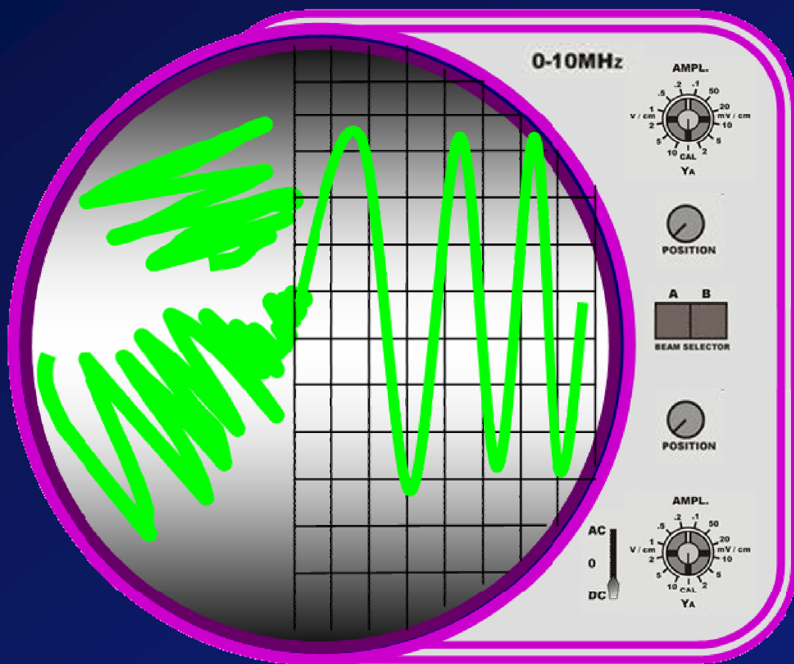
iGEM Jamboree
MIT – Nov 4th 2006



Engineering a Molecular Predation Oscillator

iGEM 2006 @ Imperial

Electrical Engineer

Biochemist

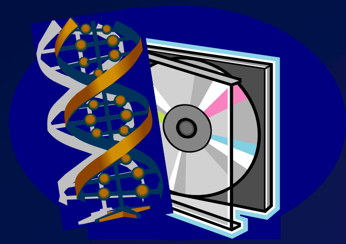
Biomedical Engineers

Biologists

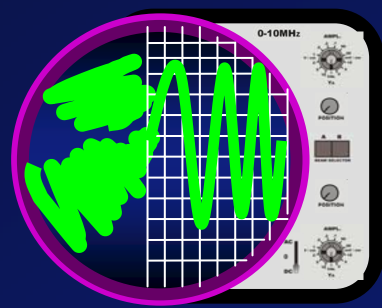
Biomedical Engineers

Biochemists

Project Ideas



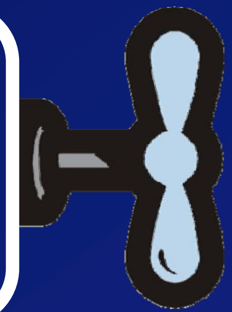
Bio-Memory



Oscillator

Bio-Clock

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

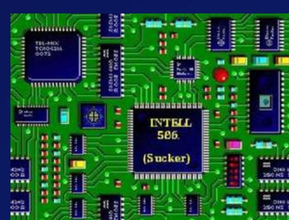


What is an Oscillator?

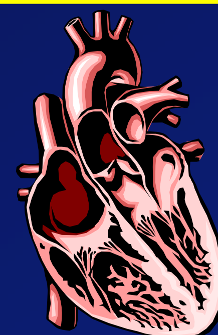
Our Definition

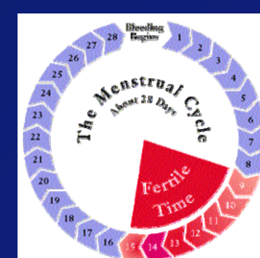
Device producing a periodic variation in time of a measurable quantity, e.g. amplitude.

Engineering



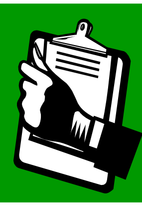
Biology



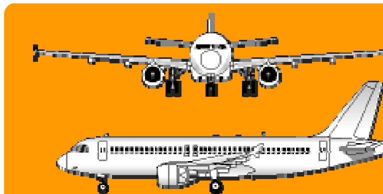


The Engineering Approach

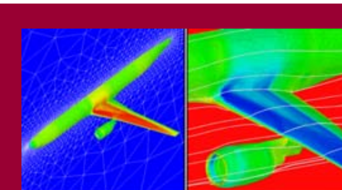
Specifications



Design



Modelling



Implementation

Testing/Validation

Stand…

E… g

…actice

Certified

The Main Challenges

Main challenges of past oscillators:

- Unstable
- Noisy
- Inflexible

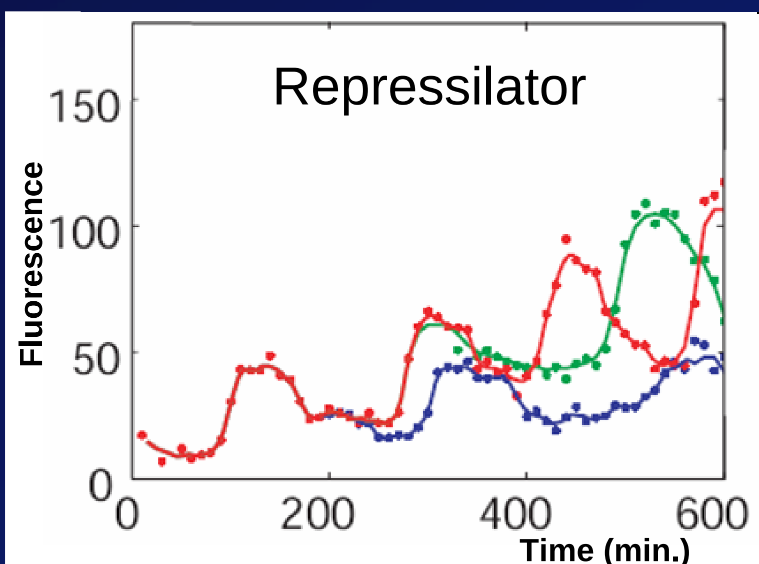


Figure Reference : Michael B. Elowitz & Stanislas Leibler *Nature* 2000

Requirement for a typical engineering oscillator

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

Our Specifications:

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

Our Initial Design Ideas

Based on

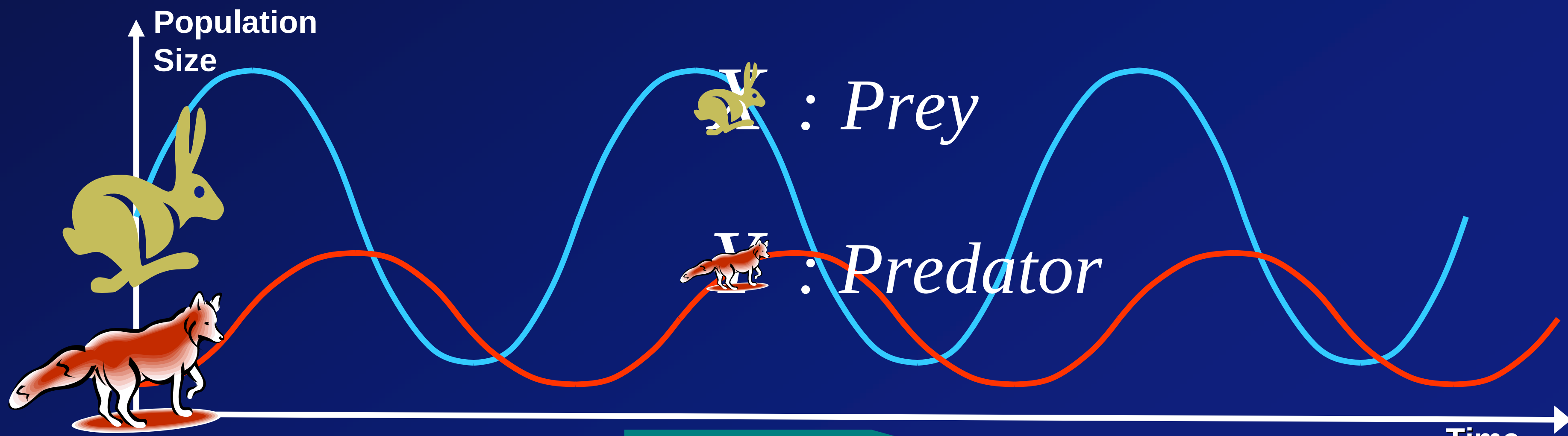
- Large populations of molecules to reduce influence of noise
- Oscillations due to population dynamics
- A well characterized model

Molecular Predator - Prey

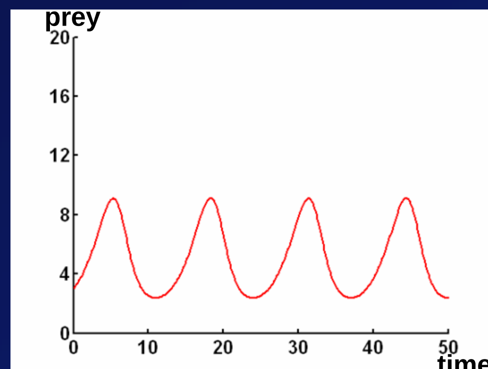
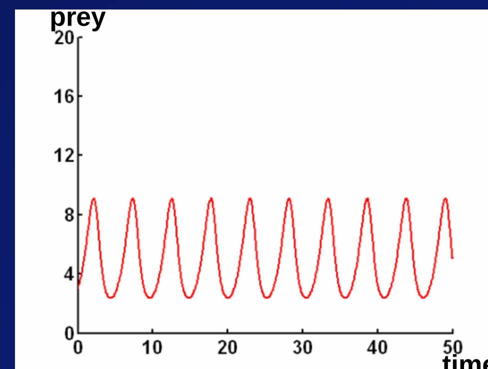
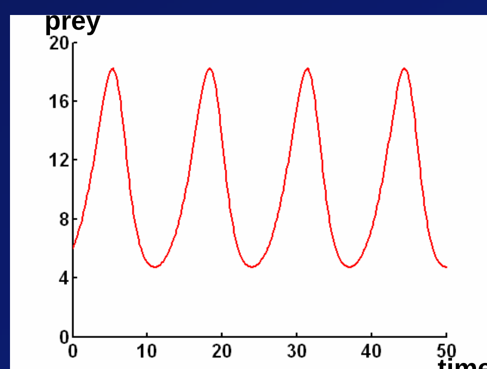
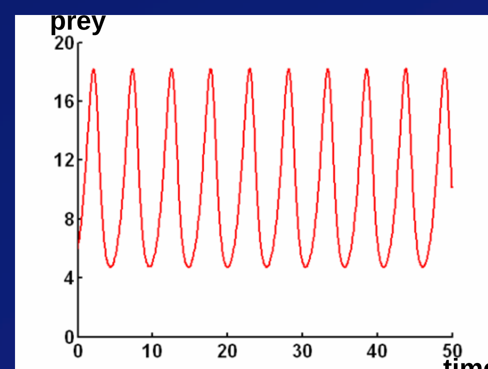
The Lotka-Volterra Model

$$\frac{dX}{dt} = \text{Prey Growth} - \text{Prey Killing by Predator}$$

$$\frac{dY}{dt} = \text{Predator Growth} - \text{Predator Death}$$



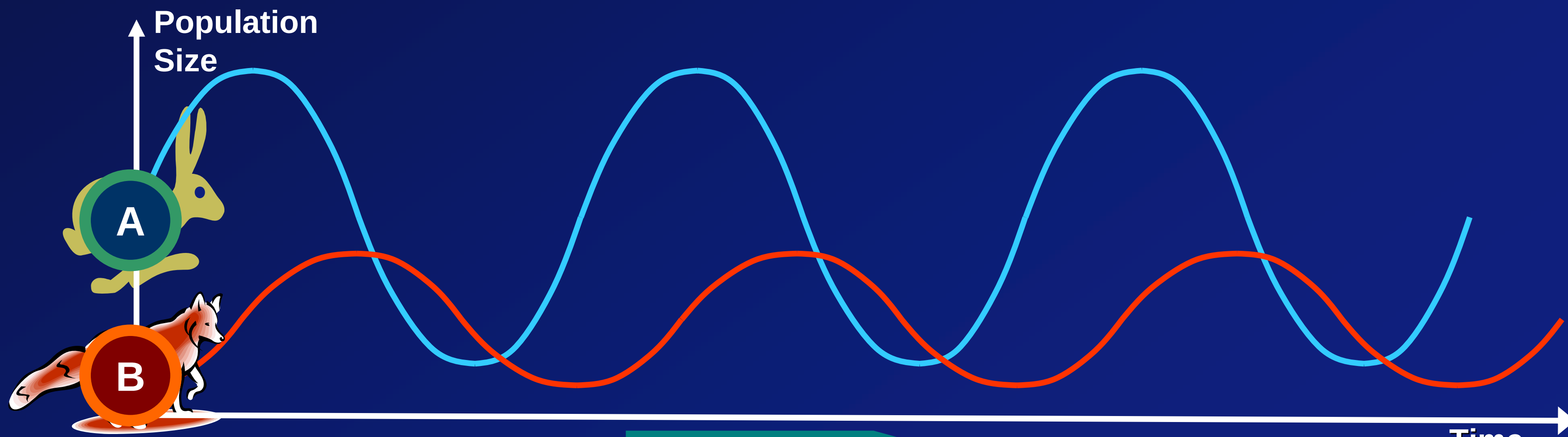
Typical LV Simulations

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

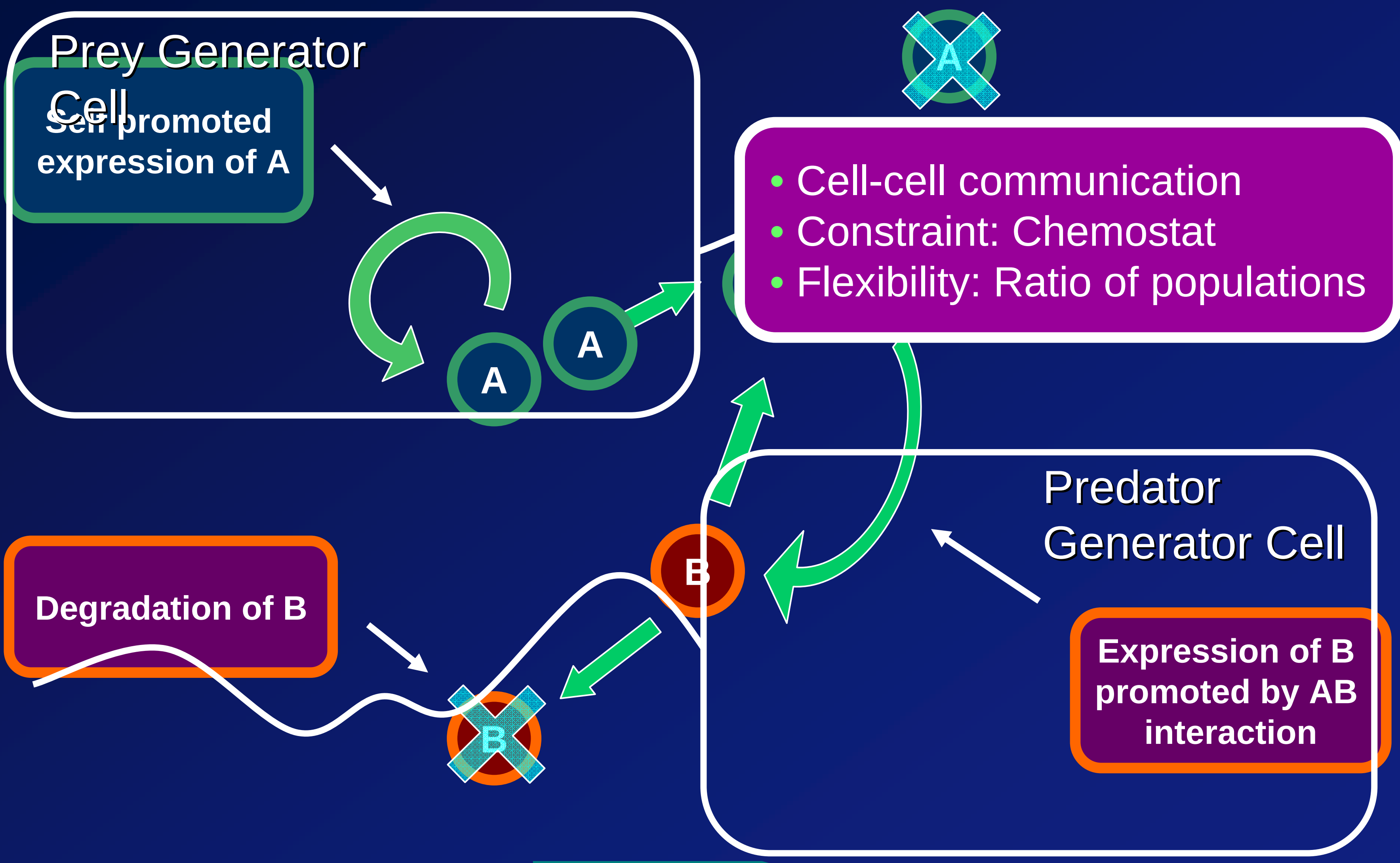
Required Biochemical Properties

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

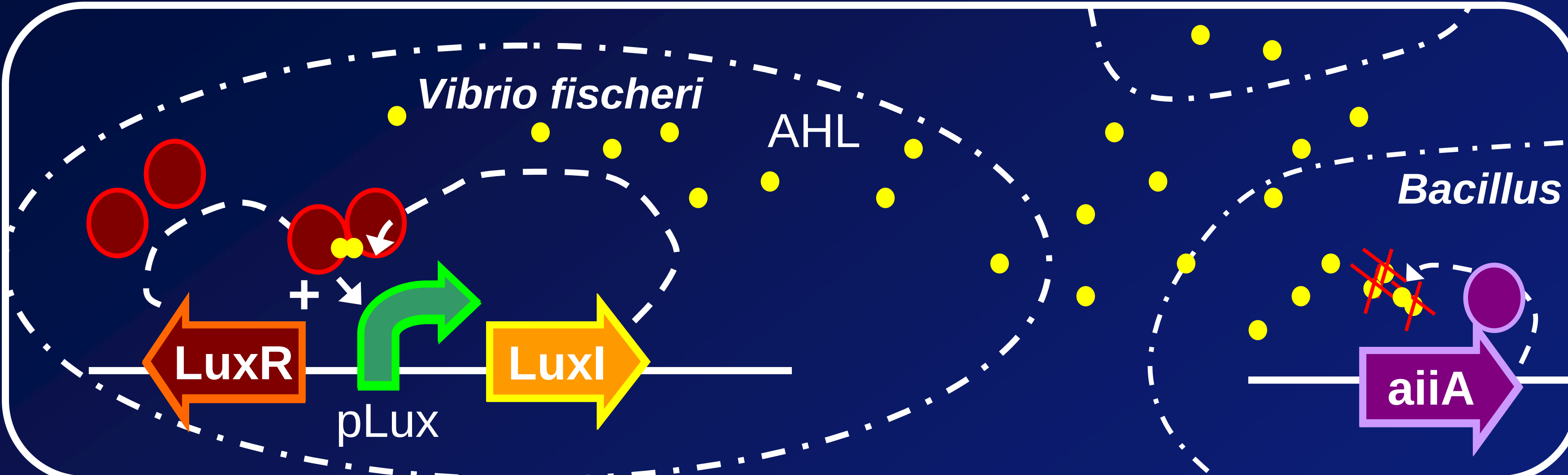
$$\frac{dB}{dt} = \text{Expression of B promoted by AB interaction} - \text{Degradation of B}$$



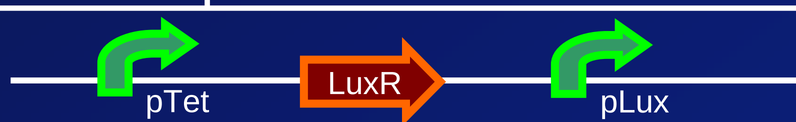
Molecular System



Quorum sensing/quenching



BioBricks available

BBa_C0062	Forms a complex with AHL to activate pLux	BBa_C0061	Makes AHL
pLux BBa_R0062	pLux Promoter	BBa_C0160	Degrades AHL
 BBa_F2620		AHL->Pops Receiver	

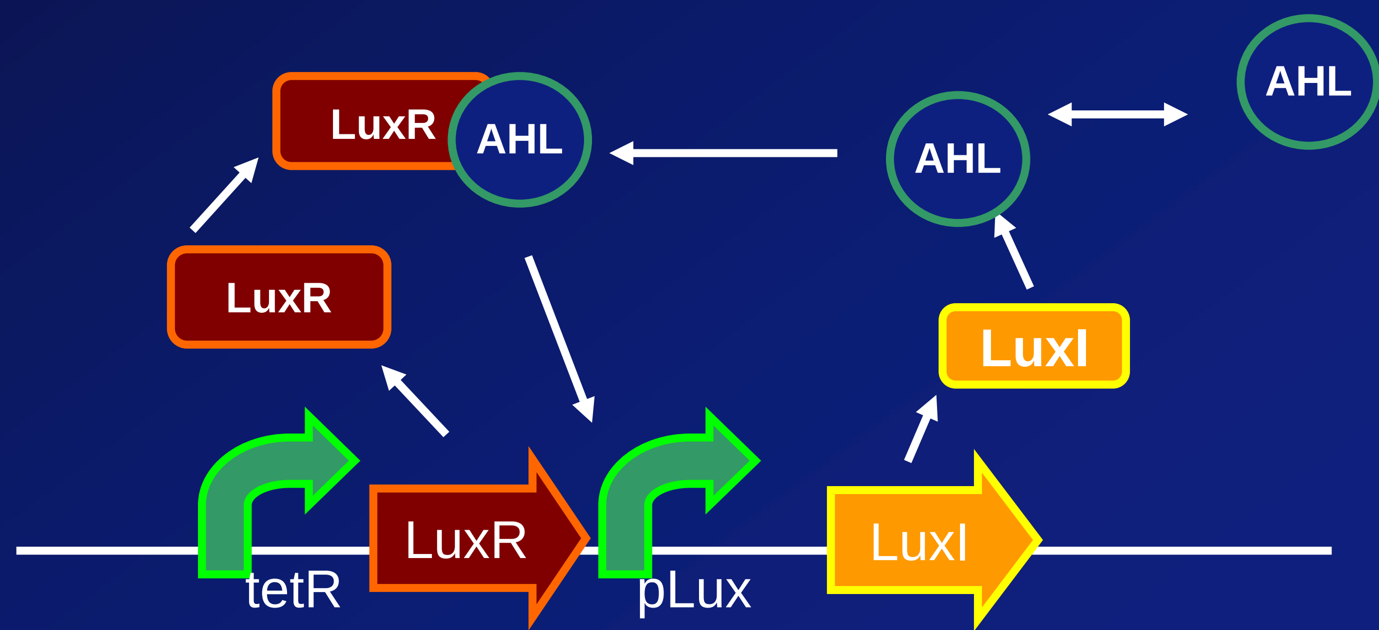
Designing the Prey Generator

Required Dynamic

Self promoted expression of A ✓

Useful BioBricks

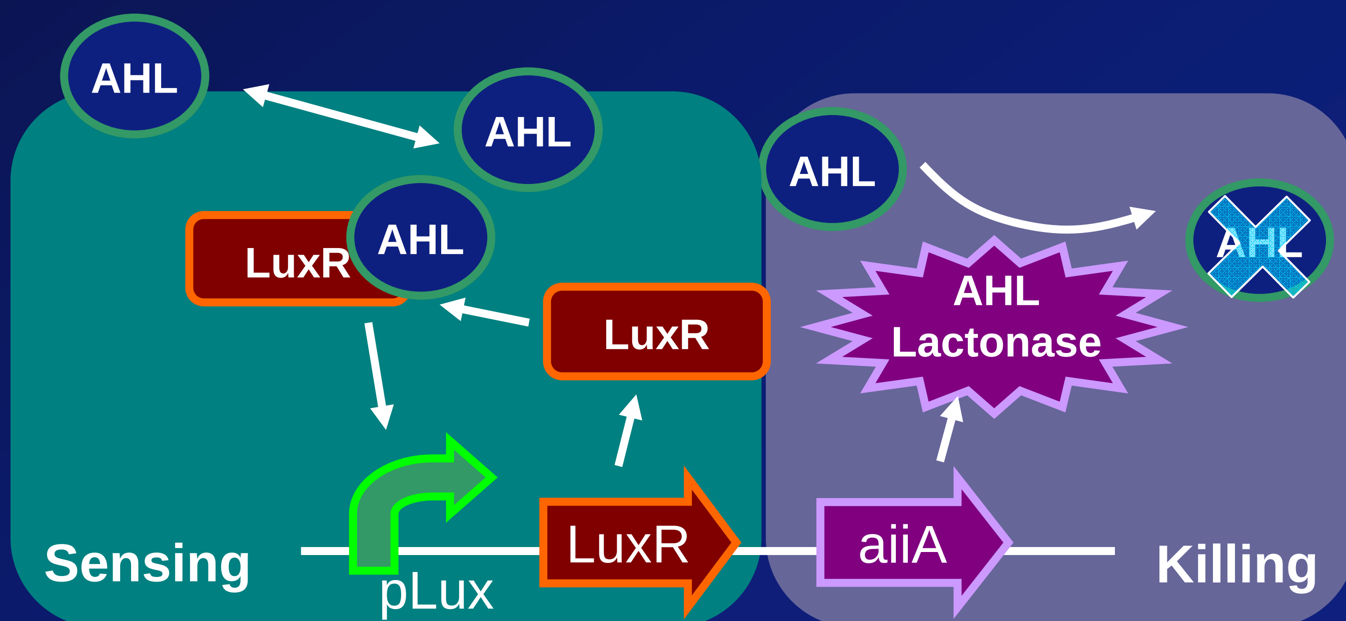
Final Construct



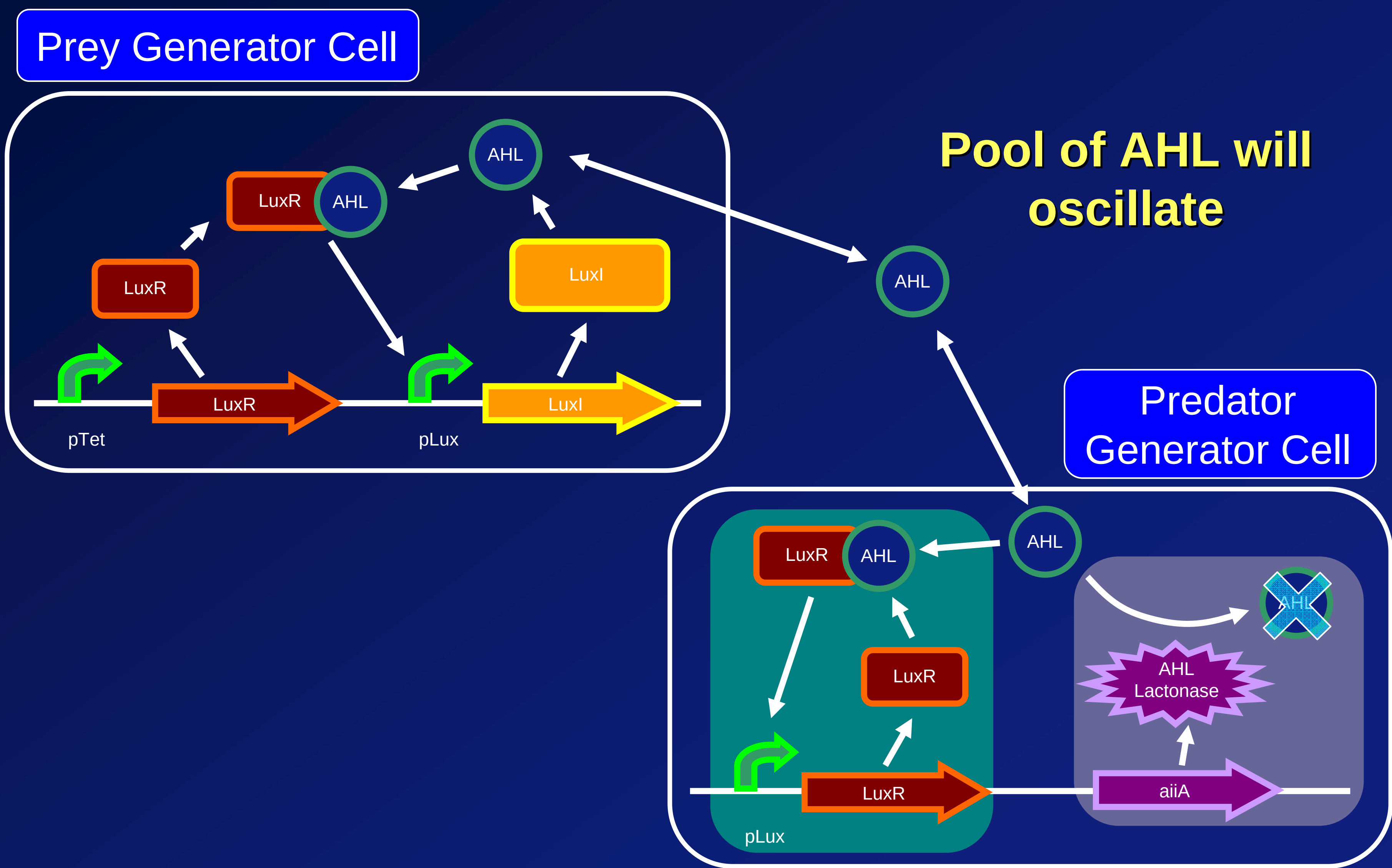
Designing the Predator Generator

Required Dynamic	Expression of B promoted by AB interaction ✓	Degradation of A by B ✓	Degradation of B ✓
Useful BioBricks	pLux R0062; LuxR C0062	aiiA C0060	Natural degradation

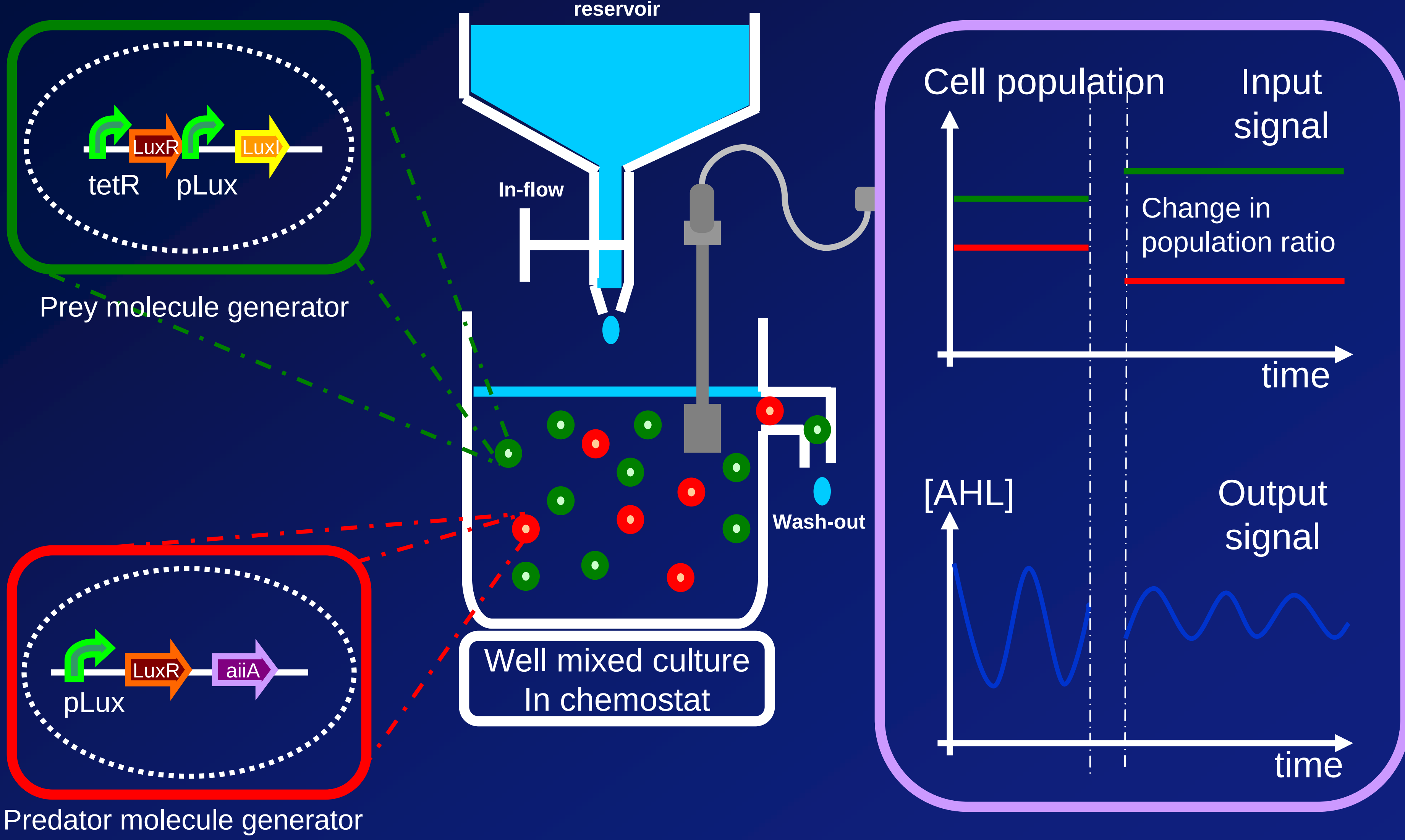
Final Construct



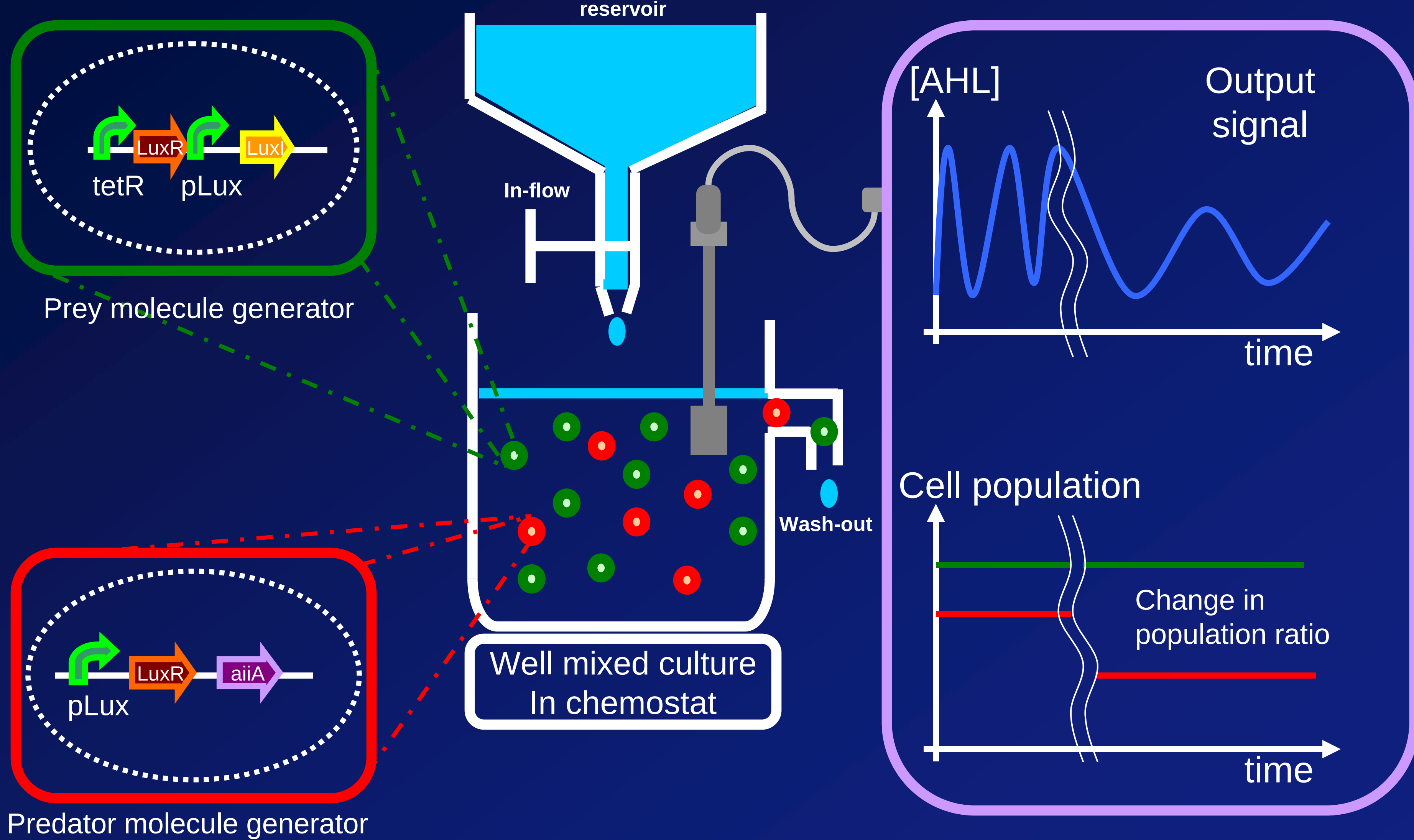
System Overview



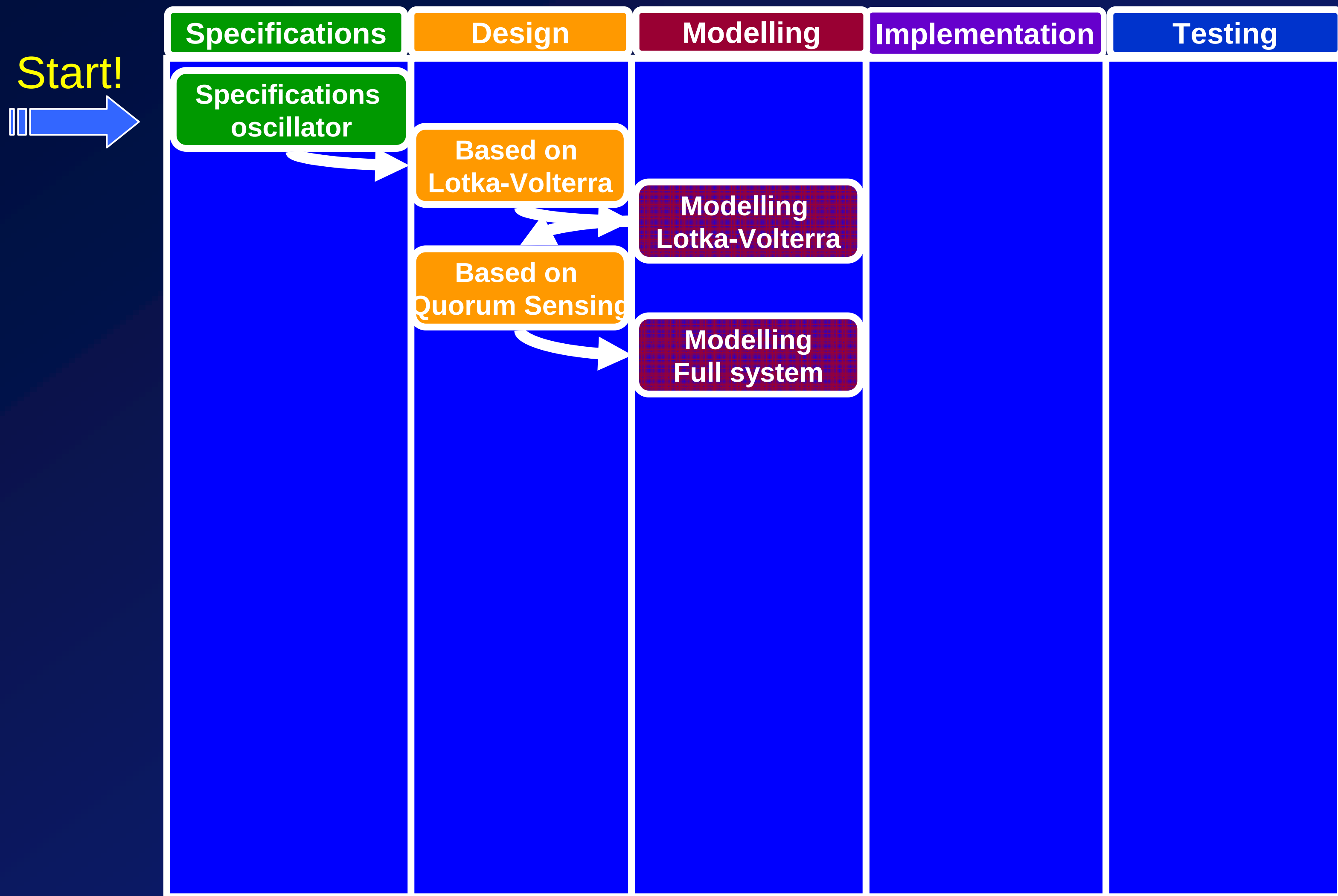
Full System set-up



Full System set-up



Path to Our Goal



Modelling the Full System

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

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

$\frac{d\,[\text{aiiA}]}{dt}$ = Expression of aiiA − 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**

$$\frac{d[AHL]}{dt} = \frac{a[AHL]}{a_0 + [AHL]} - \frac{b[aiiA][AHL]}{b_0 + [AHL]} - \text{Degradation of AHL}$$

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

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

Modelling the Full System

	Gene Expression	Enzymatic Reaction	Degradation

$$\frac{d[AHL]}{dt} = \frac{a[AHL]}{a_0+[AHL]} - \frac{b[aiiA][AHL]}{b_0+[AHL]} - e[AHL]$$

$$\frac{d[LuxR]}{dt} = \frac{c[AHL][LuxR]}{c_0+[AHL][LuxR]} - d_1[LuxR]$$

$$\frac{d[aiiA]}{dt} = \frac{c[AHL][LuxR]}{c_0+[AHL][LuxR]} - d_2[aiiA]$$

.CoLi

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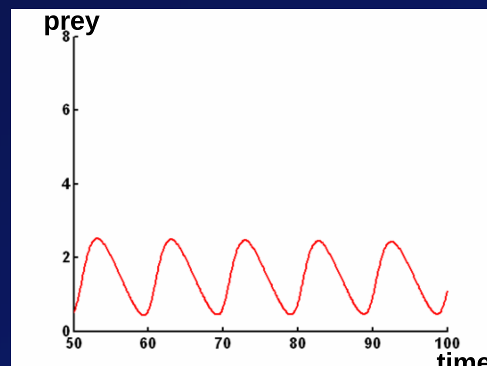
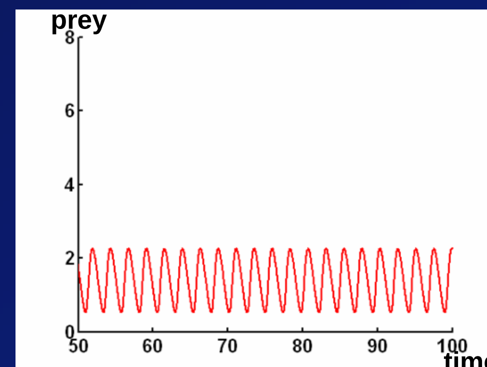
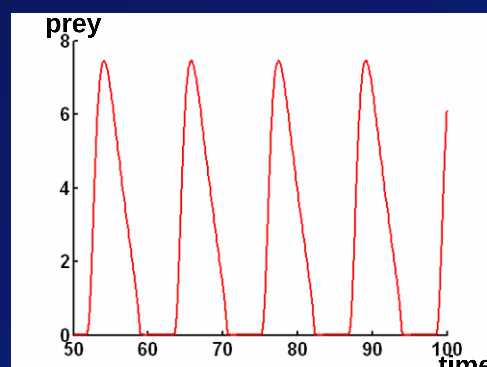
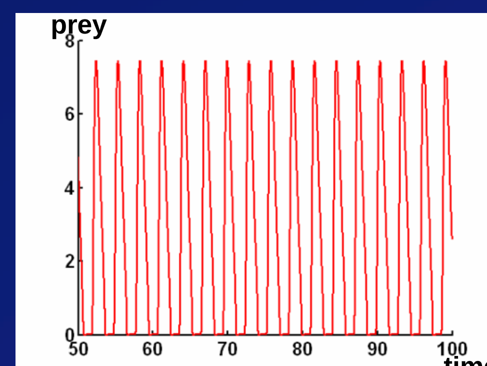
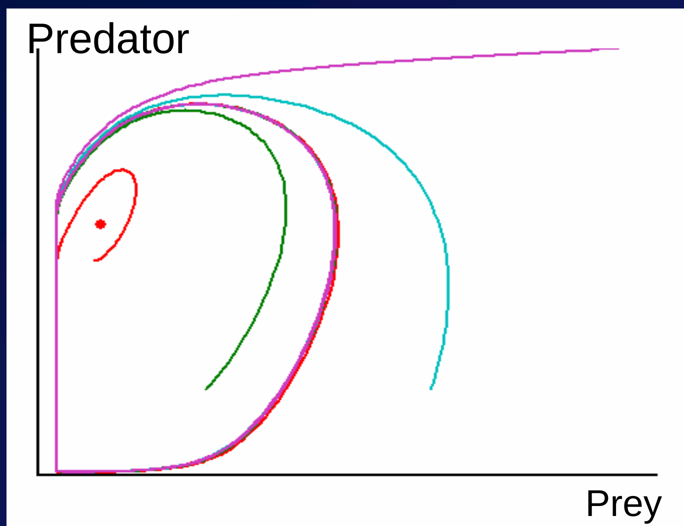
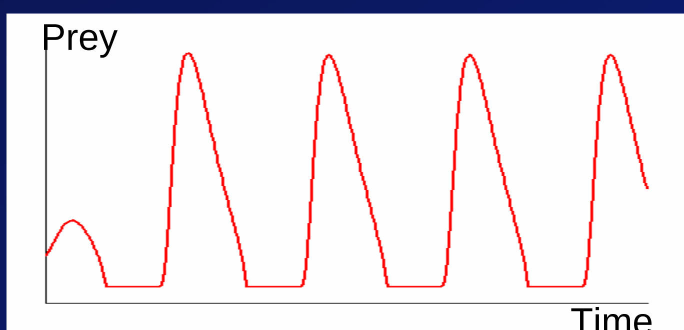
Full System Simulations

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

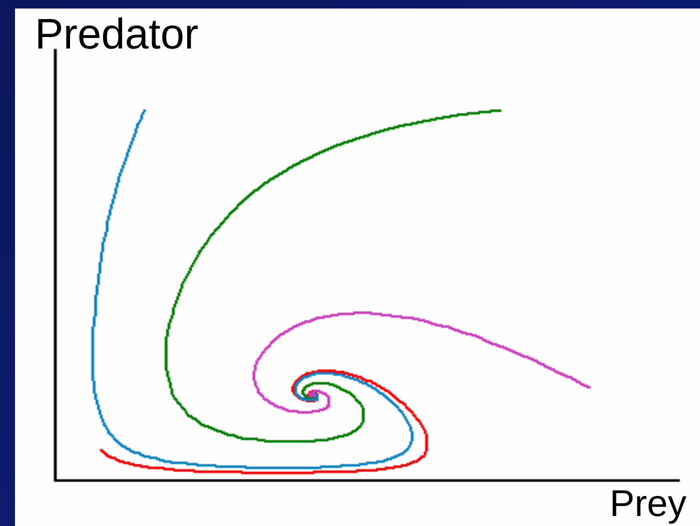
Typical System Behaviours

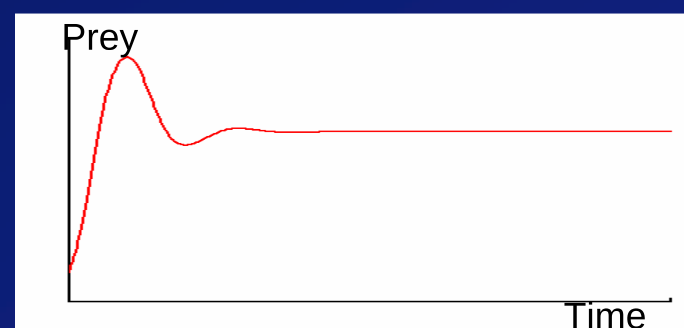
Oscillations with limit cycles





No oscillations





Modelling the Full System

	Gene Expression	Enzymatic Reaction	Degradation

$$\frac{d[AHL]}{dt} = \frac{a[AHL]}{a_0+[AHL]} - \frac{b[aiiA][AHL]}{b_0+[AHL]} - e[AHL]$$

$$\frac{d[LuxR]}{dt} = \frac{c[AHL][LuxR]}{c_0+[AHL][LuxR]} - d_1[LuxR]$$

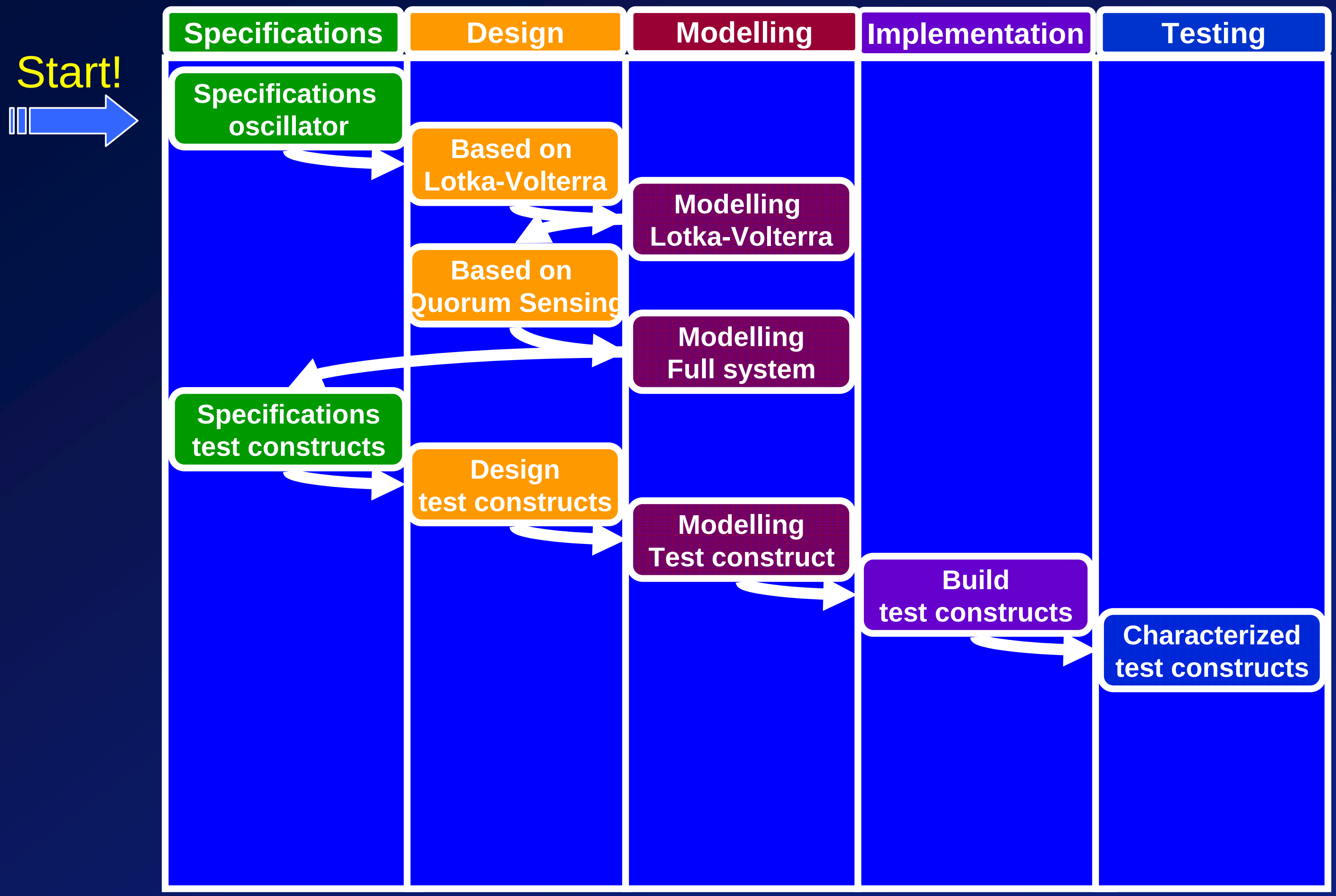
$$\frac{d[aiiA]}{dt} = \frac{c[AHL][LuxR]}{c_0+[AHL][LuxR]} - d_2[aiiA]$$

Population dependent

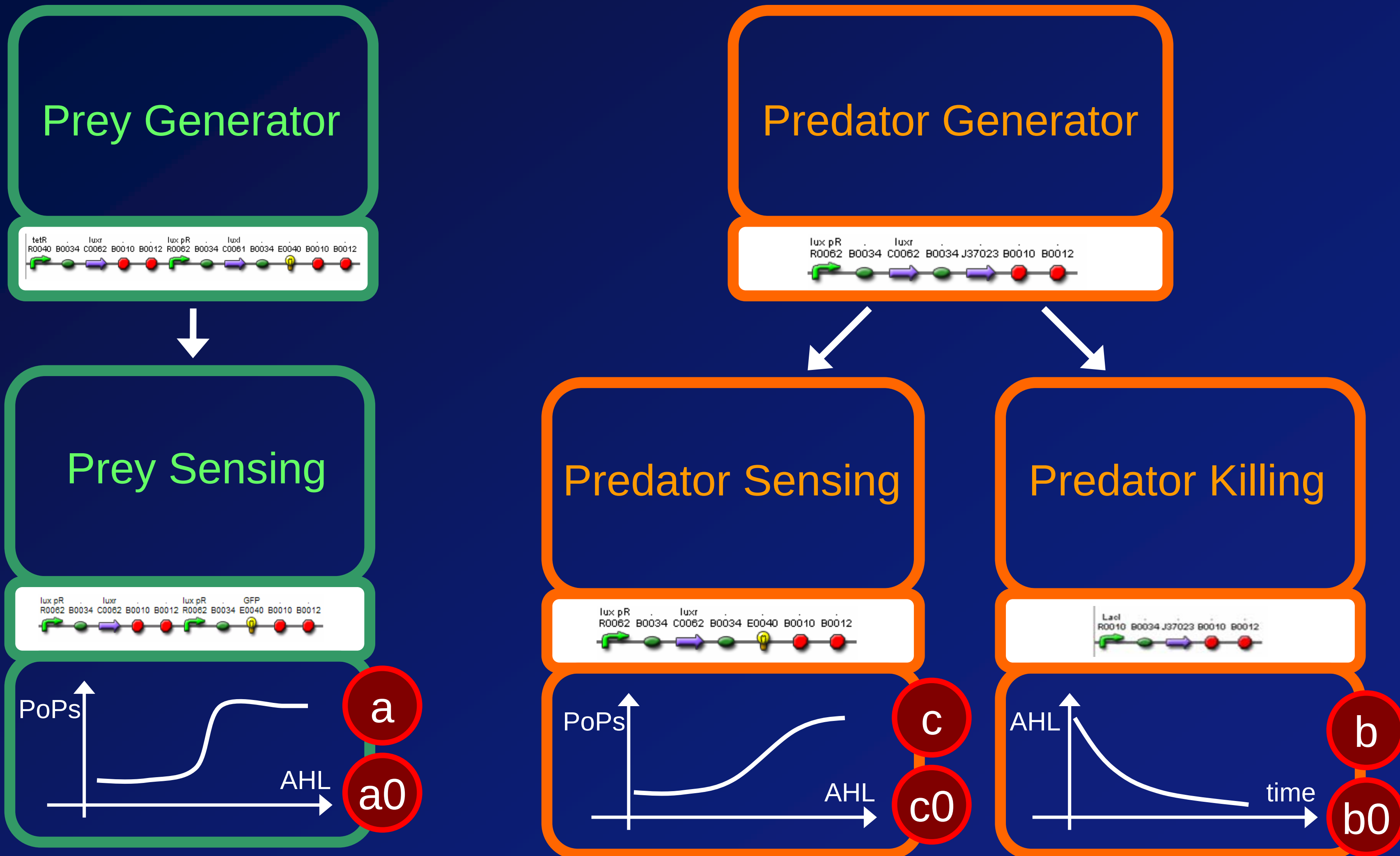
Wash-out related

Constant

Path to Our Goal



Breaking Down the Complexity



Characterization Predator Sensing

Test part

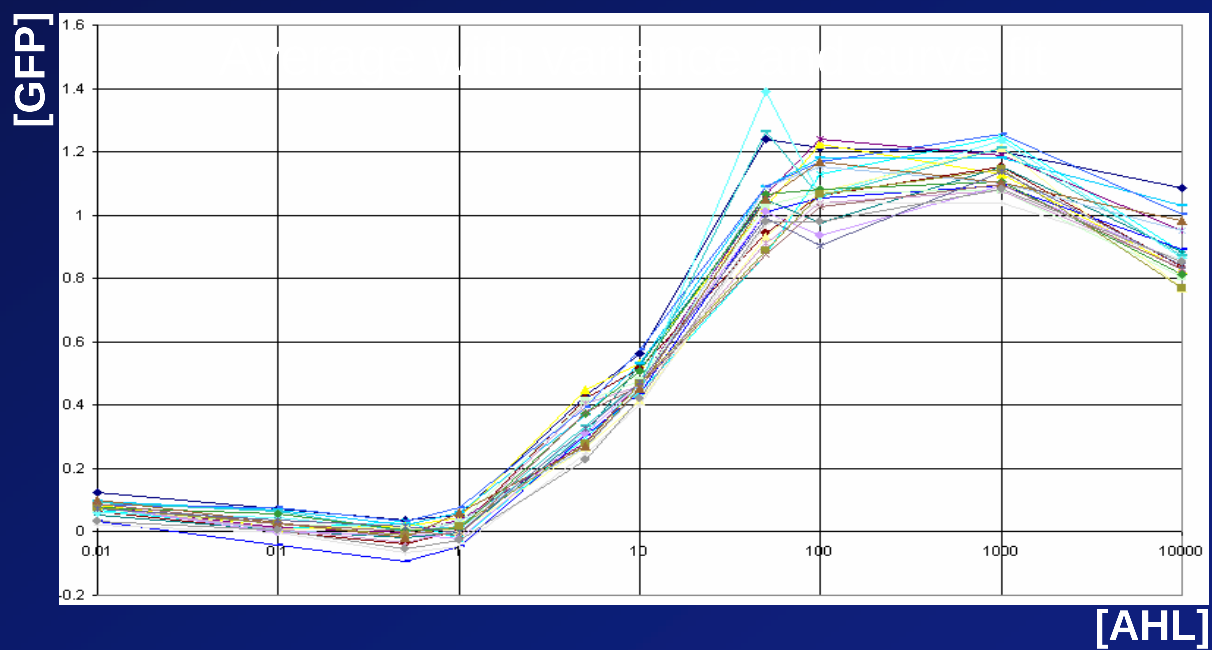
↓

Predictive model transfer function

↓

Experimental data

Experimental Data



Characterization Predator Sensing

Test part

↓

Predictive model transfer function

↓

Experimental data

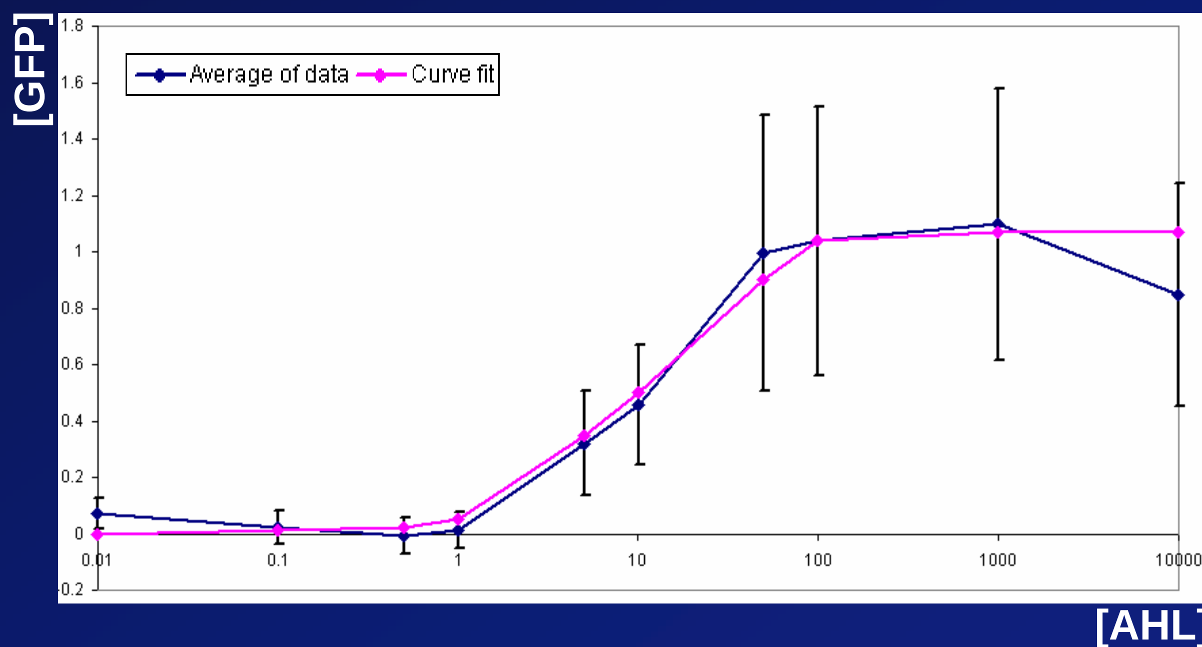
↓

Fitting model to data

↓

Parameter extractions

Average with variance and curve fitting



Implementation

Registry Catalogue Parts	Assembly process

Prey

J37034

RS+J37034

Prey with Riboswitch

J37023

J37024

AiiA Test Construct

J37025

Final predator

J37033

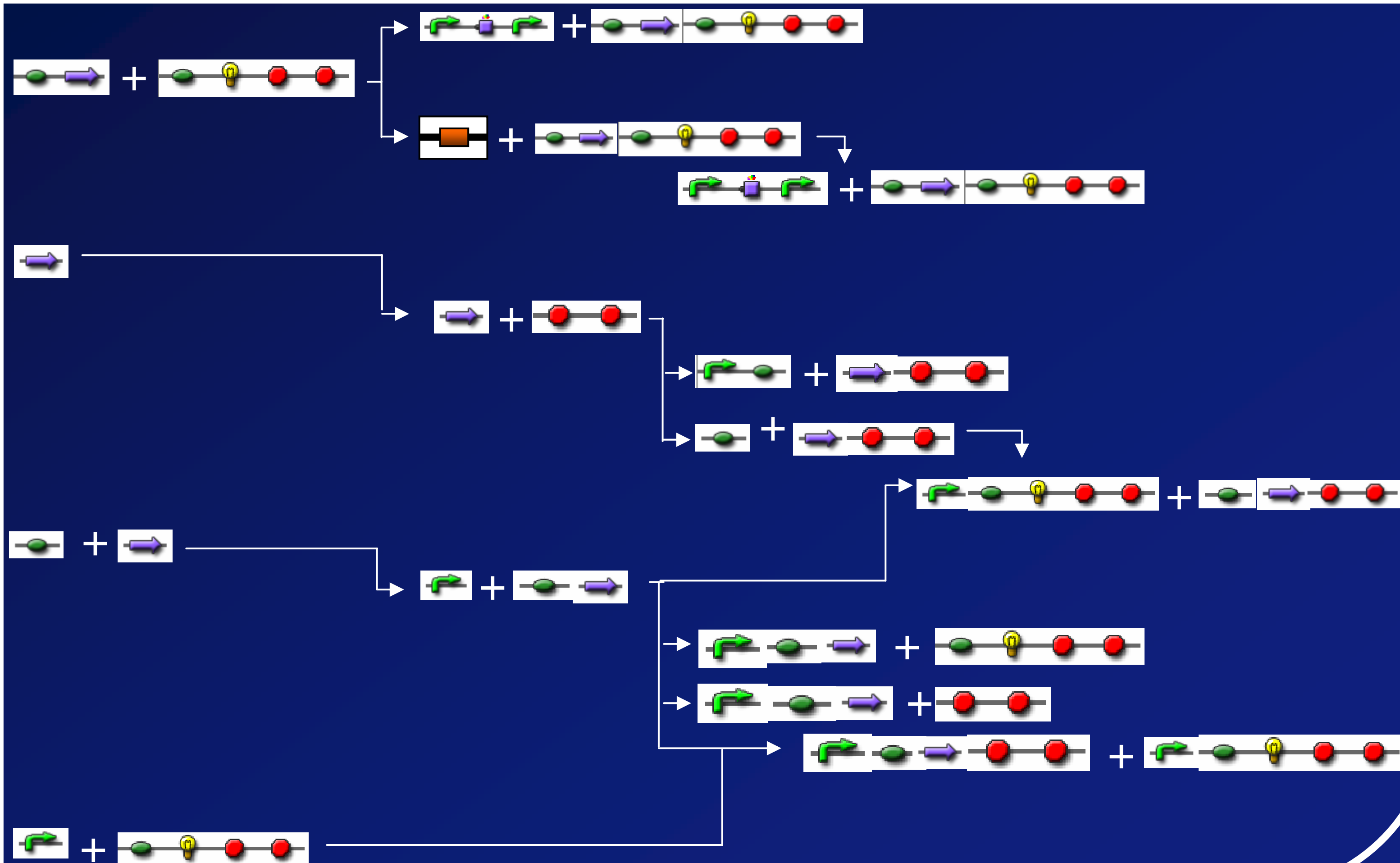
J37019

Sensing predator

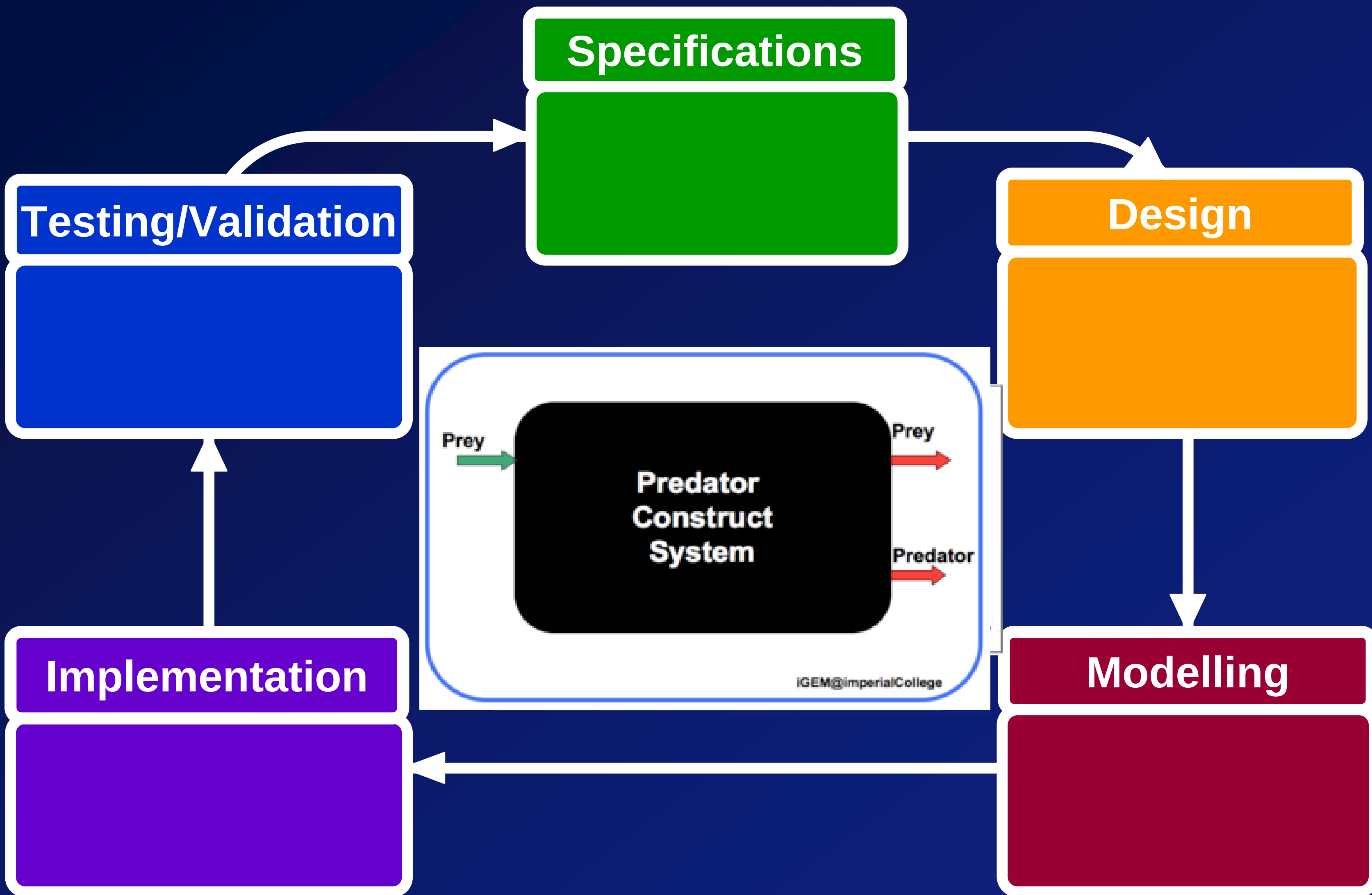
J37031

Sensing predator

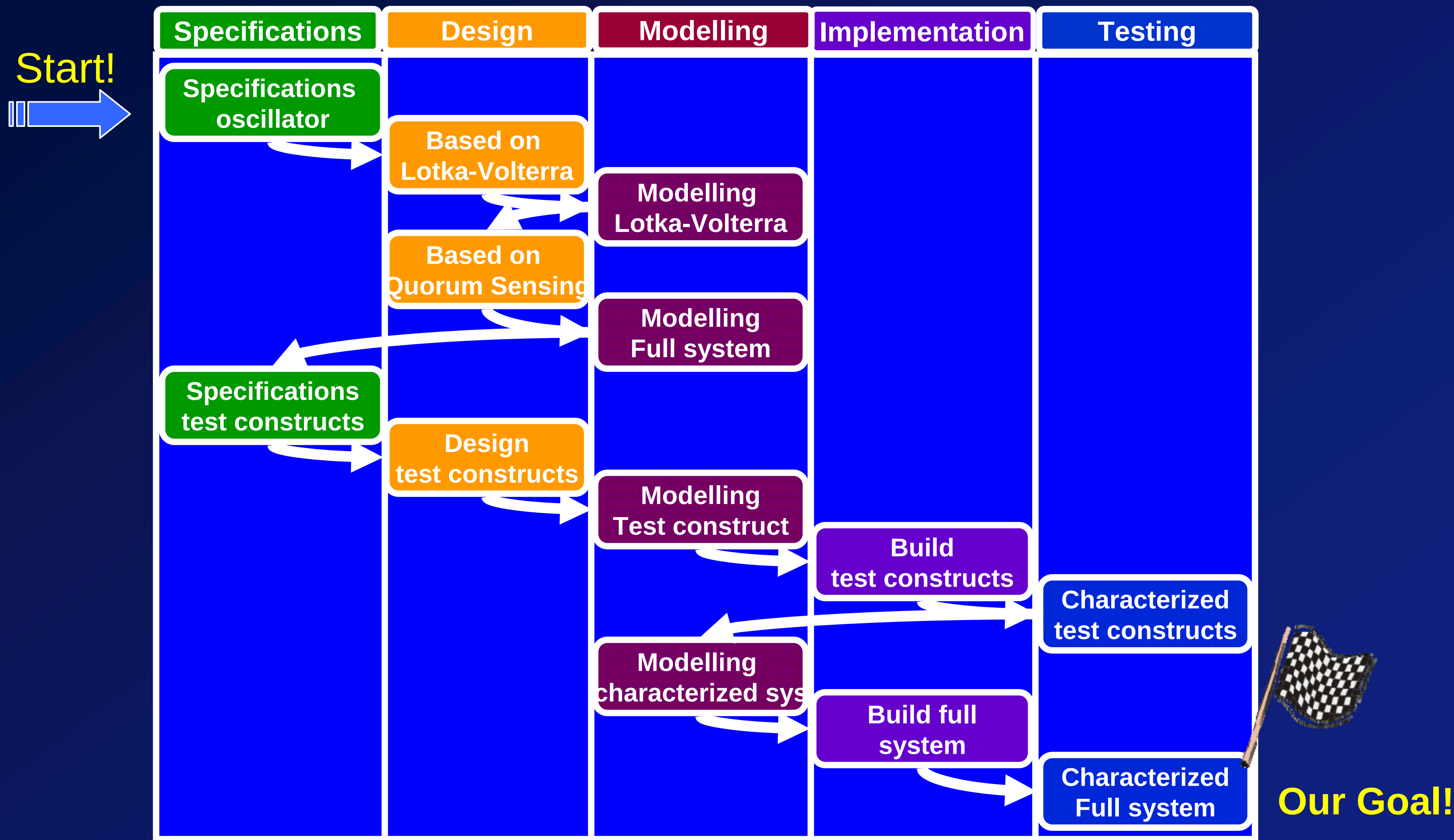
J37032



On Our Experience

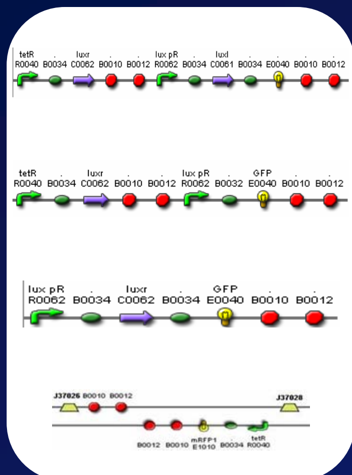


Path to Our Goal



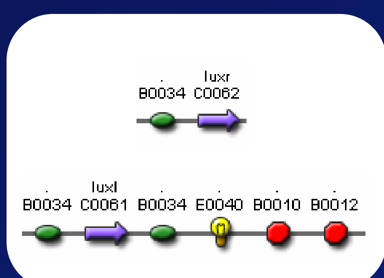
Contributions to the Registry

Functional Parts

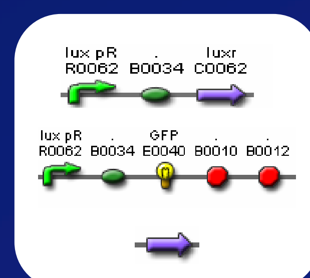


	Built	Sequenced	Tested	Characterized	Documented
Final Prey J37015	✓	✓	✓	✓	✓
Sensing Prey T9002			✓		✓
Sensing Predator J37016	✓	✓	✓	✓	✓
Cre/Lox J37027	✓	✓	✓		✓

Intermediate Parts



	Built	Sequenced
J37033	✓	✓
J37034	✓	✓



	Built	Sequenced
J37019	✓	✓
J37032	✓	✓
J37023	✓	✓

Our Wiki

- Documentation
- Communication
- Organization

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

Thank You

Imperial College London

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- Ciaran Mckeown
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