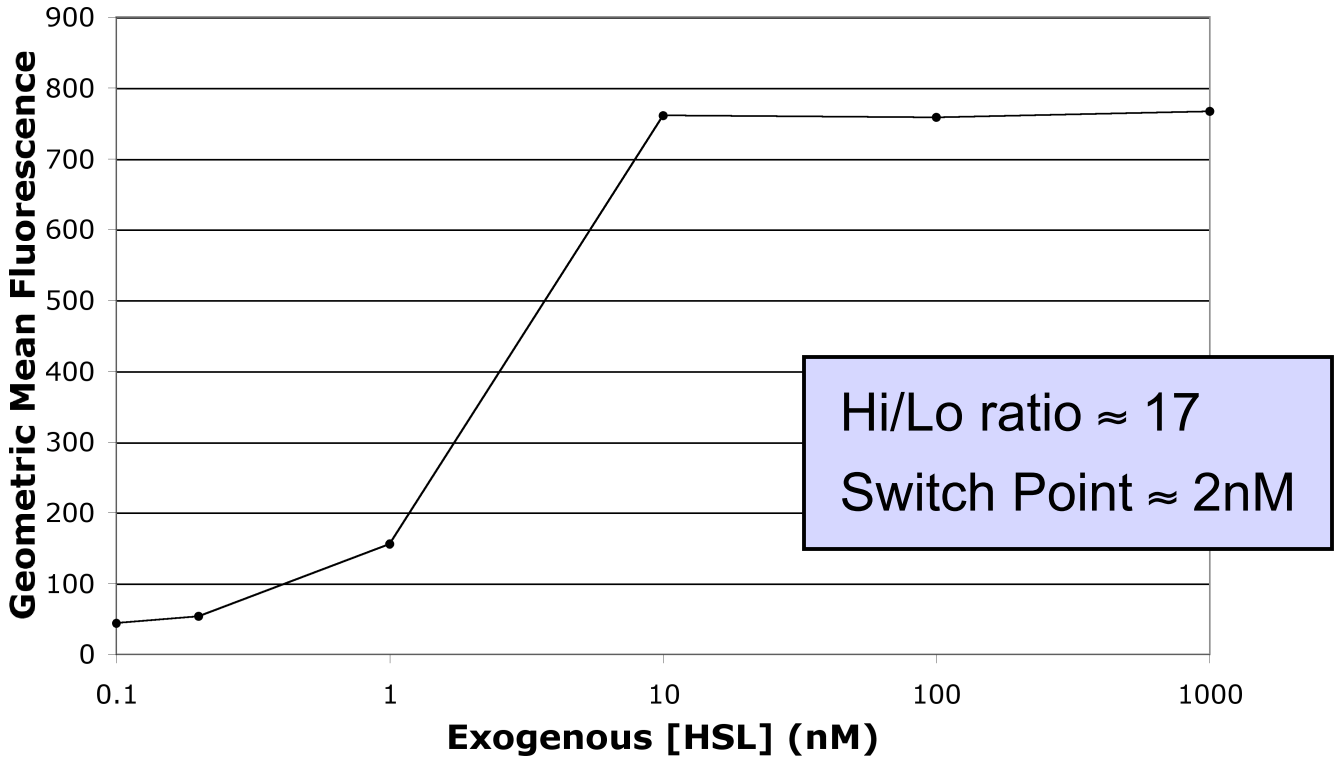
Receiver Testing



Receiver Testing

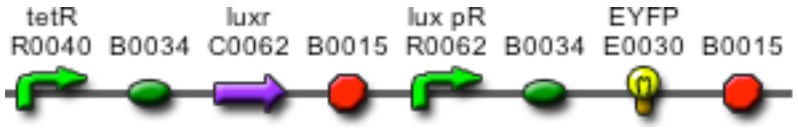
I13273 - pSB1A2

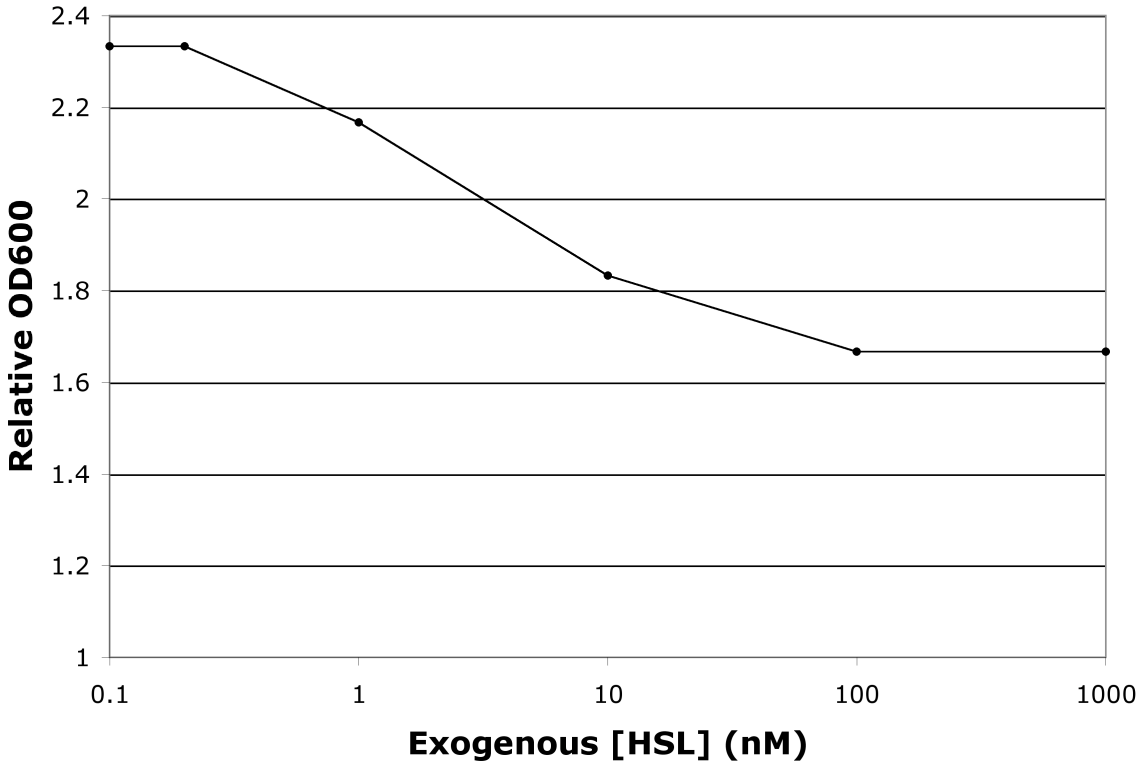




Receiver Testing

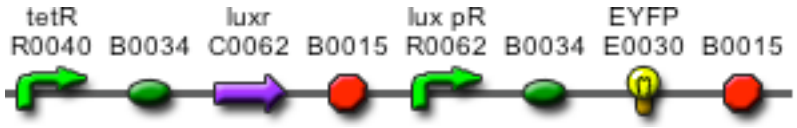
I13273 - pSB1A2 - Growth Defects

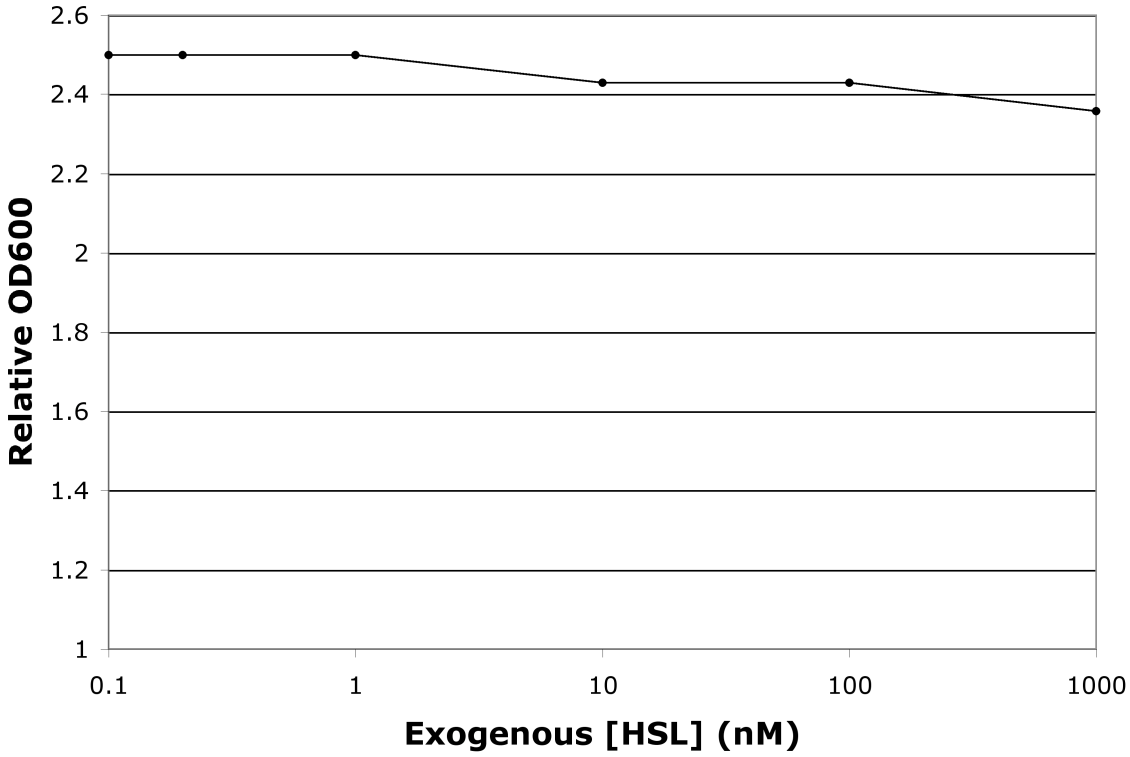




Receiver Testing

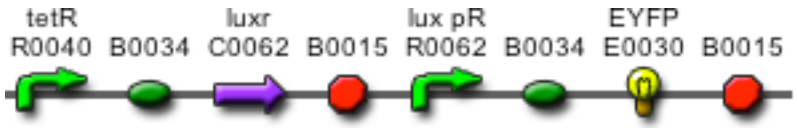
I13273 - pSB3K3 - Growth Restored

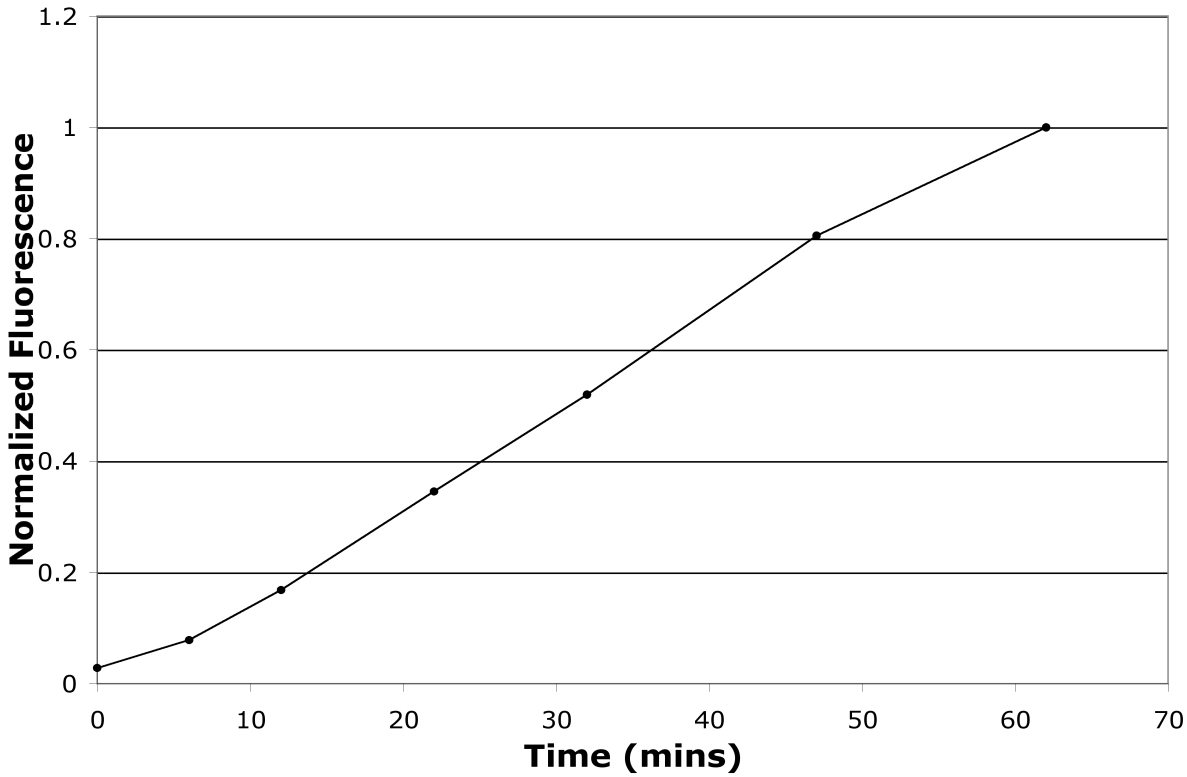




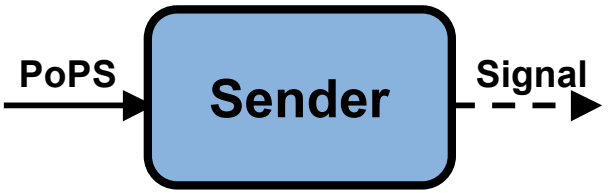
Receiver Testing

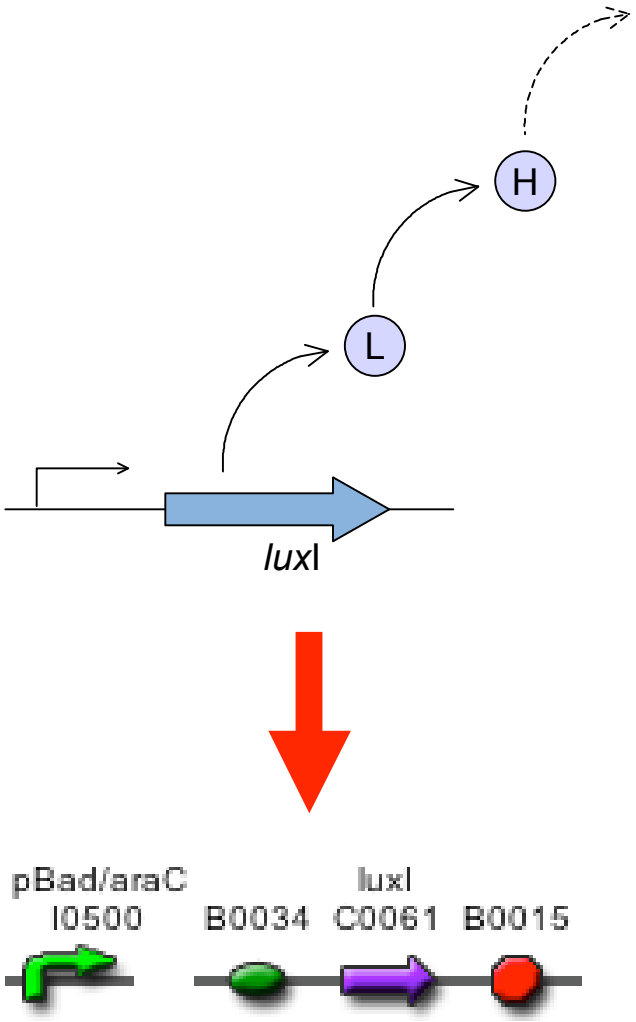
I13273 - pSB3K3 - Response Time

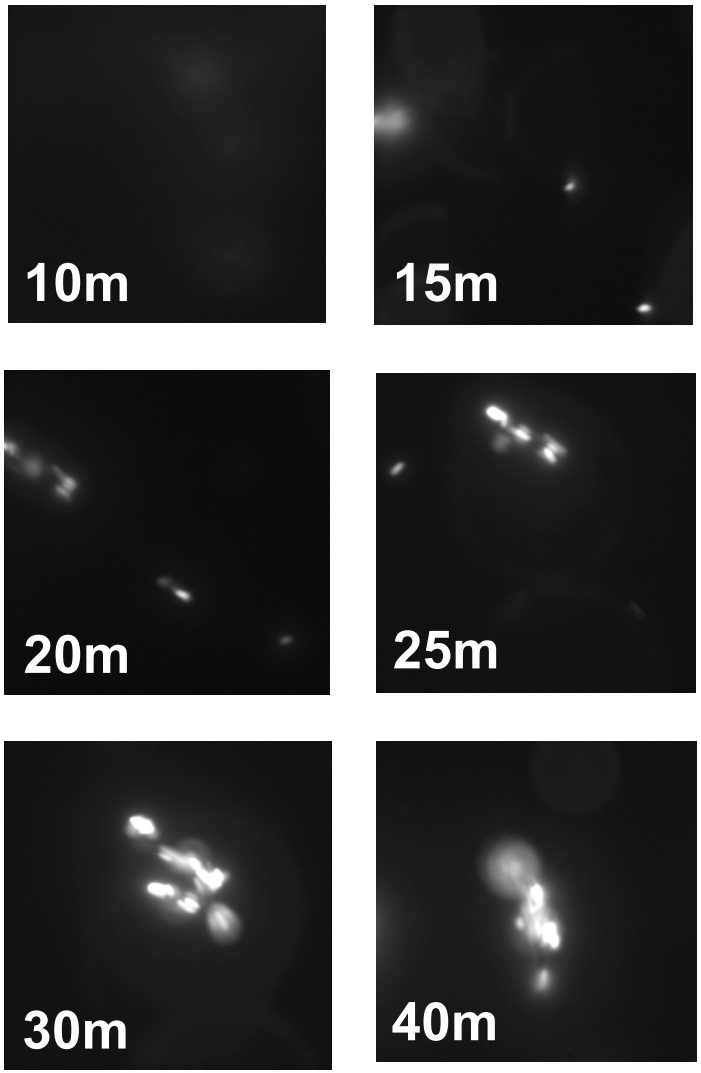




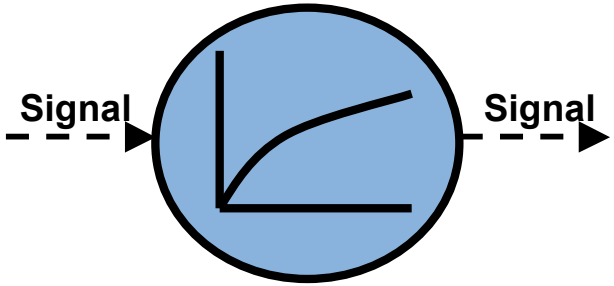
Senders


PoPS
Sender
Signal


H
L
luxI
pBad/araC
I0500
luxI
B0034
C0061
B0015

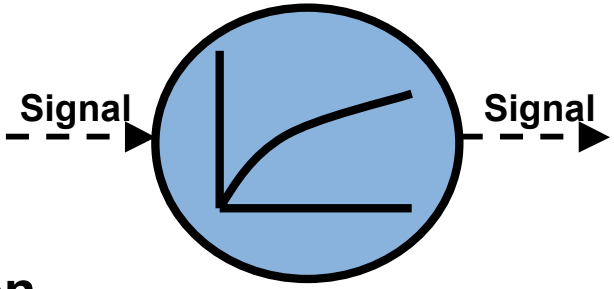

10m
15m
20m
25m
30m
40m

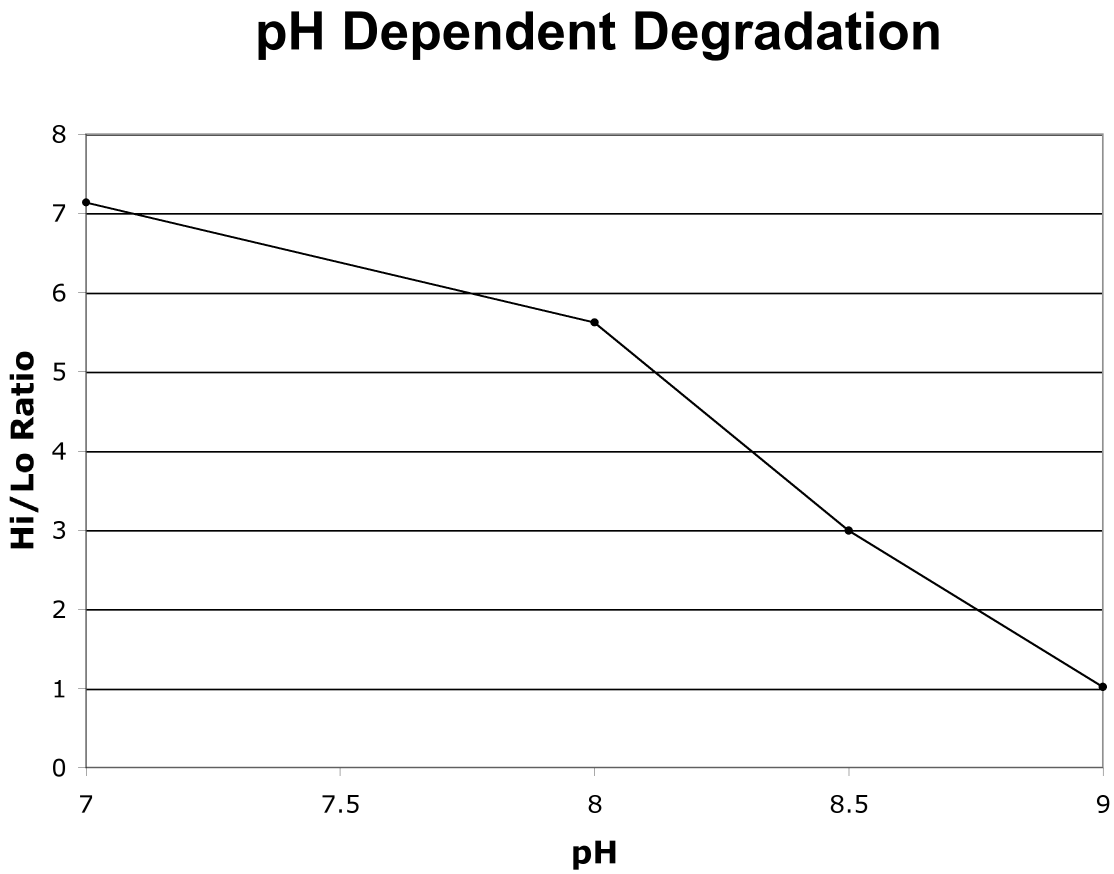
Transmission



- Diffusion rate
- Degradation due to dilution (e.g. in chemostat)
- Degradation due to raised pH
- Active enzymatic degradation - aiiA

Transmission

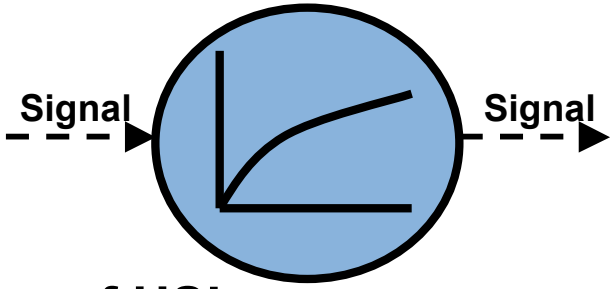




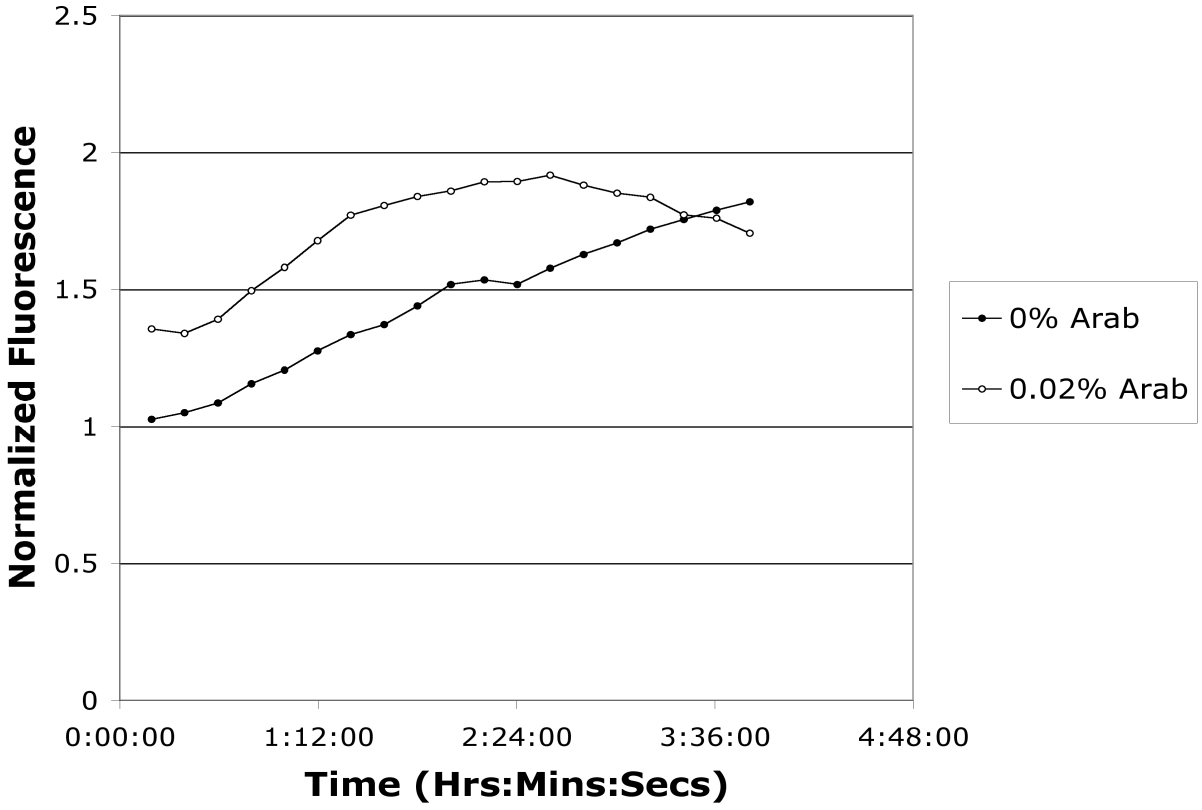
60 minute incubation of HSL at various pH

Use that HSL to activate receivers at neutral pH

Transmission



aiiA
Enzymatic intracellular Degradation of HSL

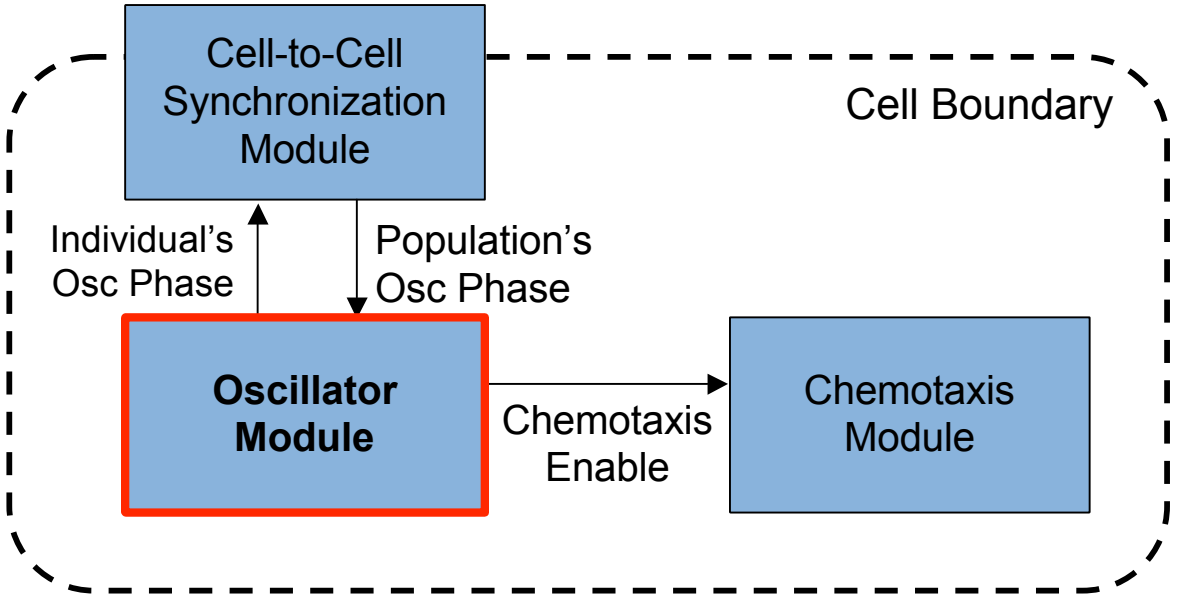


Future Work

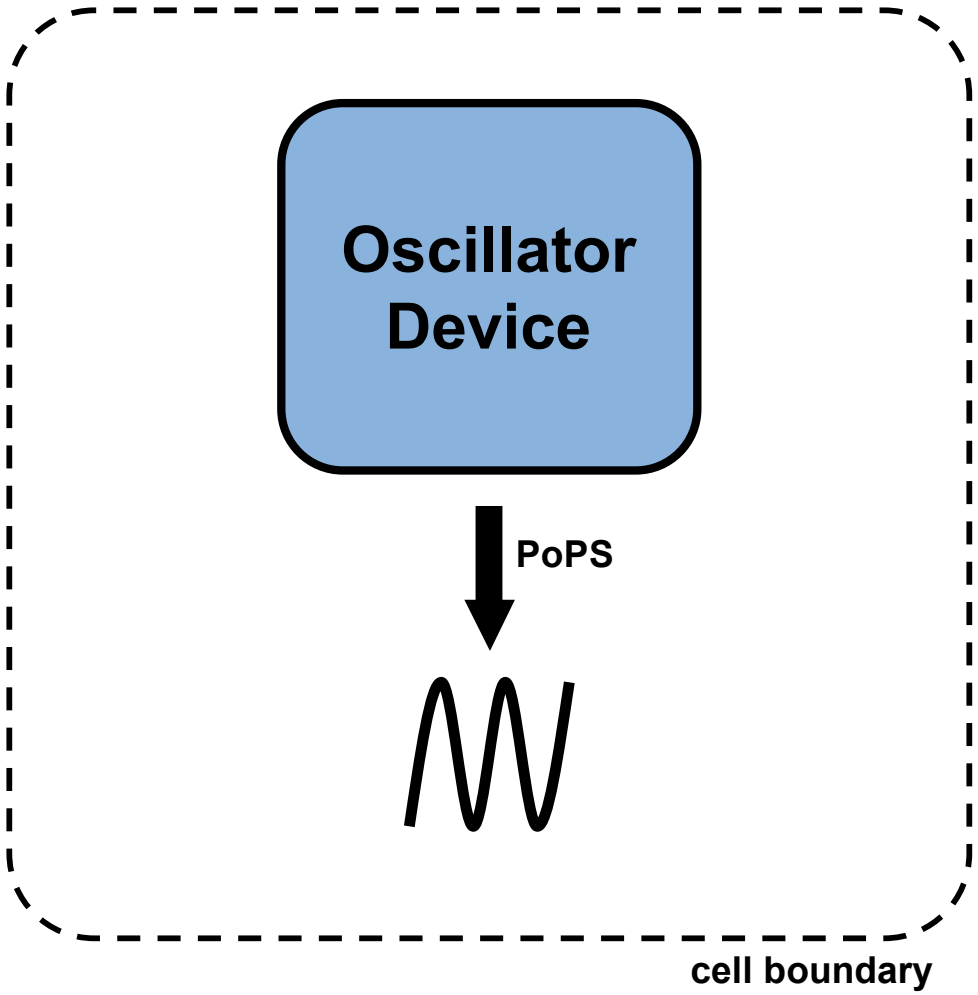
- Develop the ability to adjust receiver transfer function parameters at will
- Complete characterization of existing sender device using the receiver device
- Build and test the sender device used in the synchronized oscillator
- Continue to test the aiiA degradation mechanism
- Test under different operating conditions - microfluidic chemostat, microscope slides etc.

Oscillator Module

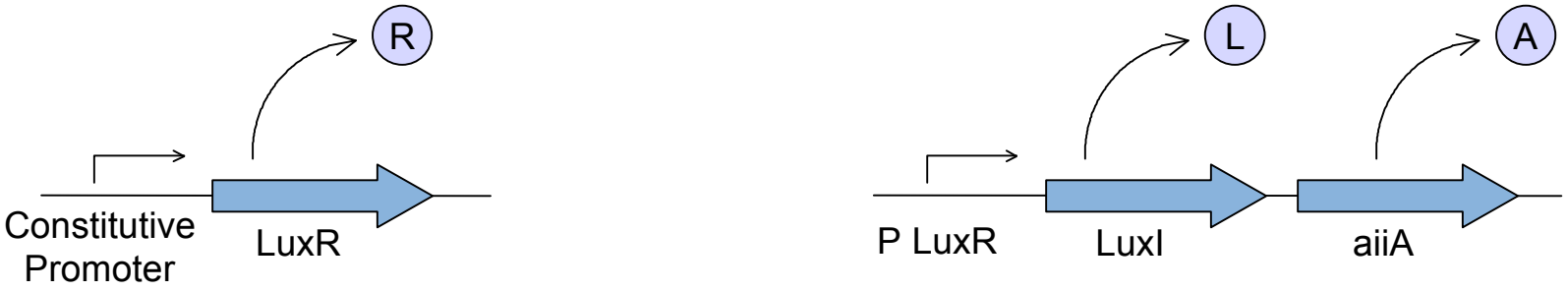
- Stand-alone Oscillator
  - Relaxation Oscillator
  - Ring Oscillator
- Synchronized Oscillator
  - Synchronators
  - Synchronized Ring Oscillator
- Future Work



Input/Output

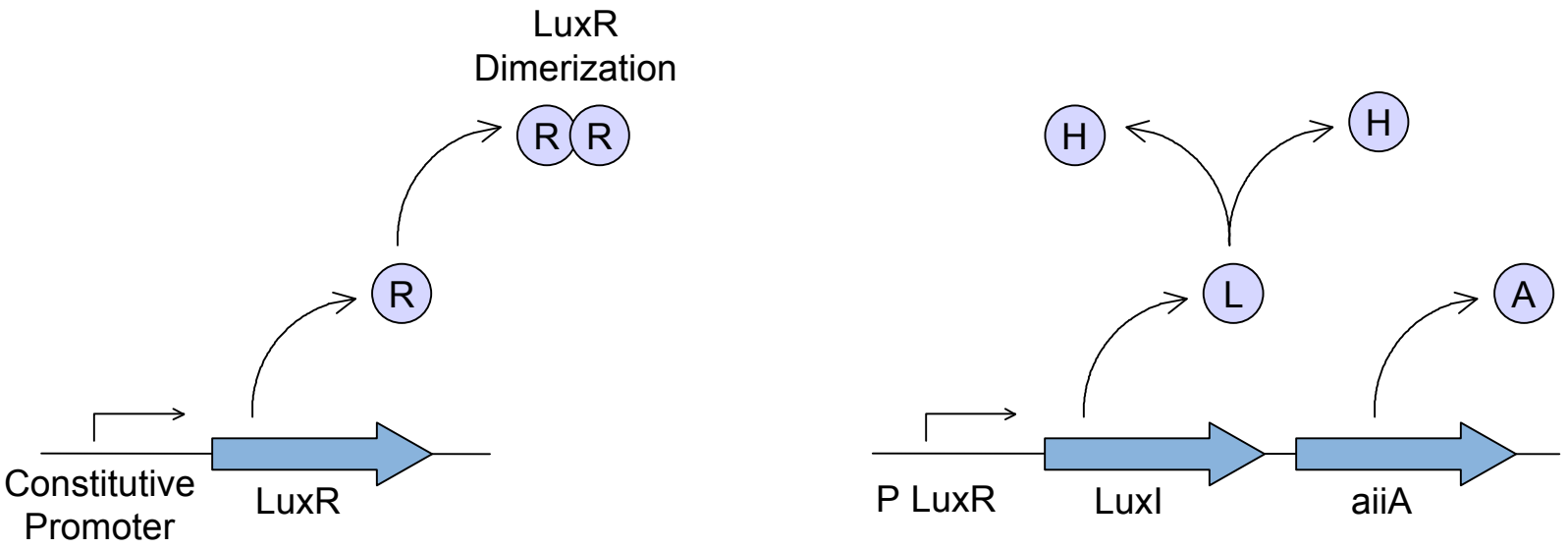


Lux/aiiA Relaxation Oscillator



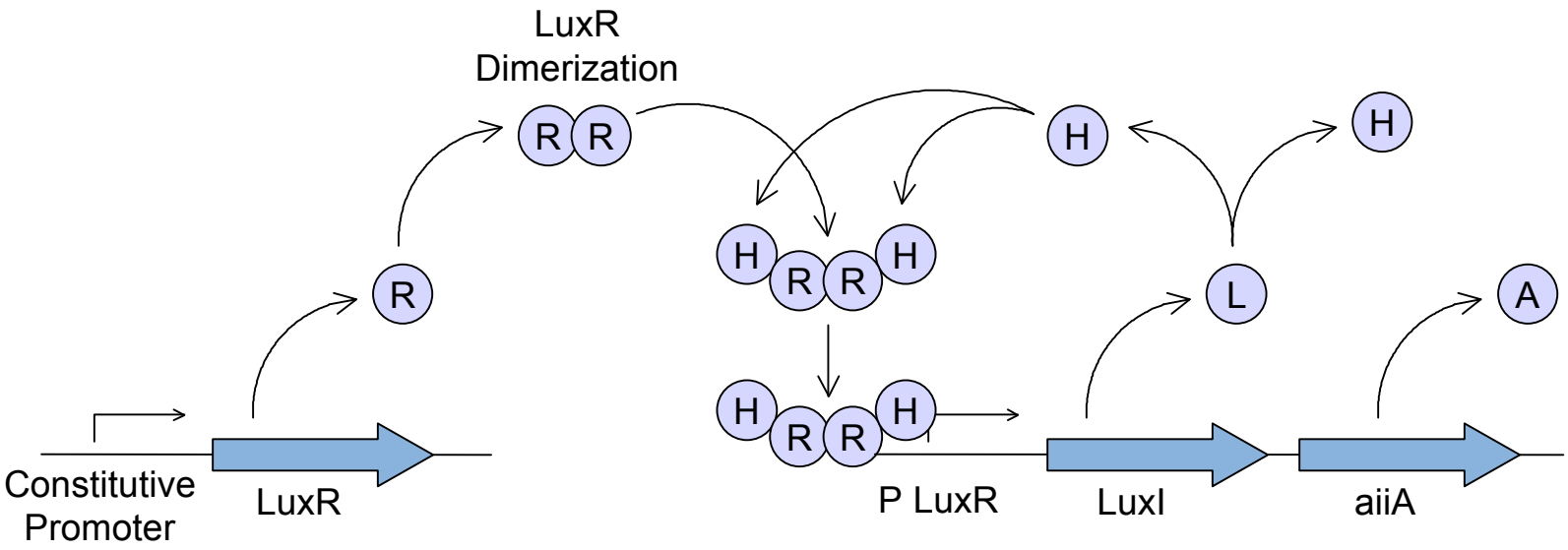
LuxR is constitutively expressed, while LuxI and aiiA are regulated by a LuxR activated promoter

Lux/aiiA Relaxation Oscillator



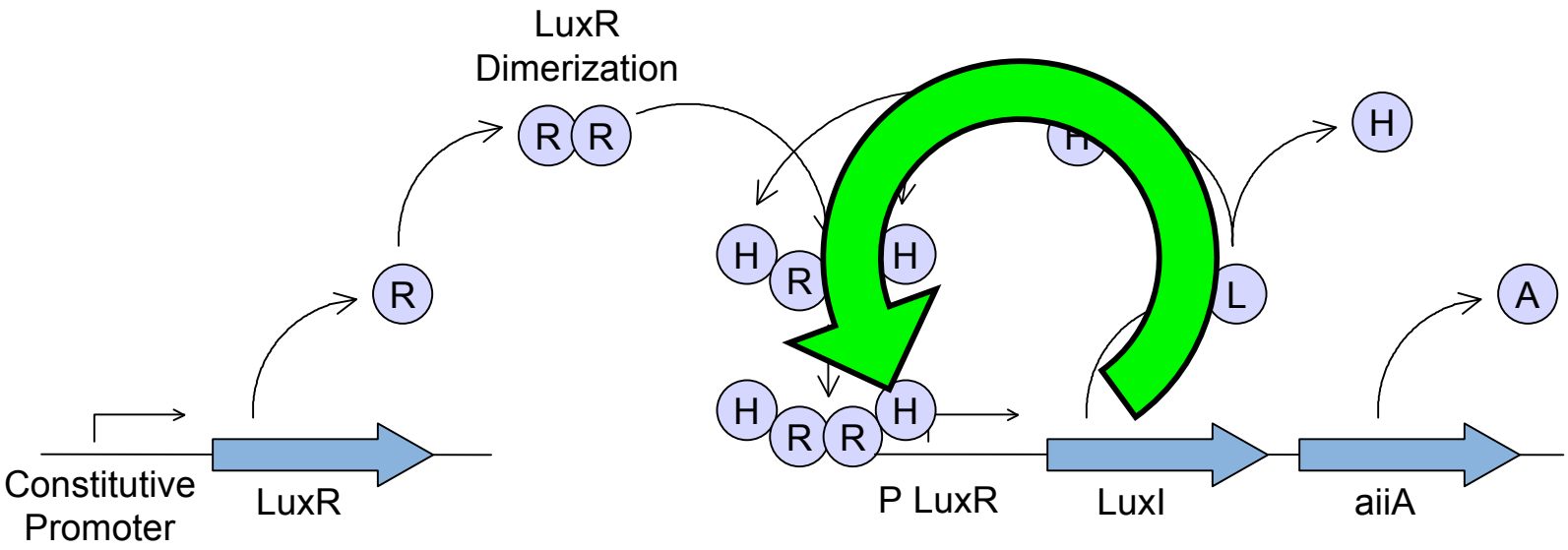
LuxR forms a dimer while LuxI synthesizes HSL

Lux/aiiA Relaxation Oscillator



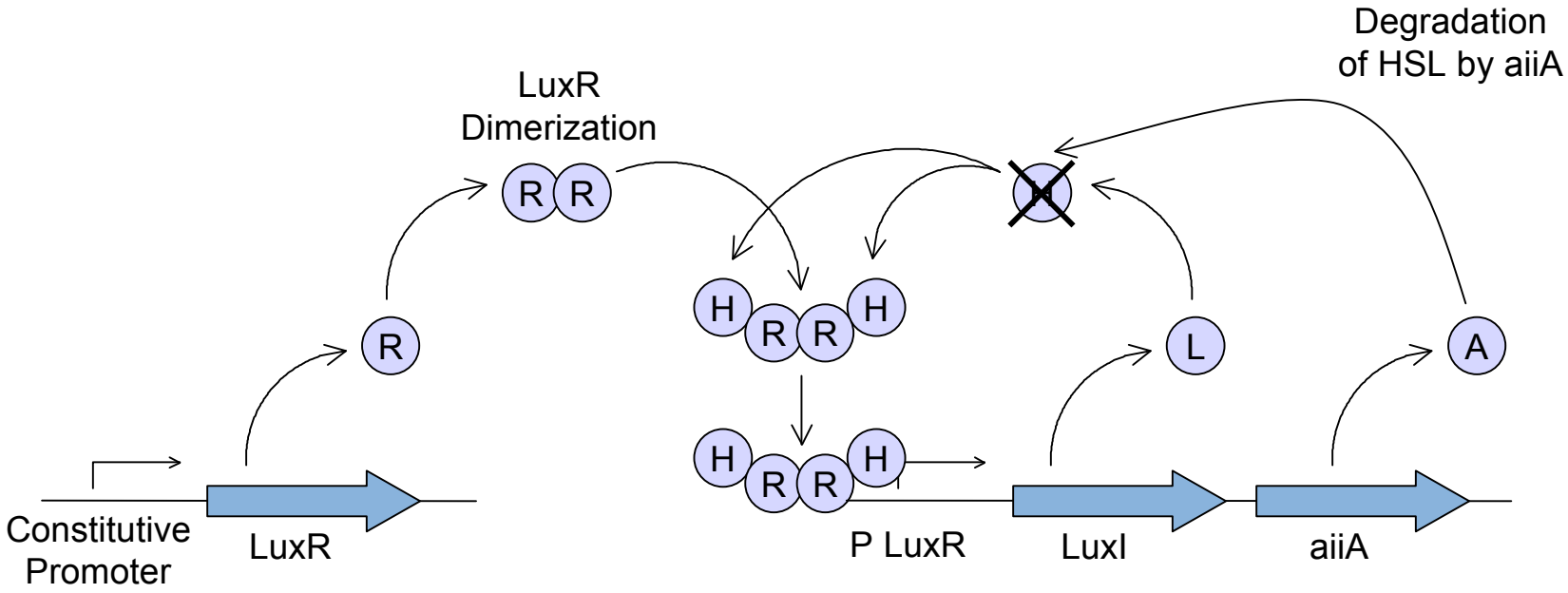
LuxR and HSL bind to form the transcriptional activator providing positive feedback

Lux/aiiA Relaxation Oscillator



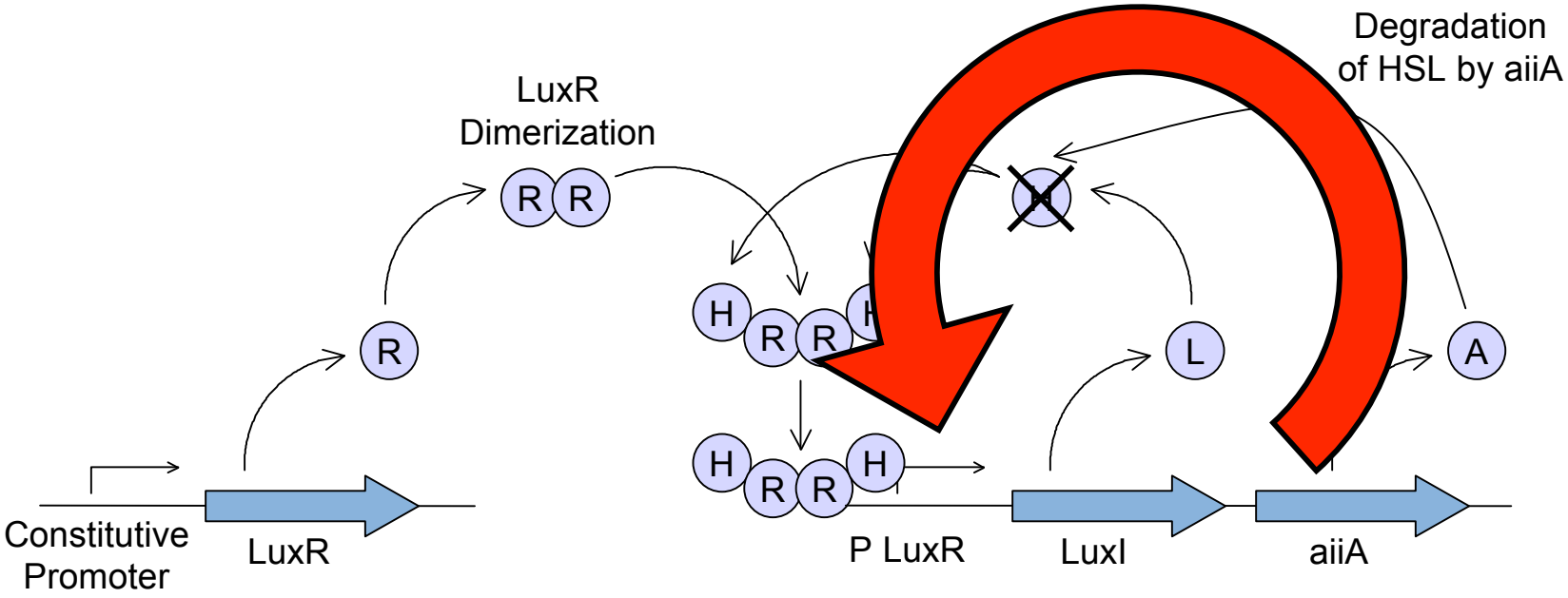
LuxR and HSL bind to form the transcriptional activator providing positive feedback

Lux/aiiA Relaxation Oscillator



aiiA degrades HSL providing negative feedback

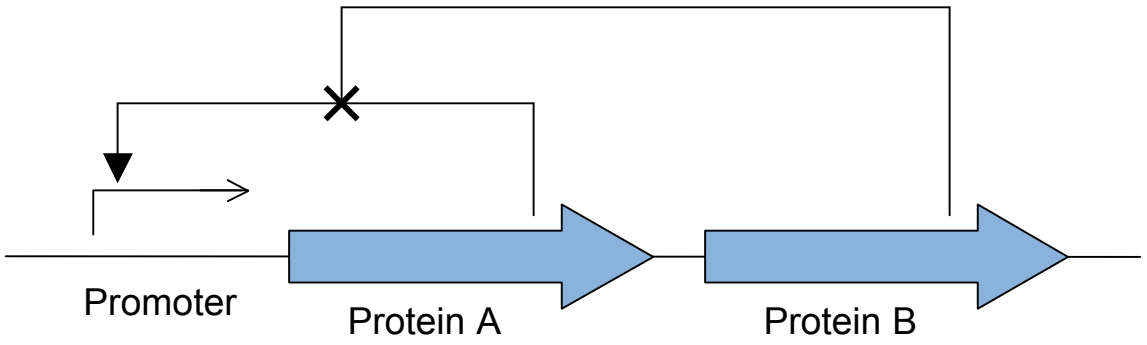
Lux/aiiA Relaxation Oscillator



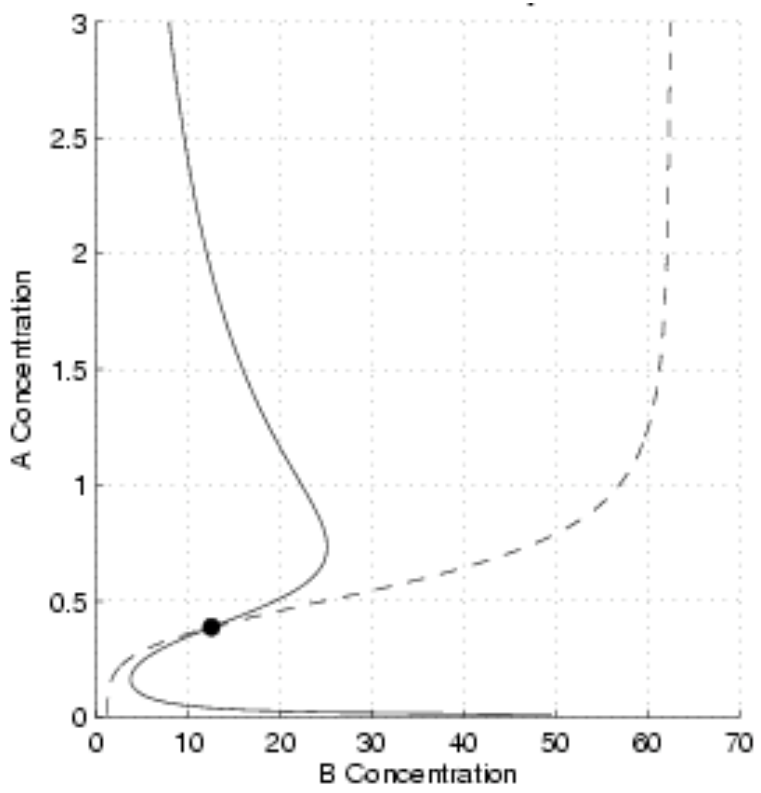
aiiA degrades HSL providing negative feedback

Simplified Relaxation Oscillator

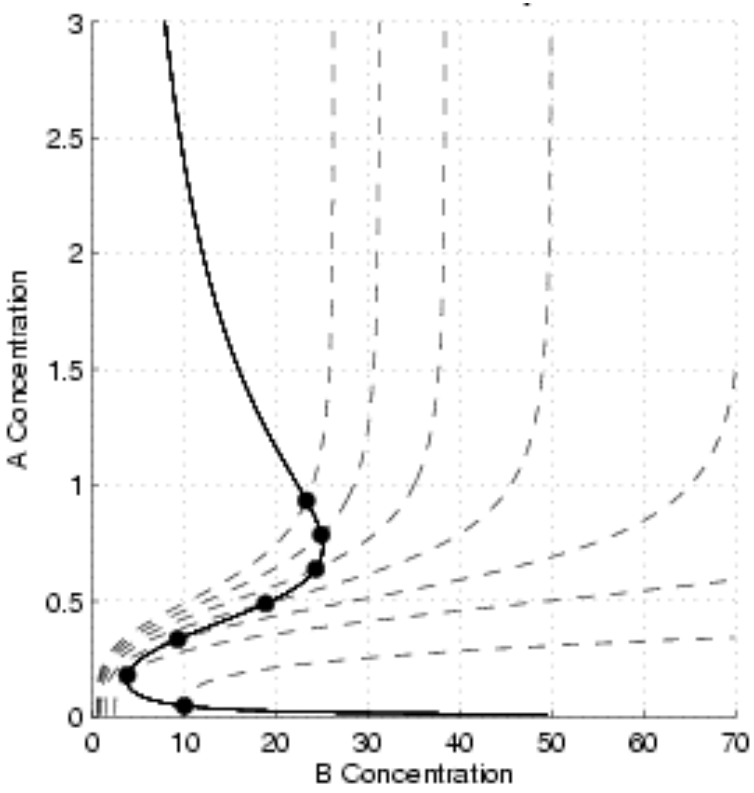
- Initial modeling work used a system of continuous differential equations to examine a simplified oscillator
- Folds the positive feedback into a single Protein A ignoring the details of LuxI, HSL, and LuxR
- Even with these simplifications, the model can give insight into what experimental constructs would be useful when building the actual Lux/aiiA oscillator



State Space Analysis

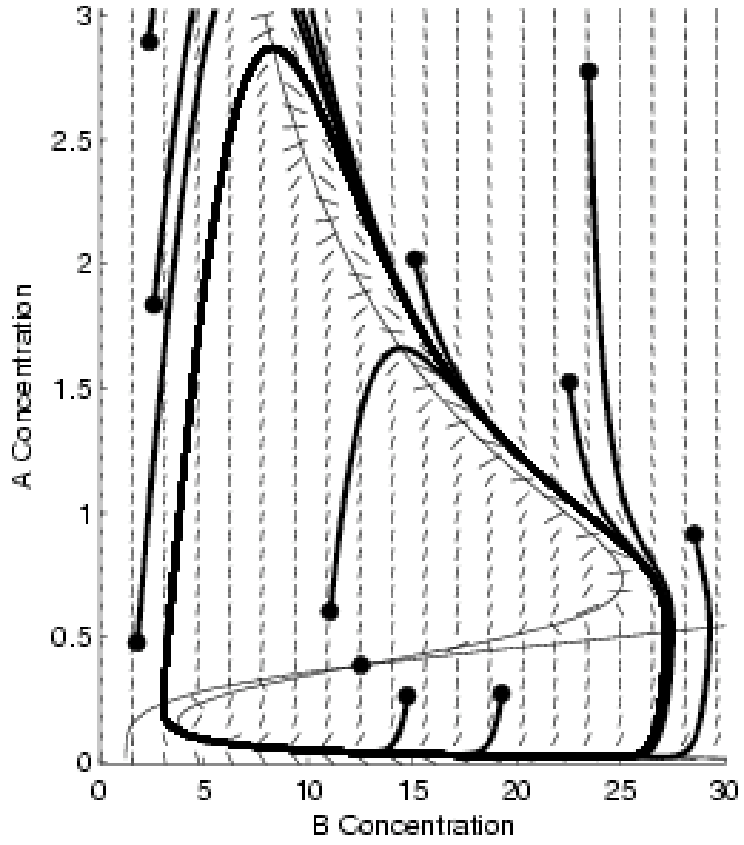


Intersection of nullclines yields system equilibrium point

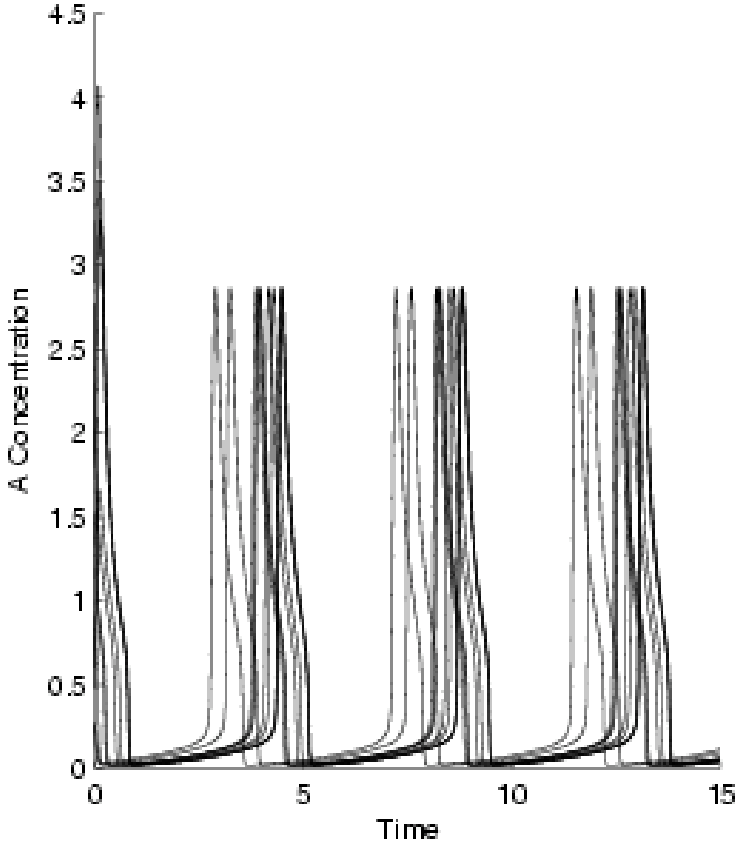


Equlibrium point changes with Protein B degradation rate

Preliminary Modeling Results



A vs B State Space

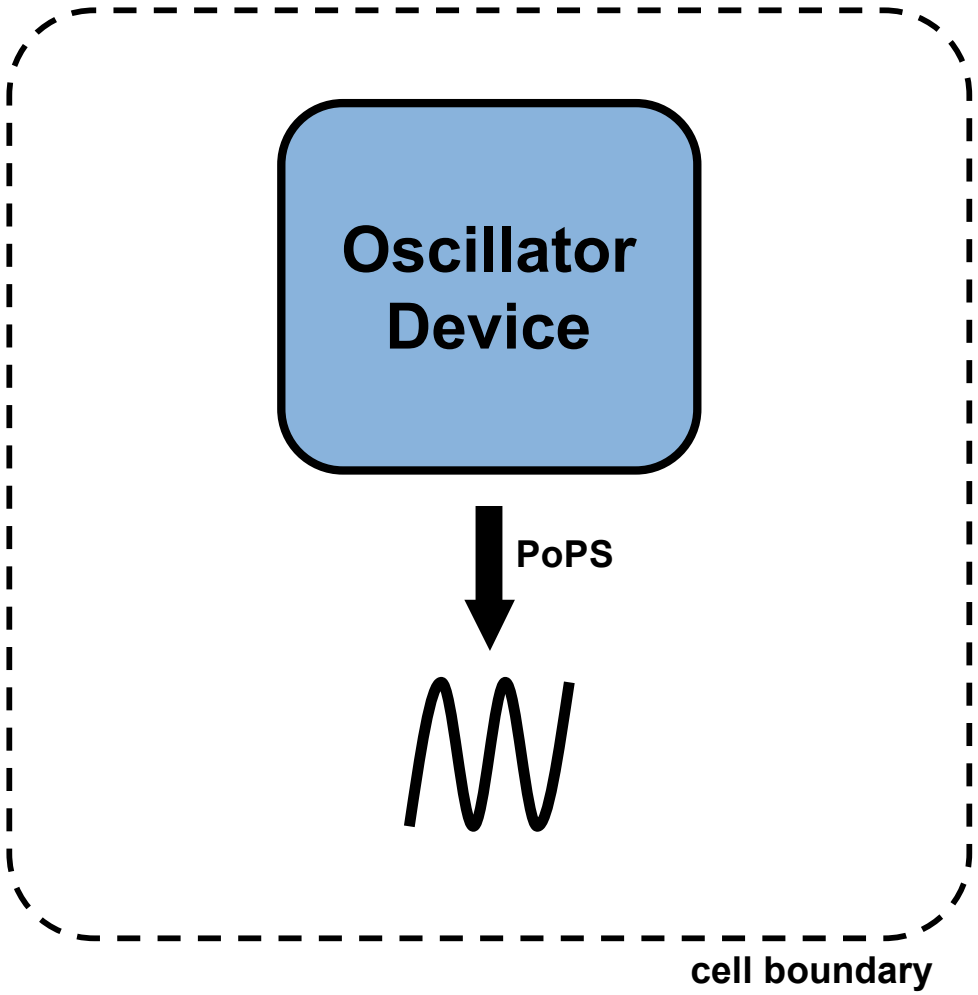


Concentration of A vs Time

Experimental Work

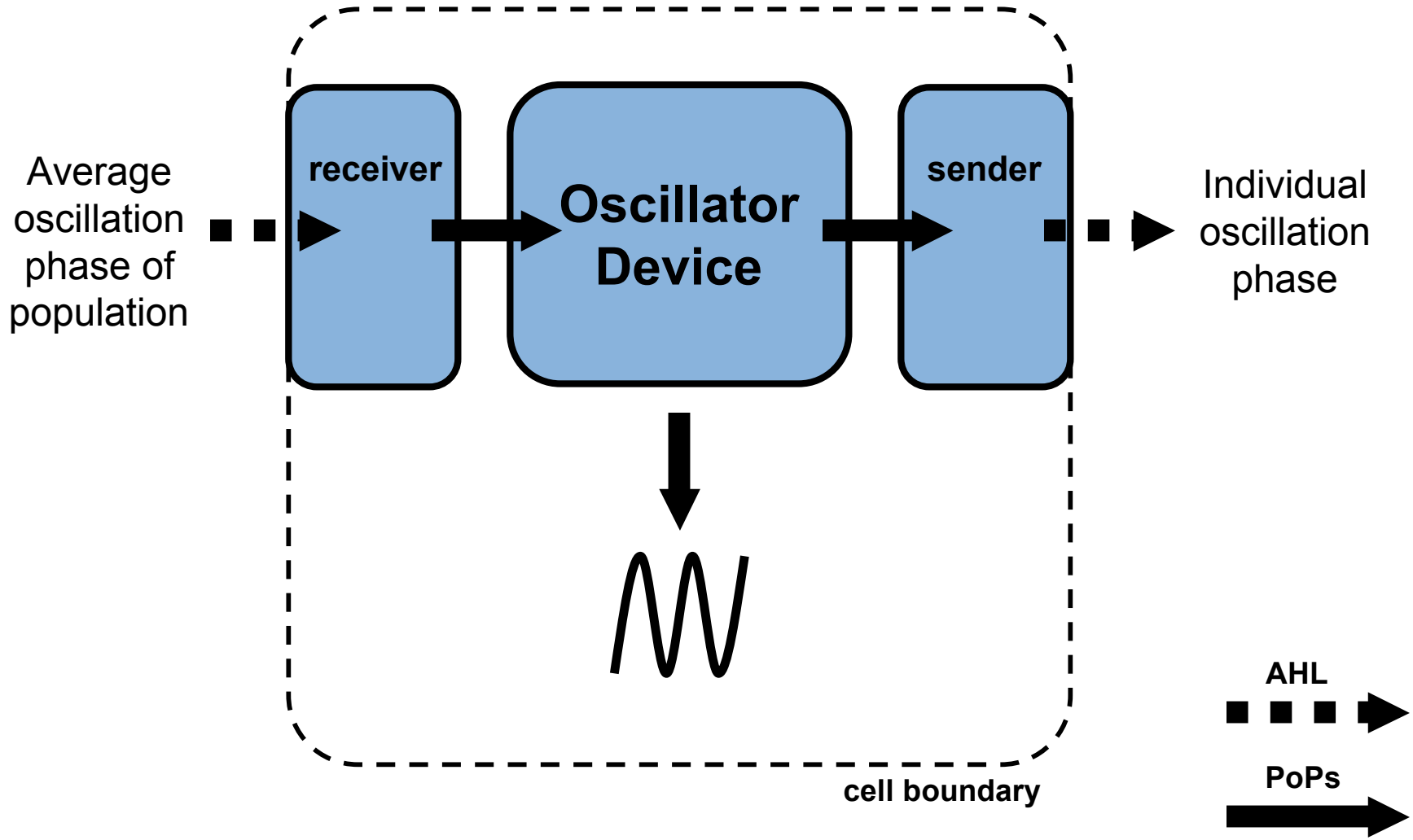
- Modeling work suggested possible test constructs
- Experimental work on the Lux/aiiA relaxation oscillator was put on hold
  - Initial results on aiiA were discouraging
  - Not enough degradation tags were available to effectively tune the aiiA degradation rate

Input/Output



Ring Oscillator

Input/Output

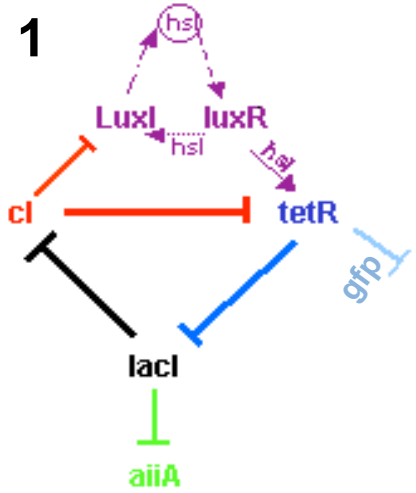


Synchronized Oscillator Options

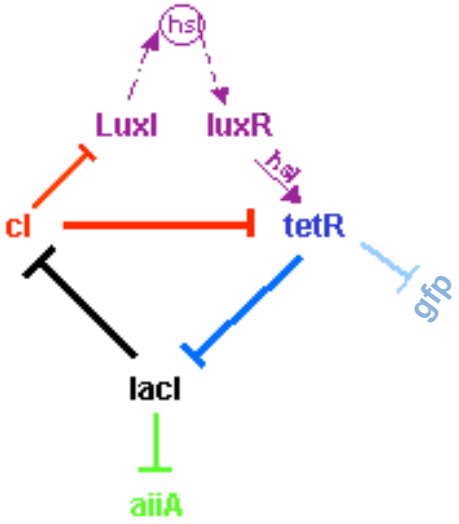
- Repressilator & Synchronization Device
  - Functional oscillator
  - Need to design synchronization device
- Synchronator
  - 4 designs available from the MIT 2003 Synthetic Biology course
  - Designed to synchronize, completely built, but untested and uncharacterized
- See-ya-lator
  - Modeled after Yankees ALCS performance

Synchronator Designs

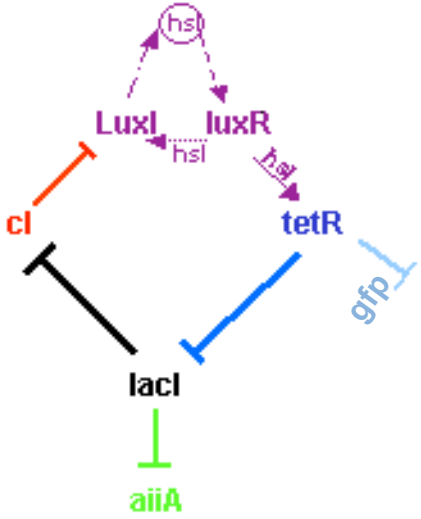
Design 1



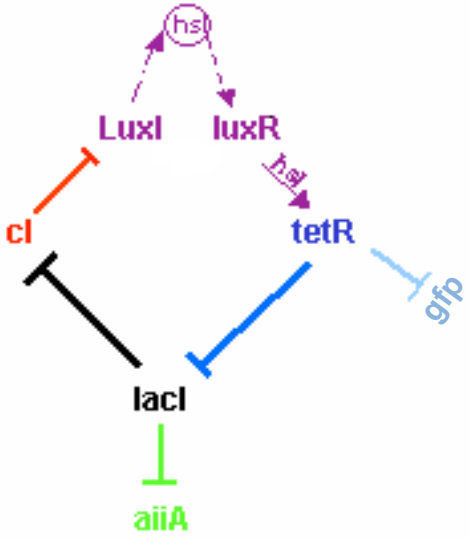
Design 2



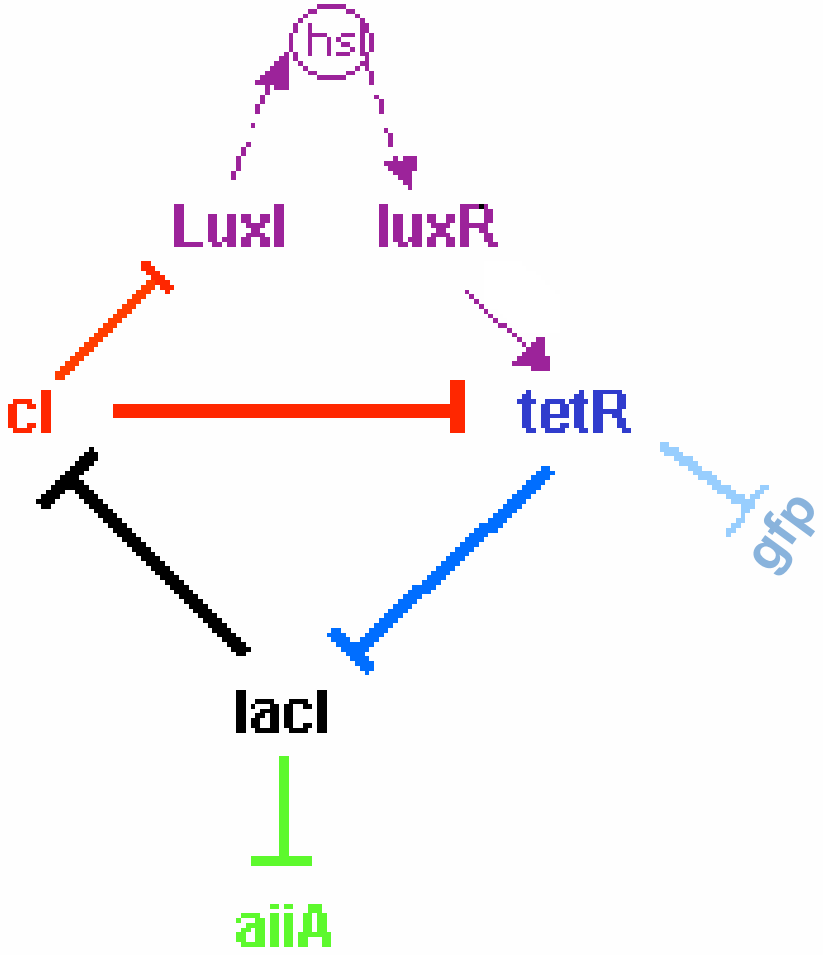
Design 3



Design 4

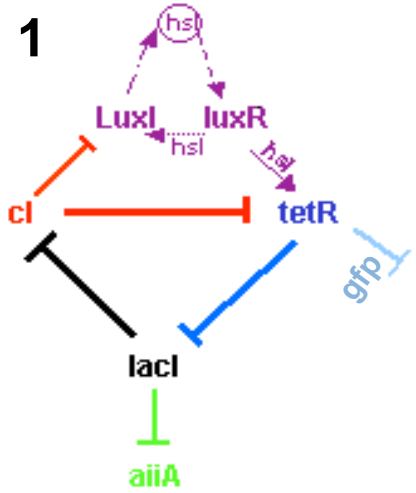


Synchronator Design 2



Synchronator Designs

Design 1



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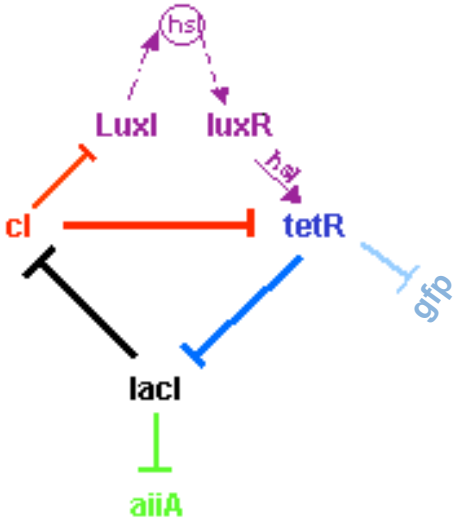

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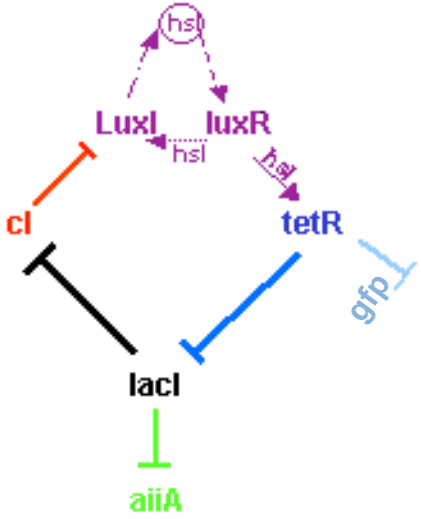

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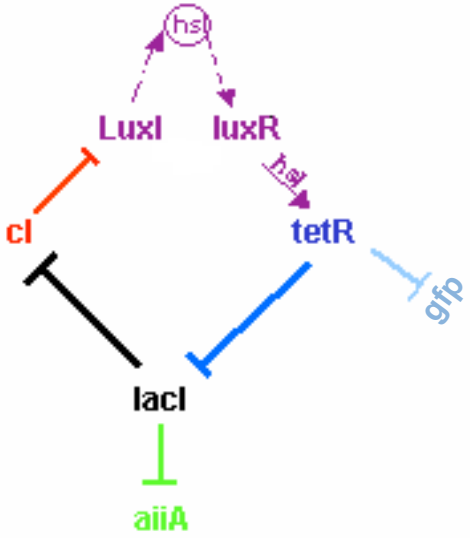
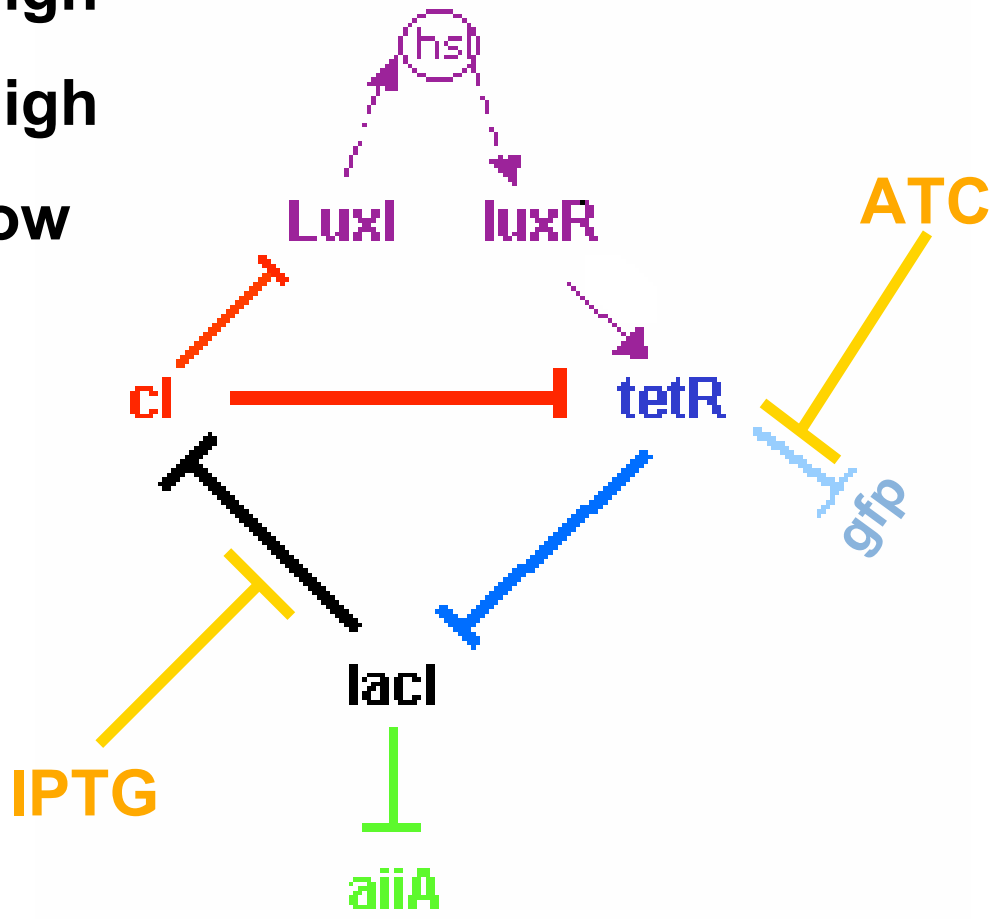


Oscillator Lockdown Experiment

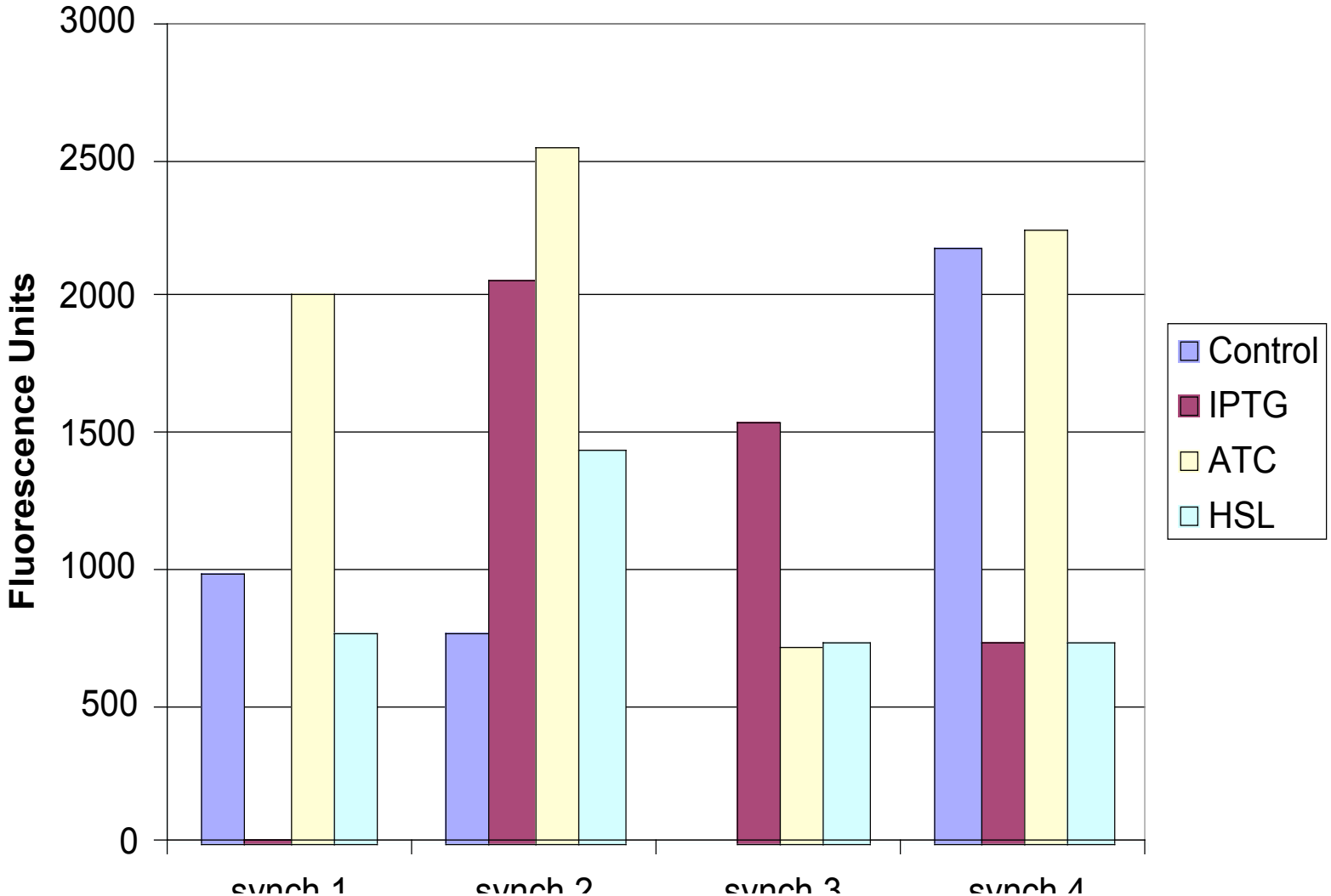
Add IPTG = GFP high

Add ATC = GFP high

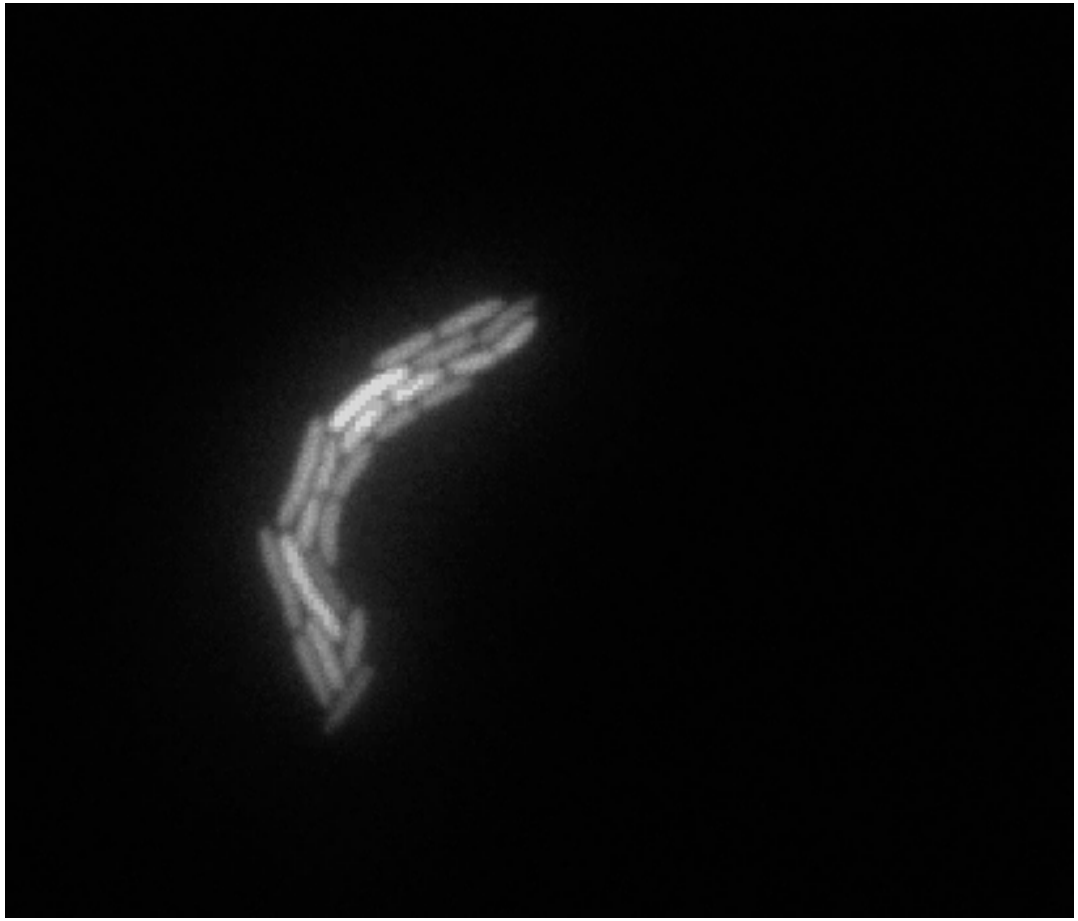
Add HSL = GFP low



Synchronator Lock-Down

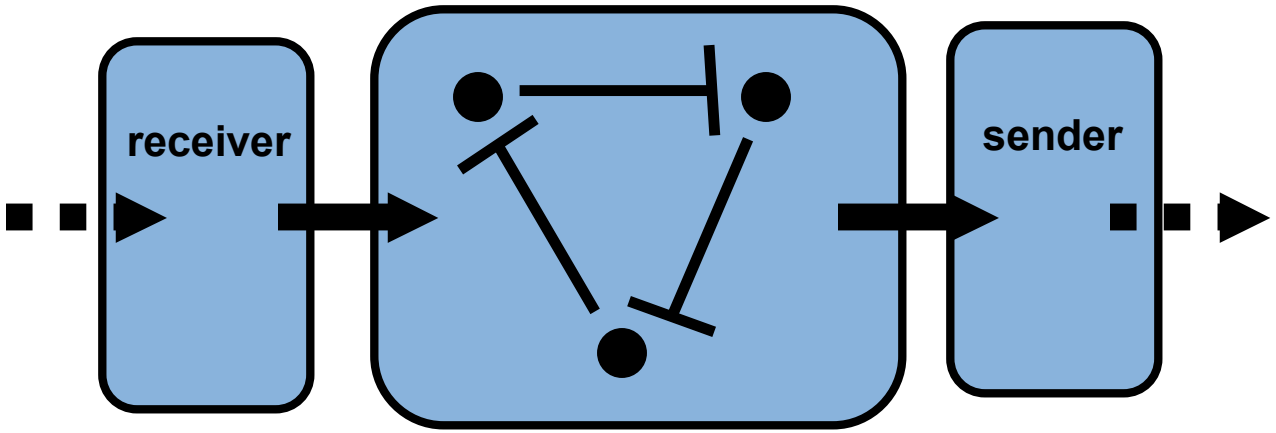


Synchronator 2 Movie



Synchronized Ring Oscillator

- Add a synchronization element to the Repressilator *(Garcia-Ojalvo,Elowitz,Strogatz, PNAS 2004)*



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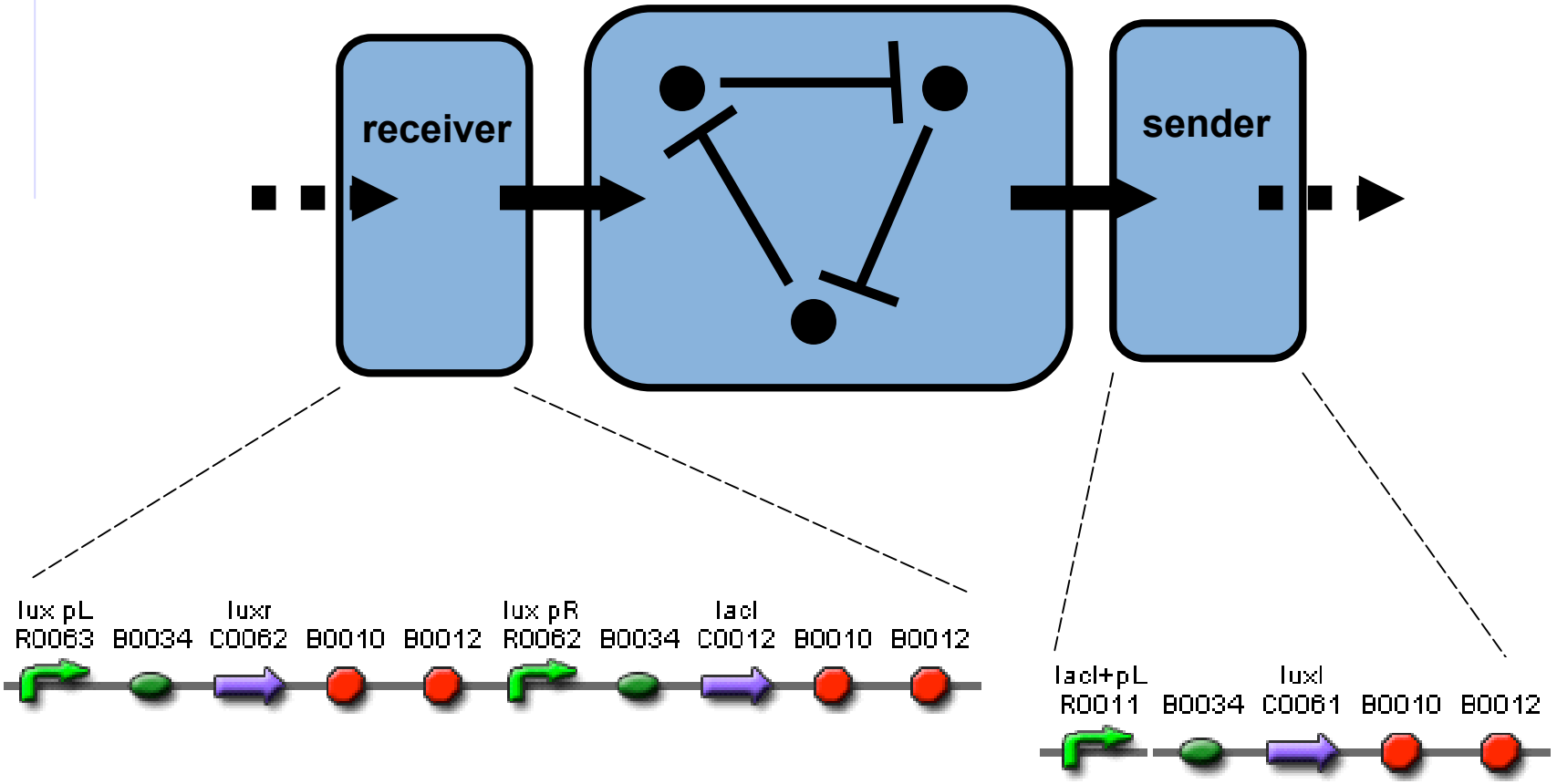
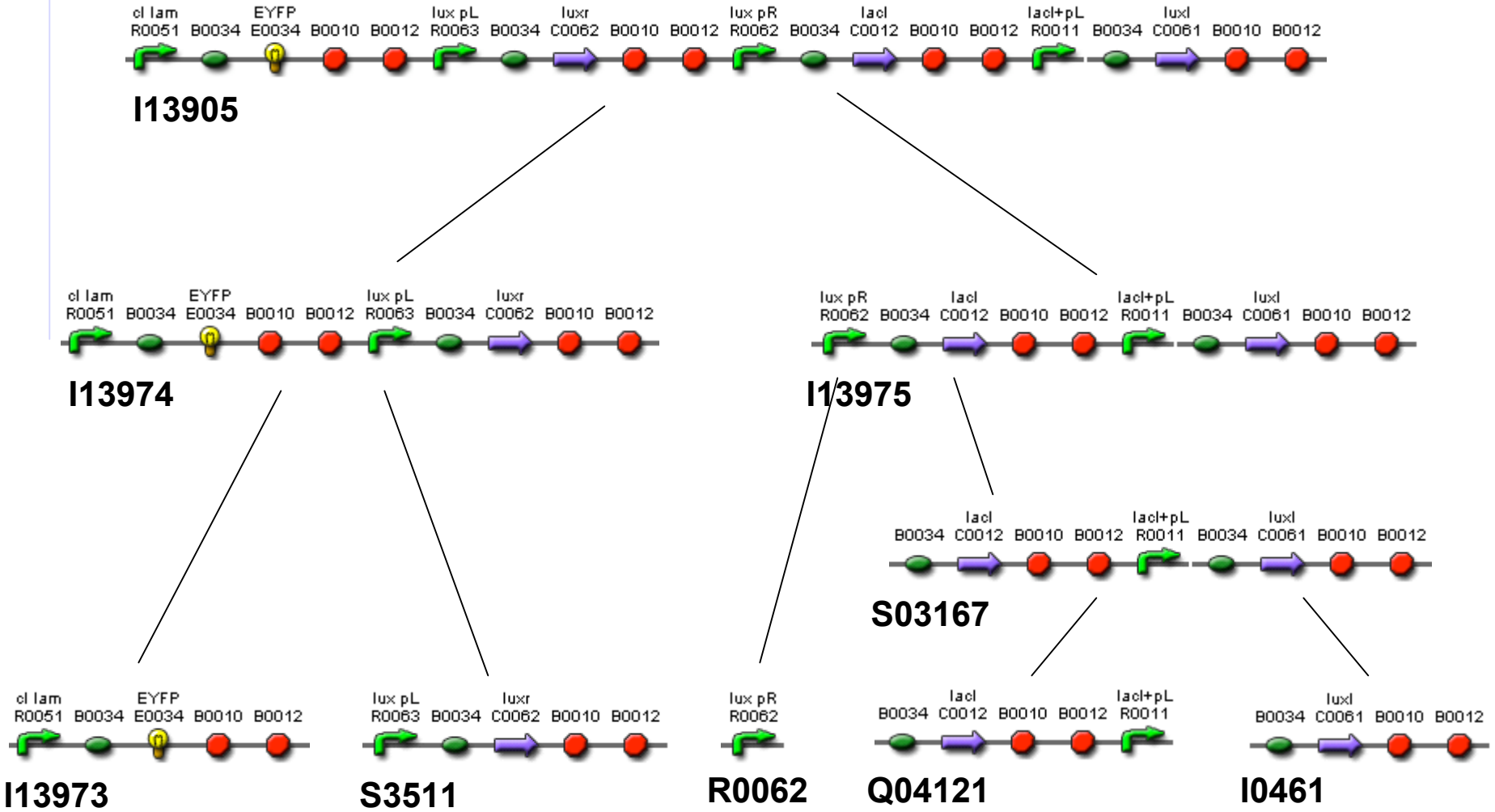


Construction of Synchronization Device

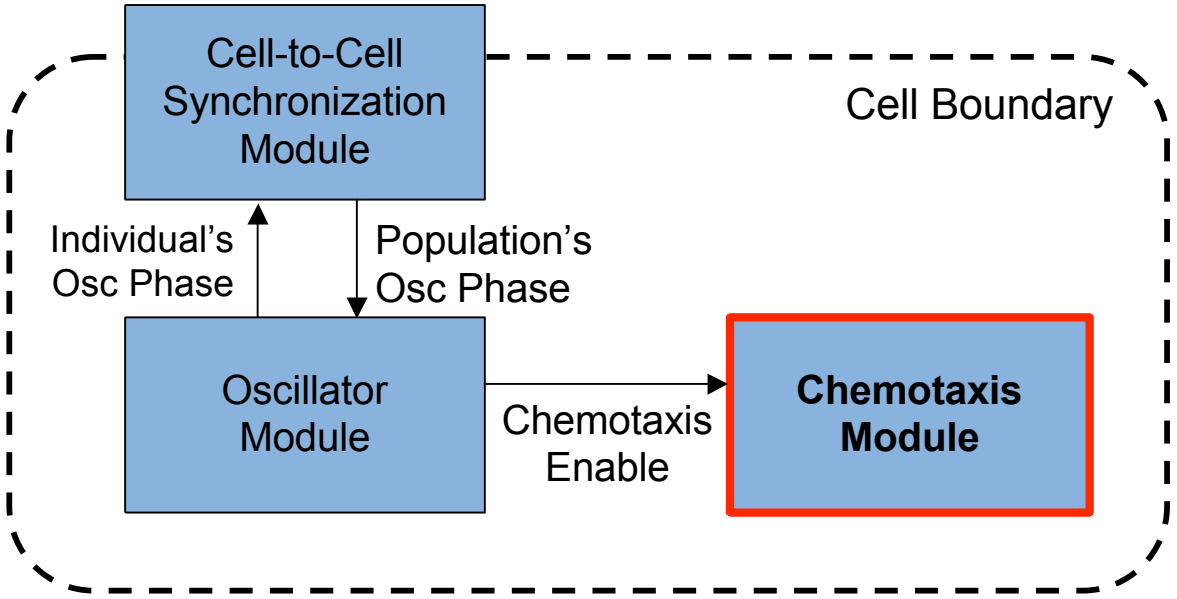


Future Work

- Synchronized Ring Oscillator
  - Lock-down experiments
  - Agarose Pad Time Lapse Movie
  - Continuous Culture (chemostat)
  - Plate Reader Time Course
- Relaxation Oscillator
  - Explore aiiA further to determine why it isn't functioning as expected
  - Build test constructs and characterize

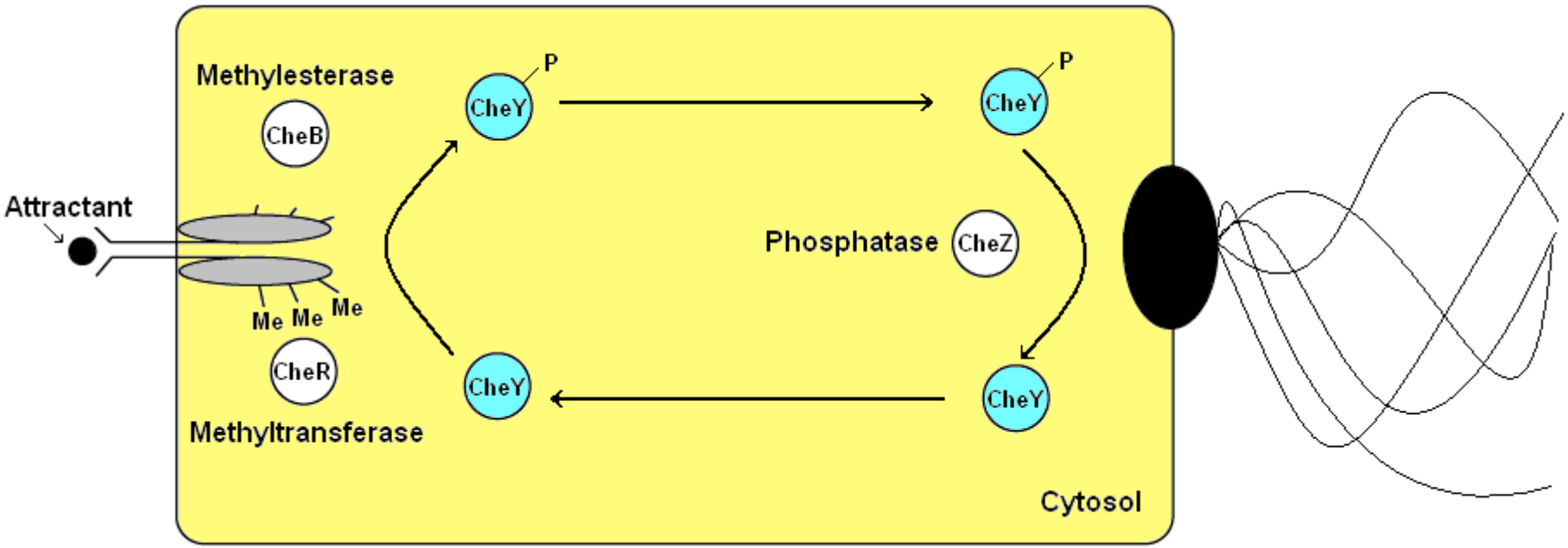
Chemotaxis Module

- Chemotaxis biology
- Chemotaxis devices
  - Restoring motility
  - Deactivating motility
- Chemotaxis assay
- Results
- Future work



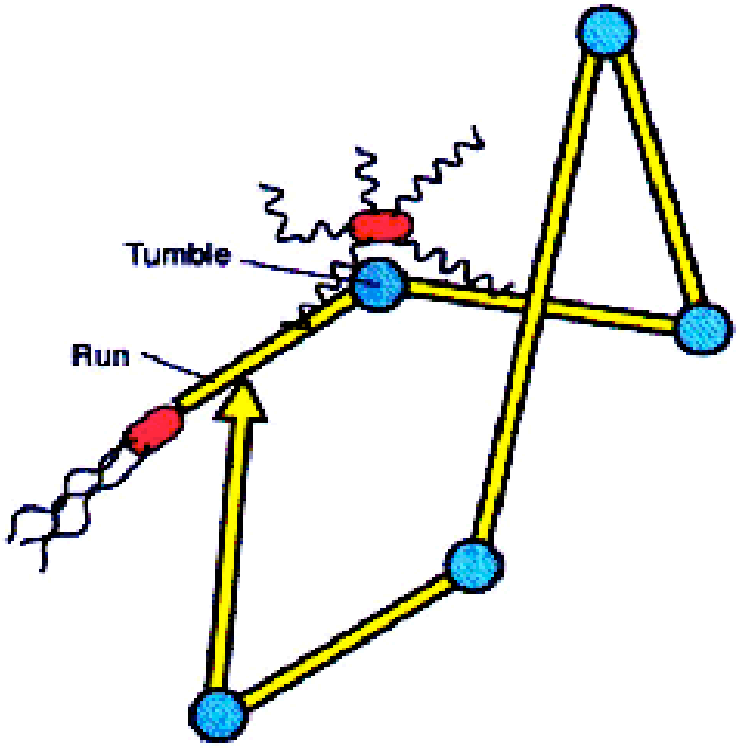
Chemotaxis in Escherichia Coli

- Four intracellular signaling proteins
  - CheB, CheR, CheY, and CheZ
  - Maintained at specific levels and ratios



Motile Behavior

- Net movement toward or away from chemicals result from the combined effect of smooth runs and tumbles
- The expression and activity of signaling proteins (CheB, CheR, CheY and CheZ) determines the frequency of tumbles and runs



http://www.jameshallsweb.co.uk/dissertation/about_chemotaxis.htm

Too Much or Too Little?

Absence of any signaling protein affects motile behavior

Genotype	Motility	Phenotype
wt	+	smooth runs and tumbles
ΔCheB	+	tumbles
ΔCheR	+	smooth runs
ΔCheY	-	none
ΔCheZ	+	smooth runs

Overexpression of any signaling molecule affects motile behavior

Genotype	Overexpression	Phenotype
wt	CheB	smooth runs
wt	CheR	tumbles
wt	CheY	tumbles
wt	CheZ	smooth runs

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Overexpression of any signaling molecule affects motile behavior

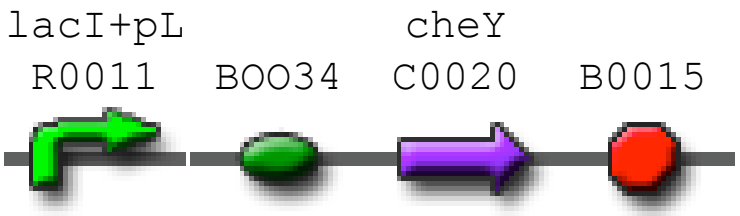
Genotype	Overexpression	Phenotype
wt	CheB	smooth runs
wt	CheR	tumbles
wt	CheY	tumbles
wt	CheZ	smooth runs

CheY concentration in RP437: **8,200 ± 310** per cell in rich media and **6,300 ± 70** per cell in minimal media

Li M, Hazelbauer G "Cellular Stoichiometery of the Components of the Chemotaxis Signaling Complex" Journal of Bacteriology, 2004, 186(12) 3687-3694

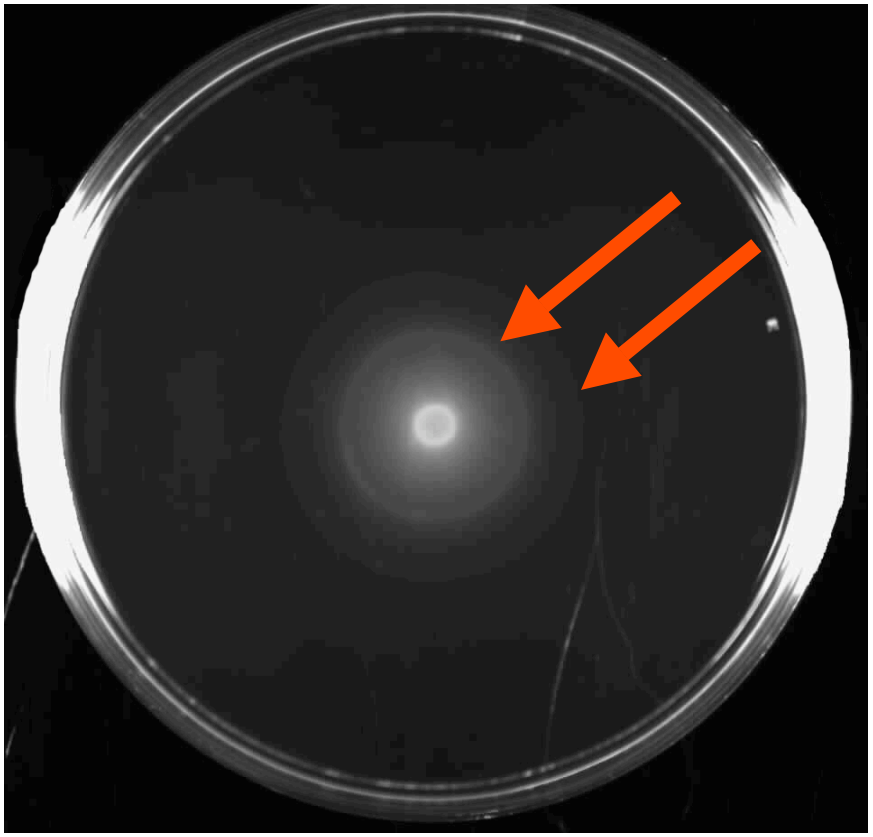
Building Chemotaxis Output

- CheY quadparts: High Copy (150-200) and Low Copy (15-20)



- Two methods for coupling to Chemotaxis
  - **Restoration** of normal chemotaxis in ΔCheY mutant strain
  - **Deactivation** of normal chemotaxis in wild type strain
- Characterizing quadpart expression
  - Time frame of protein expression
- Observing the inactivation and deactivation of bacterial chemotaxis
  - Swarm plate characterization
  - Drop assay and bacterial clustering on glass slide
  - Capillary Assay

Swarm Plate Assay

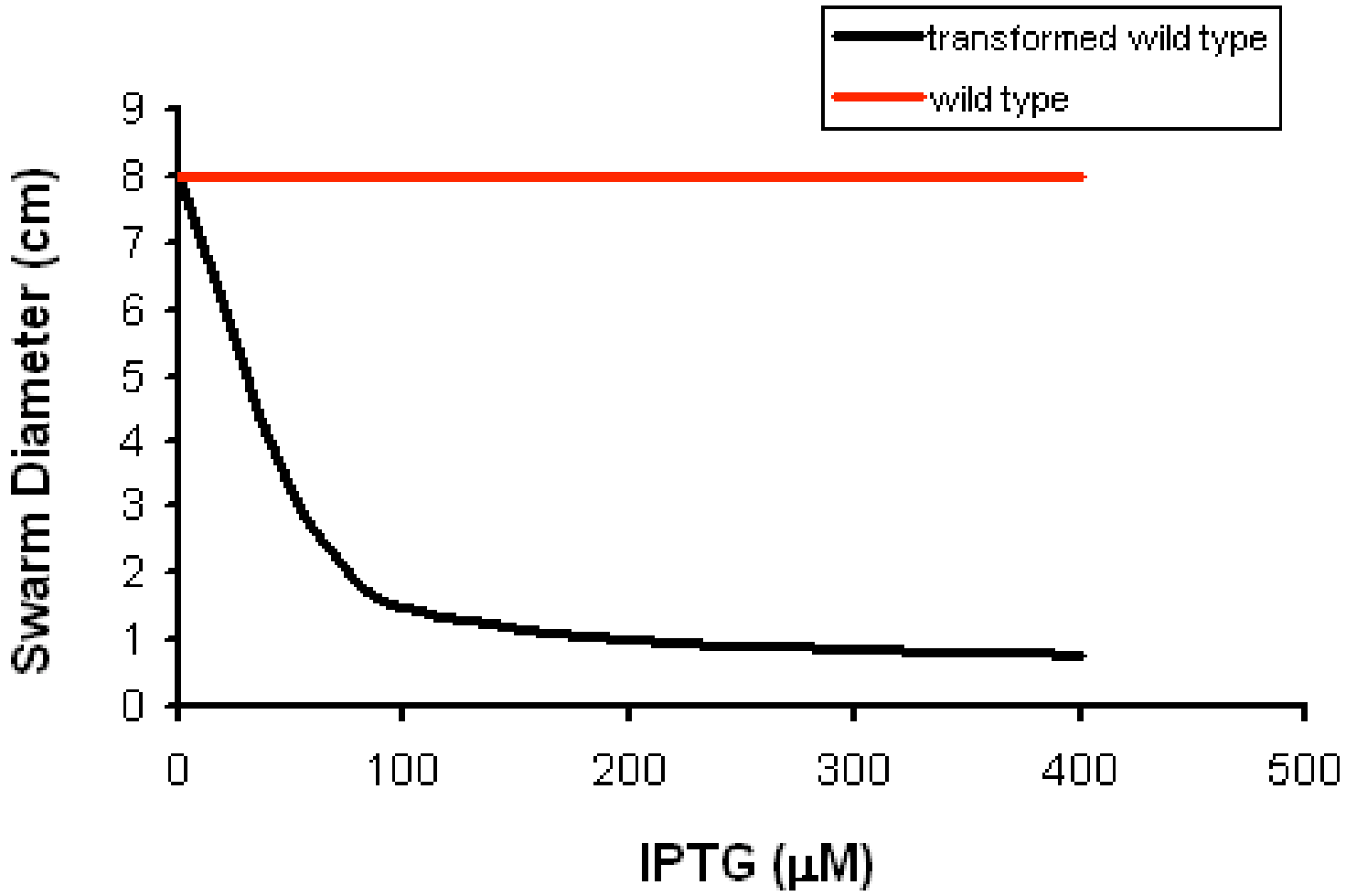


10ul of bacterial suspension

30ºC for duration of swarming

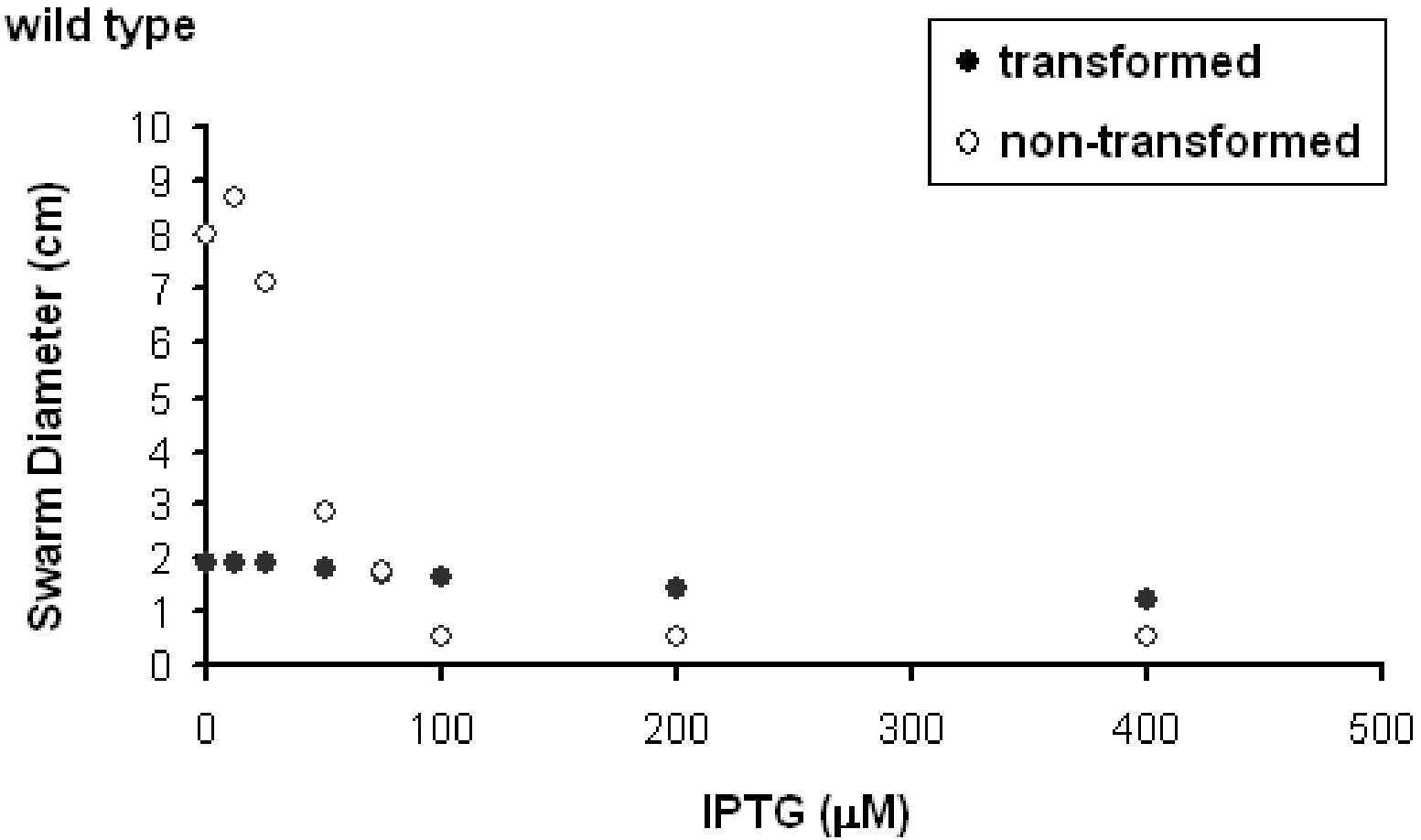
- Amino acids are bacterial chemoattractants
- Nutrient consumption produces gradient
- Ring formation on agar corresponding to particular amino acid/chemoattractant consumed by motile bacteria
- Movement away from the center (point of inoculation)

Deactivation: Expected Results



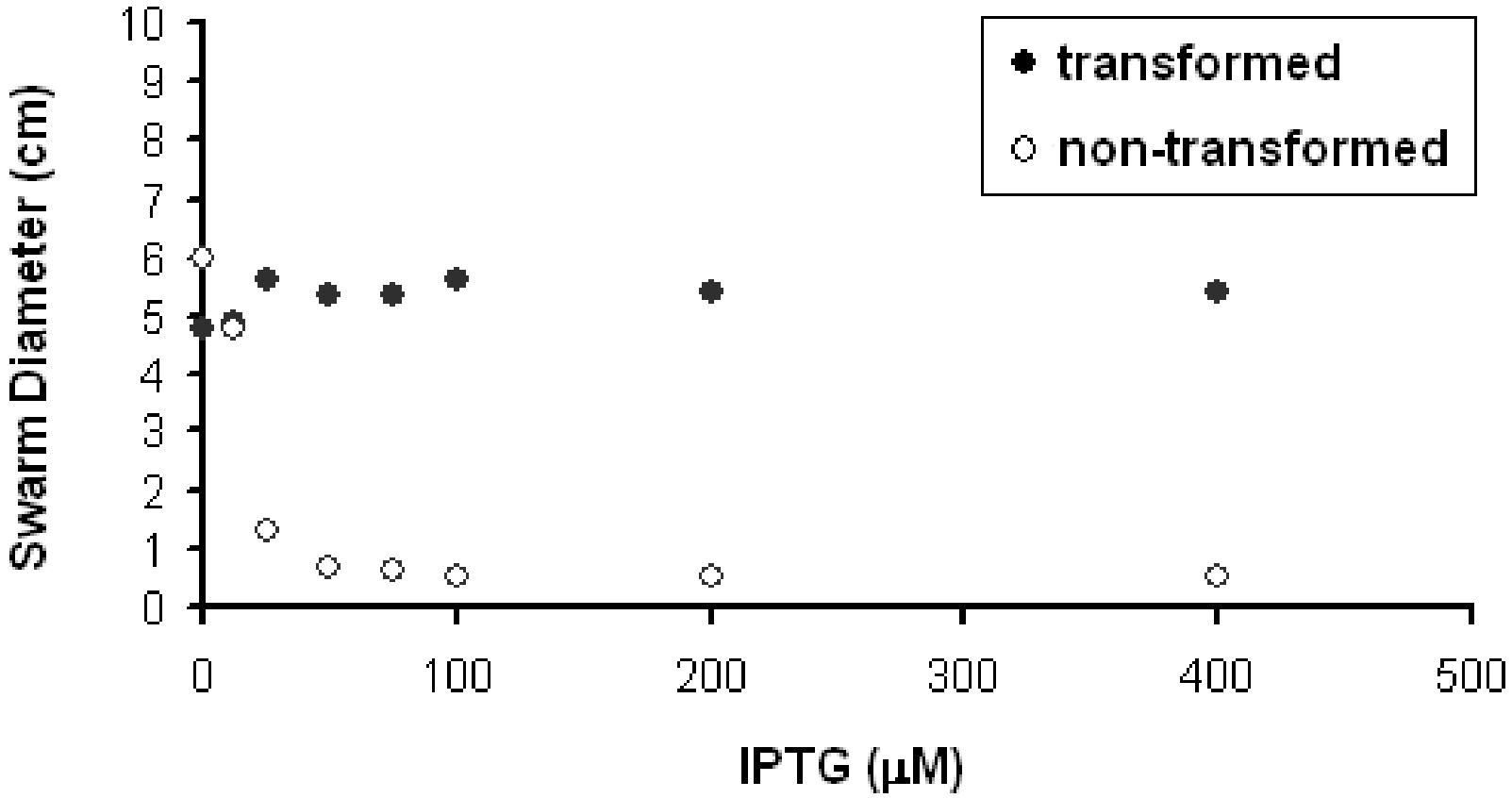
Deactivation: High Copy Results

High copy CheY expression in wild type



Deactivation: High Copy Results

High copy CheY expression in LacI- strain (wild type background)



Deactivation: Low Copy Results

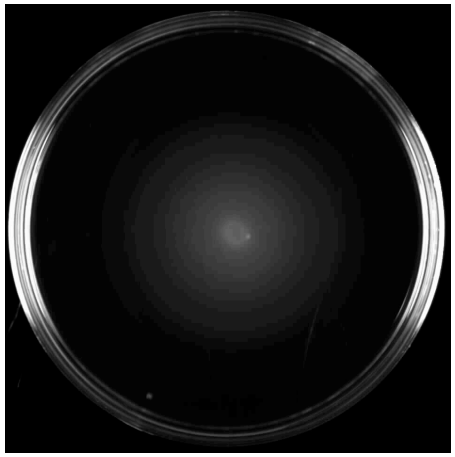
25μM IPTG induction for 2 hours, OD_{660} = 0.1

10ul spot, **16 hrs**

LacI$^-$

Expected:
Swarm

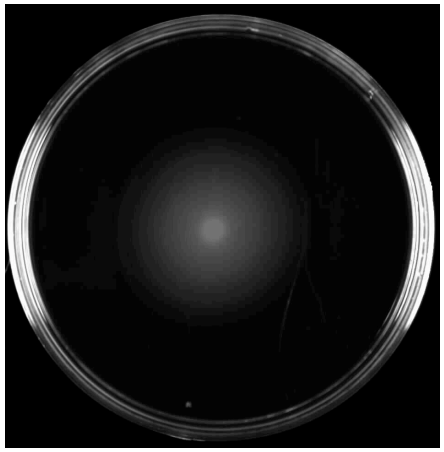
Observed:
Swarm



wild type

Expected:
Swarm

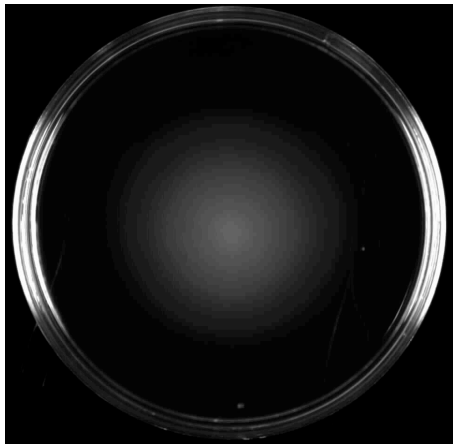
Observed:
Swarm



LacI$^-$ (transformed)

Expected:
Swarm/No rings

Observed:
Swarm/No rings



wild type (transformed)

Expected:
Swarm

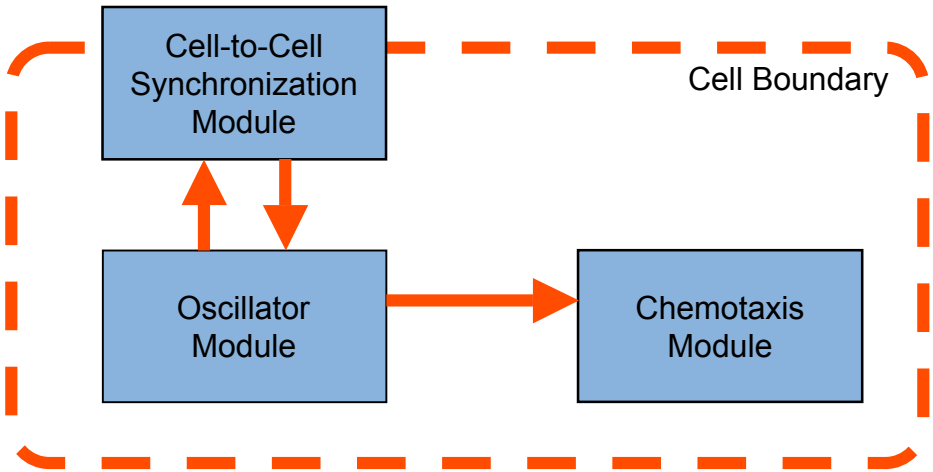
Observed:
No Swarm

Future Work

- More low copy construct (15-25 per cell) experiments
- Experimentation with ΔcheY mutants of E. coli RP437 for the restoration of motility
  - Requires the tuned expression of CheY
- Drop assay or coverslip assay to observe bacterial aggregation
  - Characterization of immediate chemotaxis response under varying levels of induction
- Coupling to population oscillator module and cell-cell signaling module

Module Integration

- Operating Conditions
  - Strain
  - pH
  - Temperature
  - Test setup
- Inter-Module Communication
  - Signal Interpretation
  - Timing



Op Conditions: Strain

Module	Known Requirements	Tested Conditions
Cell-Cell Signaling		MC4100, DH5alpha
Oscillator	LacI$^-$	MC4100
Chemotaxis	Chemotactic	RP437 (HCB33)

Future Plans

- Testing Cell-Cell signaling module in RP437
- Create LacI- version of RP437
- Test combined construct in LacI- version of RP437

Op Conditions: pH and Temp

Component	Known Requirements	Tested Conditions
Cell Cell Signaling	HSL stability is pH dependent	37° Celsius, pH 7
Oscillator		37° Celsius, pH 7
Chemotaxis	pH around 7, temperature around 30° Celsius	30° Celsius, pH 6.5-7.5

Future Plans

- Test combined system in pH7, 32° Celsius

Op Conditions: Test Setup

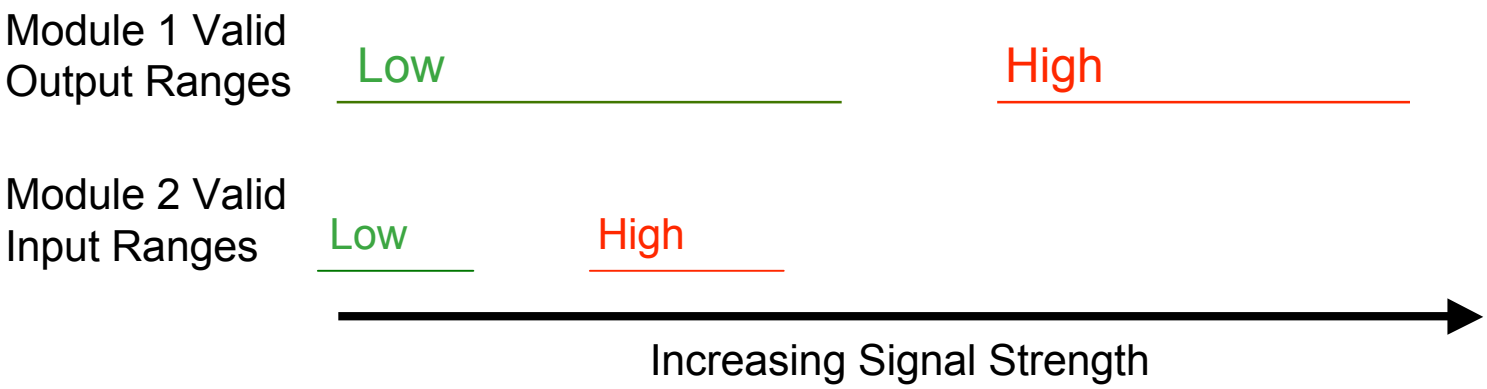
Component	Known Requirements	Tested conditions
Cell Cell Signaling		Culture tubes
Oscillator		Chemostat Agarose pads
Chemotaxis	Steady gradient of chemoattractants	Swarm plates

Future Plans

- **Plan I: Swimming Pool** for continuous observation
- **Plan II: Time Course Sampling** with time course test for chemotaxis

Inter-Module: Signal Interpretation

Signals given as a logical “1” or “0” output from one module must be interpreted as a logical “1” or “0’ input by the other modules



Future Plans

- Do signal strength characterization tests for modules
- If strengths don’t match, fine tune by swapping promoter/RBS

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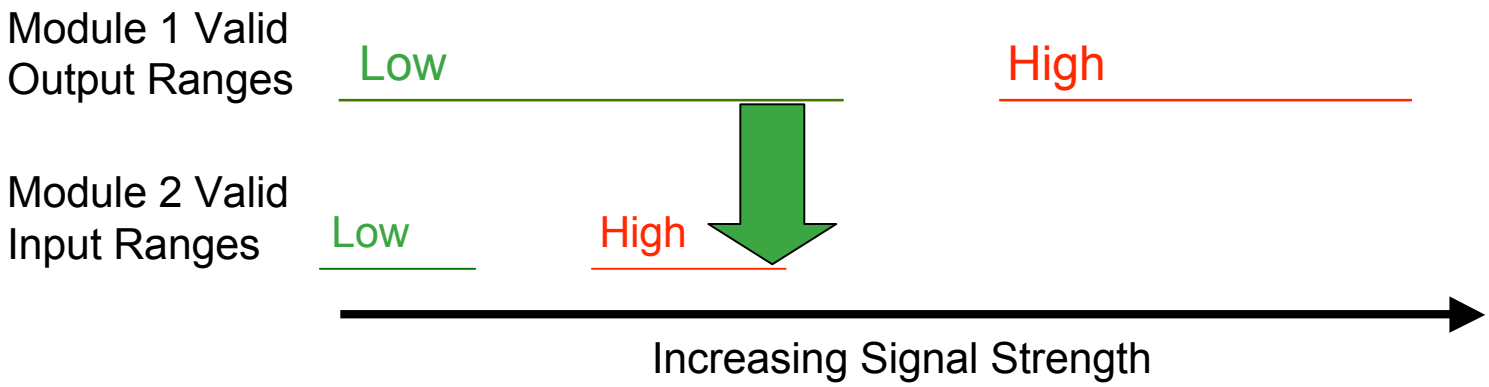

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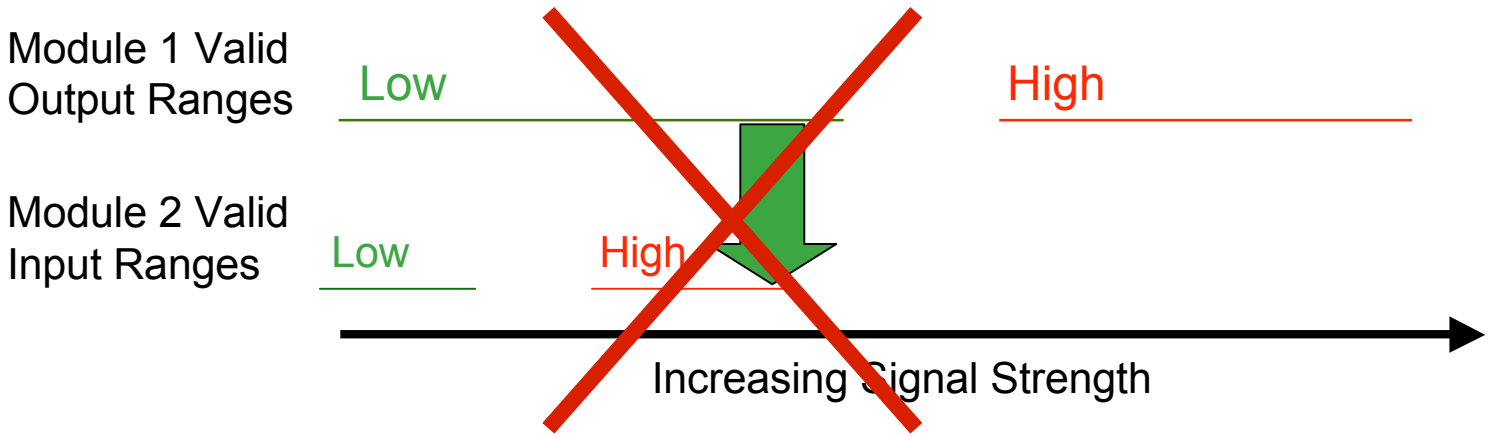


Future Plans

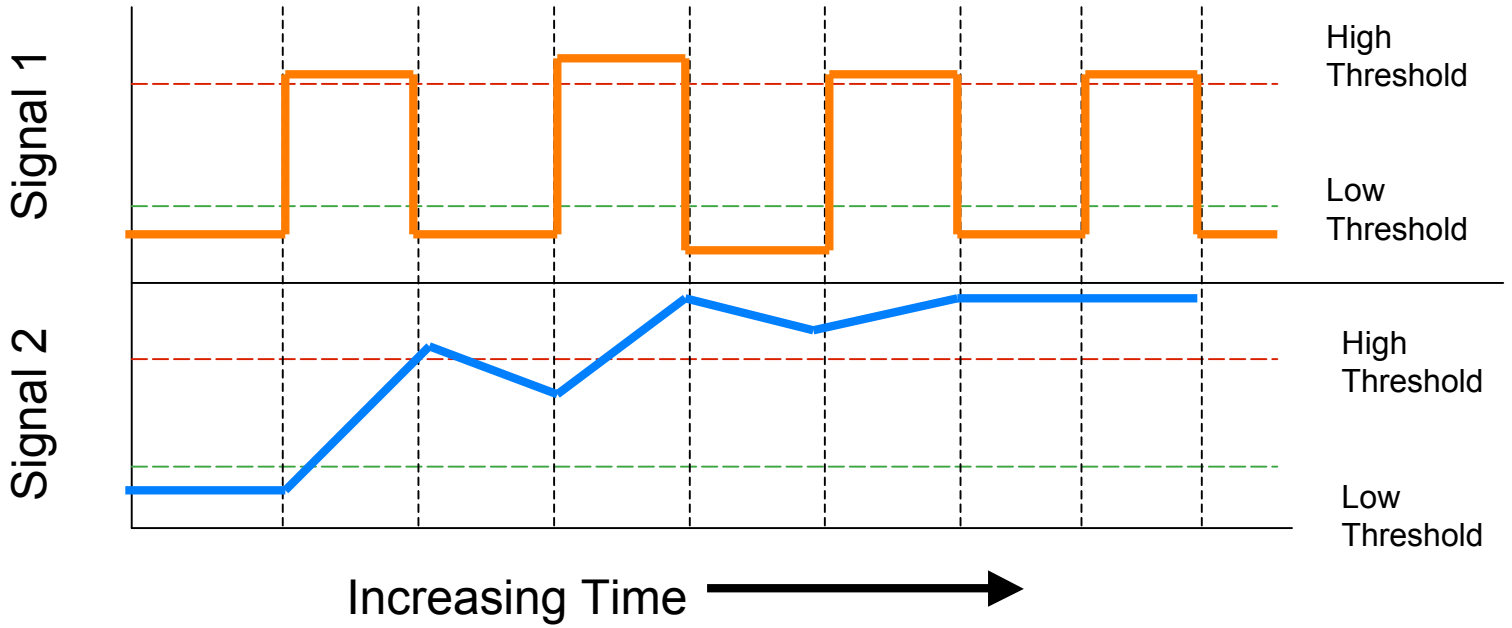
- Do signal strength characterization tests for modules
- If strengths don’t match, fine tune by swapping promoter/RBS

Inter-Module: Timing

Timing of oscillator signals must match up with timing of cell-cell signaling and chemotaxis modules

Signal 1: (Oscillator) Rise Time=0; Fall time=0; Period =2

Signal 2: (Follower) Rise Time=1; Fall time=2



Inter-Module: Timing

- Cell-to-Cell Signaling and Chemotaxis must be able to turn on and off in at **most** the amount of time it takes the Oscillator to turn on and off.
- Current Knowledge
  - Repressilator : Period ~2 hours
  - Cell-to-Cell and Chemotaxis On : transcription+protein action is **fast**
  - Cell-to-Cell and Chemotaxis Off : protein degradation/dilution is **slow**
- Future Plans
  - Try getting the HSL signals to degrade faster by operating at a higher pH (10) or in a chemostat
  - Characterize off times for chemotaxis module

Integration Summary

- Integration is HARD!
  - Operating Conditions
  - Inter-module Communication
- Still a lot of work to be done

Final Remarks

- Overview of summer accomplishments
- Advice for future summer competitions
- Key enablers for the field of synthetic biology
  - Assembly process
  - Device characterization
  - Standard operating conditions

Summer Accomplishments

- Over **200** new BioBrick parts added to the registry
- Device characterization
  - RBS measurements
  - Preliminary copy number measurements
  - Basic chemostat constructed and tested
  - Many inverter measurement constructs ready to be tested
- Cell-to-cell signaling module
  - Working Lux sender/receiver constructed with BioBrick parts
  - Characterizing receiver transfer curves
  - Verified importance of low-copy constructs
  - Characterization of cell-to-cell signaling channel

Summer Accomplishments

- Oscillator module
  - Modeling work on Lux/aiiA relaxation oscillator
  - Refined techniques for creating time-lapse movies
  - Verified repressilator ring oscillator
  - Tested previously designed Synchronators
  - New synchronized repressilator is built and ready for testing
- Chemotaxis module
  - Working swarm plate chemotaxis assay
  - Results on possibility of transcriptional control of chemotaxis
- Twelve synthetic biology students who are excited about the potential of this new field
- Five extremely frustrated advisors who are looking forward to a long winter vacation

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13273	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13053	I13277	I1466	I13026	I13114	I13657	I13920	I13606	S04002
I13721	I13200	I13279	I1468	I13027	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13280	P34060	I13028	I13611	I13665	I13922	I6401	S04010
S10000	I13202	I13283	P34160	I13029	I13613	I13667	I13930	I6402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13273	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13053	I13277	I1466	I13026	I13114	I13657	I13920	I13606	S04002
I13721	I13200	I13279	I1468	I13027	I13115	I13660	I13921	I13607	S04003
I13730	I13201	I13280	I13500	I13028	I13116	I13661	I13922	I16401	S04010
S10000	I13202	I13282	p24160	I13029	I13613	I13662	I13923	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

Over 200 Parts Added to Registry

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13273	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13053	I13277	I1466	I13026	I13114	I13657	I13920	I13606	S04002
I13721	I13200	I13279	I1468	I13027	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13284	I1469	I13028	I13661	I13666	I13922	I16401	S04010
S10000	I13202	I13293	P24160	I13029	I13613	I13667	I13939	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

166 Parts Began Assembly Process

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13273	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13039	I13277	I13916	I13026	I13114	I13655	I13920	I13606	S04002
I13721	I13200	I13279	I13917	I13027	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13280	I534060	I13028	I13611	I13665	I13922	I16401	S04010
S10000	I13202	I13282	I534160	I13029	I13612	I13666	I13939	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

68 Parts Completely Assembled

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13272	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13039	I13277	I13916	I13026	I13114	I13655	I13920	I13606	S04002
I13721	I13200	I13279	I1465	I1350	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13280	P34060	I13028	I13611	I13665	I13922	I16401	S04010
S10000	I13202	I13282	P34160	I13029	I13612	I13667	I13939	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

29 Parts Completely Working

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13272	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13039	I13277	I13916	I13026	I13114	I13662	I13907	I13606	S04002
I13721	I13200	I13279	I1306	I13027	I13115	I13664	I13931	I13607	S04003
I13730	I13201	I13280	P34060	I13028	I13611	I13665	I13922	I16401	S04010
S10000	I13202	I13282	P34160	I13029	I13613	I13667	I13939	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

13 Parts Canceled

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13272	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13039	I13277	I13916	I13026	I13114	I13657	I13920	I13606	S04002
I13721	I13200	I13279	I1466	I13027	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13280	P34060	I13028	I13611	I13665	I13922	I16401	S04010
S10000	I13202	I13282	P34160	I13029	I13612	I13667	I13939	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

69 Parts for Cell-to-Cell Module

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13272	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13656	I13906	I13605	S04001
I13712	I13039	I13277	I1466	I13026	I13114	I13657	I13920	I13606	S04002
I13721	I13200	I13279	I1467	I13027	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13280	I1468	I13028	I13611	I13665	I13922	I16401	S04010
S10000	I13202	I13282	P24164	I13029	I13613	I13667	I13939	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

22 Parts for Oscillator Module

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13272	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13039	I13277	I13916	I13026	I13114	I13663	I13920	I13606	S04002
I13721	I13200	I13279	I13917	I13027	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13280	P34060	I13028	I13611	I13665	I13922	I16401	S04010
S10000	I13202	I13282	P34160	I13029	I13612	I13667	I13939	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

11 Parts for Chemotaxis Module

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13272	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13039	I13277	I1466	I13026	I13114	I13657	I13920	I13606	S04002
I13721	I13200	I13279	I1468	I13027	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13280	P34060	I13028	I13611	I13665	I13922	I16401	S04010
S10000	I13202	I13281	C0062	I13029	I13612	I13666	I13923	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

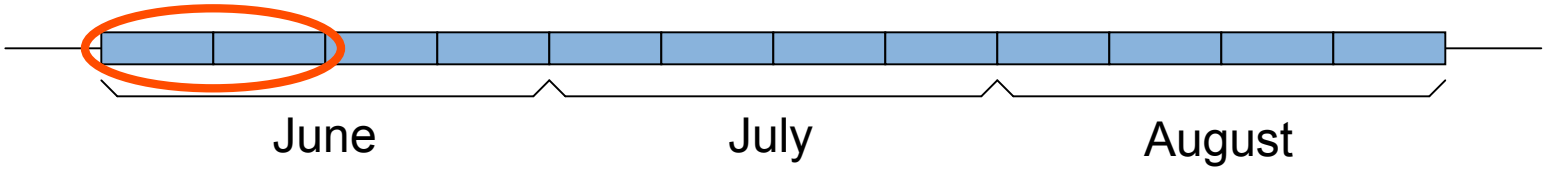
63 Parts for Device Characterization

BioBrick Parts

C0020	I13000	I13263	I13313	I13014	I13106	I13634	I13209	E0130	P0474
C0024	I13015	I13264	I13314	I13019	I13107	I13637	I13210	E0669	Q00121
C0028	I13018	I13265	I13910	I13020	I13108	I13644	I13900	I13600	Q00400
I13700	I13034	I13266	I13911	I13021	I13109	I13645	I13901	I13601	Q02121
I13701	I13035	I13270	I13912	I13022	I13110	I13647	I13902	I13602	Q02400
I13702	I13036	I13271	I13913	I13023	I13111	I13651	I13904	I13603	Q04740
I13710	I13037	I13272	I13914	I13024	I13112	I13653	I13905	I13604	S04000
I13711	I13038	I13274	I13915	I13025	I13113	I13654	I13906	I13605	S04001
I13712	I13039	I13277	I1466	I13026	I13114	I13657	I13920	I13606	S04002
I13721	I13200	I13278	I1468	I13027	I13115	I13664	I13921	I13607	S04003
I13730	I13201	I13279	I13029	I13028	I13611	I13666	I13922	I16401	S04010
S10000	I13202	I13282	I534160	I13029	I13613	I13667	I13939	I16402	I13990
G00700	I13203	I13303	I534060	I13030	I13614	I13007	I13940	I13017	
G00701	I13205	I13304	I534160	I13031	I13617	I13008	I13941	I13062	
I13001	I13206	I13305	I13207	I13032	I13621	I13009	I13942	I13072	
I13002	I13208	I13306	C0063	I13033	I13623	I13010	I13943	I13800	
I13002	I13211	I13307	C0163	I13100	I13624	I13011	I13950	I13801	
I13004	I13212	I13308	C0260	I13101	I13625	I13012	I13951	I13850	
I13005	I13213	I13309	C0261	I13102	I13626	I13013	I13974	I13851	
I0466	I13220	I13310	I0463	I13103	I13627	I13006	I13975	I13971	
I0467	I13261	I13311	I0464	I13104	I13631	C0056	I13976	I13972	
I0468	I13262	I13312	I0465	I13105	I13633	I13016	I13977	I13973	

12 New Reporter Parts

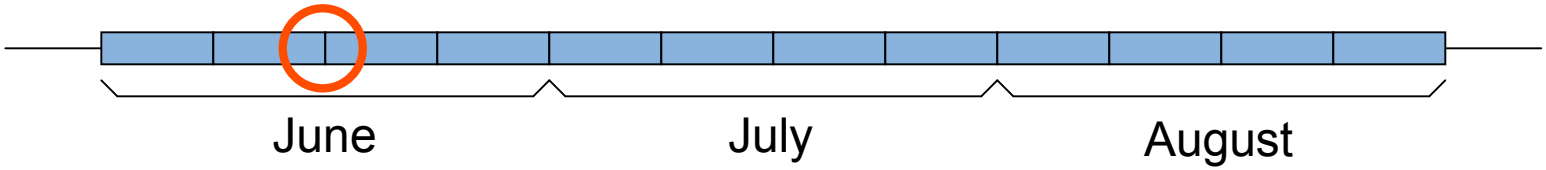
Advice for Future Competitions



Structured introductory two-week curriculum

- Daily lectures in the mornings and specific lab tutorials in the afternoons
- Students would **model**, **assemble**, and **characterize** a simple synthetic system such as a single OR gate
- Teaches synthetic biology basics and experimental lab technique as well as providing a solid foundation for initial design work

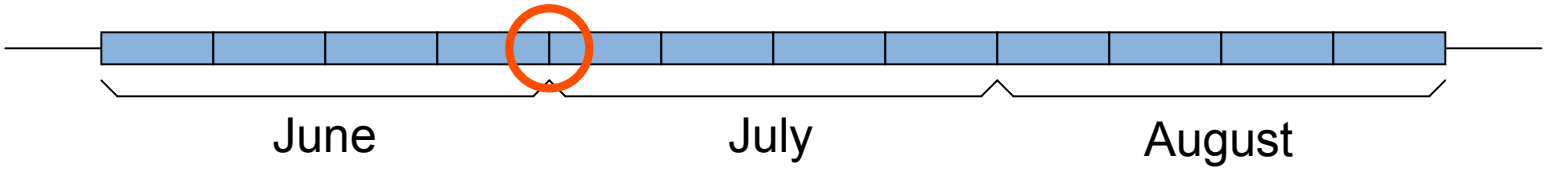
Advice for Future Competitions



More milestones and incremental deliverables

– Report on simple synthetic system

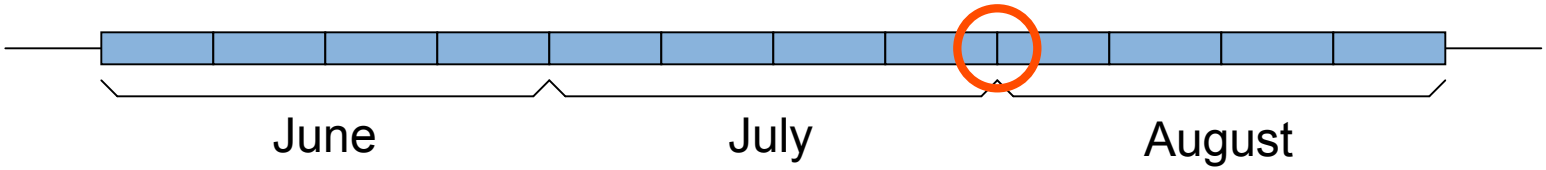
Advice for Future Competitions



More milestones and incremental deliverables

- Report on simple synthetic system
- Preliminary design specification

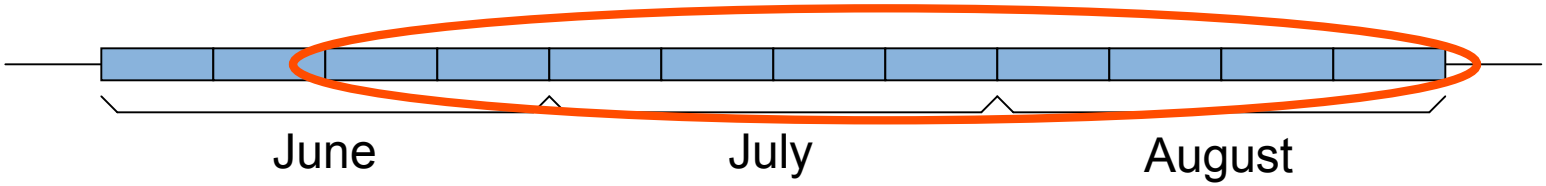
Advice for Future Competitions



More milestones and incremental deliverables

- Report on simple synthetic system
- Preliminary design specification
- Interim progress report

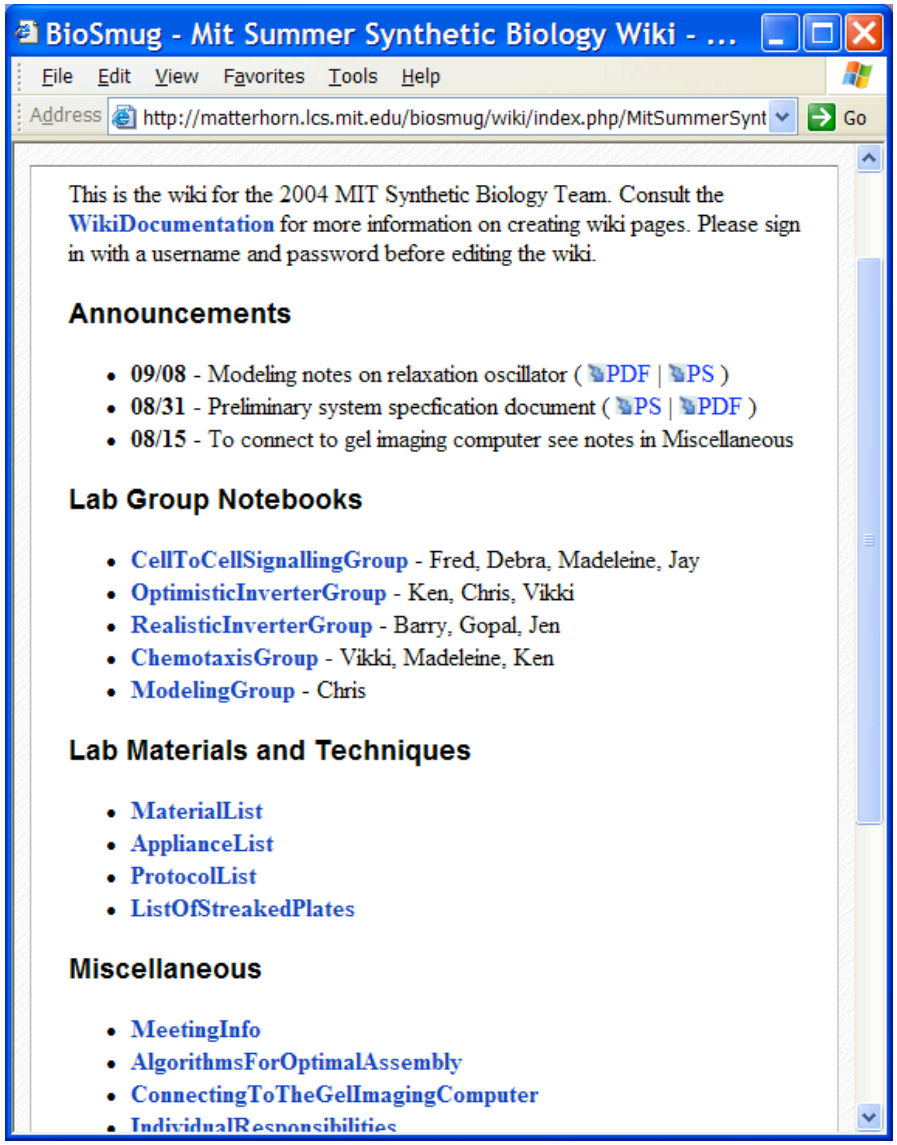
Advice for Future Competitions



More milestones and incremental deliverables

- Report on simple synthetic system
- Preliminary design specification
- Interim progress report
- Periodic logs kept by each student and lab-group

Advice for Future Competitions

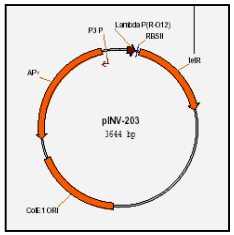


Inter-team collaboration

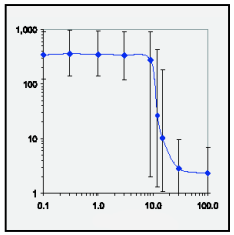
- Periodic conference calls
- Distribute design specifications and interim reports to all teams
- Logs managed in online forum accessible by all teams

Key Enablers for SynthBio

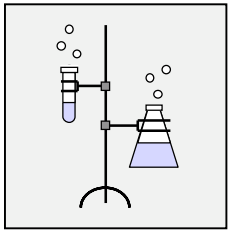
Through our summer experiences we identified three key enablers which will greatly help future work in the field of synthetic biology



Assembly Process

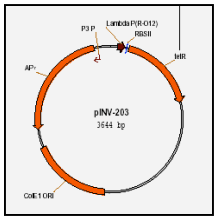


Device Characterization



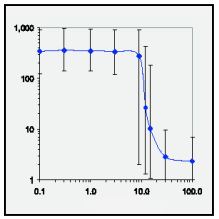
Standard Operating Conditions

Assembly Process



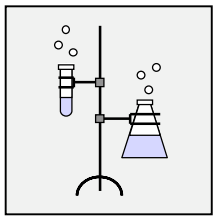
- Remarkable that a group of students with very little biology background was able to build working biological parts relatively quickly
- Even so, current assembly process placed significant constraints on what was possible
  - Took on average one week per stage
  - When assembly failed very difficult to determine why
- Assembly is an important research topic
  - Optimize each stage
  - Characterize and model error rates
  - Develop more assembly tools

Device Characterization



- Modeling work is significantly hampered by lack of useful device characterization
- Device characterization is challenging
  - What do we actually measure?
  - How do we measure it?
  - How do we make measurements repeatable?
- Accurate device characterization will enable
  - Effective parameterized models
  - More rational design
  - Easier reuse of previously developed parts

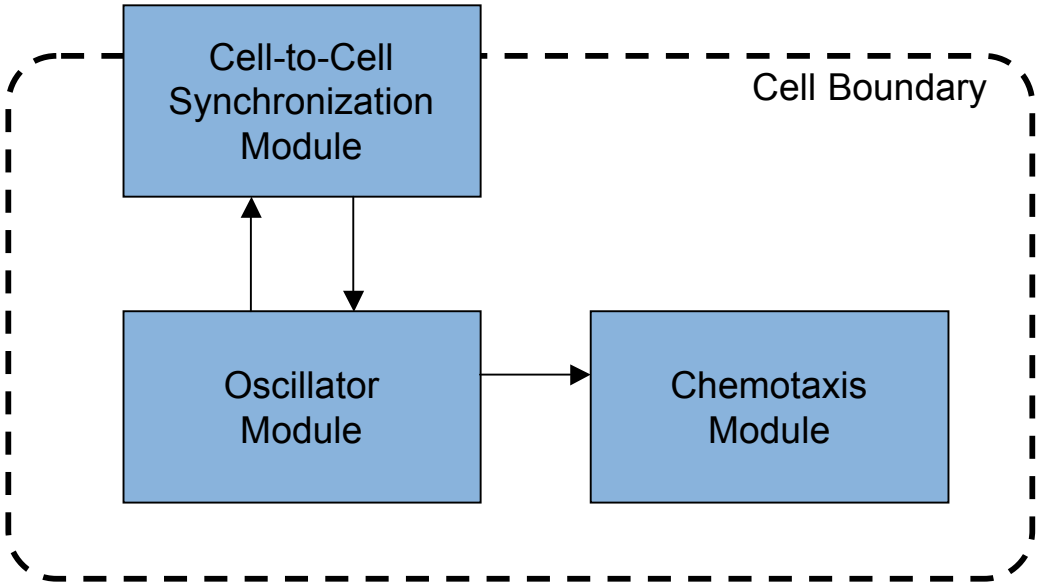
Operating Conditions



- Currently no standard operating conditions
  - Strain, media, growth phase are usually documented but they still vary with each experiment
  - Standard conditions enable easier result comparisons
  - Standard operation conditions also make it easier to predict how future systems will behave
- Standard operating conditions is challenging
  - Difficult to choose a single set of conditions since different experiments have different requirements
  - Continuous culture using chemostat is an attractive possibility but needs more work

Conclusions

- We have designed and made strong progress towards building a **synchronized chemotactic oscillator**
- The **assembly process**, **device characterization**, and **standard operating conditions** are key enablers which will greatly benefit the field of synthetic biology



Conclusions

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- The **assembly process**, **device characterization**, and **standard operating conditions** are key enablers which will greatly benefit the field of synthetic biology