Sync, Switch, and Self-assemble

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Synchronization of cell cycle oscillators

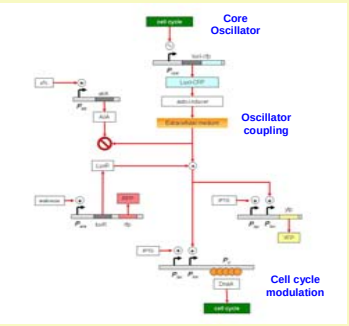
Theoretical work predicts that weak coupling of cellular oscillators should result in their synchronization [Garcia-Ojalvo et al., 2005]. We have constructed a multi-cell system to validate this prediction.

Components of this multi-cell system include:

Core oscillator: The *E.coli* cell cycle

Oscillator coupling: *Vibrio* quorum sensing machinery

Cell cycle modulation: DnaA sequestration

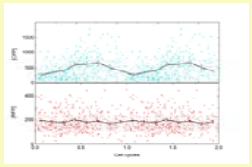


The synchronization circuit

Testing Pcya, the cell cycle dependent promoter

The LuxI-cfp fusion protein is expressed from a cell cycle dependent promoter Pcya. This should result in an oscillatory output of the autoinducer.

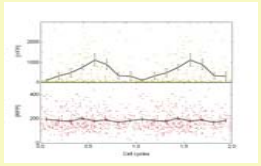
CFP expression in each cell is plotted against cell length, which is a measure of the cell cycle phase. RFP expression plotted as a control.



Testing the quorum-sensing module

The oscillatory output (AI) is fed into the quorum sensing module in order to achieve cell coupling.

YFP expression in each cell is plotted against cell length, which is a measure of the cell cycle phase. RFP expression plotted as a control.



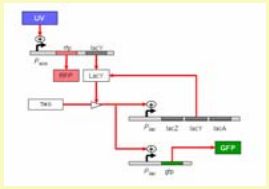
Pcya generates an oscillatory output. This signal can be fed through the quorum sensing circuit, retaining its oscillatory character.

Converting transient UV exposure into a persistent response

The regulatory network involved in uptake and utilization of lactose exhibits bistability over a range environmental conditions [Ozbudak et al., 2004].

Transient exposure to UV radiation leads to bursts of transcriptional activities in single cells from UV responsive SOS promoter (e.g. recA) [Friedman et al., 2005].

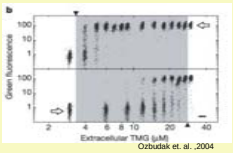
We have coupled the recA promoter to the natural bistable network in order to convert transient UV pulse input into a persistent GFP expression.



The UV-switch circuit

Testing bistability in the UV-switch circuit

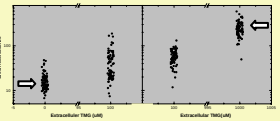
A bistable network is hysteretic. Environmental history determines state of the network at intermediate inducer concentrations.



Ozbudak et. al. ,2004

The response of cells with uninduced (lower) or induced (upper) histories, when grown in different TMG concentrations.

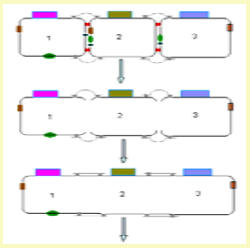
The UV-switch circuit does not show hysteresis. Cells show intermediate induction at intermediate inducer concentrations.

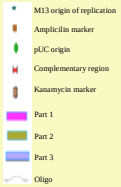


The response of cells with uninduced (left) or induced (right) histories, when grown at an TMG concentration.

The presence of LacI binding sites on the plasmid pSB2K3 decreases free LacI concentration, leading to loss of bistability of the *lac* network. We are correcting this by changing the plasmid backbone.

Construction of a network using DNA self-assembly





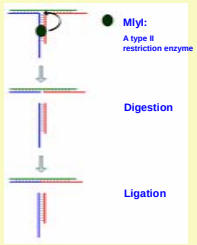
Generate single stranded DNA encoding each part using the M13 phage system.

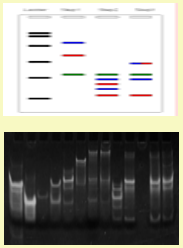
Arrange the parts in the correct order using staplers (complementary oligo-nucleotides).

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

Ligate the cut ends and transform.

Testing digestion and ligation





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.

It is essential that digestion and ligation occur efficiently at such a junction.

Each step is being tested by running the samples on Urea PAGE.

Conclusions

Sync:	Pcya produces an oscillatory PoPs output, which can be propagated through several stages of a genetic cascade without degradation.
Switch:	Synthetic components can interact with the host in unanticipated ways. Extrinsic *lacI* binding sites destroy bistability of the lactose uptake module.
Self-assemble:	Preliminary results indicate that type-II restriction enzymes can carry out digestion at oligonucleotide T-junctions.

Teams

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Switch:	Balaji, Divya, Garrit, Gopakumar, Krithiga, Mehrab
Self-assemble:	Senthil, Panic, Mukund

These projects were funded by NCBS.

Cloning services were provided by Bangalore Genei.

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