Design of a Modular Inducible Circuit for Pulsatile Population Control of Engineered Bacteria

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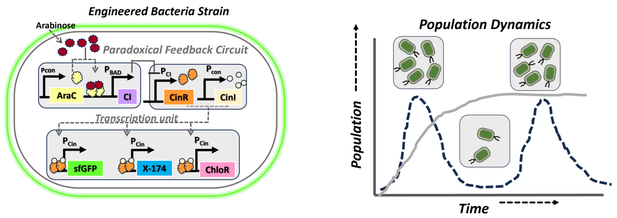
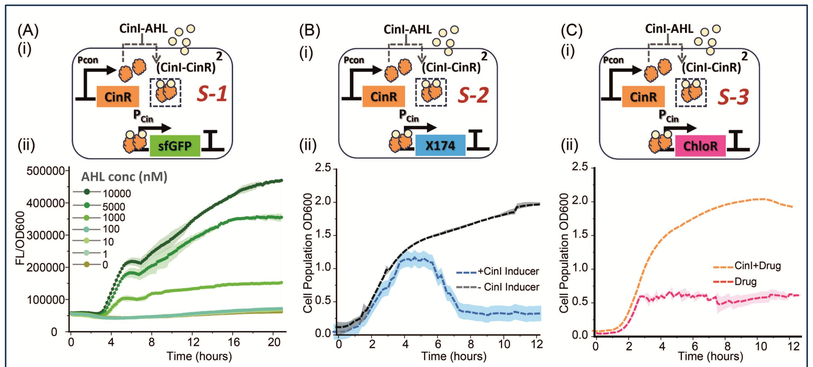
Background overview

- Synthetic biology enables the precise **reprogramming of living systems** using modular genetic circuits
- **Microbial control** unlocks next-gen therapies for colonization, drug delivery, and immunity
- Smart circuits time- control bacterial **growth**, **function**, and **self-clearance**
- Our designed genetic circuit integrates:
  - **Arabinose**-inducible **CI repression**
  - **CinI/CinR-AHL** quorum sensing
  - **ΦX174** lysis-based synchronized cell death
- **Positive-negative feedback** drives growth with arabinose and triggers lysis without it.
- Cyclic **growth and collapse** enable precision-timed delivery and biosensing



Single Gene Circuit

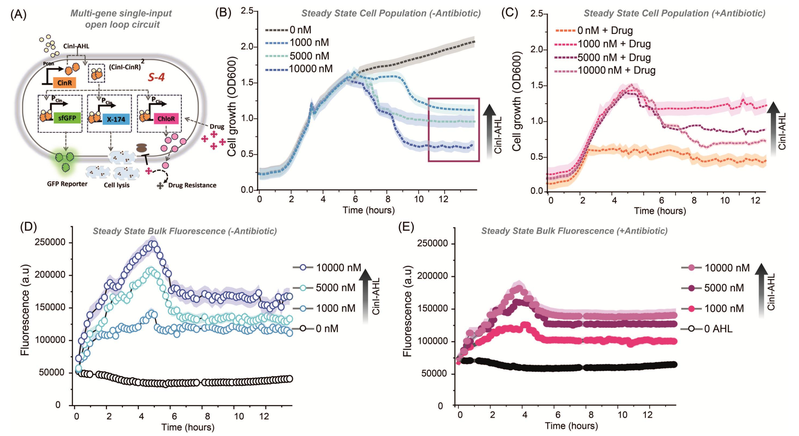


- Individual single gene transcriptional circuits ***S-1, S-2 and S-3*** generated via Golden gate and Gibson Assembly
- ***S-1***, in which the expression of sfGFP is driven by the CinR–AHL responsive promoter (P_{Cin})
- **(A-ii)** shows the fluorescence response of the circuit across AHL concentration gradient
- **(B-ii)** displays cell growth curves with and without CinI induction, triggering cell lysis.
- **(C-ii)** cell growth in the presence of chloramphenicol, with and without CinI–AHL induction.

Reference

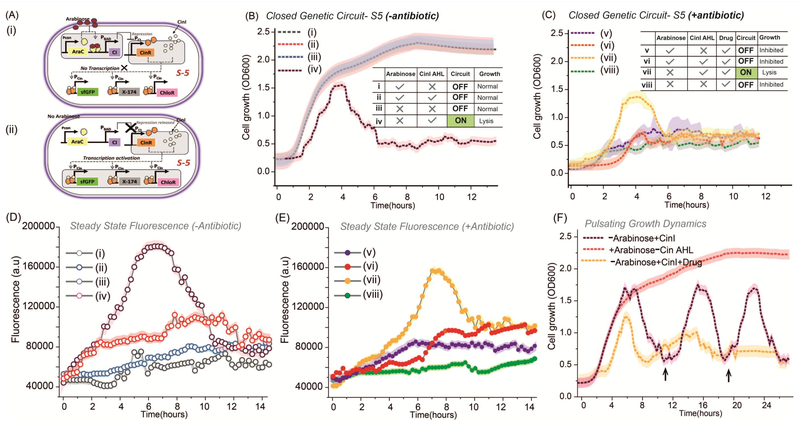
- Huang, Y. & Mayalu, M. N. Biomolecular Control Circuit With Inherent Bi-Stability Is Applicable for Automatic Detection of Gut Infection. *IEEE Control Syst. Lett.* **7**, 2251–2256 (2023).
- Mayalu, M. N. & Murray, R. M. Theoretical Design of Paradoxical Signaling-Based Synthetic Population Control Circuit in E. coli. at https://doi.org/10.1101/2020.01.27.921734 (2020)
- Roy R., Mayalu M. N. Design of a Modular Inducible Circuit for Pulsatile Population Control of an Engineered Bacteria (uploaded in bioRxiv)

Multi-gene Single Input Open Loop: Steady State Dynamics



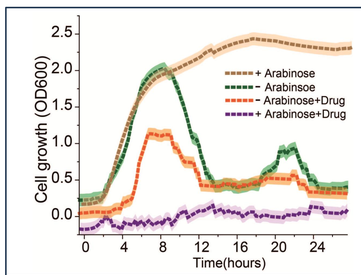
- **Different steady states** observed across different CinI-AHL concentration, post cell lysis.
- Extent of cell lysis and **steady states affected in presence of 20X drug**.
- Presence of **drug imposes burden** to the growing cells

Multi-gene Dual Input Open loop: Steady State Dynamics



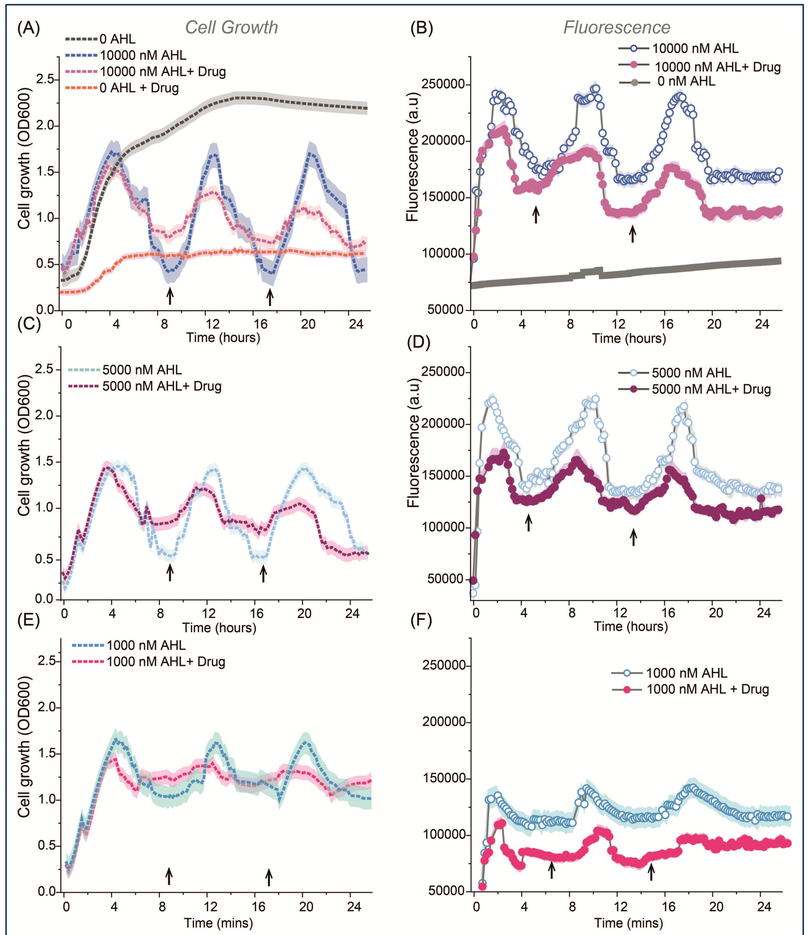
- In **absence of arabinose** the genetic circuit **activates transcription** expression of the effector genes, triggering both lysis and drug resistance.
- Arabinose is the **"OFF" switch** in the genetic circuit.
- **Pulsatile dynamics** observed here in absence of the arabinose.

Close loop AHL-feedback circuit dynamics



- This paradoxical feedback circuit incorporates **endogenous AHL synthesis** to create a fully autonomous loop.
- **Removal of arabinose**, **activates transcriptional** cascade.
- Activated circuit undergoes cell lysis
- **Spontaneous, self-sustained** pulsatile dynamics
- **Partial damping** of the oscillatory behavior observed over time.

Pulsating Behaviour of Single input multi-gene open loop



- **Pulsatile population pattern** observed across AHL-gradient in the engineered cells.
- **Stepwise population collapse and regrowth**, coinciding with oscillatory gene expression
- **Elevated AHL levels** lead to **increased lysis events**, resulting in more pronounced dips in cell growth.
- Drug treatment (20X chloramphenicol) imposing an additional **selective bottleneck**
- **Fluorescence output** follows the same cyclical pattern, supporting the **synchronization of gene expression** with population dynamics

Conclusion & Future Work

- Built a **modular genetic circuit** combining inducible regulation, quorum sensing, and paradoxical feedback..
- Synchronized **growth-lysis cycles** by transitioning from an open-loop to a closed-loop.
- Created a **self-regulating platform** for therapeutics, probiotics, and biosensing.
- ,**Future: (1) Microfluidic platforms** to monitor real-time population dynamics and spatial control under defined environmental conditions.

  (2) Exploring system tunability by varying antibiotic types, concentrations and AHLs

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