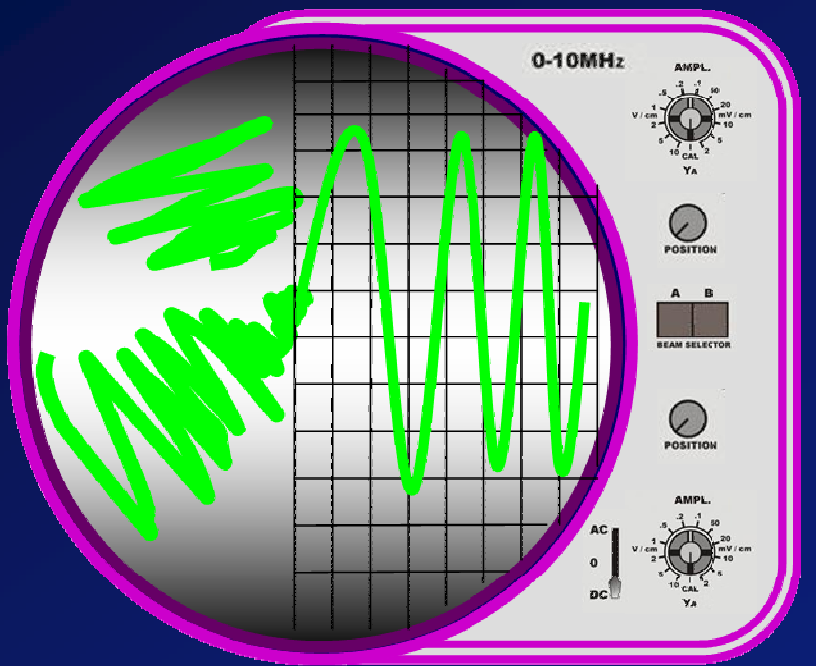


Engineering a Molecular Predation Oscillator

iGEM 2006 @ Imperial

Biomedical Engineers

Electrical Engineer

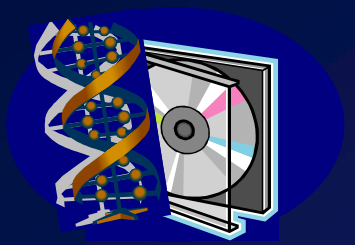
Biochemist

Biologists

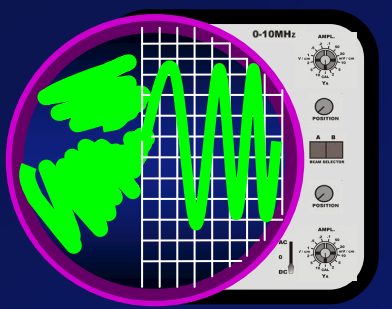
Biomedical Engineers

Biochemists

Project Ideas



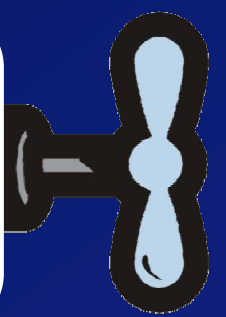
Bio-Memory



Oscillator

Bio-Clock

- Feasibility
- Originality of Design
- BioBrick Availability
- Future Impact

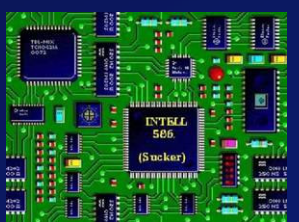


What is an Oscillator?

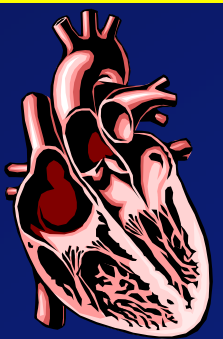
Our Definition

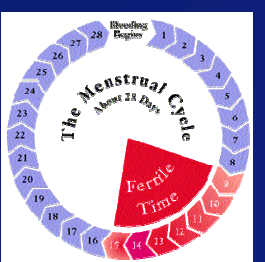
Device producing a periodic variation in time of a measurable quantity, e.g. amplitude.

Engineering



Biology

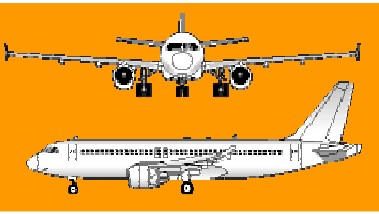




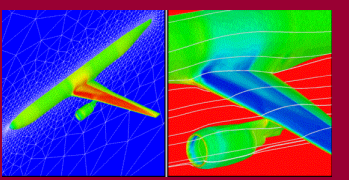
The Engineering Approach

Specifications

Design



Modelling



Implementation

Testing/Validation

Stand

Certified

ctice

The Main Challenges

Main challenges of past oscillators:

- Unstable
- Noisy
- Inflexible

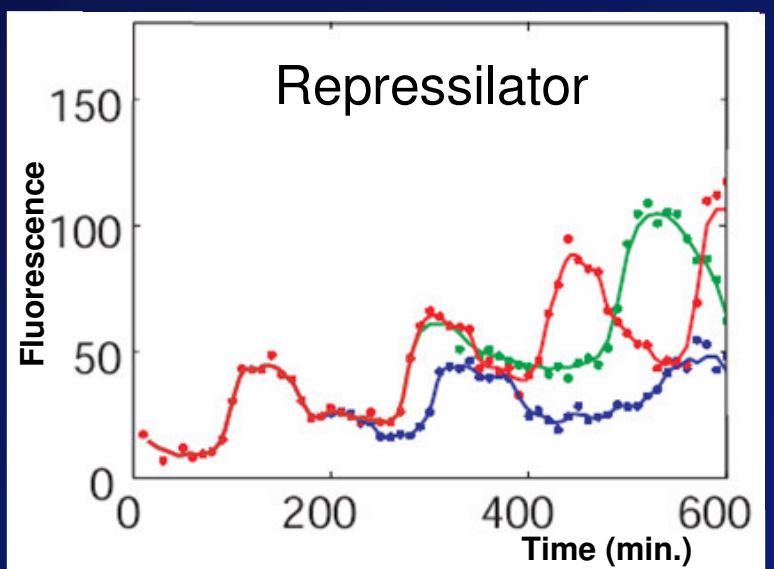


Figure Reference : Michael B. Elowitz & Stanislas Leibler *Nature* 2000

Requirement for a typical engineering oscillator

- Sustained Oscillations
- High Signal to Noise Ratio
- Controllable Oscillations
- Standardized Device for Easy Connectivity

The Main Challenges

Main challenges of past oscillators:

- Unstable
- Noisy
- Inflexible

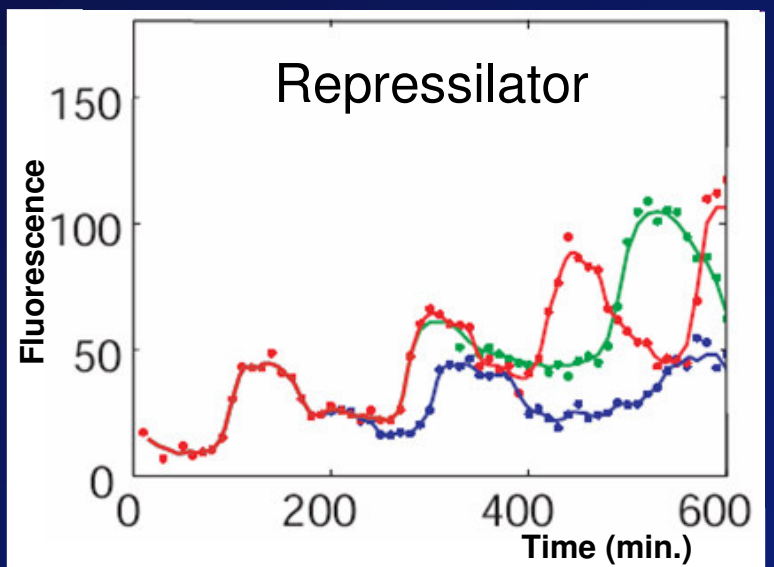


Figure Reference : Michael B. Elowitz & Stanislas Leibler *Nature* 2000

Our Specifications:

- Stability: >10 periods
- SNR: High
- Flexibility: Controllable Amplitude and Frequency
- Modular Design
- Easy Connectivity

Our Initial Design Ideas

Based on

- Large populations of molecules to reduce influence of noise
- Oscillations due to population dynamics
- A well characterized model

Molecular Predator - Prey

The Lotka-Volterra Model

$$\frac{dX}{dt} = aX - bXY$$

$$\frac{dY}{dt} = cXY - dY$$

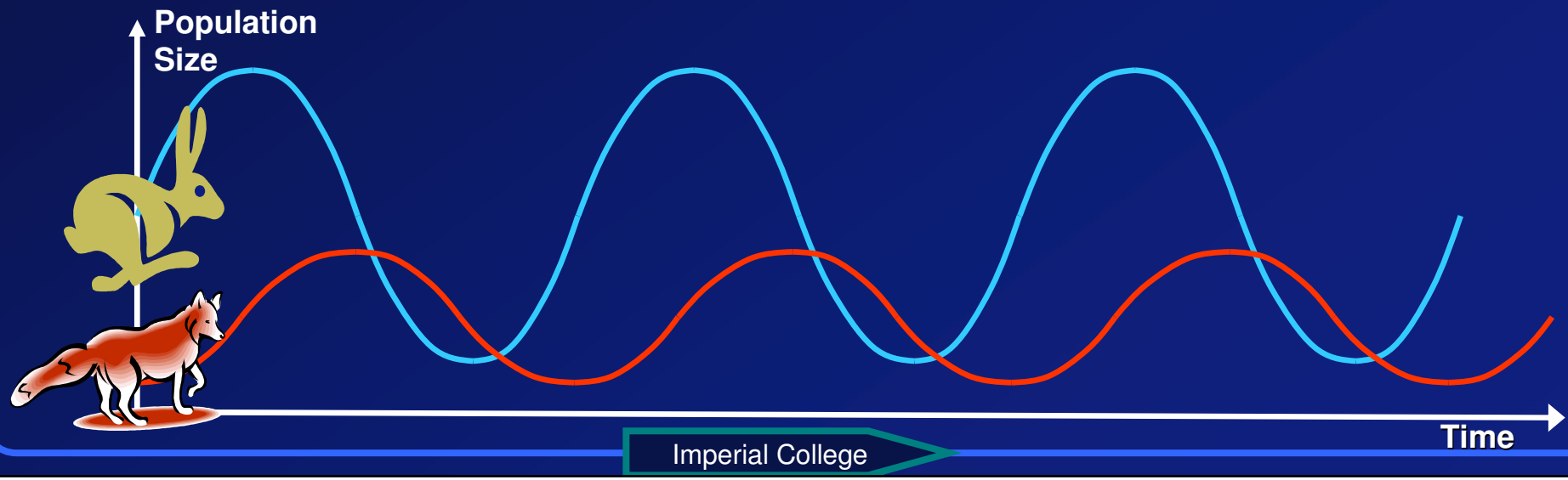
X : *Prey*

Y : *Predator*

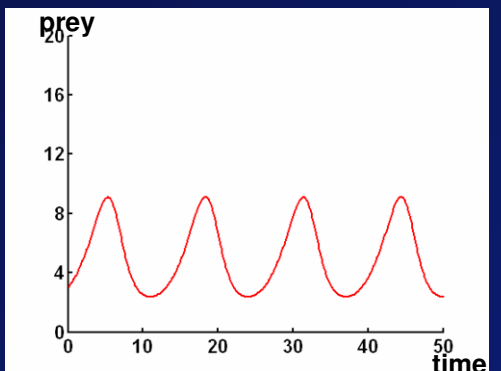
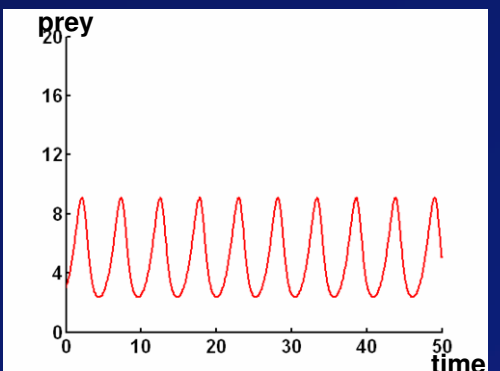
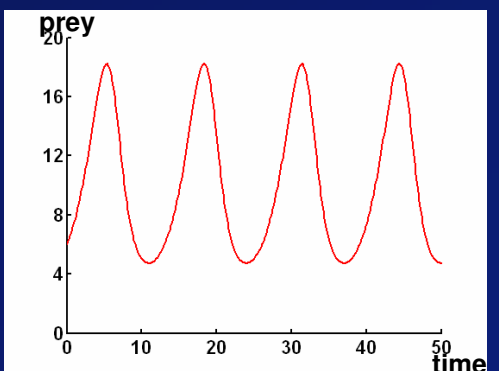
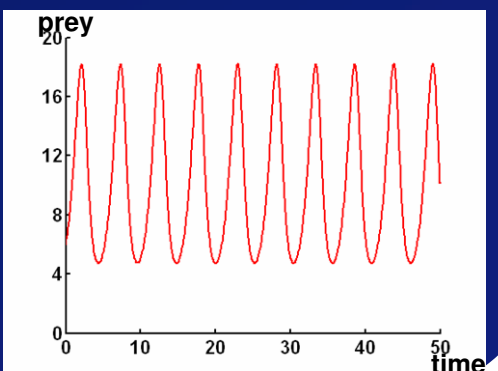
The Lotka-Volterra Model

$$\frac{d\,\text{rabbit}}{dt} = \text{Prey Growth} - \text{Prey Killing by Predator}$$

$$\frac{d\,\text{fox}}{dt} = \text{Predator Growth} - \text{Predator Death}$$



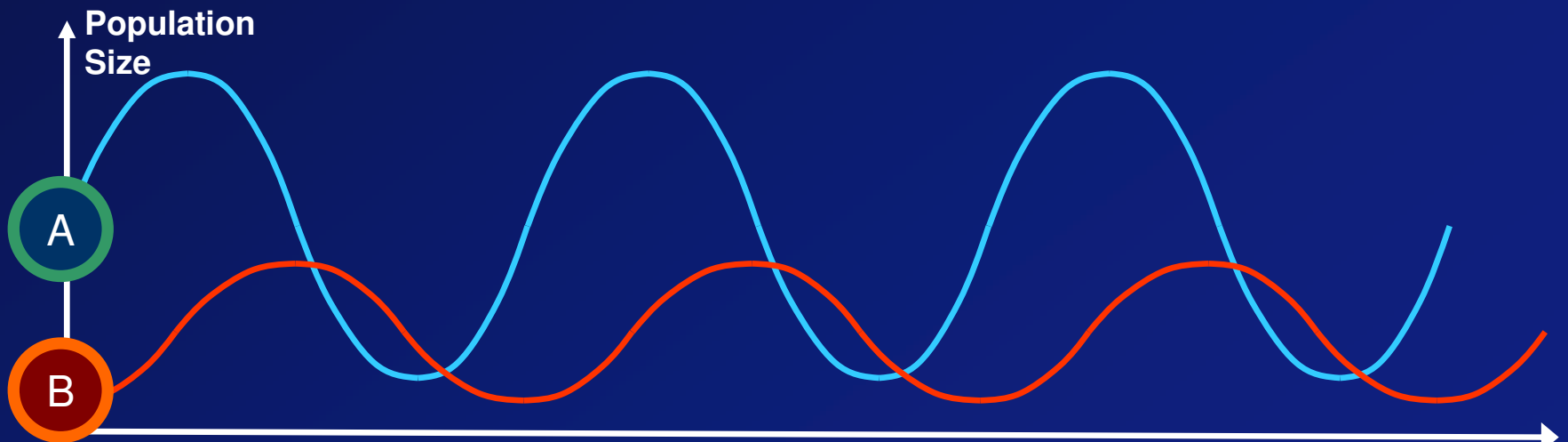
Typical LV Simulations

Graph of Prey vs. Time	Low Frequency	High Frequency
Small Amplitude		
Large Amplitude		

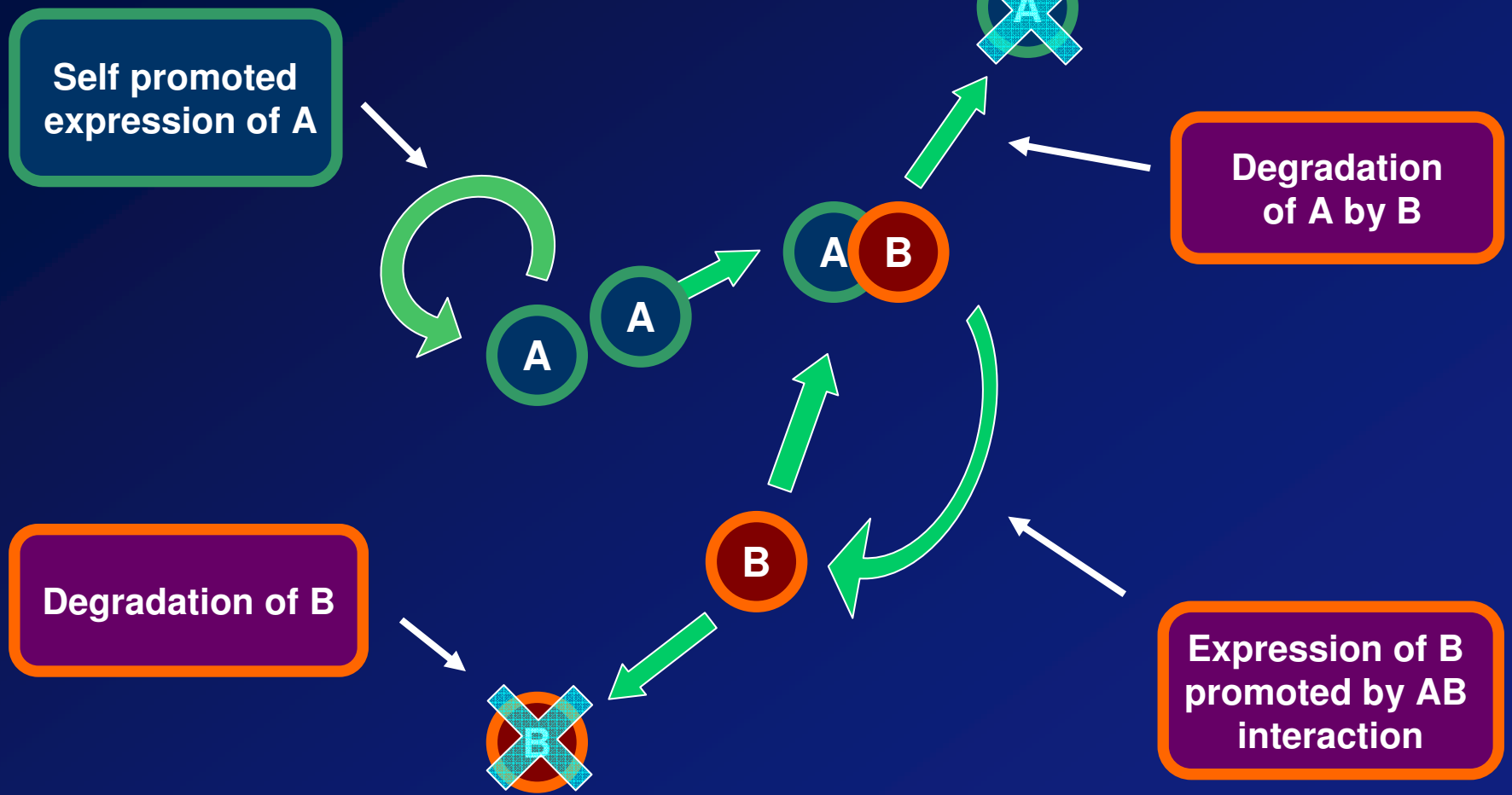
Required Biochemical Properties

$$\frac{d[A]}{dt} = \text{Self promoted expression of A} - \text{Degradation of A by B}$$

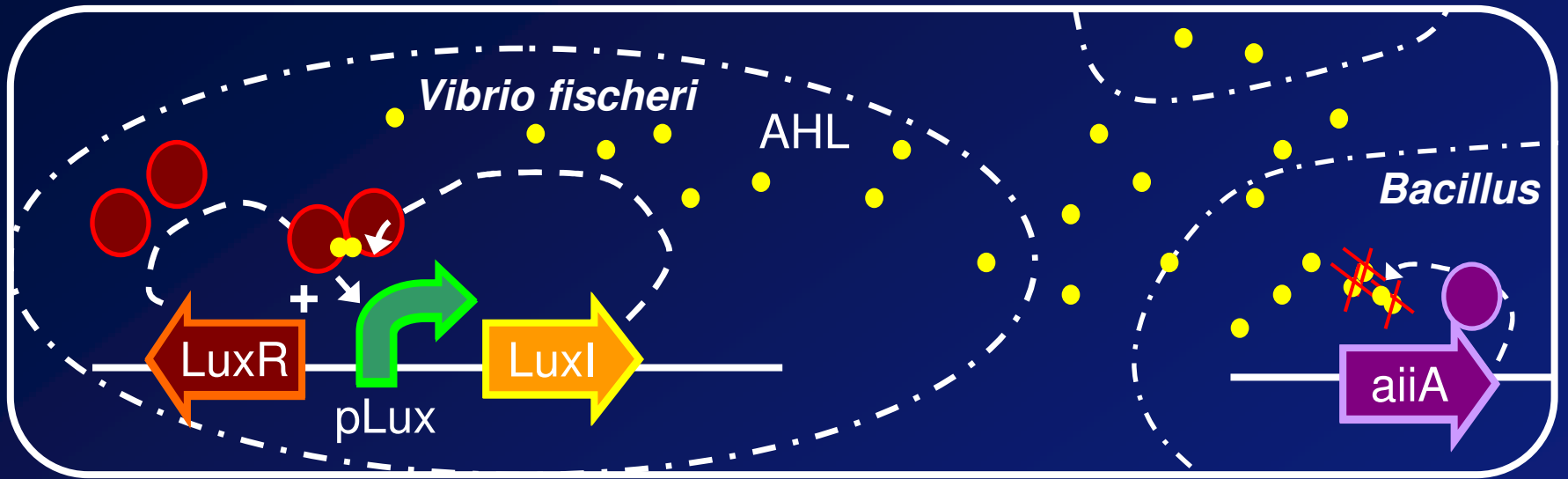
$$\frac{d[B]}{dt} = \text{Expression of B promoted by AB interaction} - \text{Degradation of B}$$



Molecular System



Quorum sensing/quenching



BioBricks available

C0062	Forms a complex with AHL to activate pLux	C0061	Makes AHL
pLux R0062	pLux Promoter	C0160	Degrades AHL
 F2620		AHL->Pops Receiver	

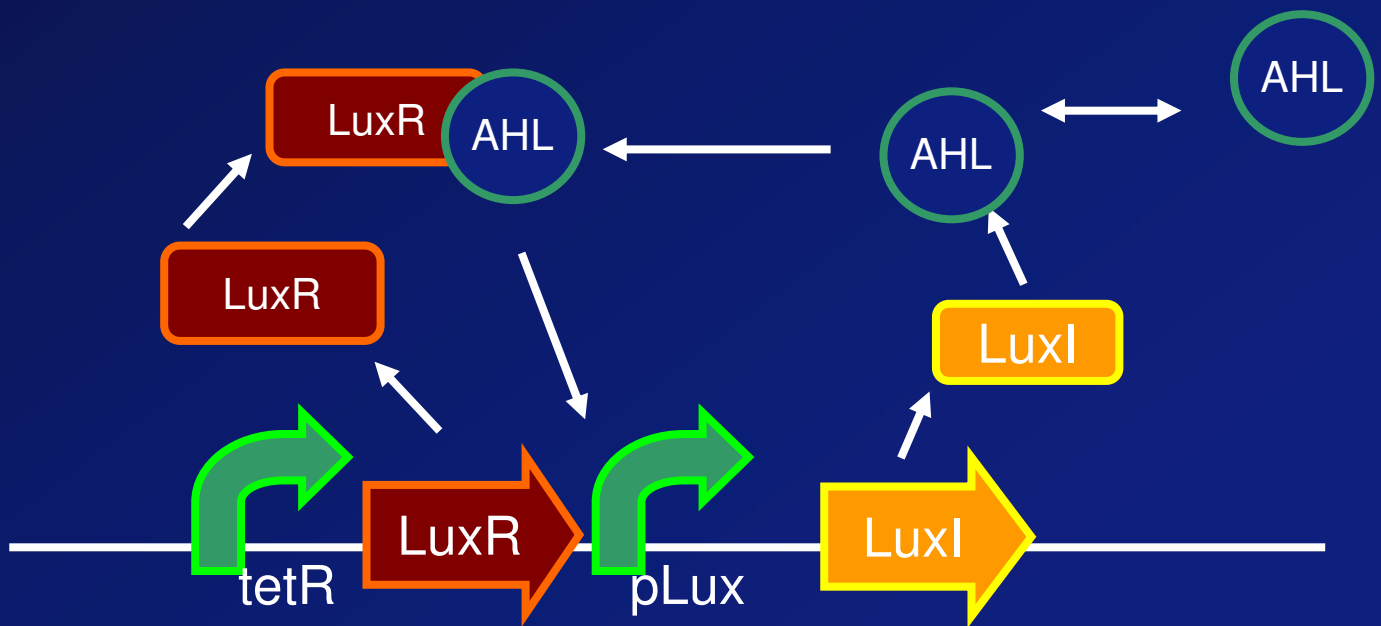
Designing the Prey Generator

Required Dynamic

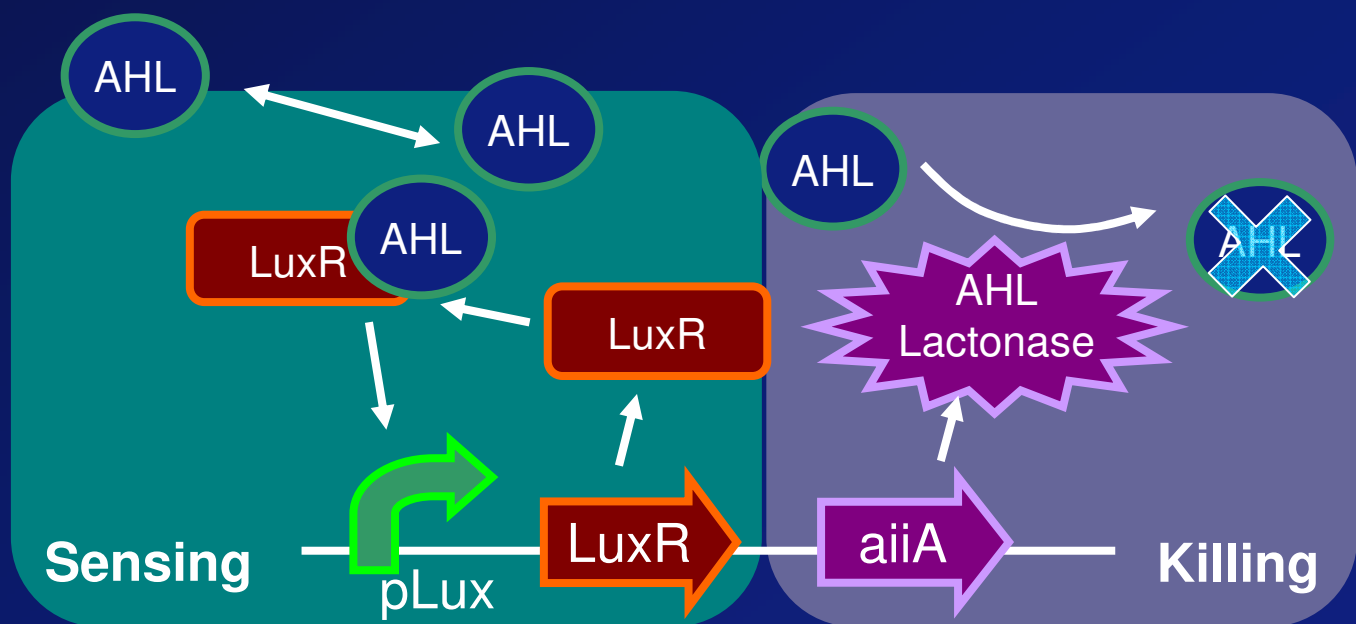
Self promoted expression of A ✓

Useful BioBricks

Final Construct

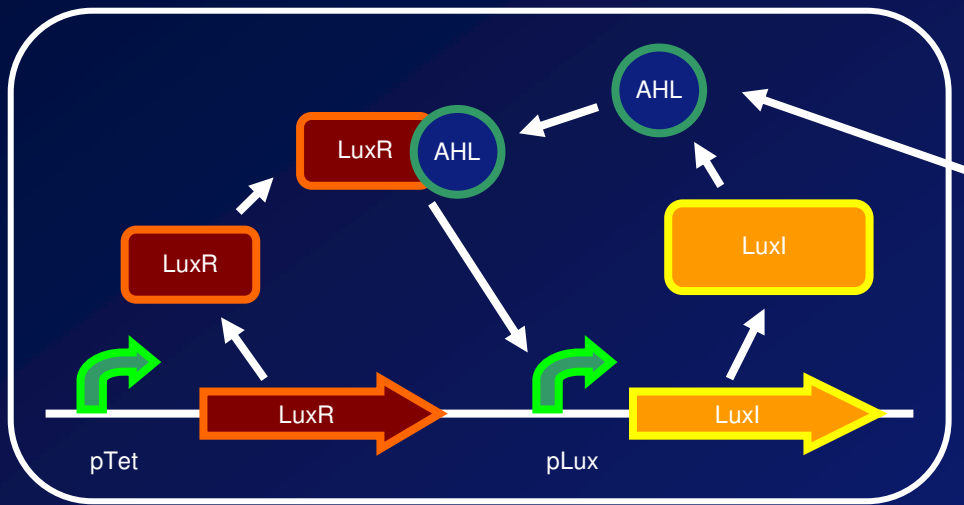


Designing the Predator Generator

Required Dynamic	**Expression of B promoted by AB interaction** ✓	**Degradation of A by B** ✓	**Degradation of B** ✓
Useful BioBricks	pLux R0062; LuxR C0062	aiiA C0160	Natural degradation
Final Construct			

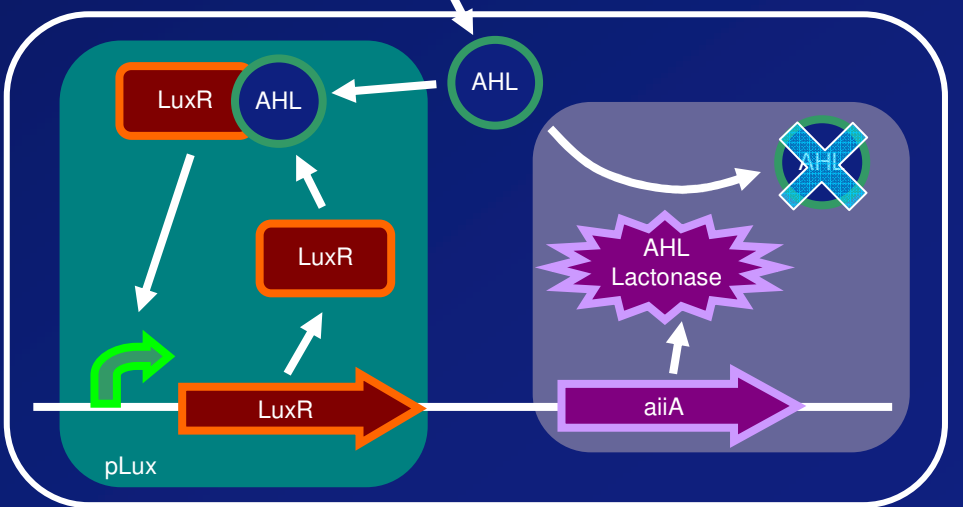
System Overview

Prey Generator

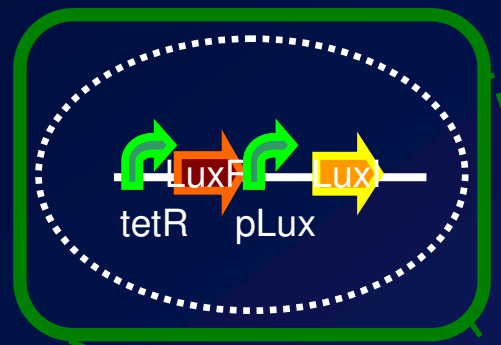


Pool of AHL will oscillate

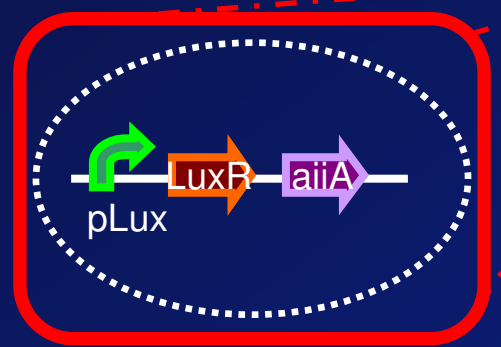
Predator Generator



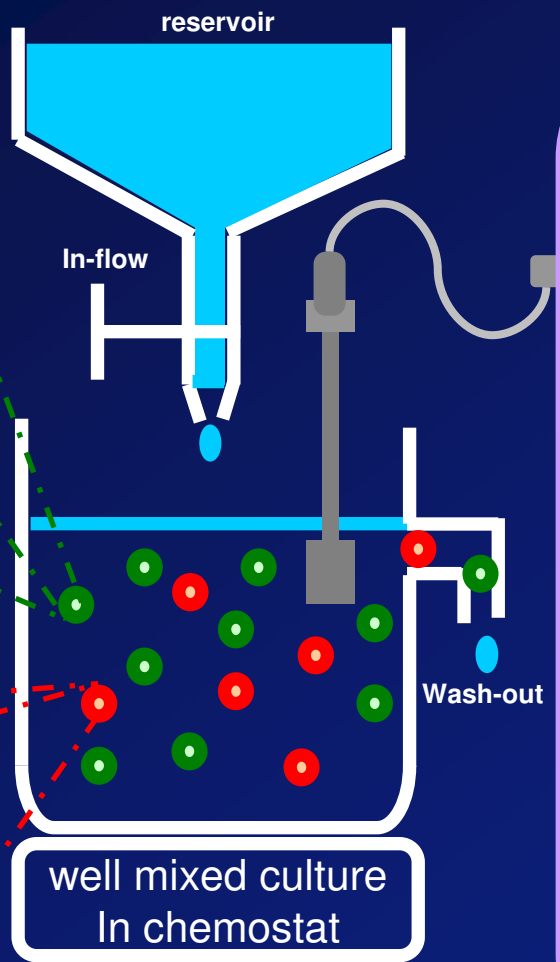
Full System set-up

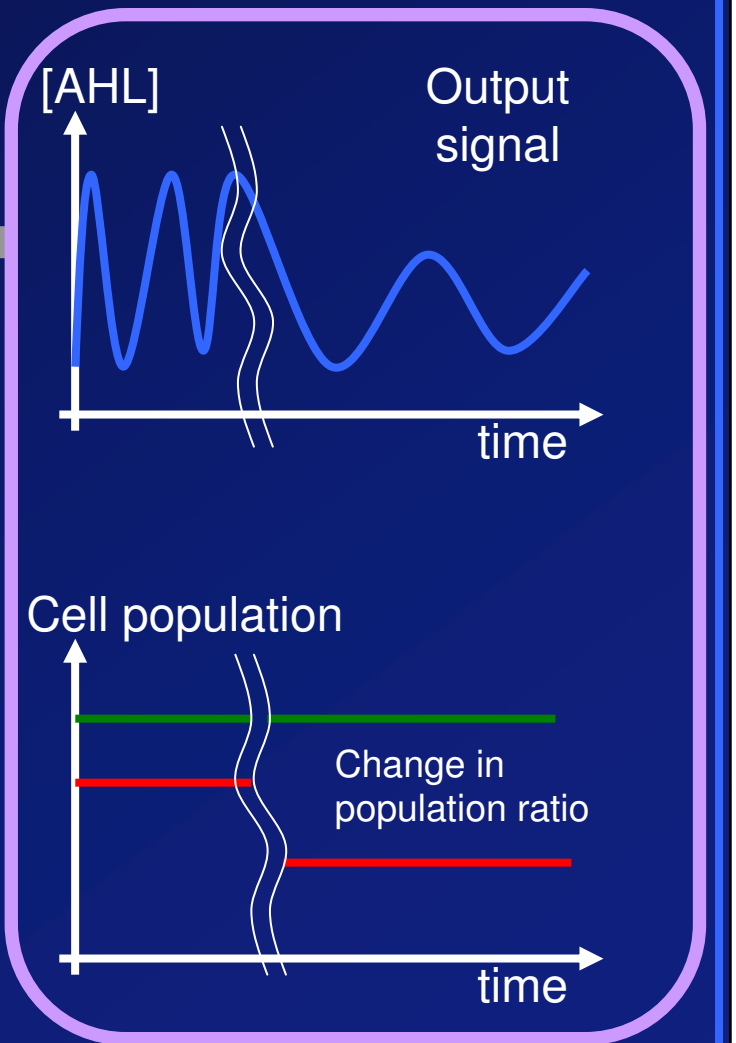


Prey molecule generator

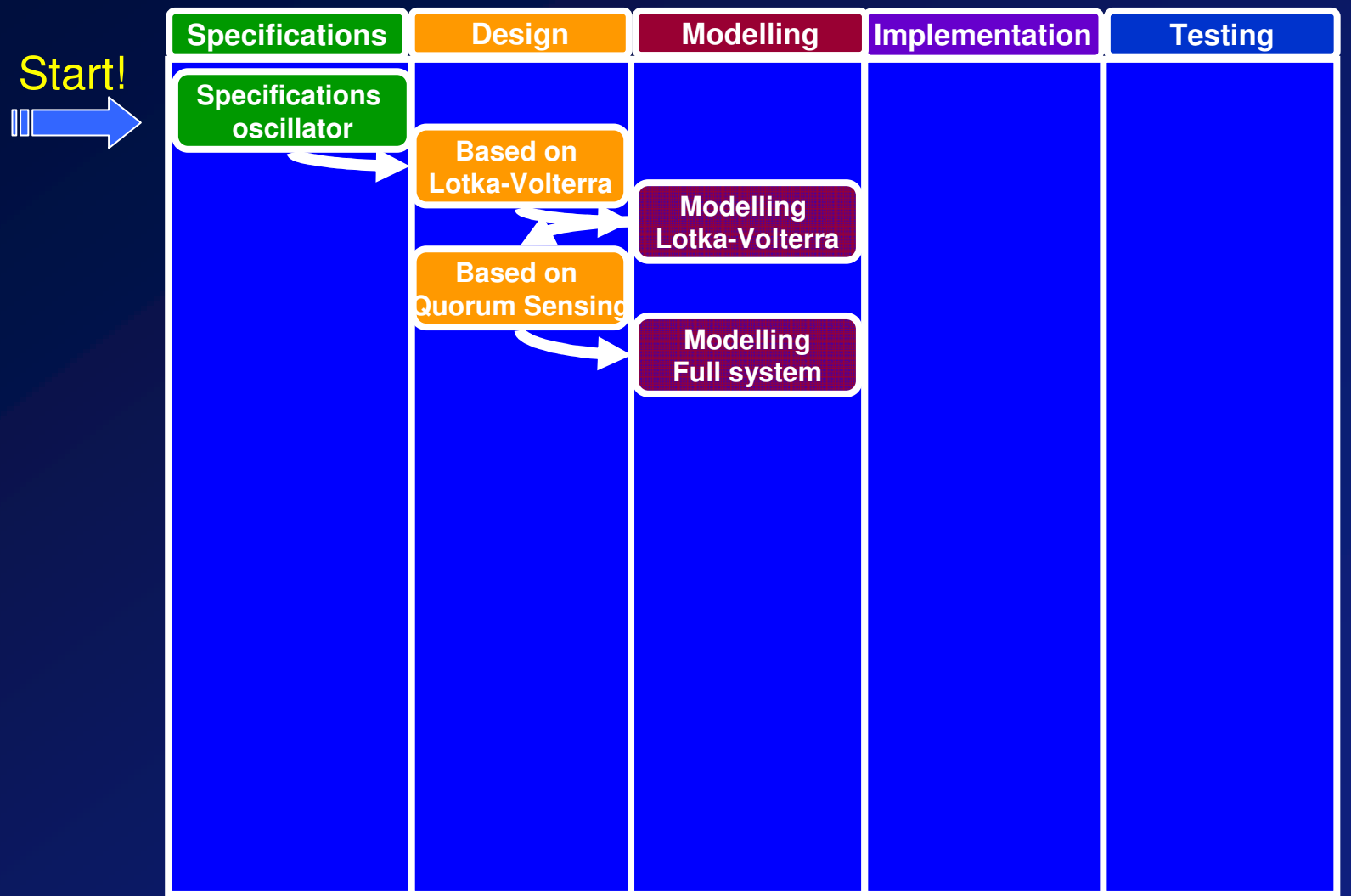


Predator molecule generator





Path to Our Goal



Modelling the Full System

$$\frac{d[\text{AHL}]}{dt} = \text{Self promoted expression of AHL} - \text{Degradation of AHL by aiiA} - \text{Degradation of AHL}$$

$$\frac{d[\text{LuxR}]}{dt} = \text{Expression of LuxR} - \text{Degradation of LuxR}$$

$$\frac{d[\text{aiiA}]}{dt} = \text{Expression of aiiA} - \text{Degradation of aiiA}$$

Modelling the Full System

$$\frac{d[AHL]}{dt} = \text{Self promoted expression of AHL} - \text{Degradation of AHL by aiiA} - \text{Degradation of AHL}$$

$$\frac{d[LuxR]}{dt} = \text{Expression of LuxR} - \text{Degradation of LuxR}$$

$$\frac{d[aiiA]}{dt} = \text{Expression of aiiA} - \text{Degradation of aiiA}$$

Modelling the Full System

Gene Expression

$$\frac{d[AHL]}{dt} = \frac{a[AHL]}{a_0 + [AHL]} - \text{Degradation of AHL by aiiA} - \text{Degradation of AHL}$$

$$\frac{d[LuxR]}{dt} = \frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]} - \text{Degradation of LuxR}$$

$$\frac{d[aiiA]}{dt} = \frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]} - \text{Degradation of aiiA}$$

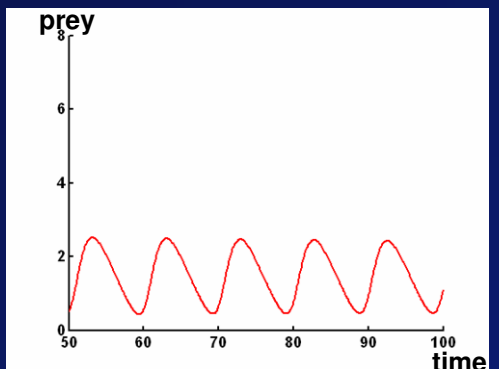
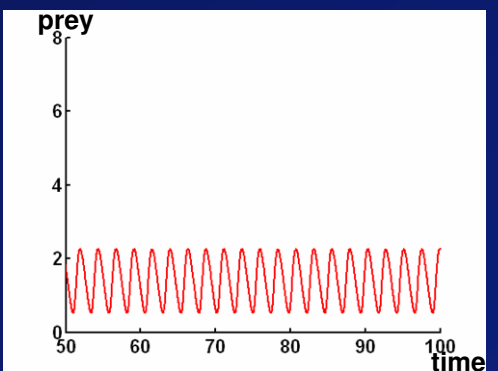
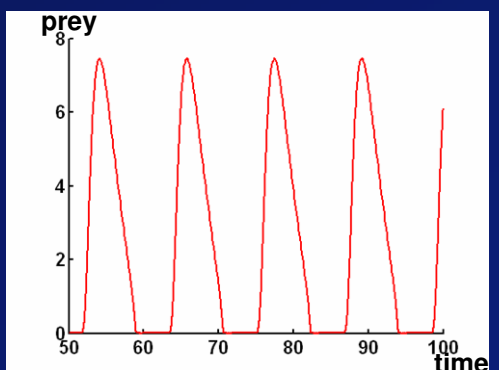
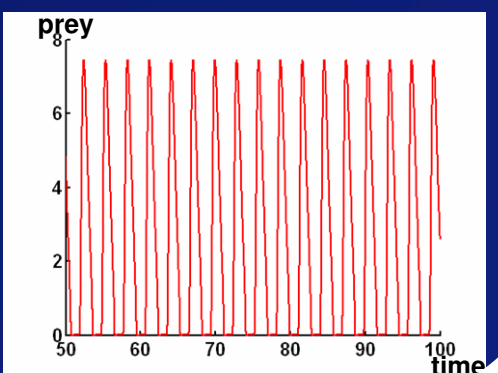
Modelling the Full System

	Gene Expression		Enzymatic Reaction	
$\frac{d[AHL]}{dt} =$	$\frac{a[AHL]}{a_0 + [AHL]}$	$-$	$\frac{b[aiiA][AHL]}{b_0 + [AHL]}$	$-$ Degradation of AHL
$\frac{d[LuxR]}{dt} =$	$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$	$-$	Degradation of LuxR	
$\frac{d[aiiA]}{dt} =$	$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$	$-$	Degradation of aiiA	

Modelling the Full System

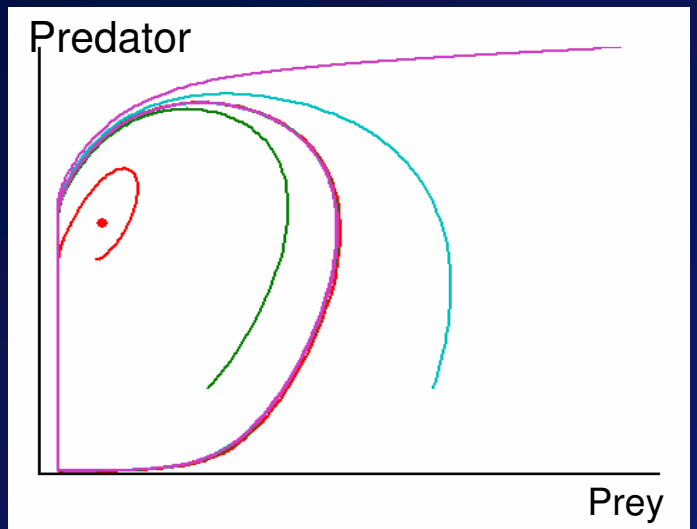
	Gene Expression	Enzymatic Reaction	Degradation
$\frac{d[AHL]}{dt} =$	$\frac{a[AHL]}{a_0 + [AHL]}$	$- \frac{b[aiiA][AHL]}{b_0 + [AHL]}$	$- e[AHL]$
$\frac{d[LuxR]}{dt} =$	$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$	$- d_1[LuxR]$	
$\frac{d[aiiA]}{dt} =$	$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$	$- d_2[aiiA]$	

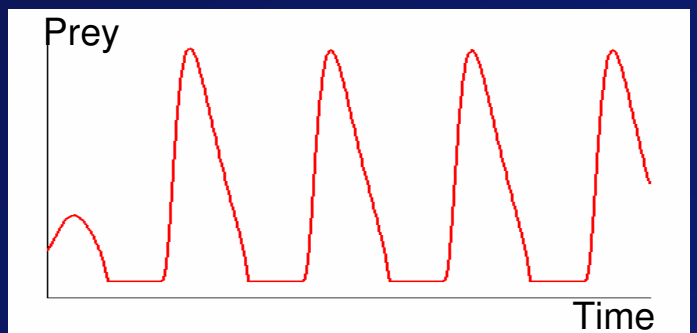
Full System Simulations

Graph of Prey vs. Time	Low Frequency	High Frequency
Small Amplitude		
Large Amplitude		

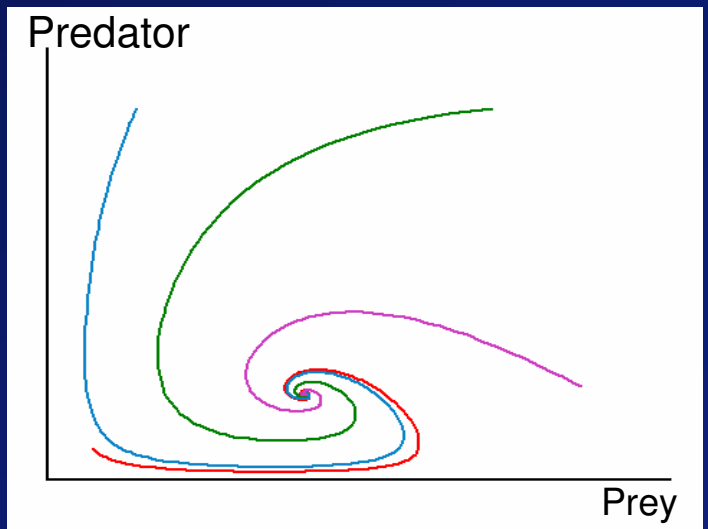
Typical System Behaviours

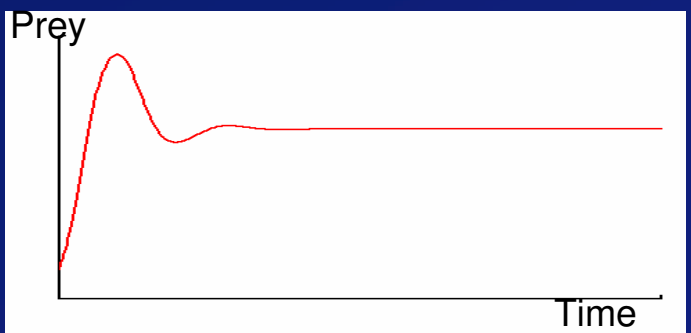
Oscillations with limit cycles



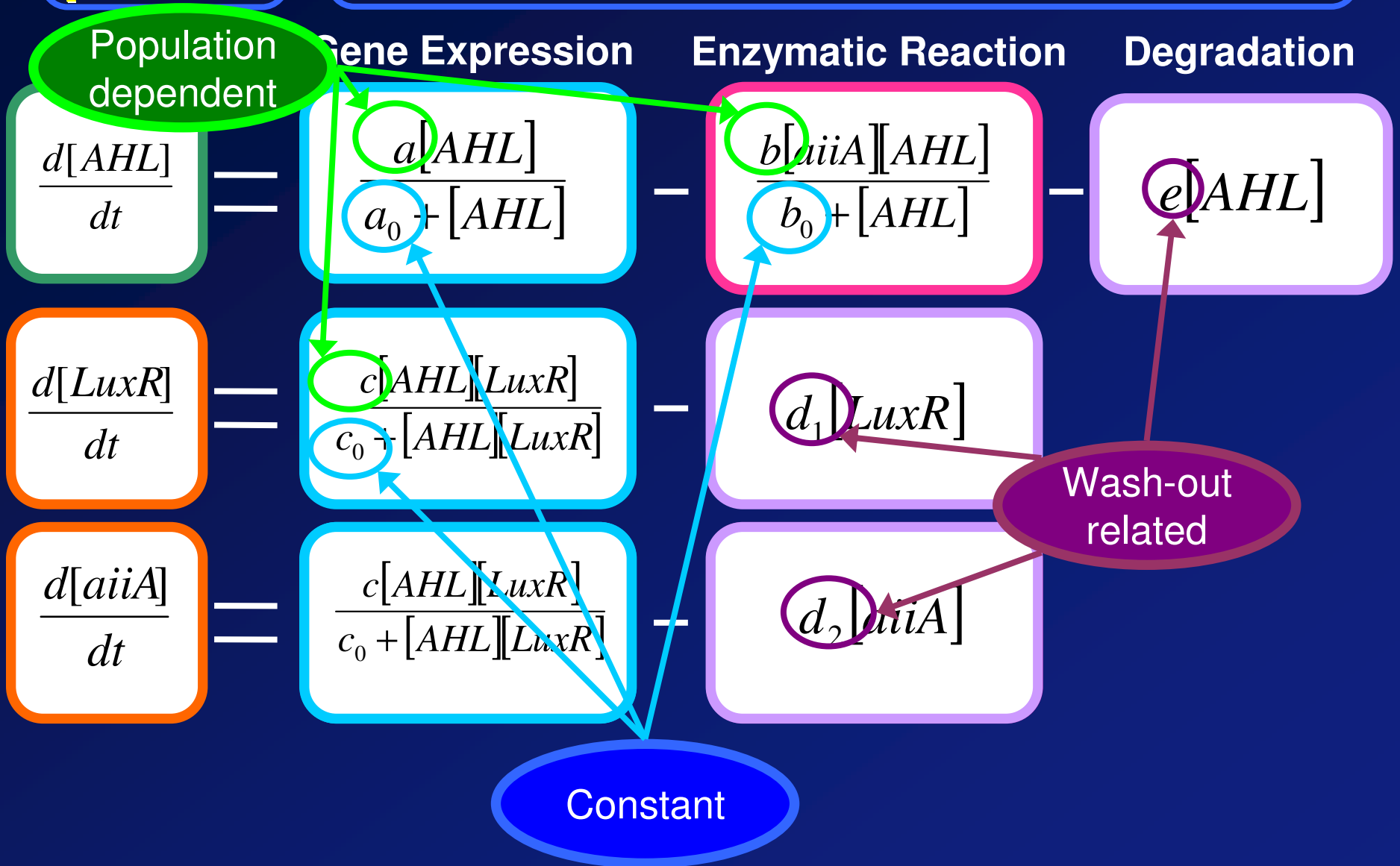


No oscillations

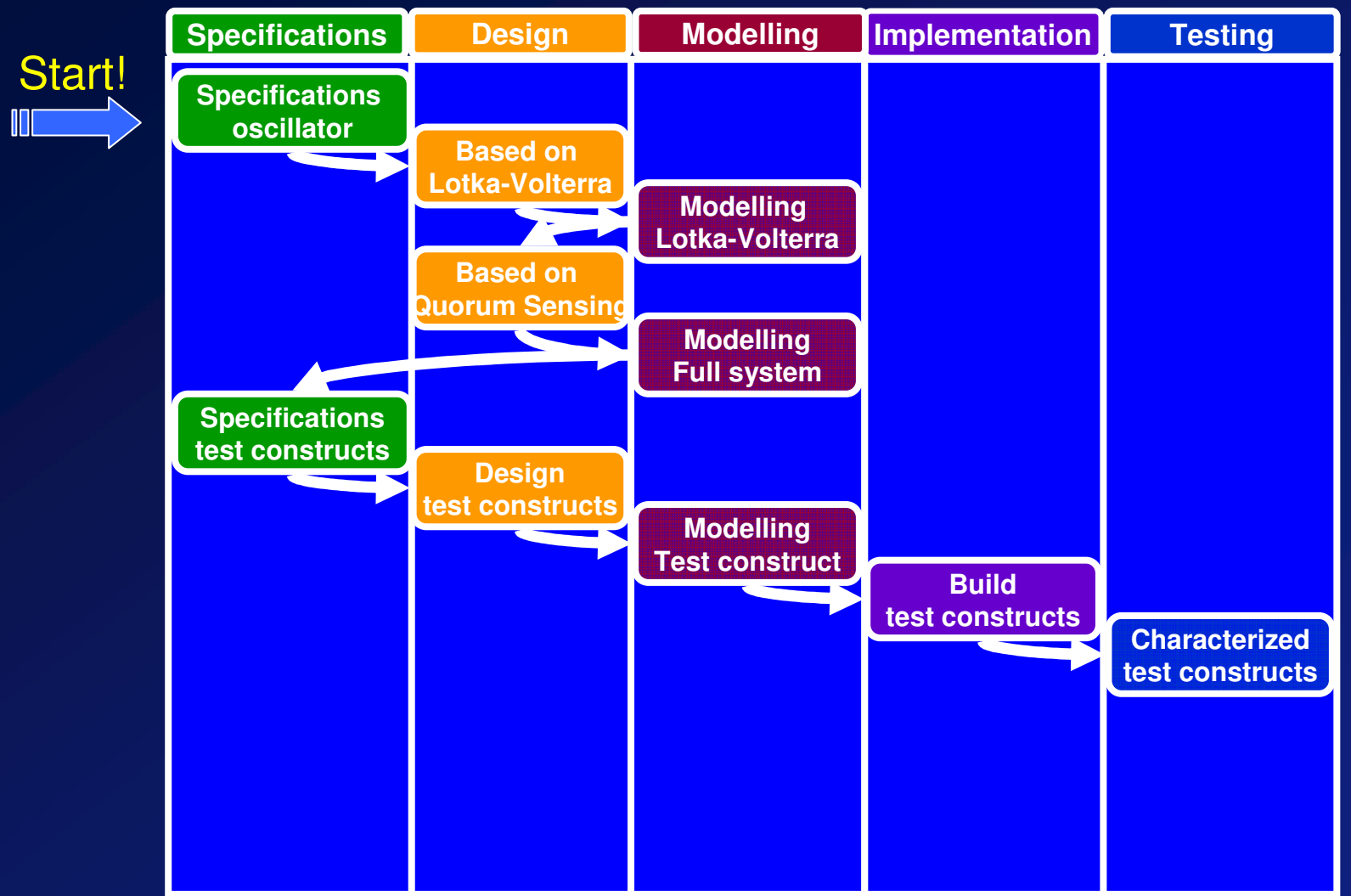




Modelling the Full System

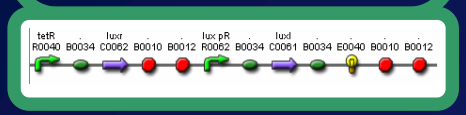


Path to Our Goal

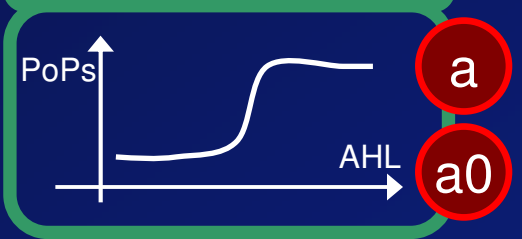


Breaking Down the Complexity

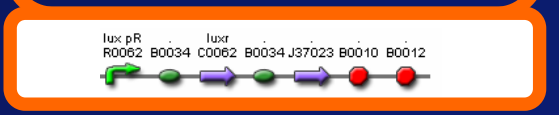
Prey Generator



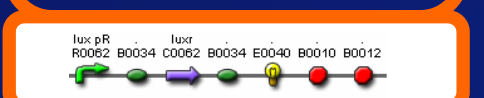
Prey Sensing



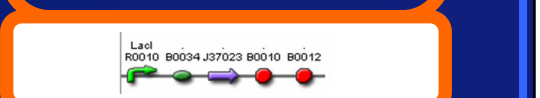
Predator Generator



Predator Sensing



Predator Killing



Characterizing Predator Sensing

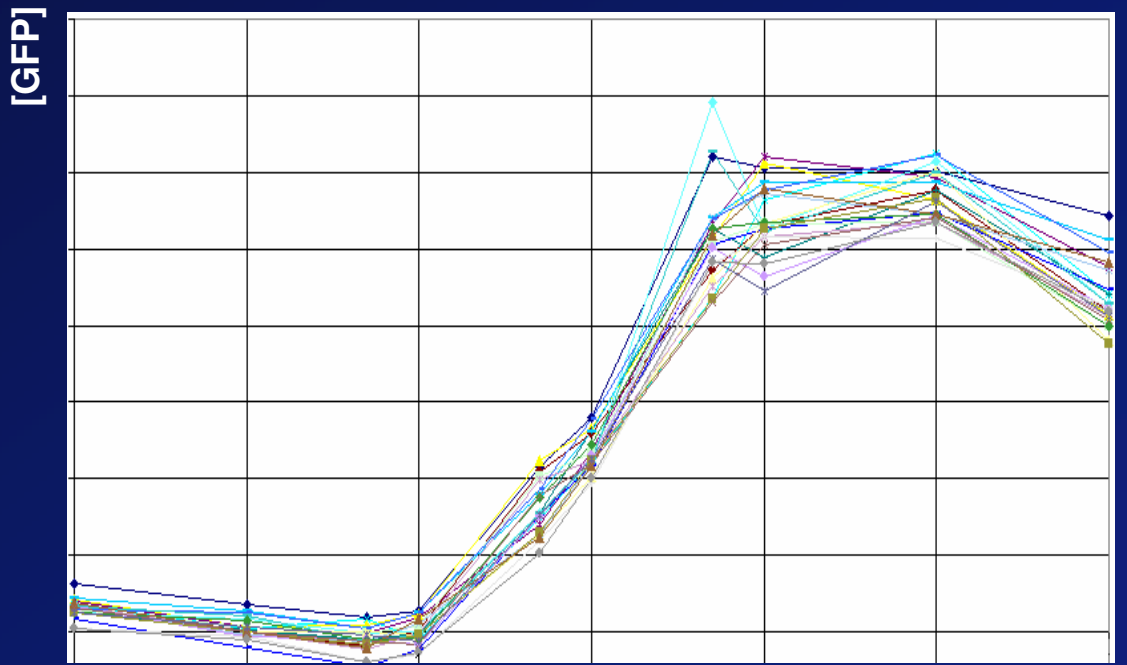
Test part

↓

Predictive model transfer function

↓

Experimental data



Experimental Results

Characterizing Predator Sensing

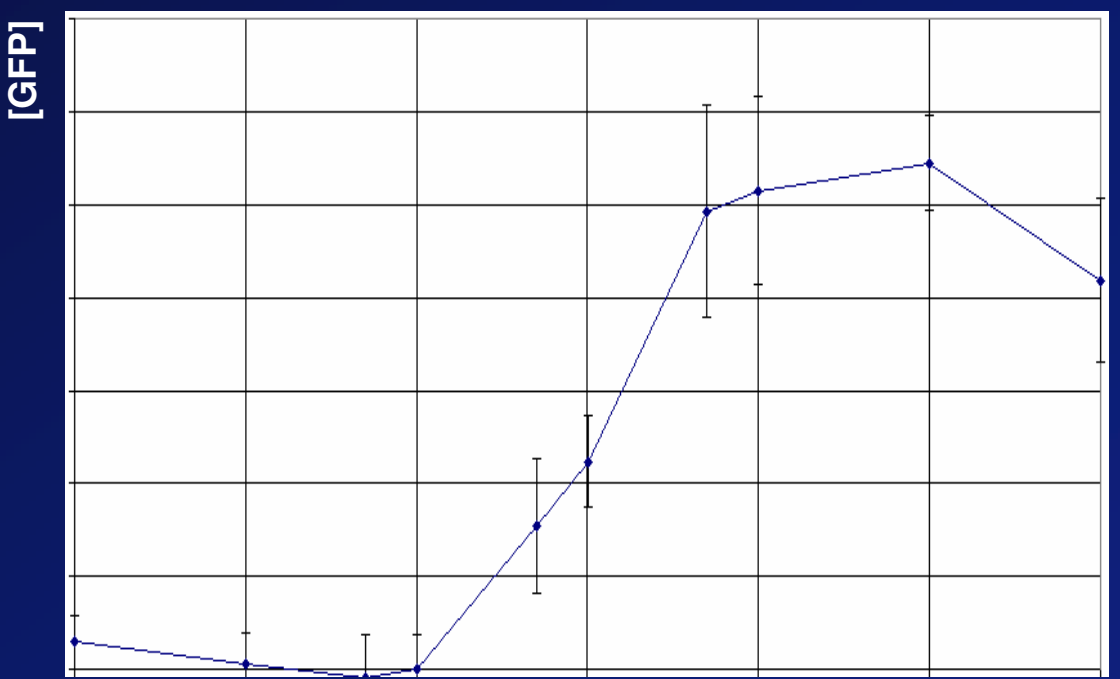
Test part

↓

Predictive model transfer function

↓

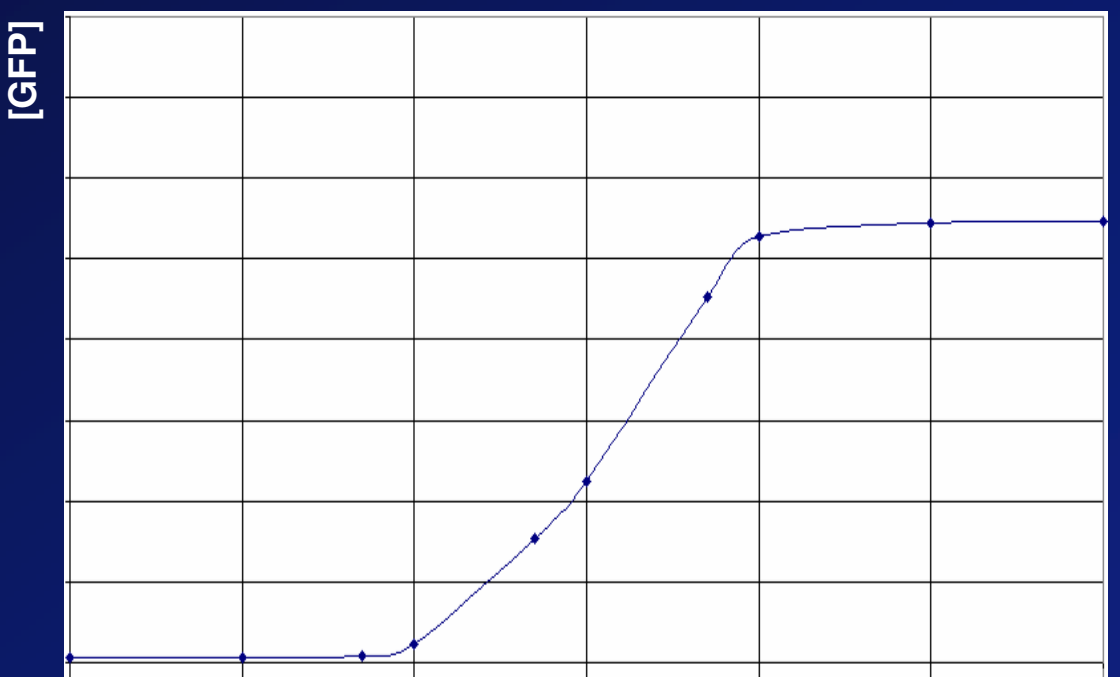
Experimental data



Average and variance

Characterizing Predator Sensing

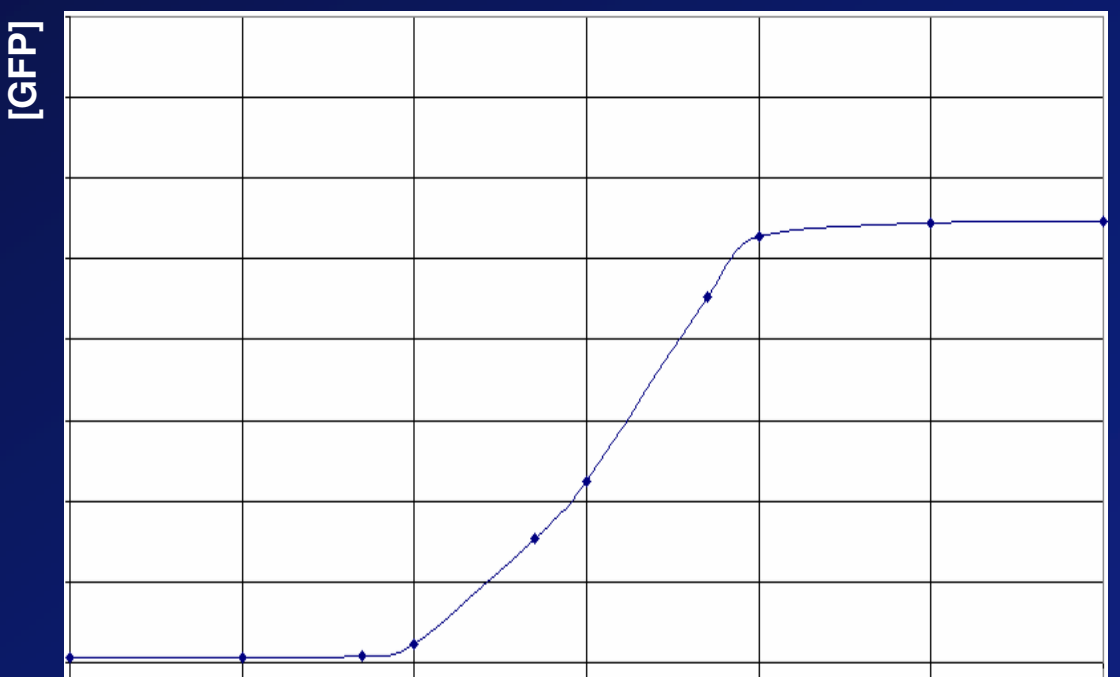
- Test part
- Predictive model transfer function
- Experimental data
- Fitting model to data



Fitted curve for parameter extractions

Characterizing Predator Sensing

- Test part
- Predictive model transfer function
- Experimental data
- Fitting model to data
- Parameter extractions



Fitted curve for parameter extractions

Implementation

Assembly process

Registry Catalogue Parts

Prey

J37034

RS+J37034

Prey with Riboswitch

J37023

J37024

AiiA Test Construct

J37025

Final predator

J37033

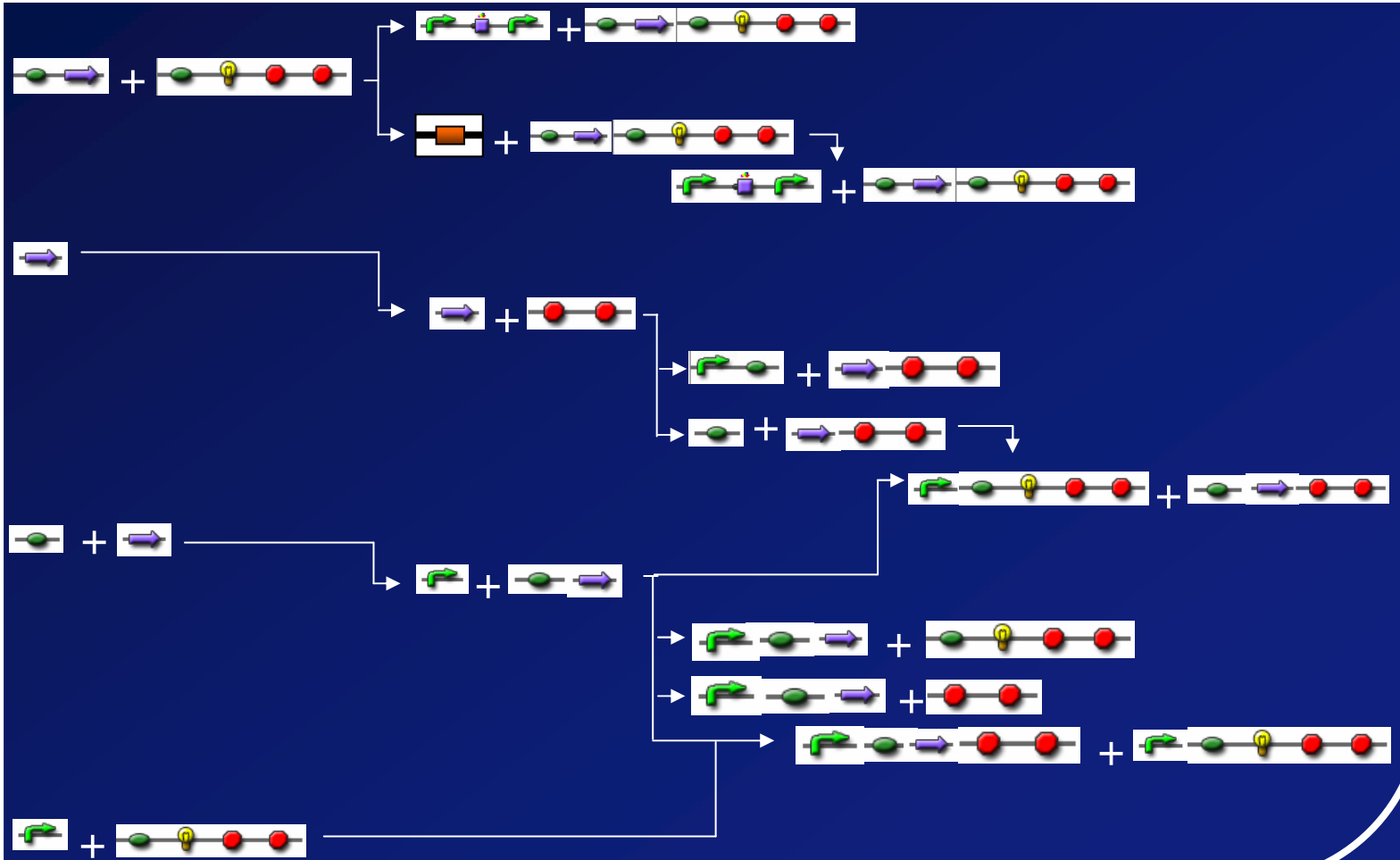
J37019

Sensing predator

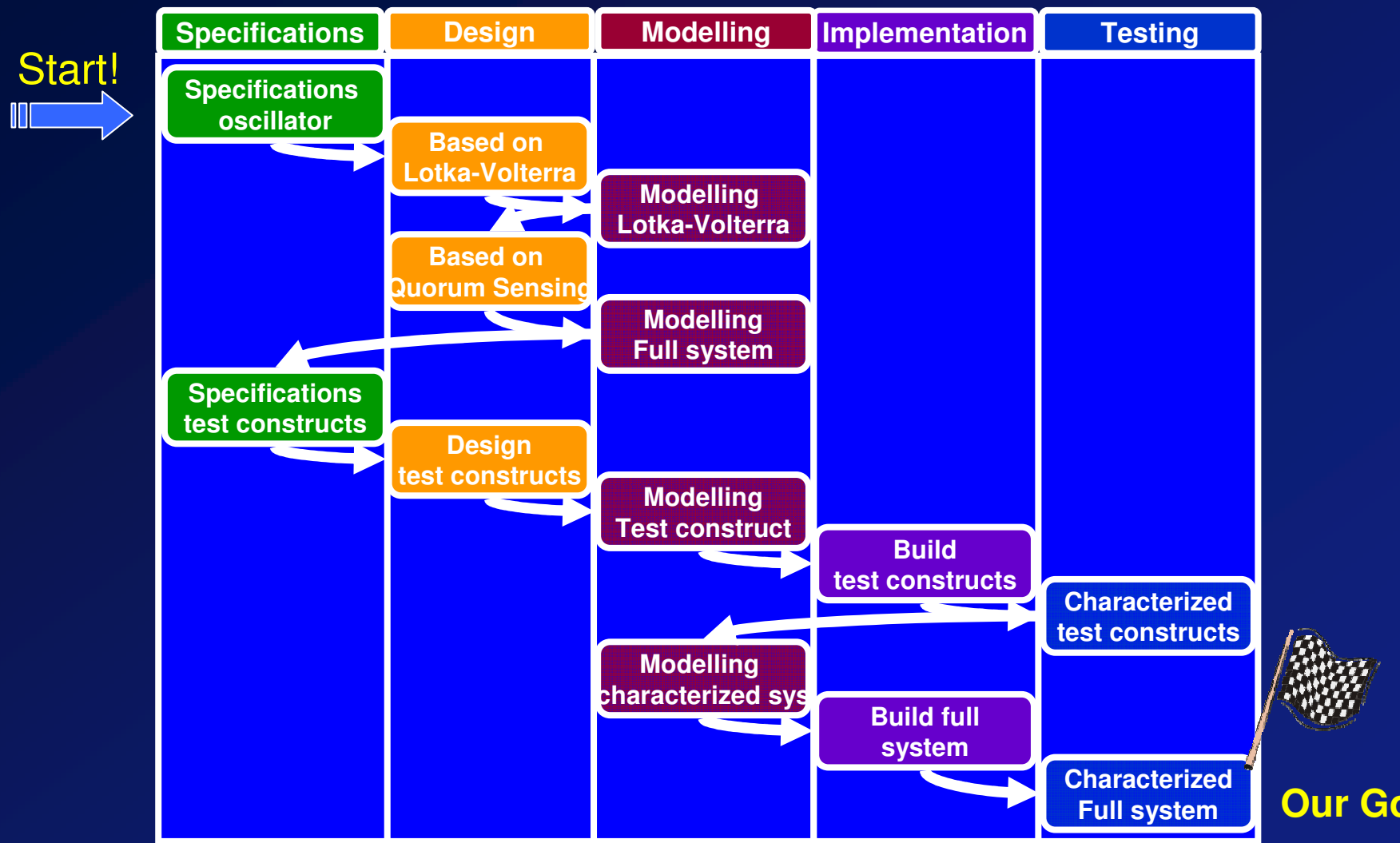
J37031

Sensing predator

J37032

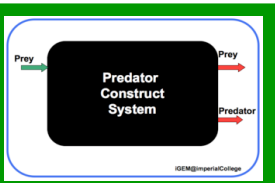


Path to Our Goal

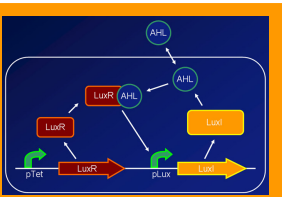


On Our Experience

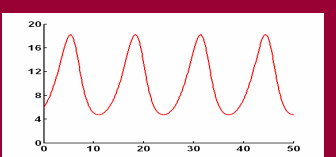
Specifications



Design

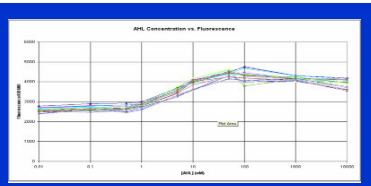


Modelling



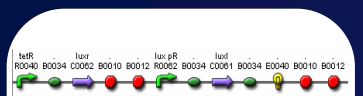
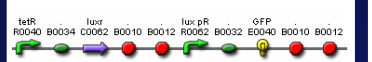
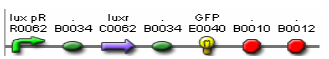
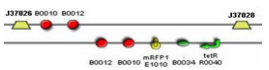
Implementation

Testing/Validation

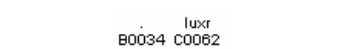
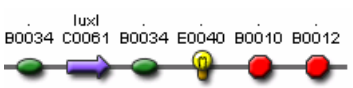


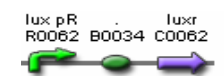
Contributions to the Registry

Functional Parts

		Built	Sequenced	Tested	Characterized	Documented
Final Prey J37015		✓	✓	✓	✓	✓
Sensing Prey T9002				✓		✓
Sensing Predator J37016		✓	✓	✓	✓	✓
Cre/Lox J37027		✓	✓	✓		✓

Intermediate Parts

		Built	Sequenced
J37033		✓	✓
J37034		✓	✓

		Built	Sequenced
J37019		✓	✓
J37032		✓	✓
J37023		✓	✓

Our Wiki

Imperial College London

THE I.CoLi REPORTER

iGEM

The newsletter of Imperial College London iGEM 2006

Our Contributions | To Do List | Biological Oscillator Parts | Modelling | Protocols | Resources

Breaking News [edit]

- 30 Oct 2006: 300+ pages documentation on OWW. New iGEM page
- 27 Oct 2006: Team Poloshirts arrived.
- 26 Oct 2006: BioBricks sent to M.I.T.
- 25 Oct 2006: Presentation of the iGEM 2006 project at the Bioengineering Departmental Seminar.
- News Archive

Location of our visitors

Visits since 6 Aug 2006

Zoom

Updated daily

Imperial College London iGEM Project(s) 2006 edit

Engineering a Molecular Predation Oscillator.

PoPs Blocker | Biological to Electrical Interface

Celebrities [edit]

Get to know the I.Coli team and their advisors:

The Undergrads [show]

The Advisors [show]

Acknowledgements

Activities [edit]

- Event Calendar
- MIT Jamboree
- BioSysBio Conference, UK
- UK iGEM Teams Meeting in Cambridge

In the Wetlab [edit]

- Lab Notebook
- Protocols
- Lab Status
- Testing
- Sequencing of Parts
- Primers

Education [edit]

- Brainstorming
- Journal Club
- Lecture Notes
- Bibliography/Papers
- Resources

Entertainment [edit]

- Photos
- Logo & Team Shirts
- Inspirational Quotes
- Project Discussion
- Sandbox

- Documentation
- Communication
- Organization

http://openwetware.org/wiki/IGEM:IMPERIAL/2006

Thank You

Imperial College London

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- David Featherbe
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- **Faculty of Engineering**
- **Faculty of Natural Sciences**