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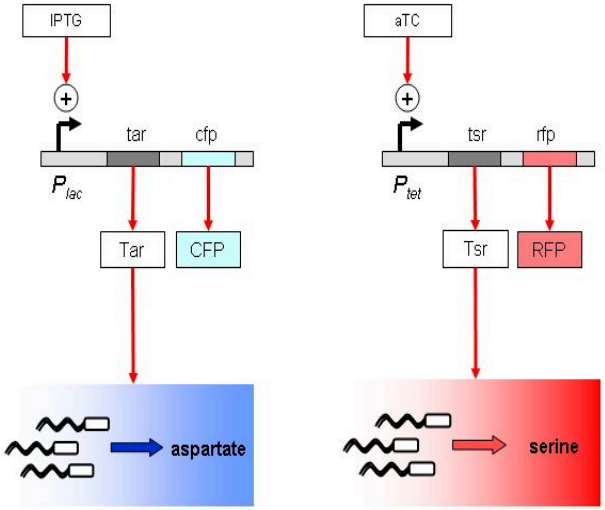
iGEM 2006 PRESENTATION

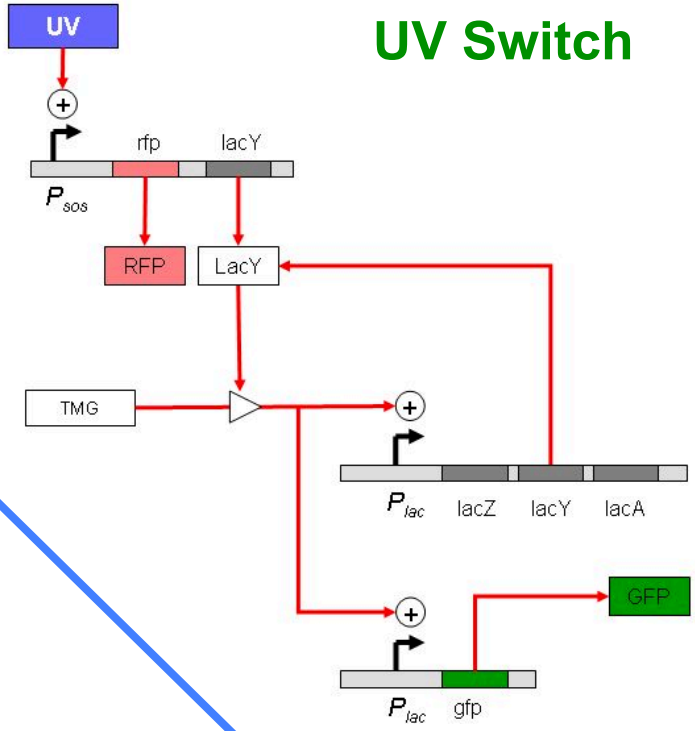
The NCBS Team :
Adil, Aparna, Ashesh, Dhanya, Krithiga, Ruchi, Sugat and Mukund

www.ncbs.res.in/events/

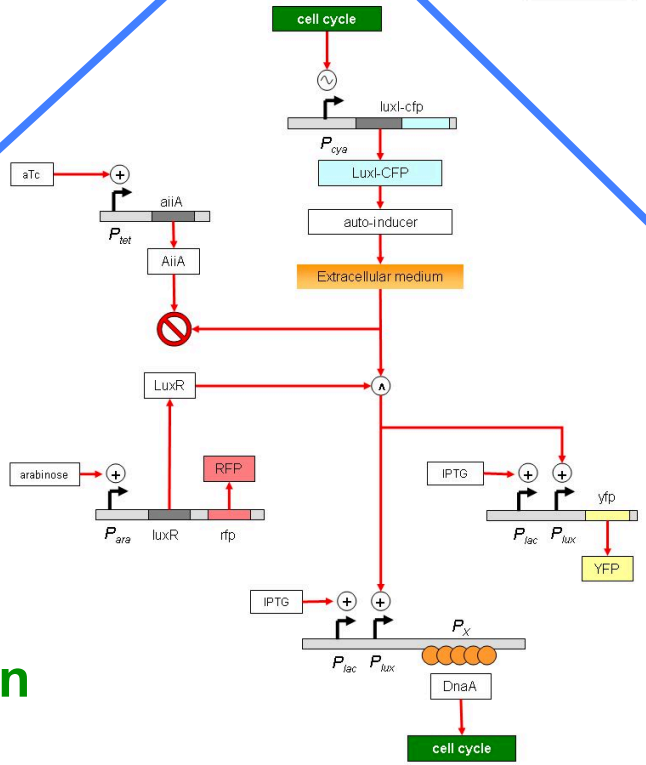
The Living Networks



2D Chemotaxis



UV Switch



Cell-Cycle Synchronization

2-D CONTROL OVER CHEMOTAXIS

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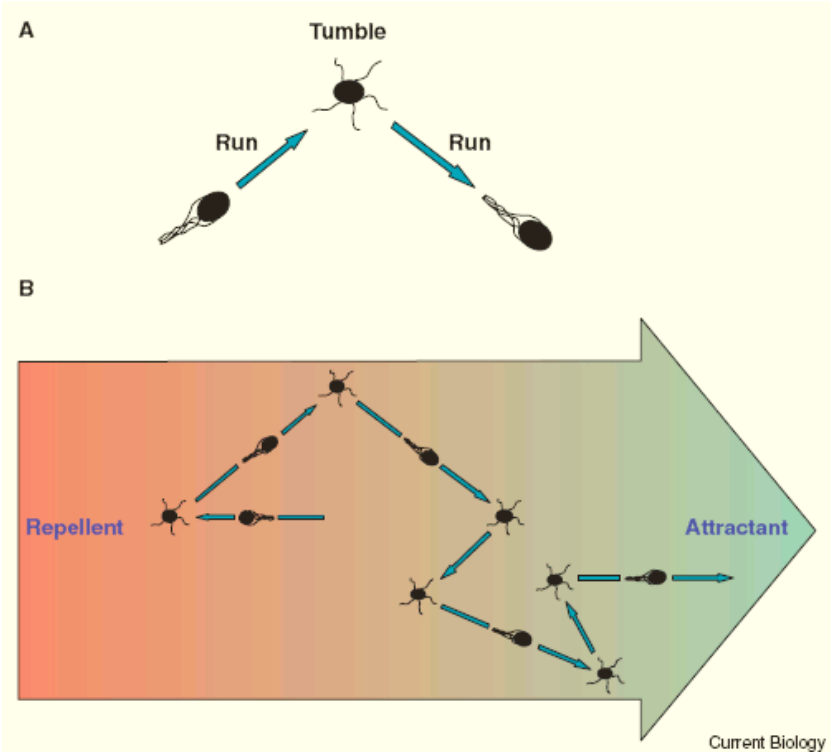

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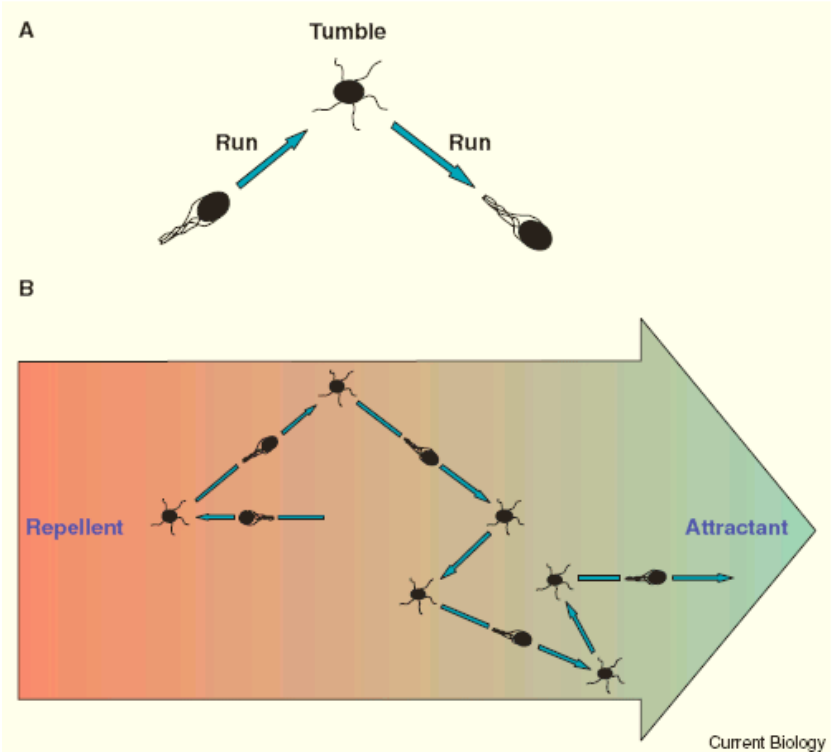
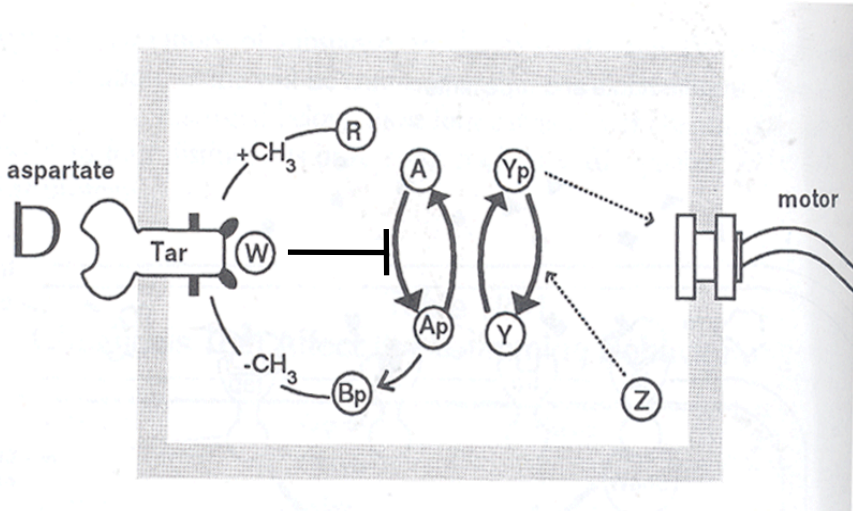


Chemosensory receptors :
Tar or Tsr



Bray D, Cell Movements (Garland, 1992)

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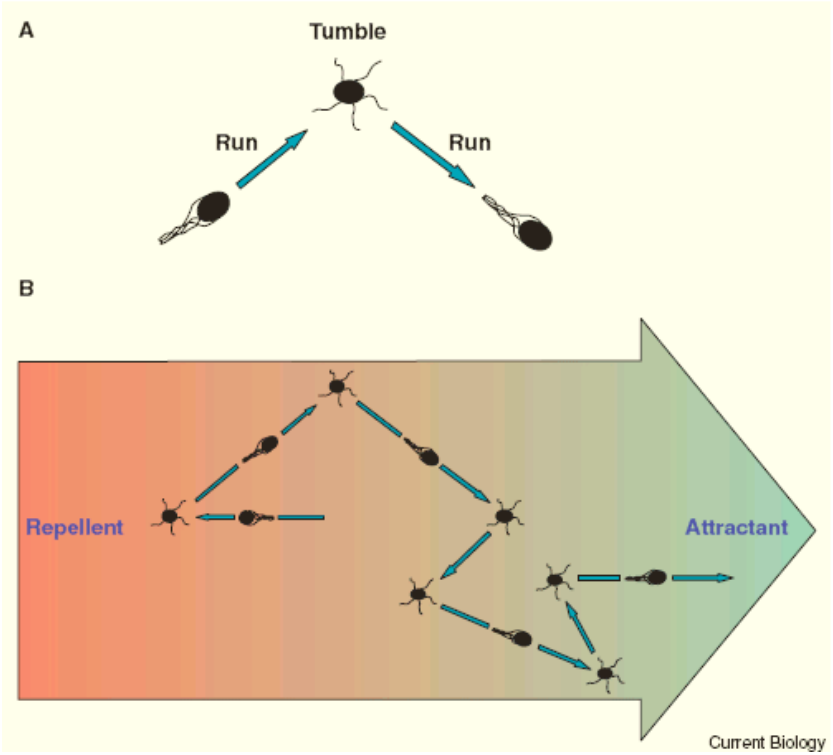

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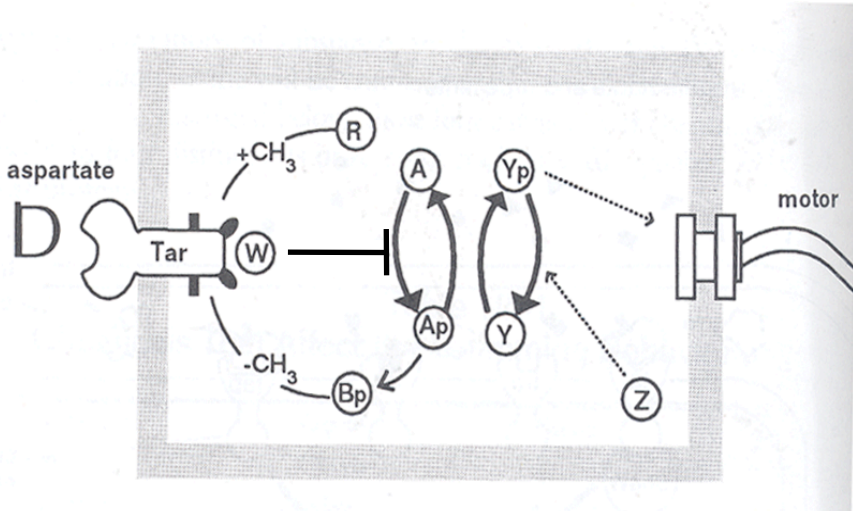
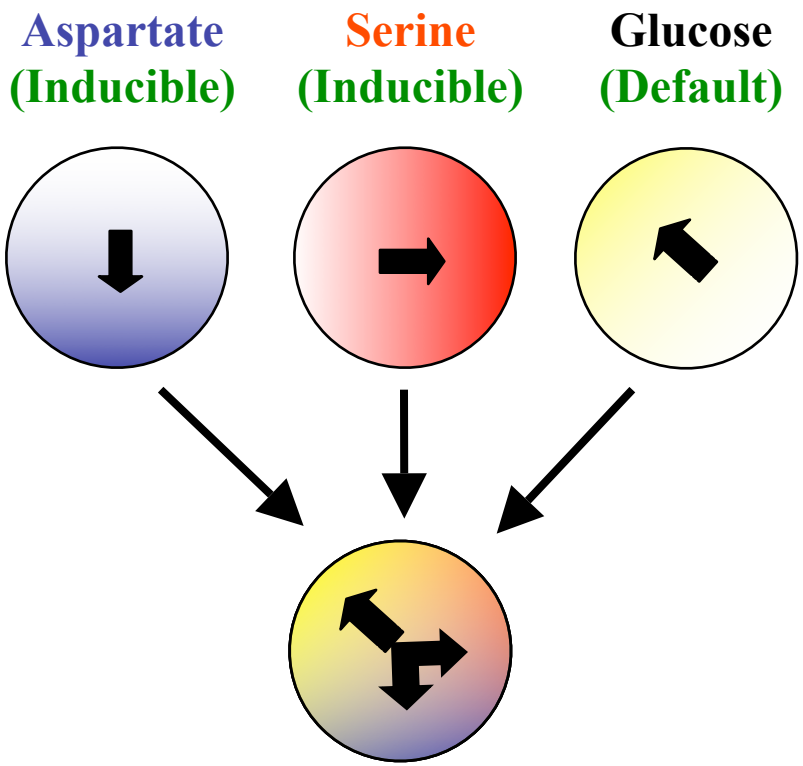


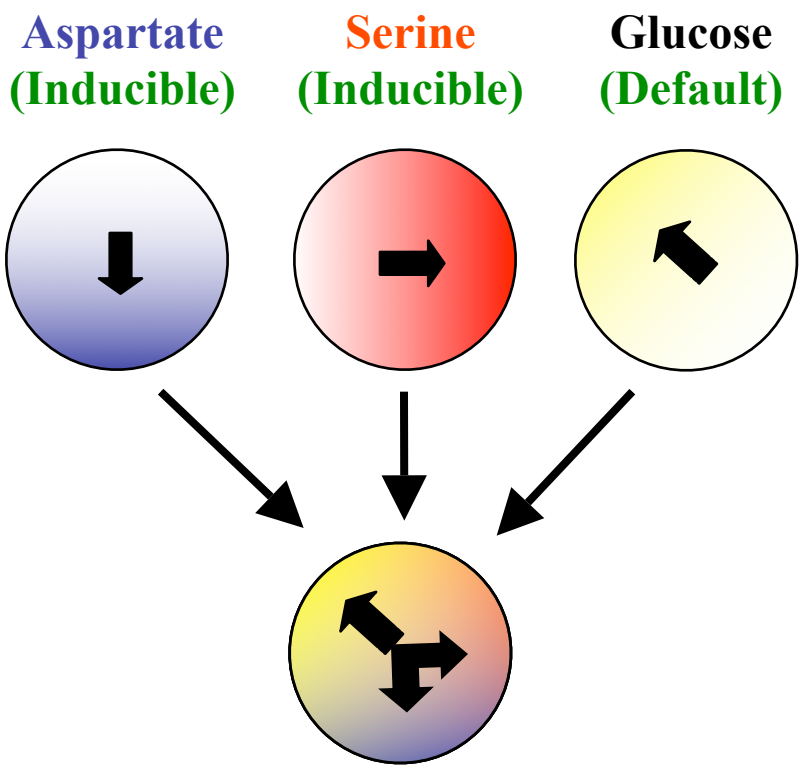
Bray D, Cell Movements (Garland, 1992)

Can we achieve 2-D control by hacking into this system?

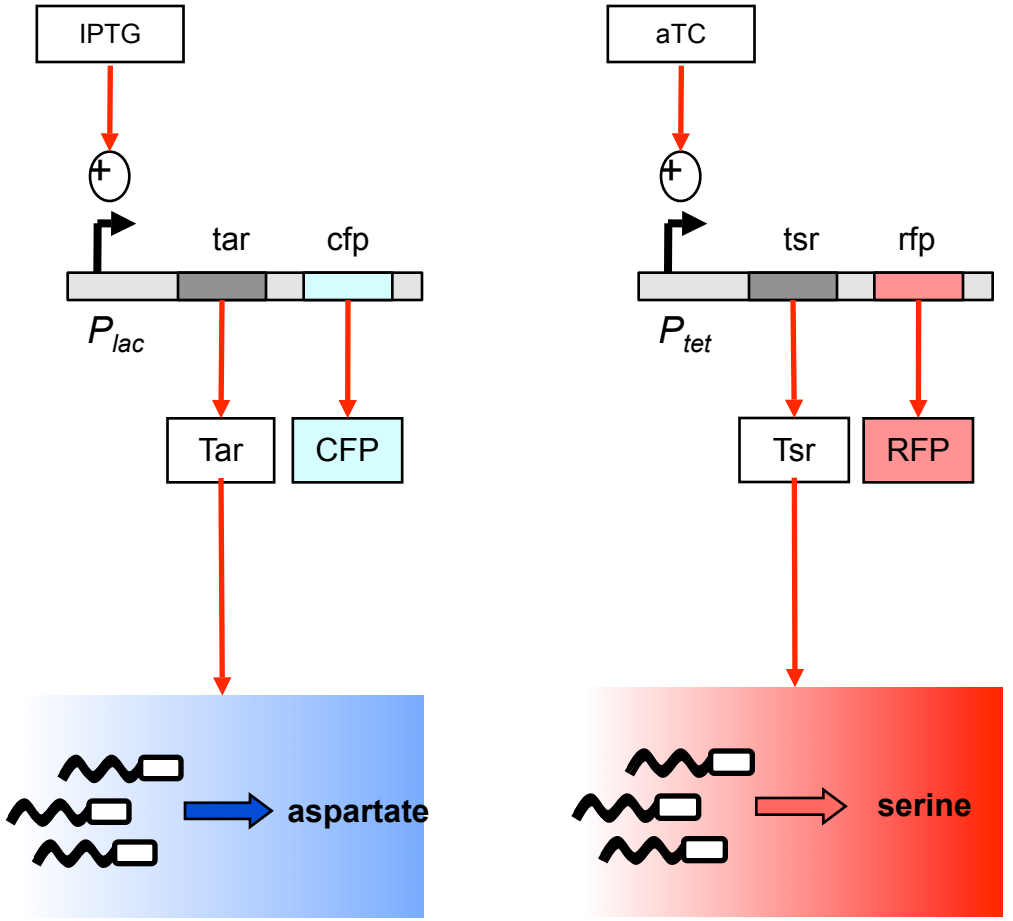
Tri-Gradient System



Tri-Gradient System



Constructs



Transform *tar, tsr* null strain UU1250

UU1250 : Gift from Sandy Parkinson

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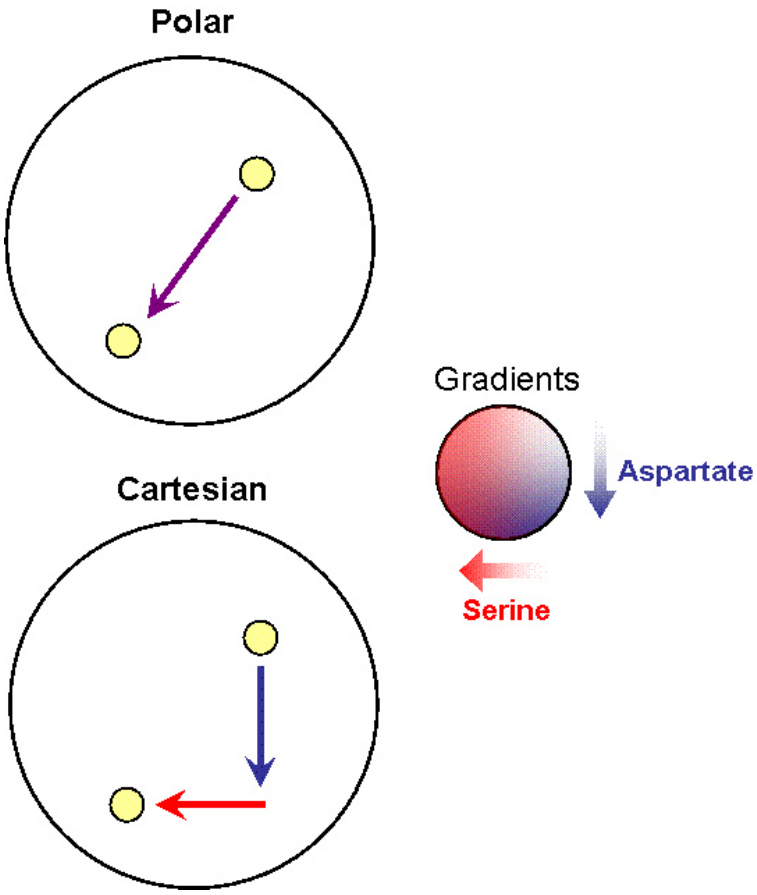
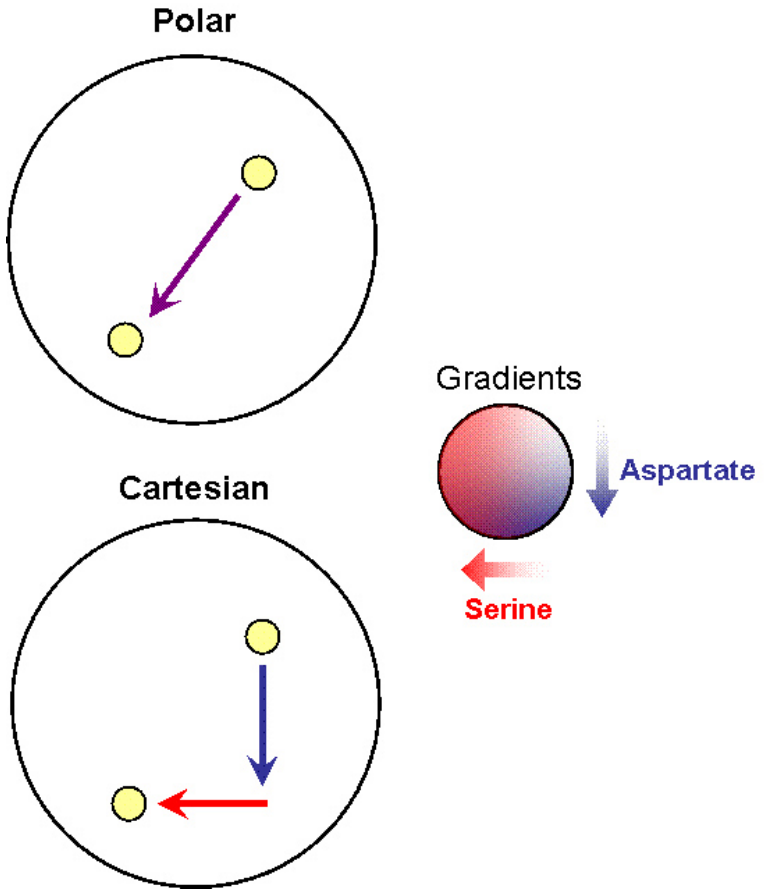
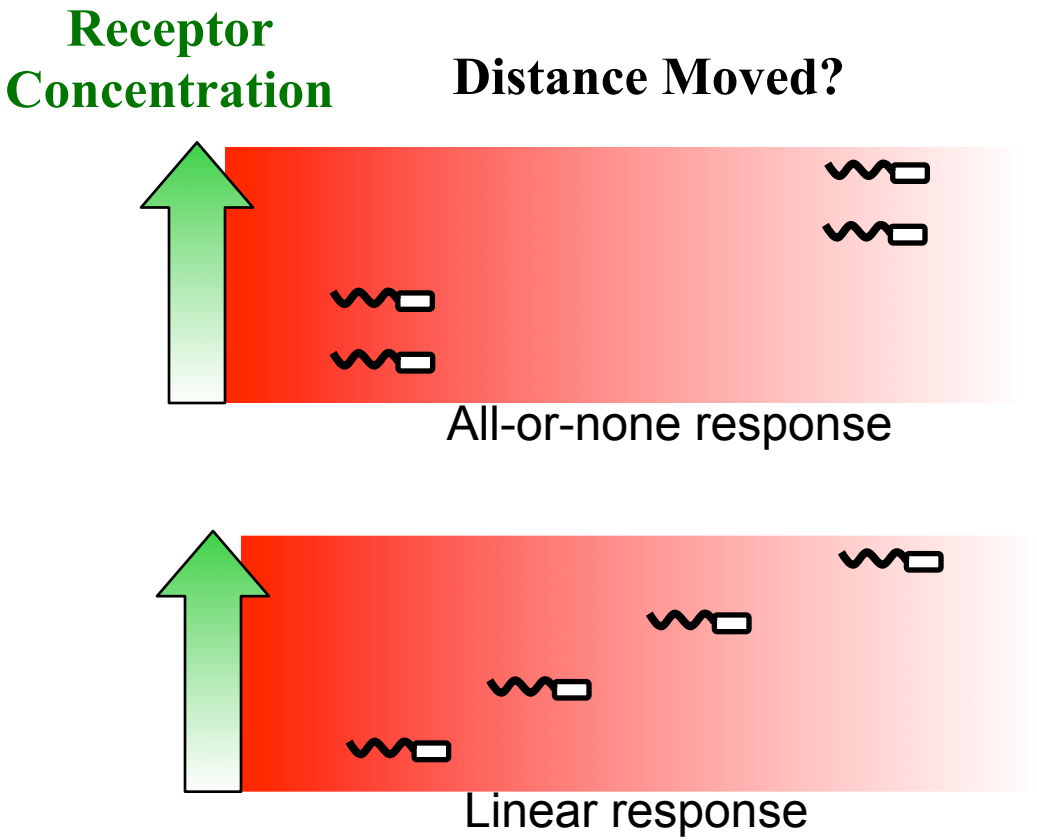
Motion-control Strategies



Motion-control Strategies



Response to a single gradient



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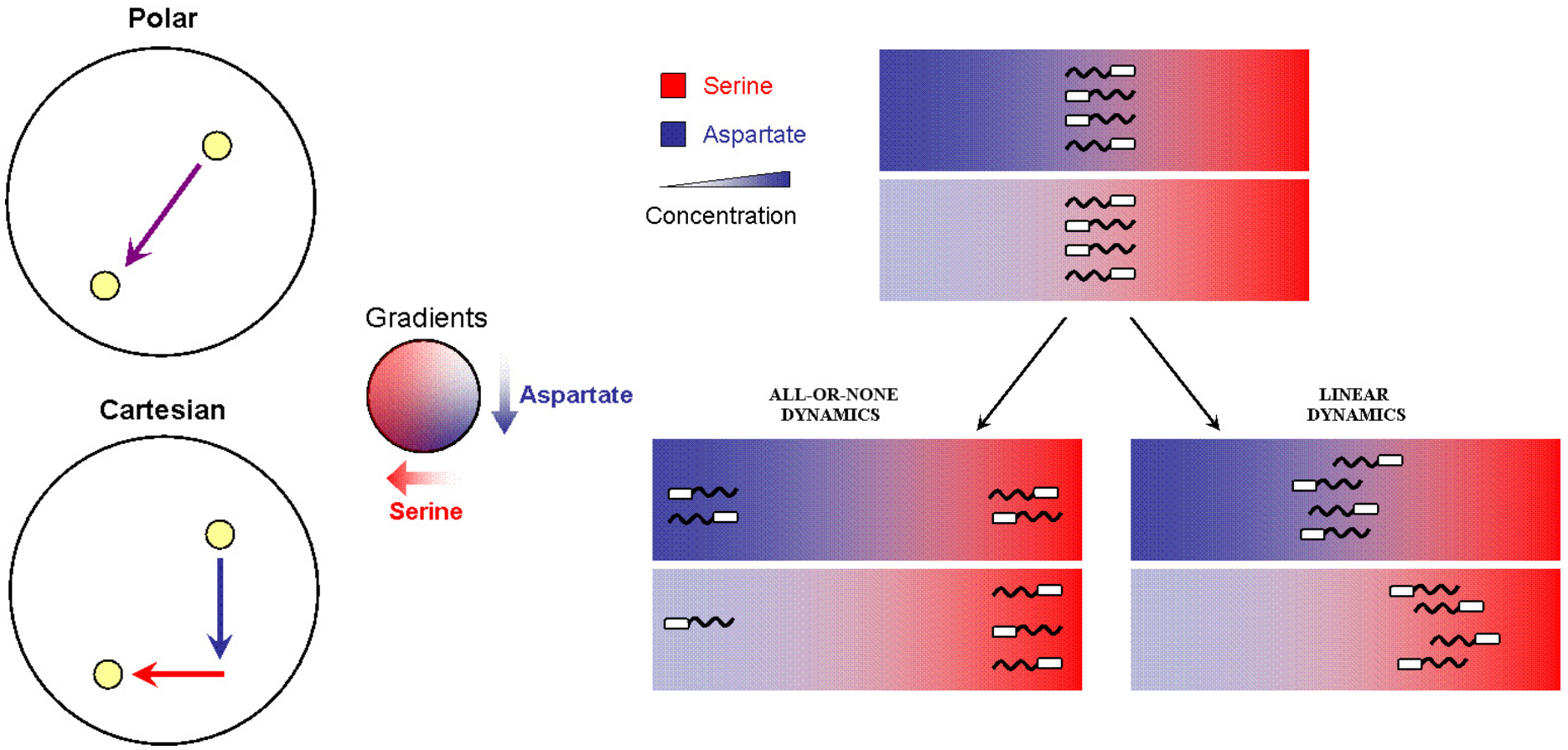
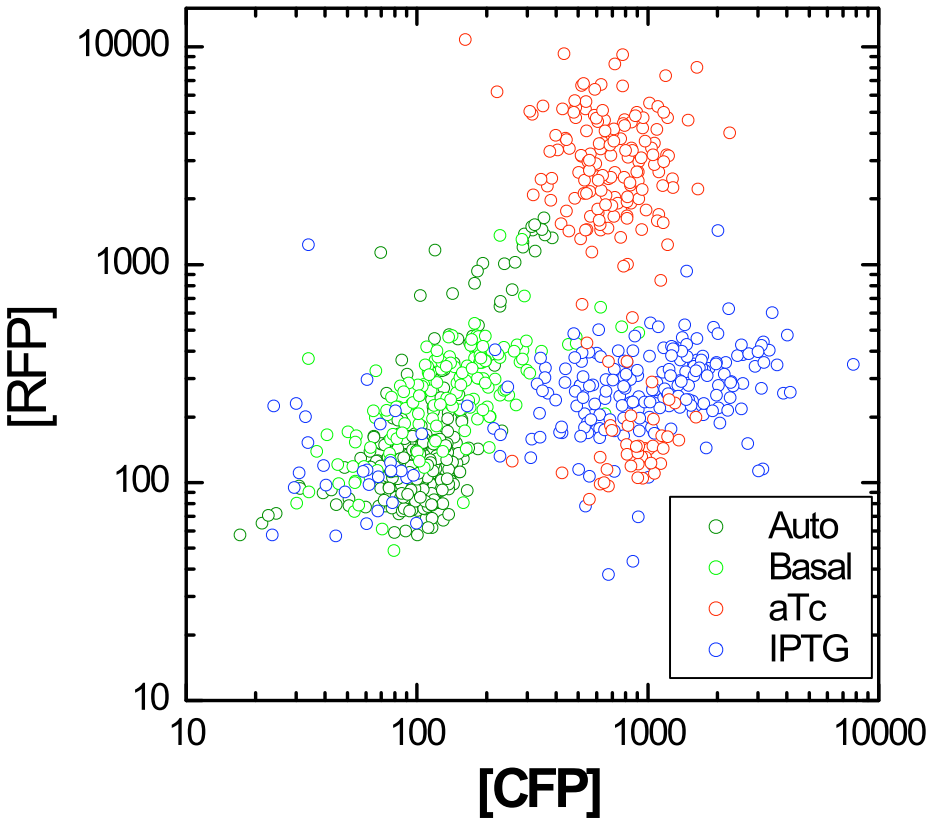
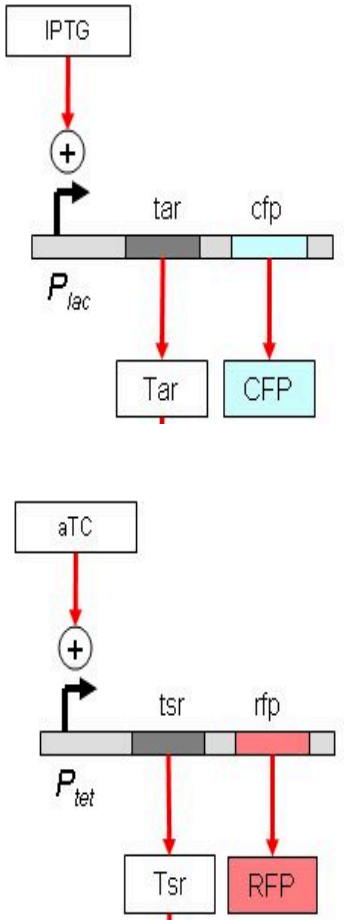


Experiments
Induction Results





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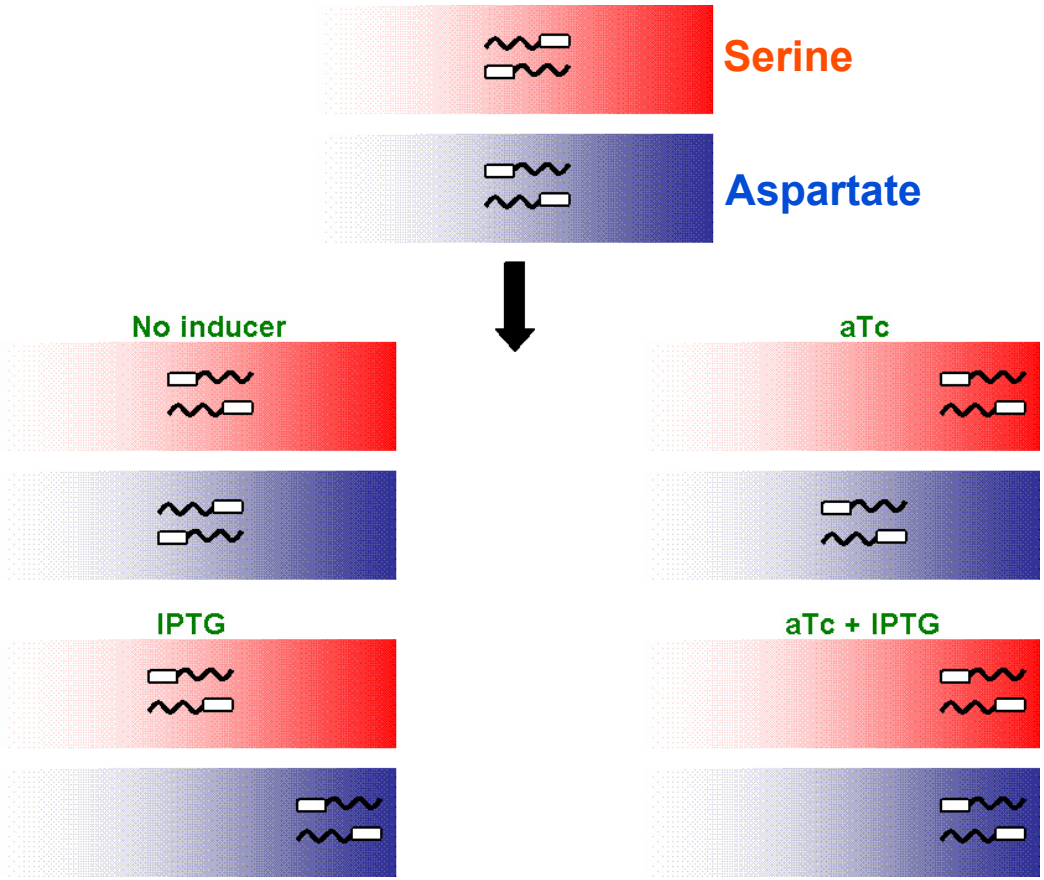
Assays for Chemotaxis

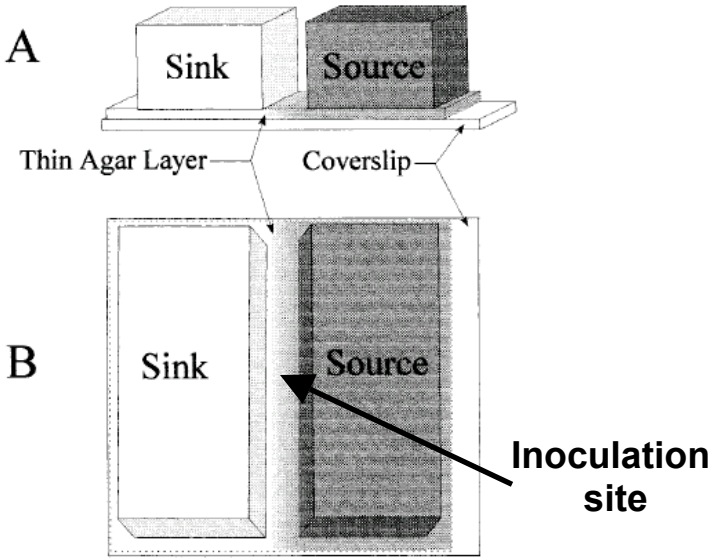
Predicted Outcome



Setting up Gradients

Setting up Gradients

Bridge Setup (Microscopic)



Tieman et al (1996)
J Bacteriology

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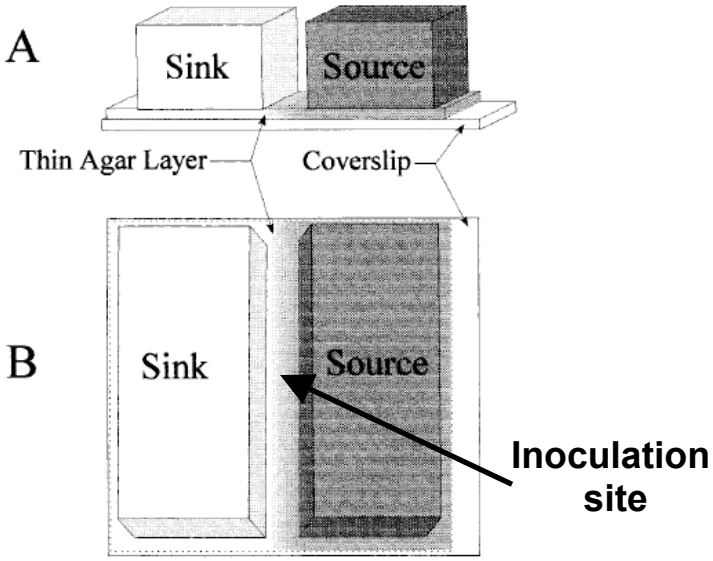

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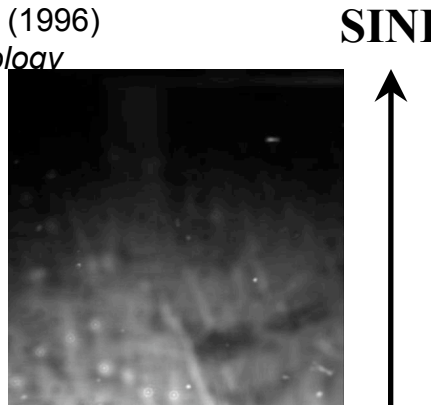

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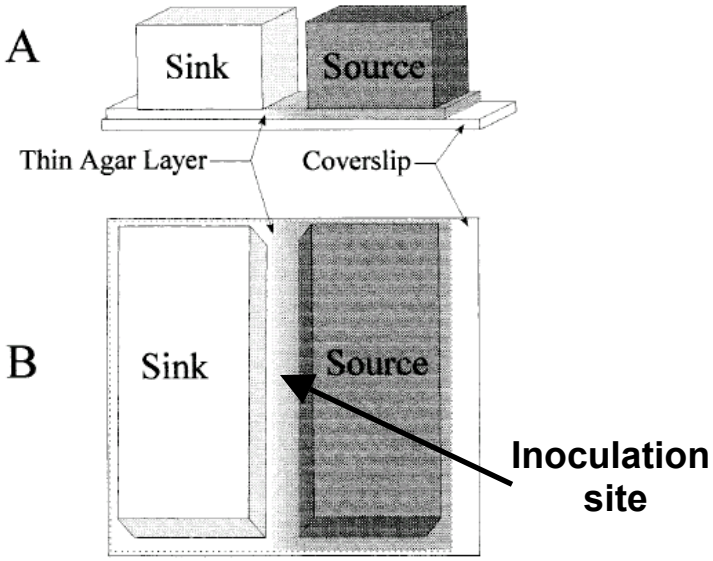

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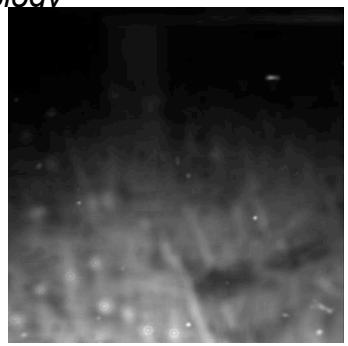
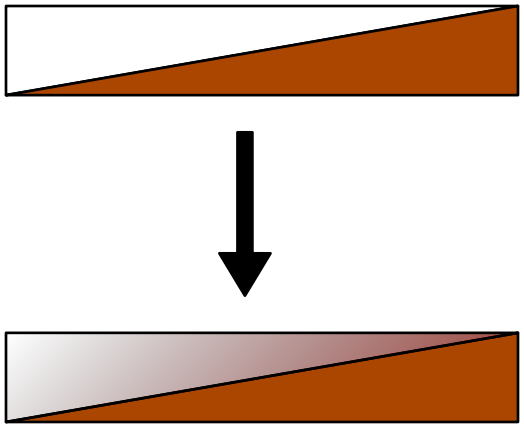


Slant Plate (Macroscopic)



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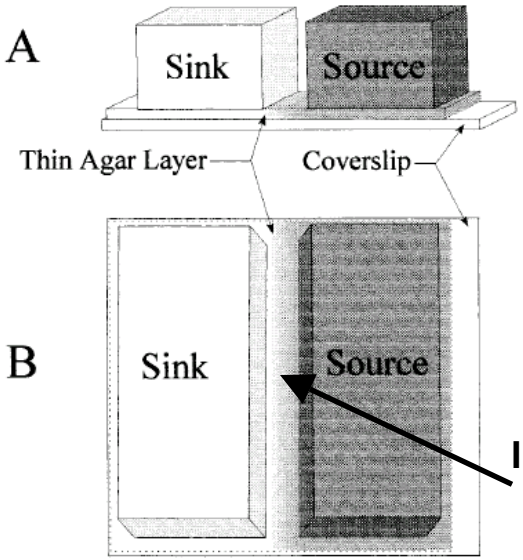
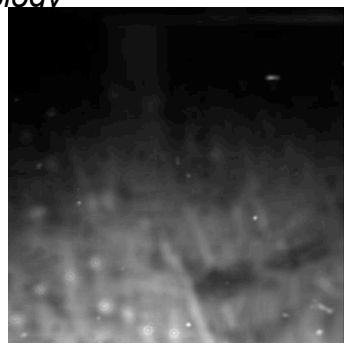


Inoculation site

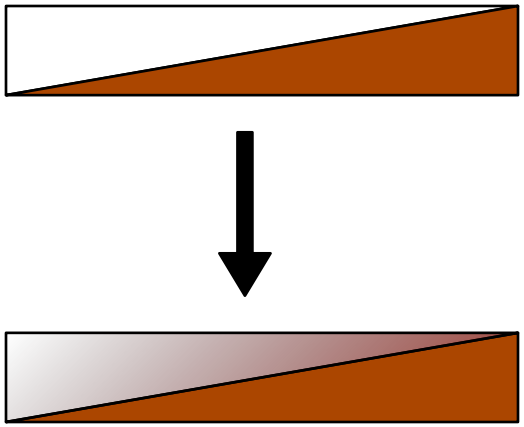
Tieman et al (1996)
J Bacteriology



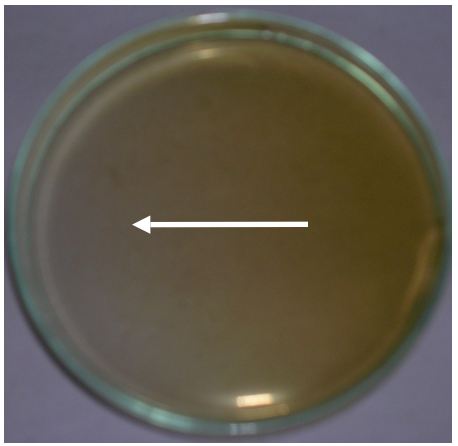
SINK

SOURCE (Rhodamine)

Slant Plate (Macroscopic)

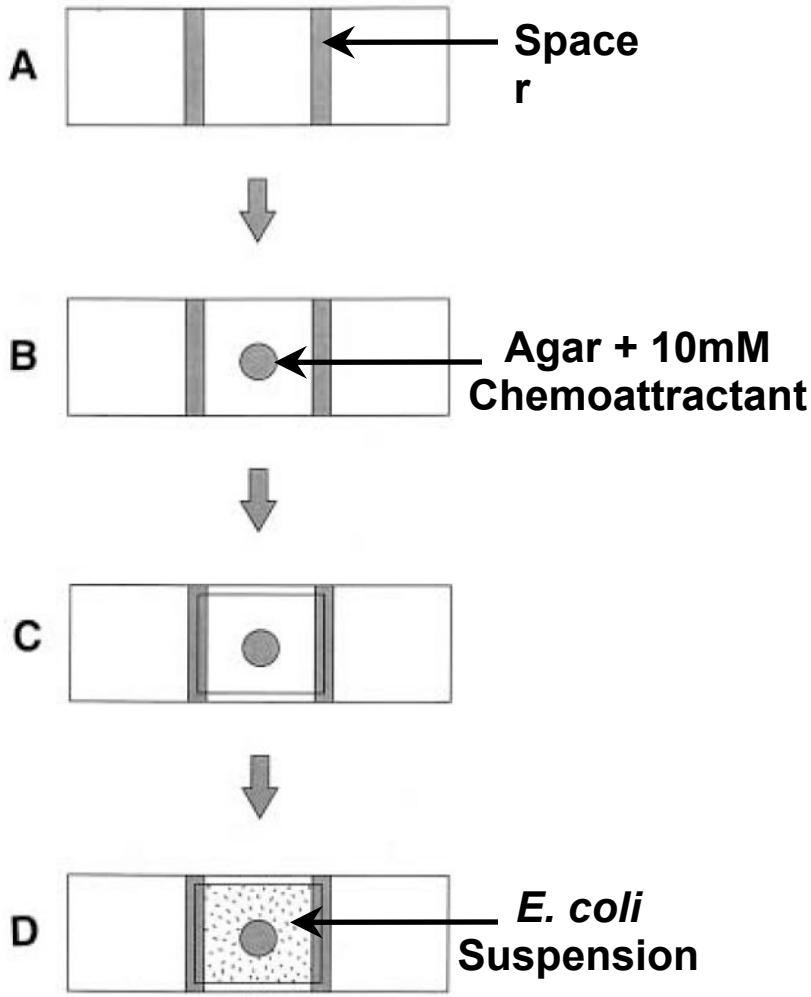


$KMnO_4$ gradient



Assays for Chemotaxis

Agar Plug Assay (Microscopic)



Yu & Alam (1997) FEMS Microbiol Lett

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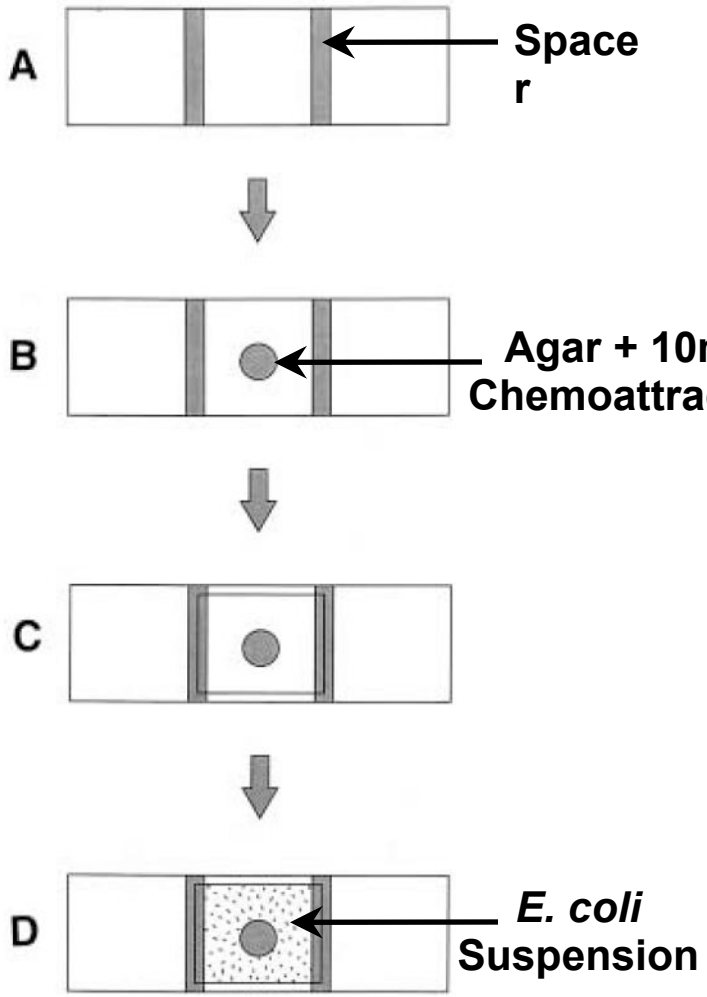

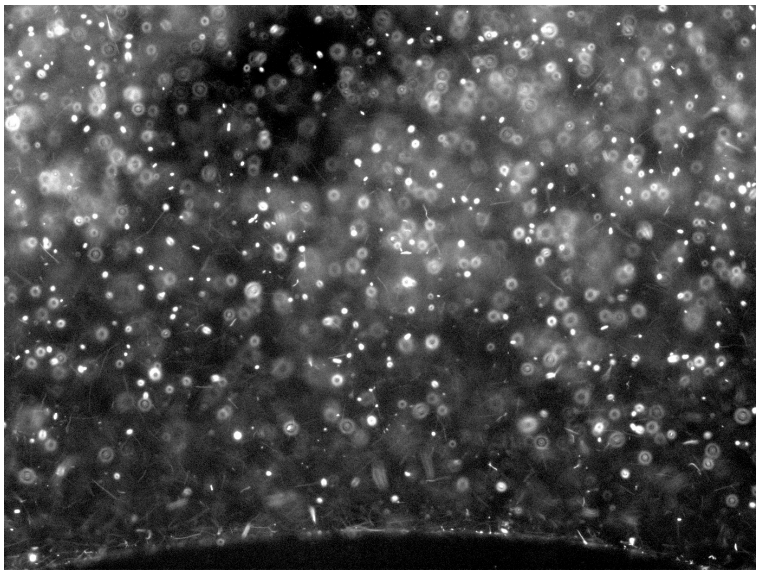

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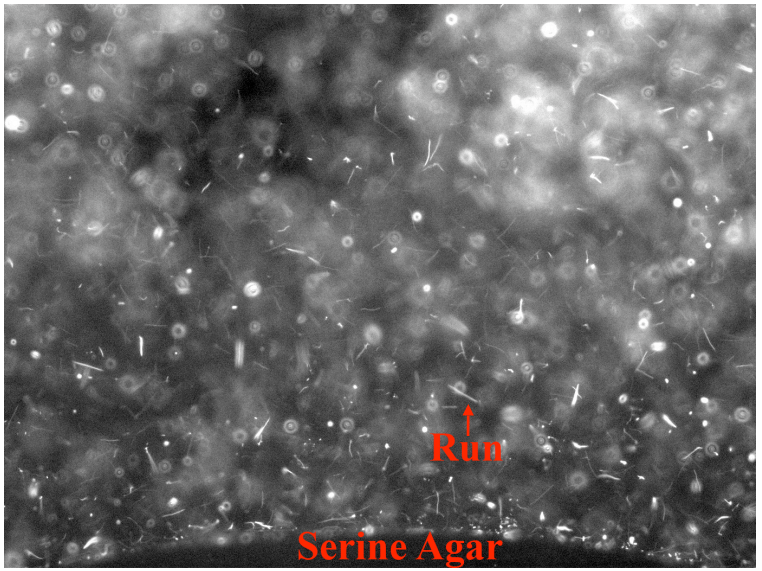


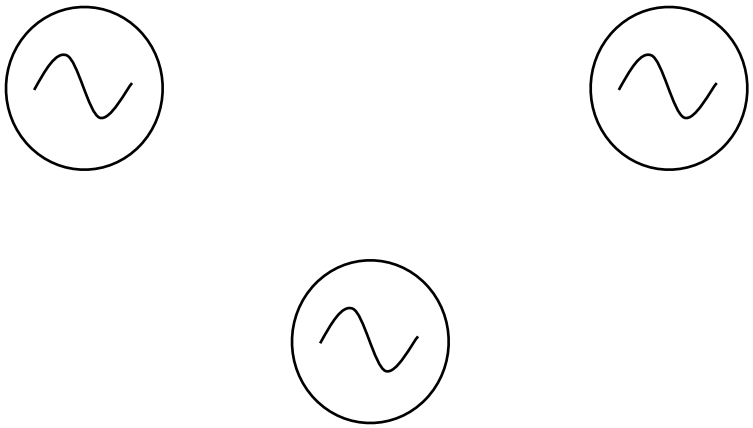
29 min

Yu & Alam (1997) FEMS Microbiol Lett

What next?

- Yet to establish that the constructs rescue chemotaxis
- Resolving cross-induction issue
- Fine tuning of Plug Assay
- Dual gradient experiments
- Construction of chemotaxis model incorporating receptor interactions

SYNCHRONIZATION OF CELL CYCLE OSCILLATORS



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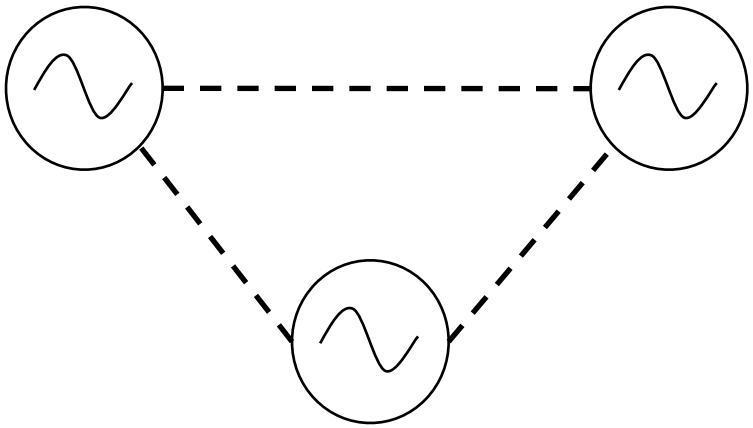

A multi-cell system:

Core oscillator: The *E.coli* cell cycle
Oscillator coupling: *Vibrio* quorum sensing machinery
Cell cycle modulation: DnaA sequestration

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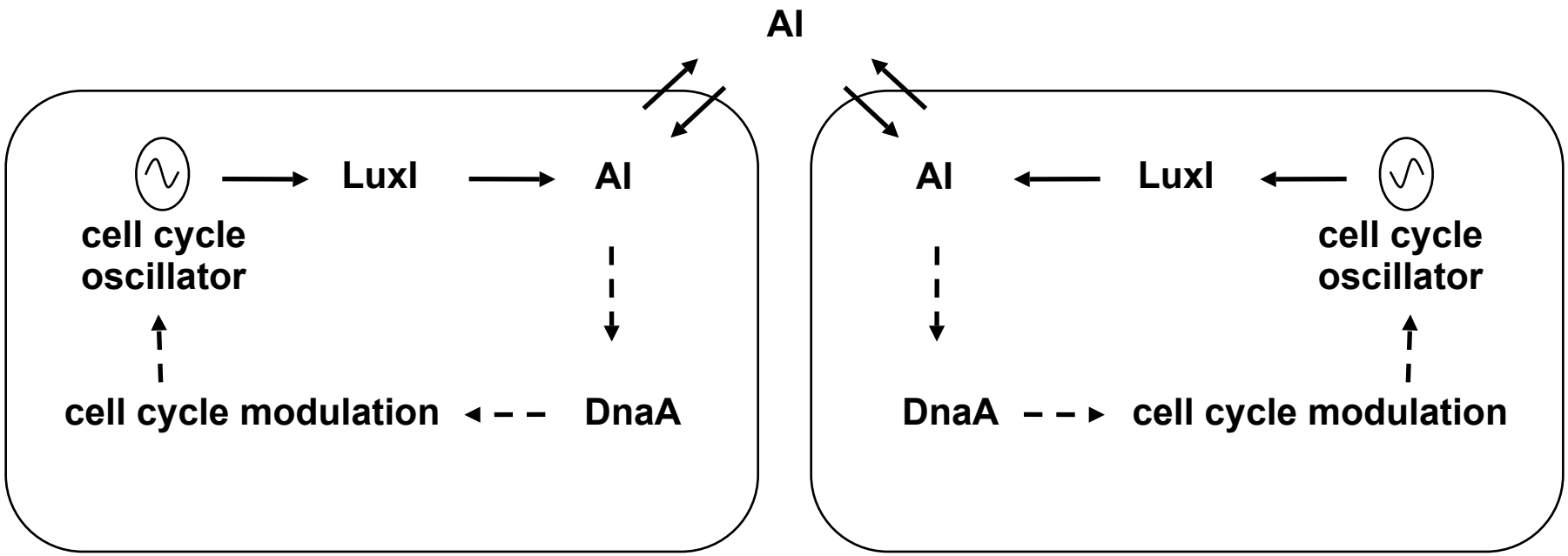
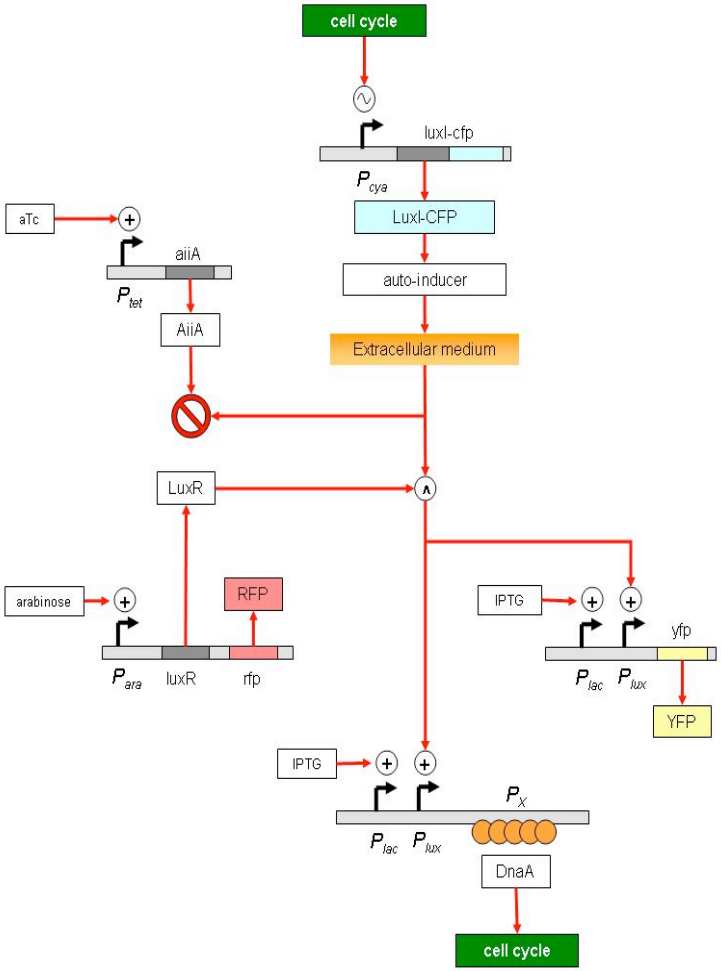


Design and Features:

Modular assembly allows us to test and modify intermediates independently.



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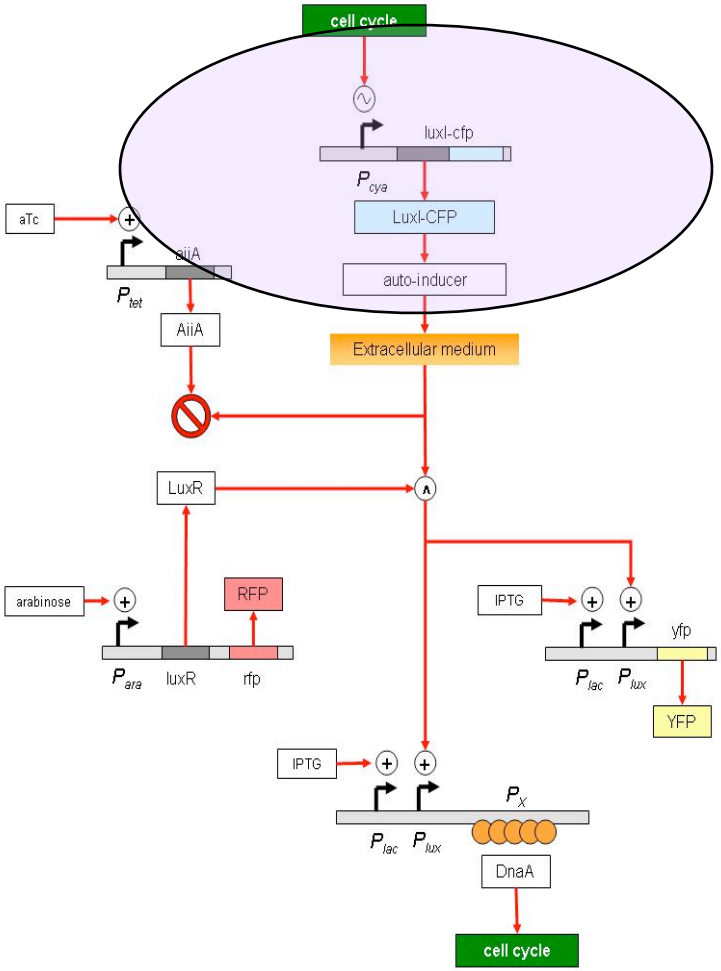
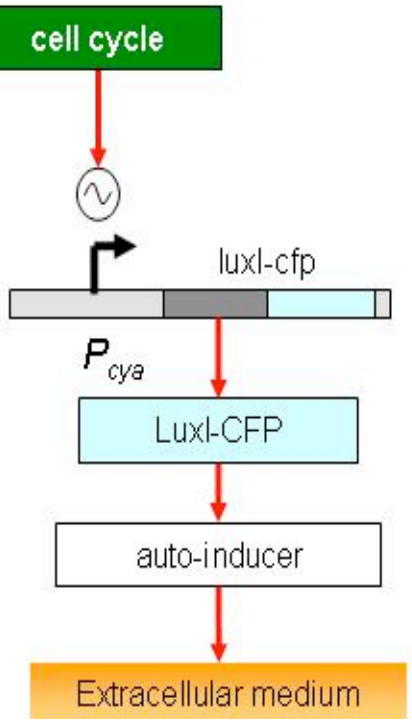


Oscillatory sender module



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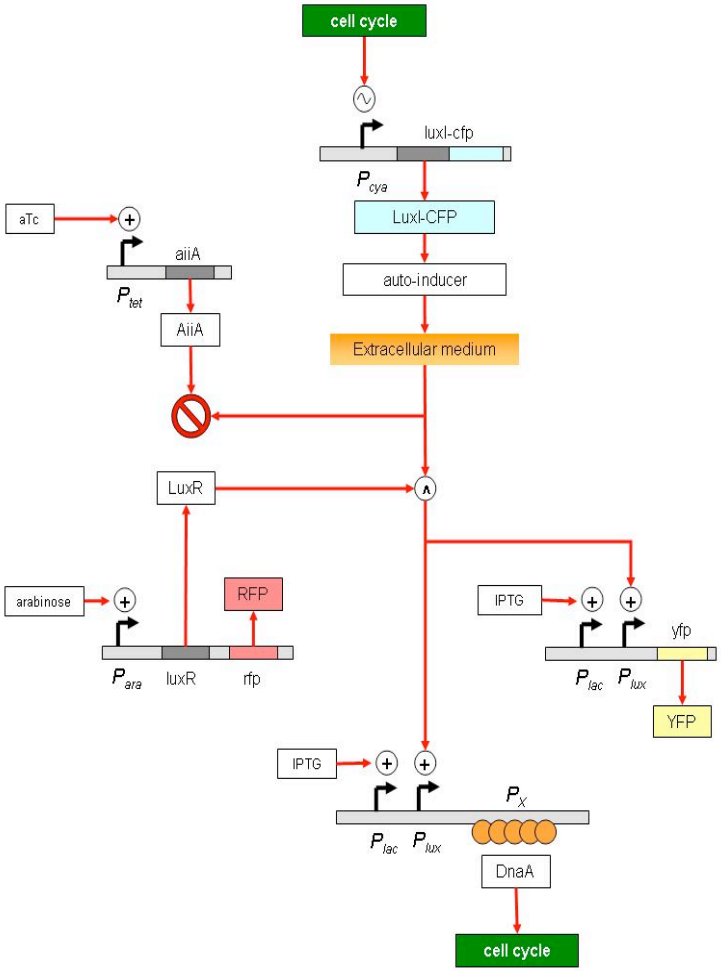

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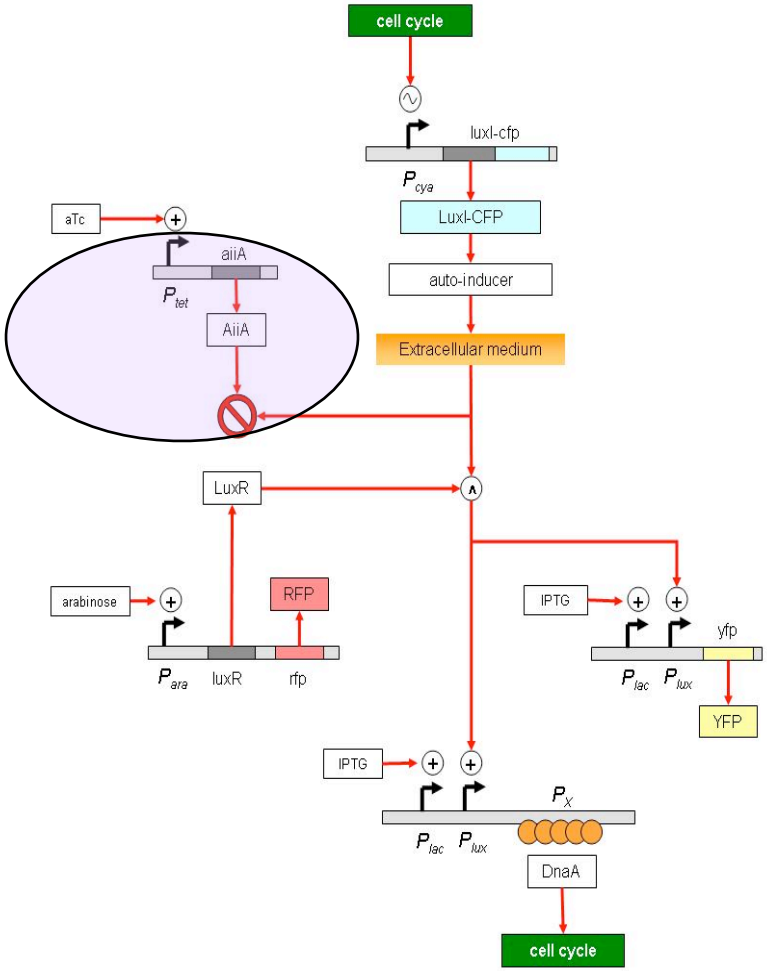
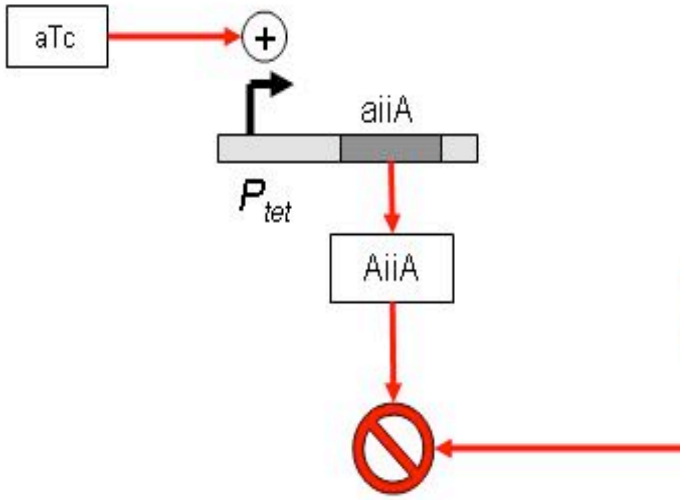


AI degradation module



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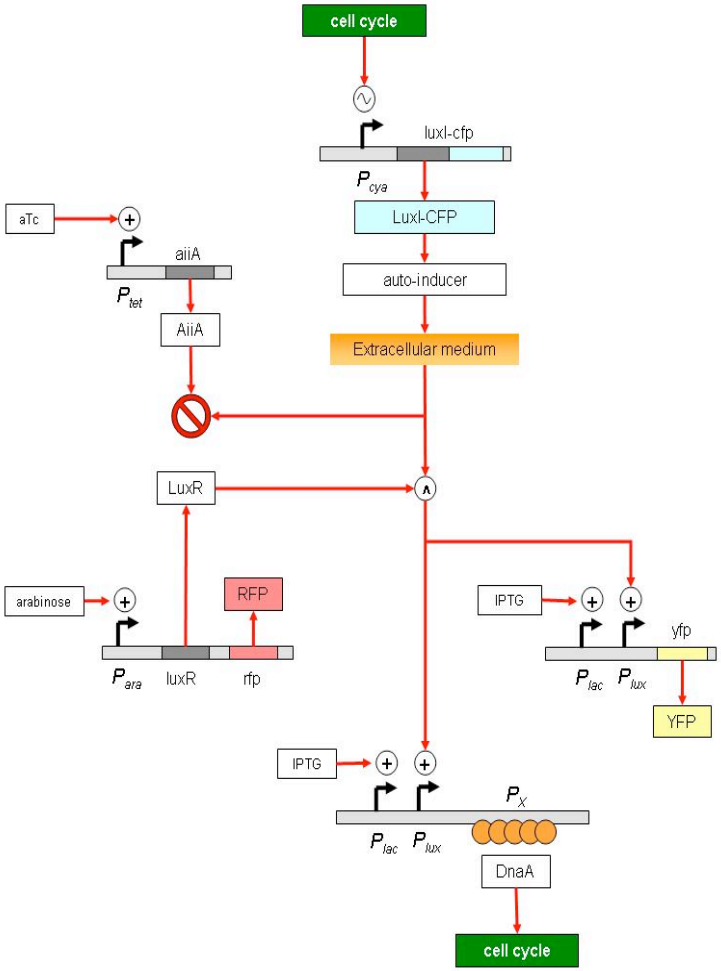

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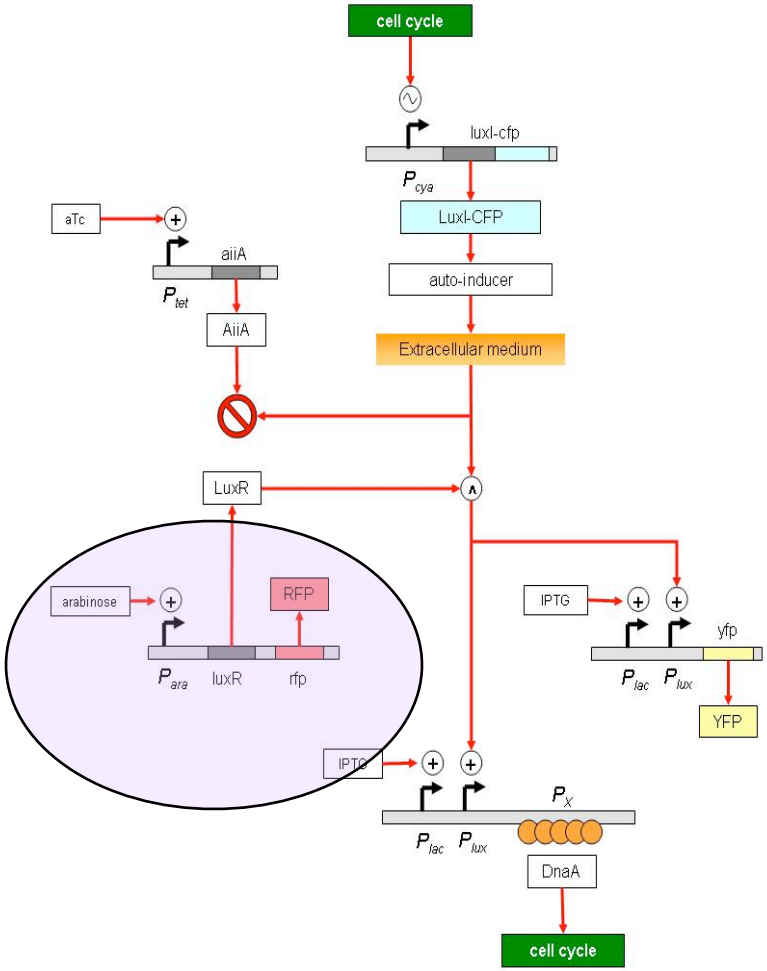
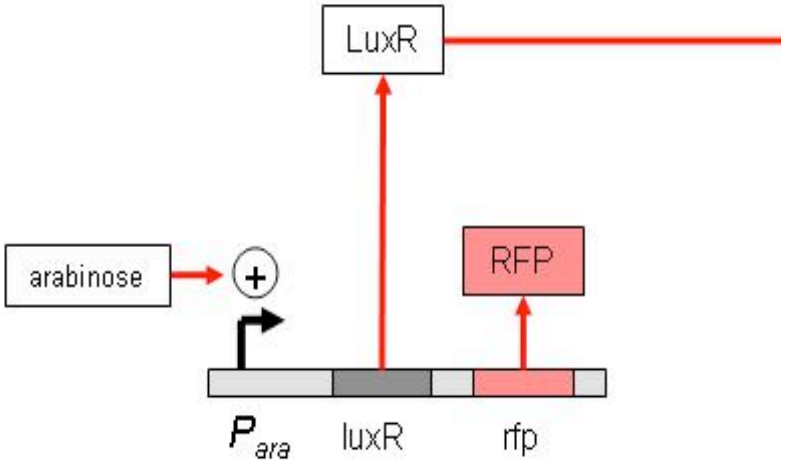


Receiver module



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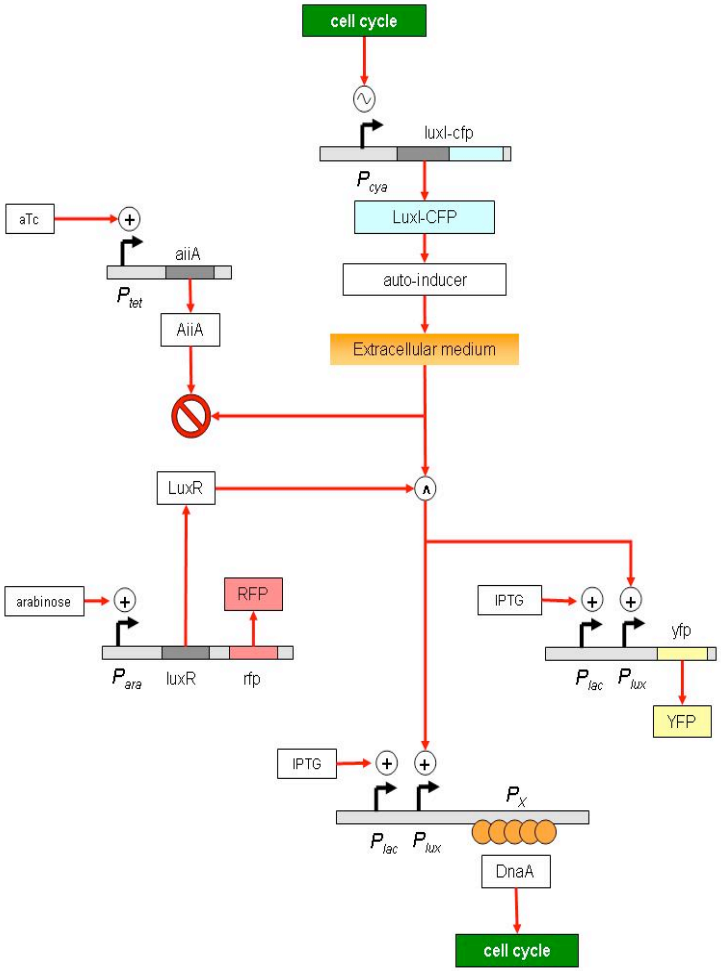

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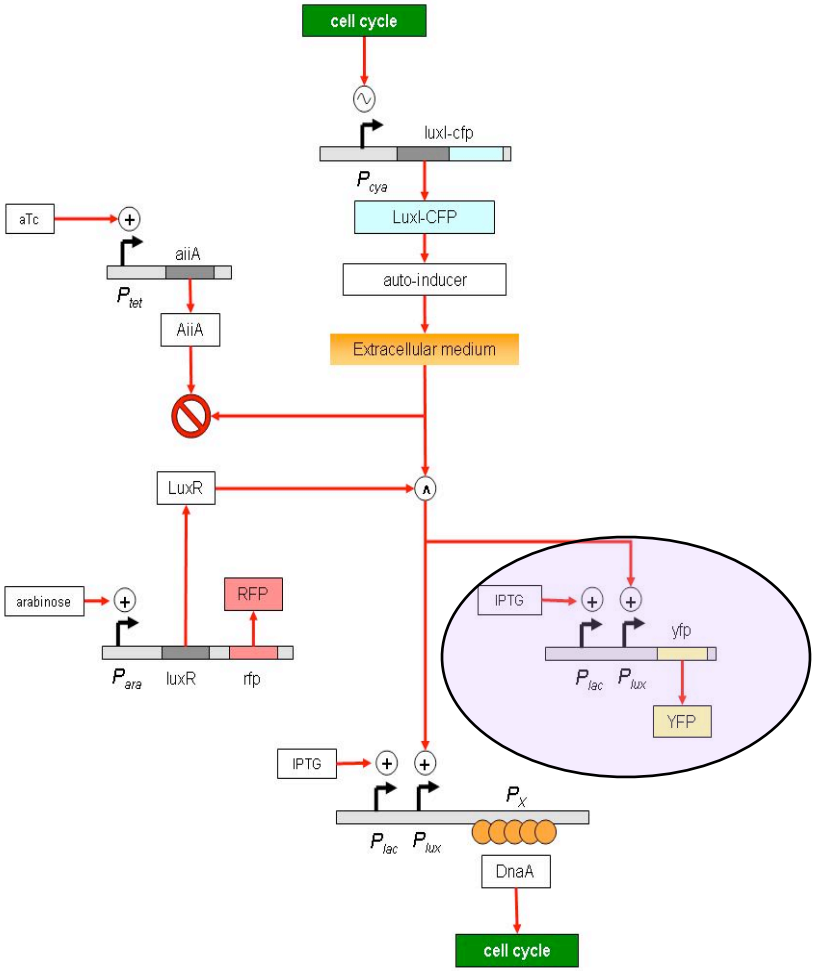
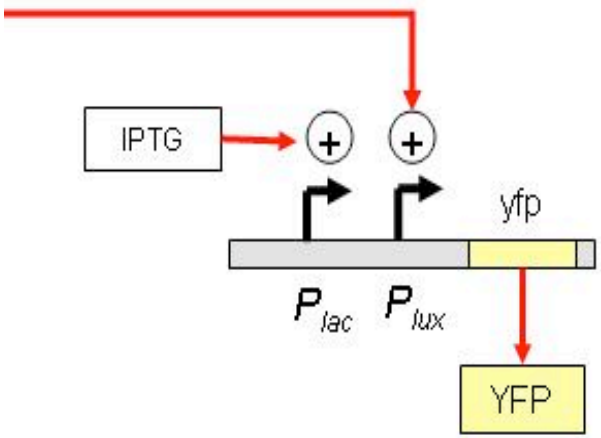


Oscillation readout module



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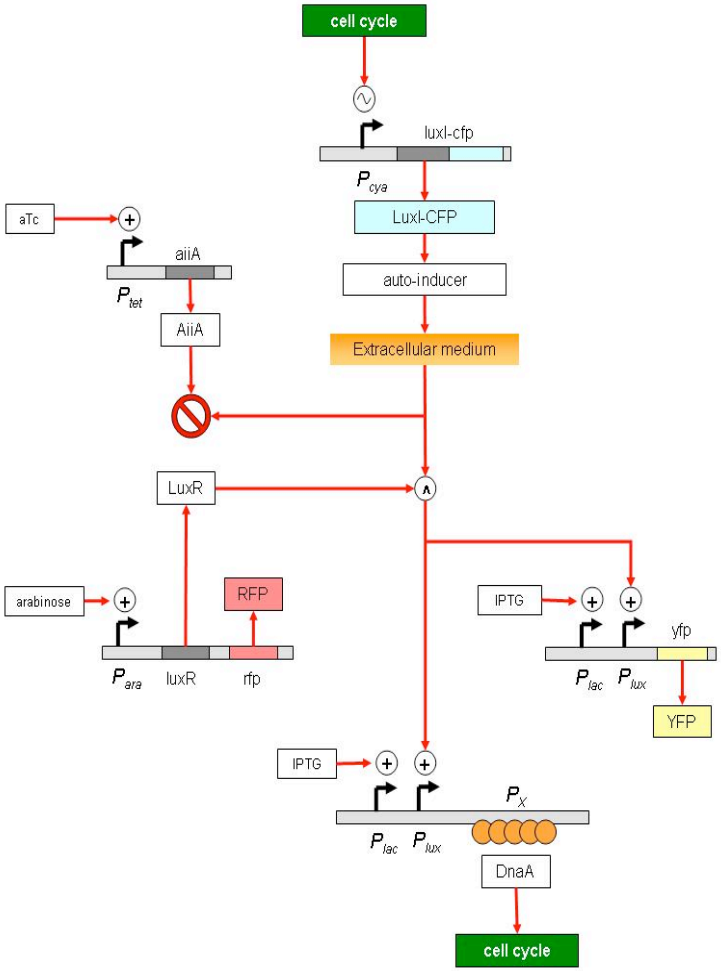

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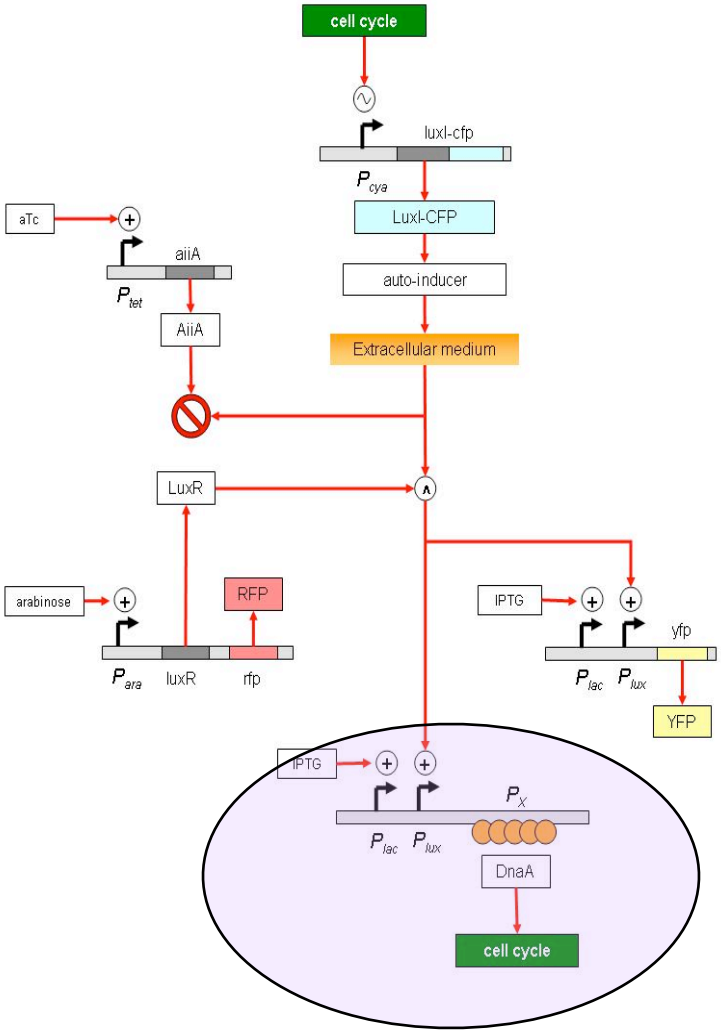
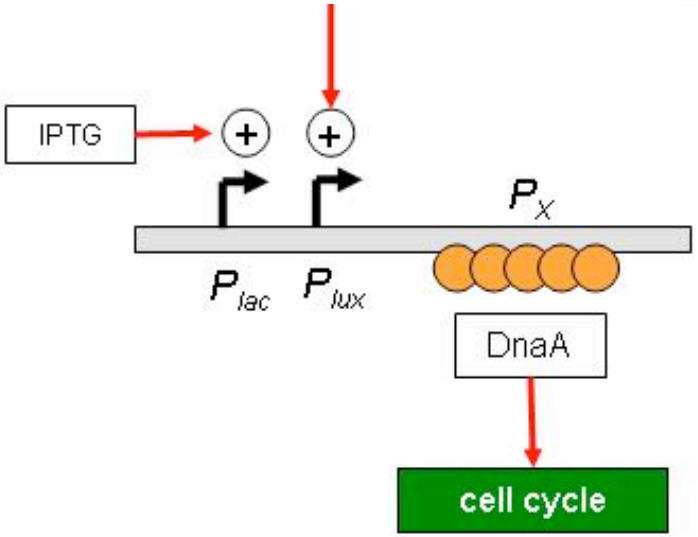


Cell cycle modulation



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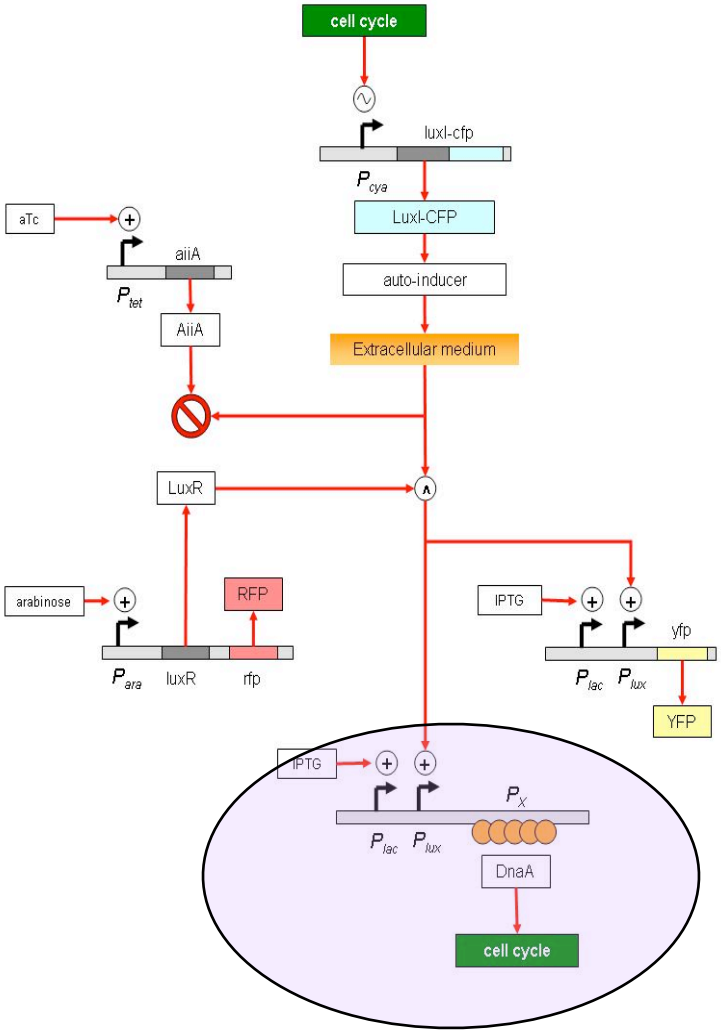

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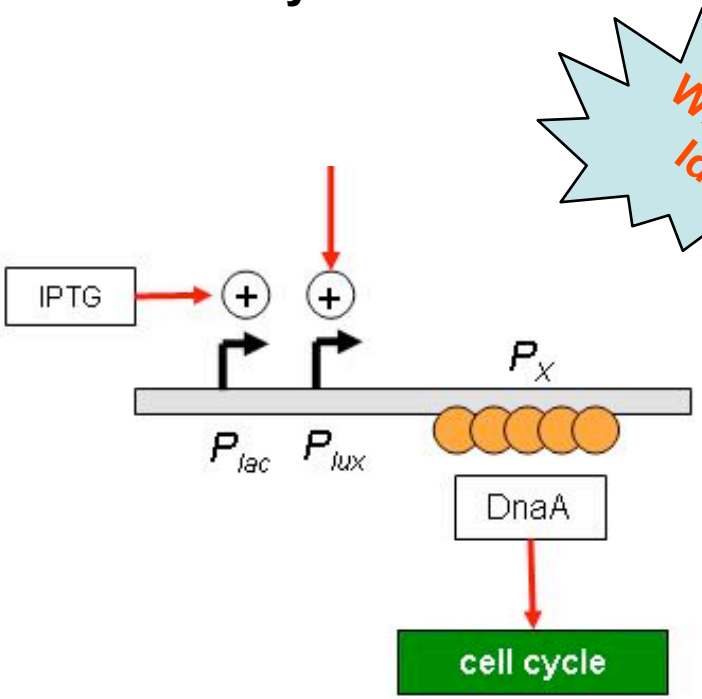
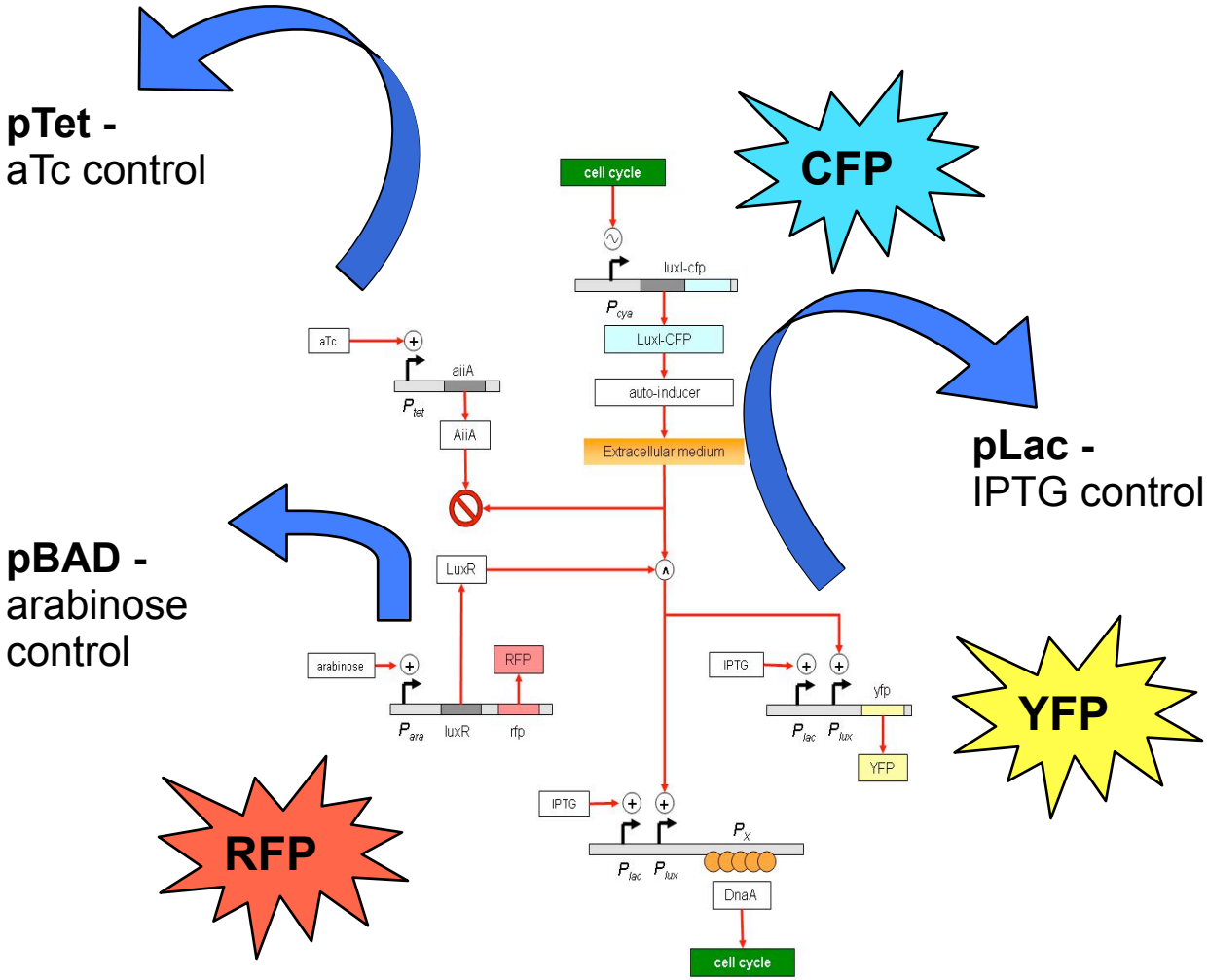


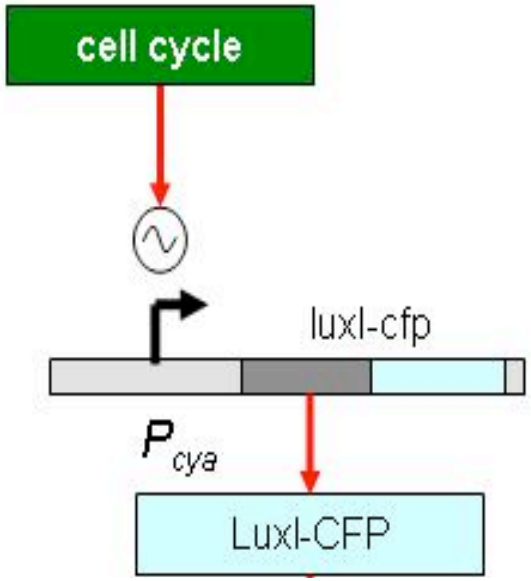
Whacky Idea

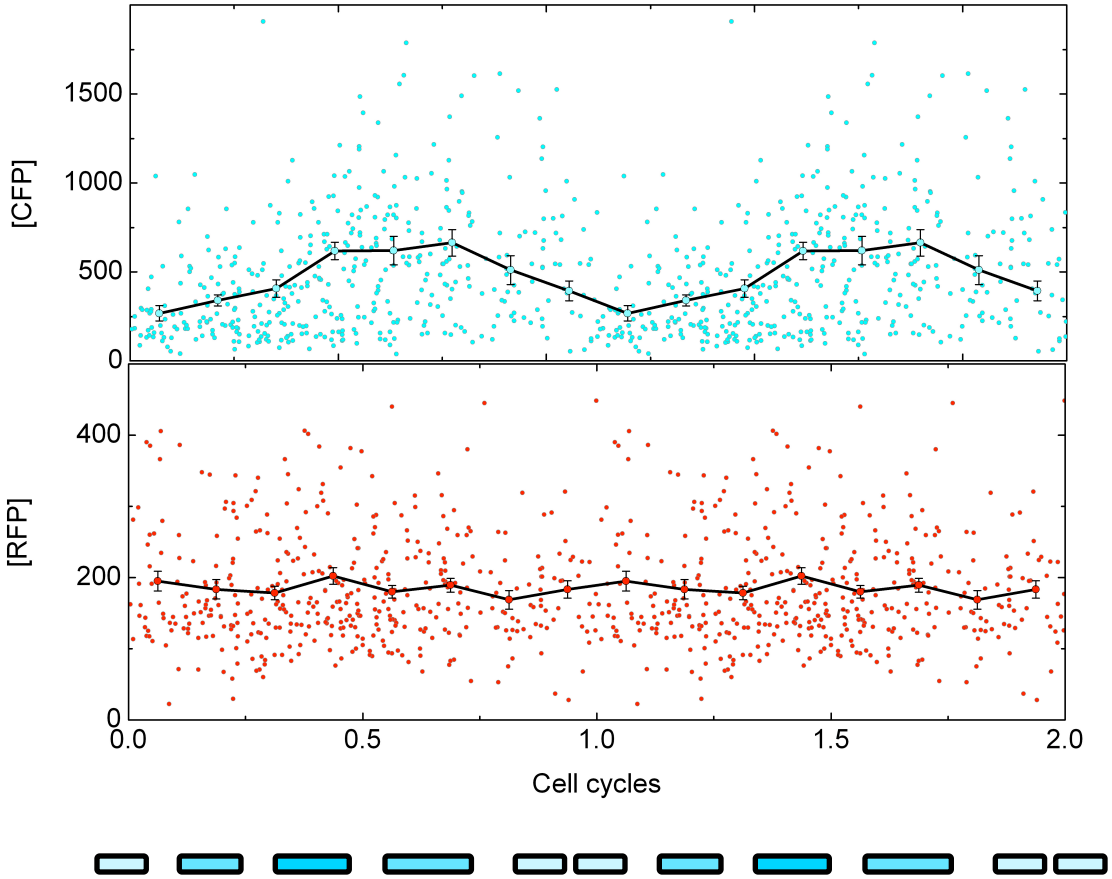
Multiple controls to tweak the system and multiple readouts



Core oscillator: The *E.coli* cell cycle

To test oscillations, we have used cell length as a correlate of cell cycle phase.

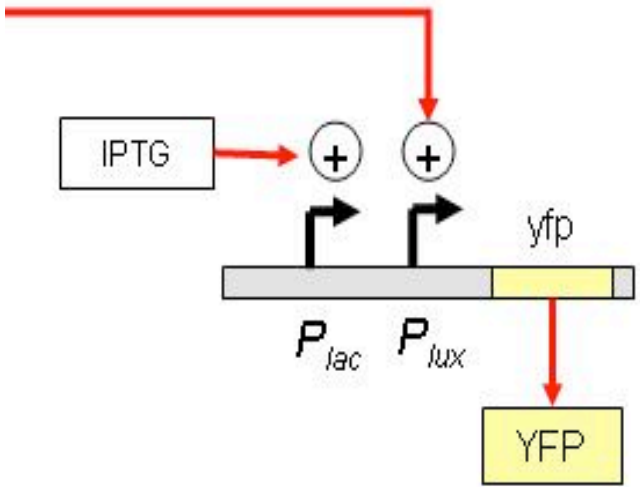


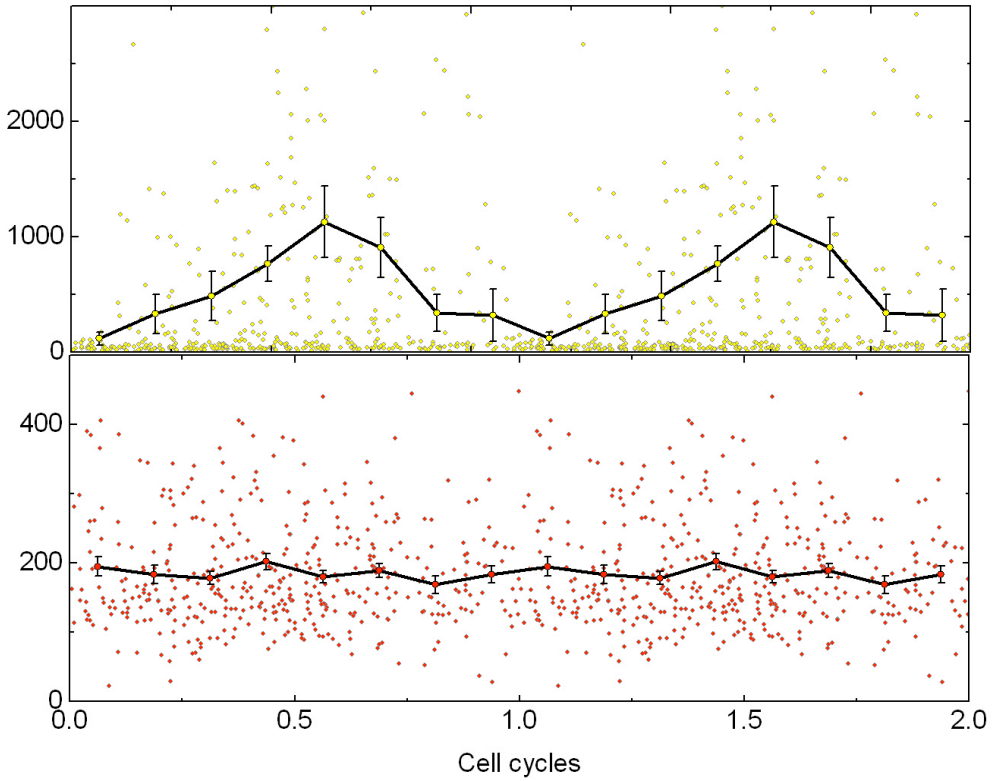


Pcya produces an oscillatory PoPs output

Oscillator coupling: *Vibrio* quorum sensing machinery

We tested Propagation of the oscillatory signal using YFP expression as readout.





The oscillatory signal can be propagated through the quorum sensing circuit without degradation.

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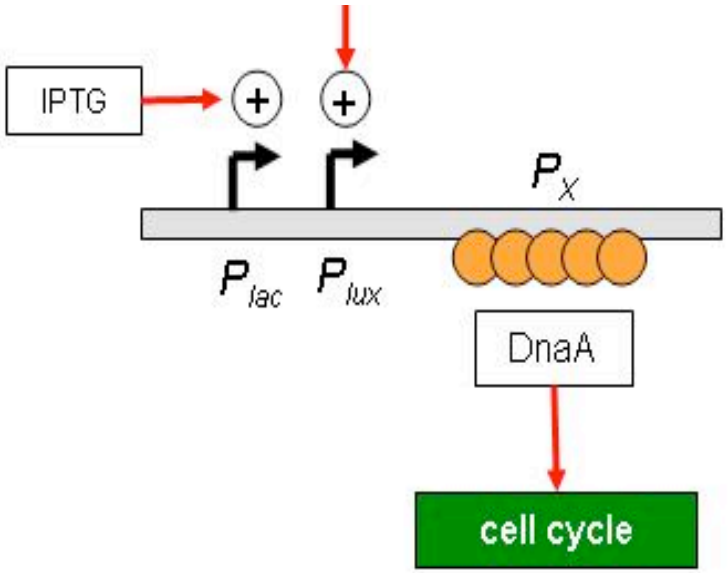
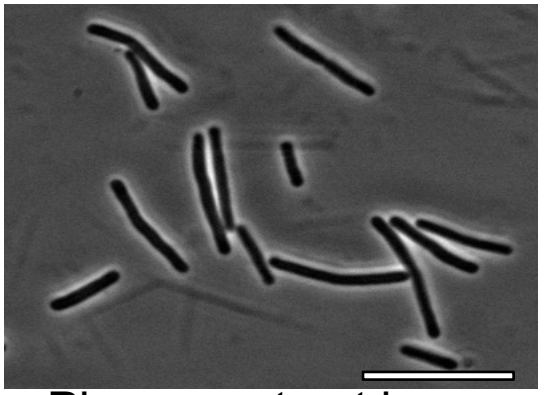
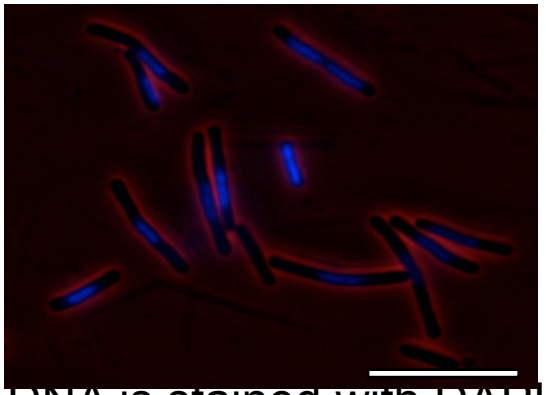
Cell cycle modulation: DnaA sequestration

We tested effect of DnaA sequestration on growth rate of cells, cell morphology and DNA localization in those cells.





Phase contrast image



DNA is stained with DAPI.

Presence of DnaA binding sites did not affect growth rate of cells, though cells grew bigger and had DNA accumulated at central region.

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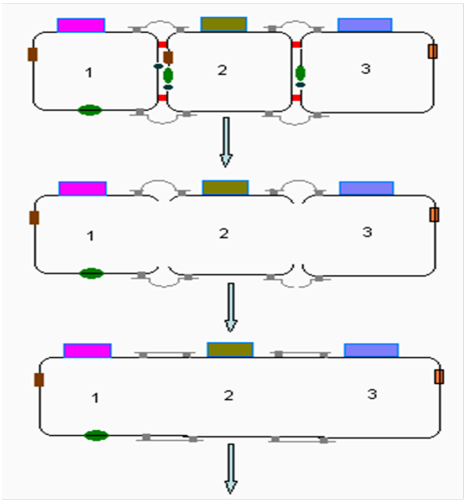
Conclusions

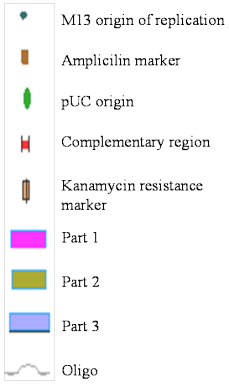
- A part with oscillatory PoPs output
- LuxI-CFP fusion is functional
- Oscillations are transmitted without degradation through quorum sensing
- pX alone is not sufficient to affect cell cycle progression

Network Construction by DNA self-assembly

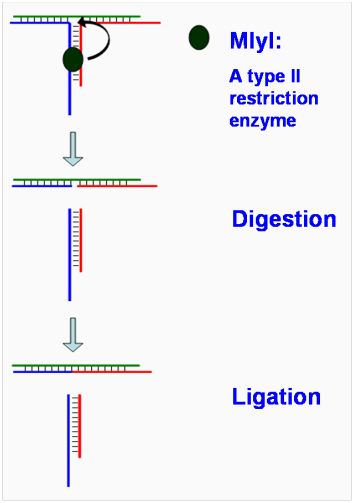
The strategy:

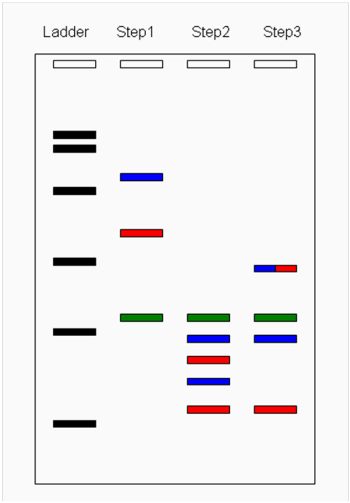


Double stranded DNA in bacteria



Testing digestion and ligation





Generate single stranded DNA encoding each part using the M13 phage system.

Arrange the parts in the correct order using staplers (complementary oligo-nucleotides).

Use type II restriction enzyme to cut at interfaces without leaving extra bases.

Ligate the cut ends and transform.

We are using a T-junction of three oligo-nucleotides to validate the strategy.

The junction resembles the T-junction that is expected to be formed by DNA self-assembly.

MlyI- a type II enzyme would cut at the junction. The fragments will ligate as they are held by an oligo.

Each step can be tested by running the samples on Urea PAGE.

Acknowledgements

- Our iGEM ambassador - Reshma
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Living Networks 2.0: Sticky pieces!
June 2007

Focus on protein and DNA self-assembly