Optogenetic control of gene expression and growth in *E. coli*

Microbial growth control and biotechnological applications workshop
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Andreas Milias-Argeitis

Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, the Netherlands

a. milias.argeitis@rug.nl

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Outline

1. Motivation:
   - Forward engineering in biology
   - Optogenetics and feedback control

2. Control of gene expression in yeast

3. Control of gene expression and growth rate in *E. coli*

4. Single-cell control of transcription

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Forward engineering: programming system behavior

Synthetic biochemical networks:

- Use libraries of existing (or new) components (proteins, sequences)
- Design systems with novel/improved functionality
- Integrate in host cell

Ideally, a process equivalent to circuit design; in practice:

- Incomplete parts characterization
- Unforeseeable host-circuit interactions – unexpected crosstalks
- Lack of robustness to environmental changes

How to overcome these problems?

- Improved reverse-engineering
- Implement robust designs (possibly bio-inspired)
- **Implement feedback control**

Programming system behavior: external control of gene expression

Why control gene expression externally?

1. Biological applications (reverse engineering)

2. Biotechnological applications

Goal: control gene expression in a targeted, reversible, dynamic and minimally invasive manner

Plenty of chemically inducible systems available. Problems:

- One-way, delayed and slow induction
- Unwanted pleiotropic effects / toxicity

Alternatively: use **light** as an actuator (**optogenetics**)

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Optogenetics in brief

Definition:

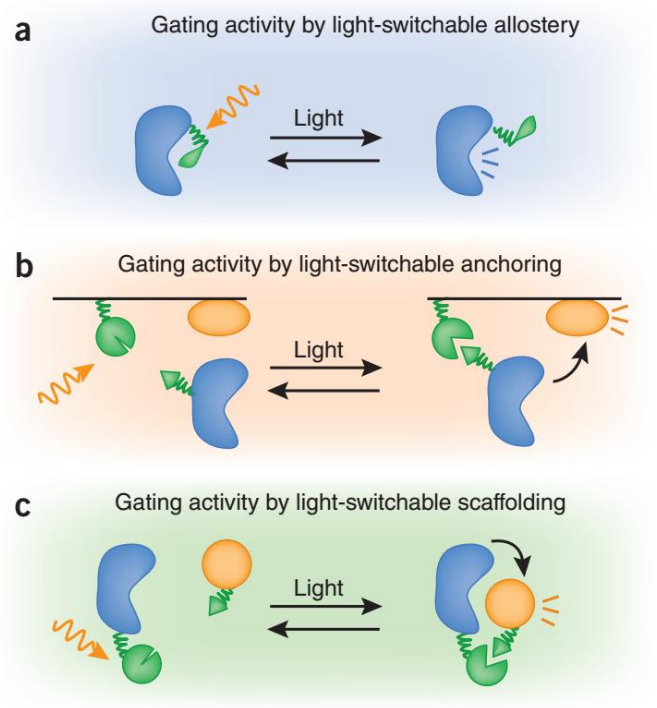
Biological techniques involving the use of light to control that have been genetically modified to express **light-sensitive** proteins

Advantages:

Rapid, targeted, low-cost and precise spatio-temporal modulation of protein function, low toxicity, no pleiotropic effects

Applications:

- Gene expression
- Signaling
- Protein localization
- Cell migration
- Neuronal control
- …



J. Toettcher, C. Voigt, O. Weiner, W. Lim. "The promise of optogenetics in cell biology: interrogating molecular circuits in space and time", *Nature Methods* 8(1), 2011

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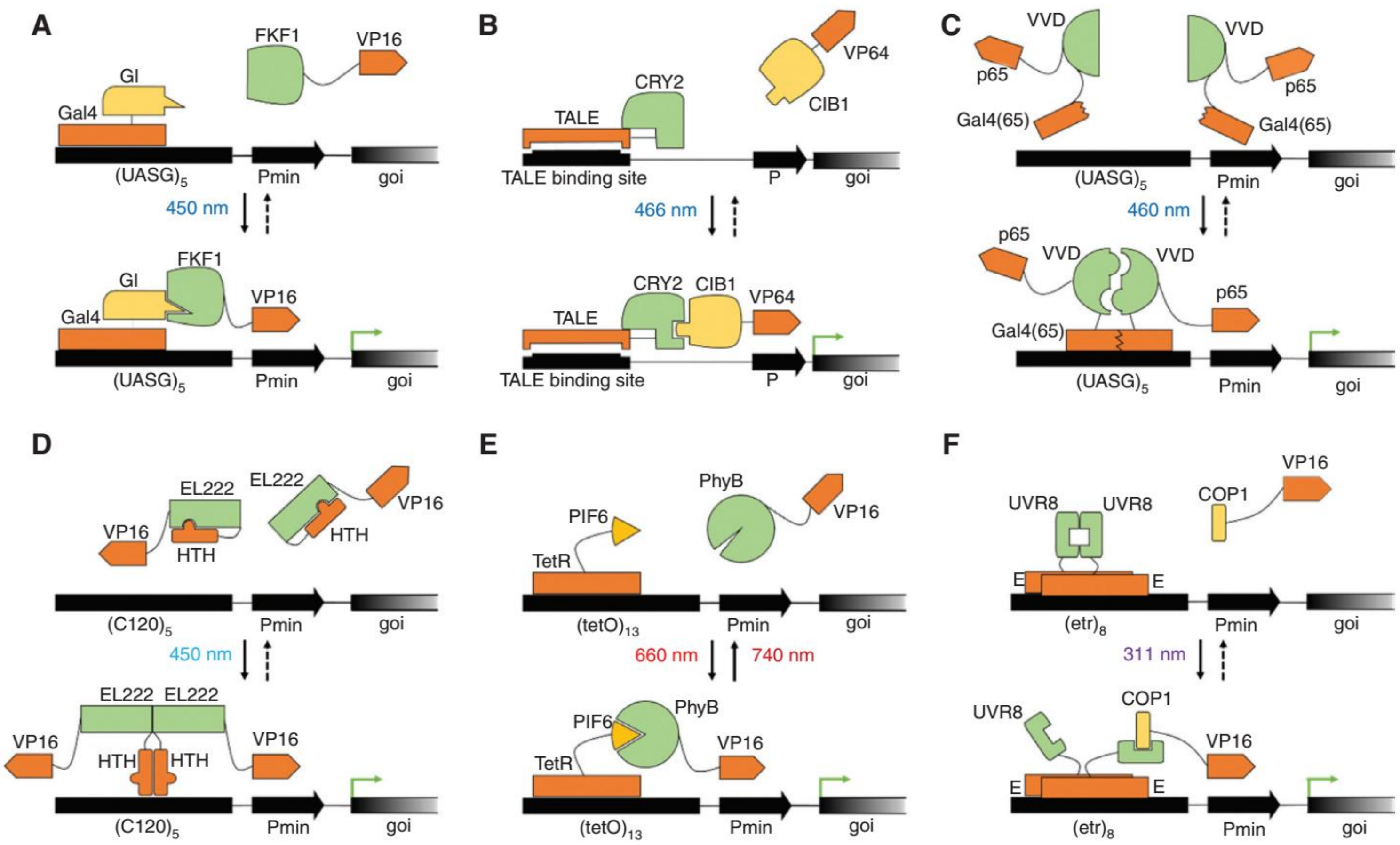
Optogenetic tools for gene expression



Müller, K., Naumann, S., Weber, W., & Zurbriggen, M. D. (2015). Optogenetics for gene expression in mammalian cells. *Biological chemistry*, *396*(2), 145-152.

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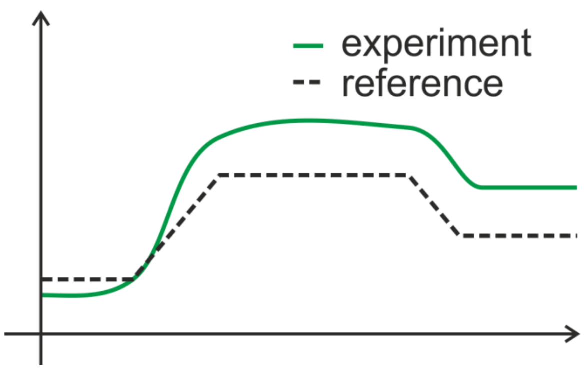
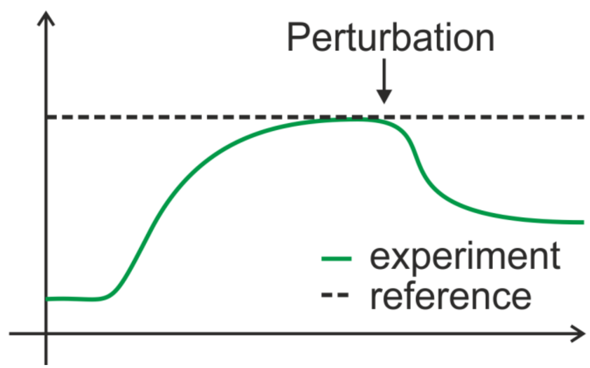
An intuitive approach: "open-loop" control

1. Build a model of the controlled system
2. Compute the input sequence that achieves a reference output
3. Apply to the system

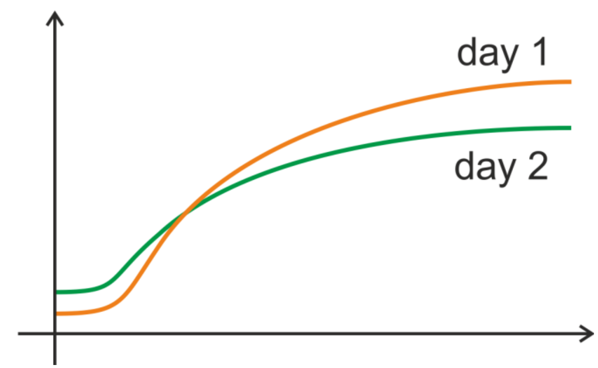
Not as straightforward as it seems…



Model mismatch



Perturbations



Day-to-day variability

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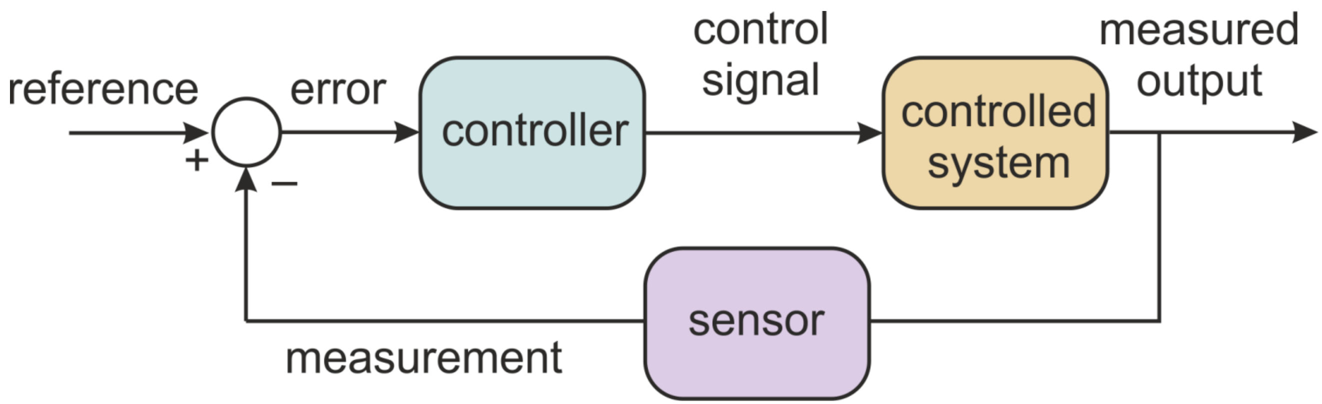
Our approach: "closed-loop" optogenetic control

1. Measure the system output in real time
2. Compare it against a desired tracking objective
3. Compute the necessary adjustments of the system input



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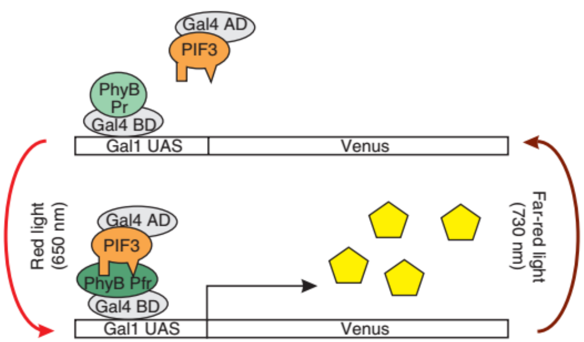
Proof-of-concept: optogenetic control of gene expression in yeast

The PhyB/PIF3 system

a



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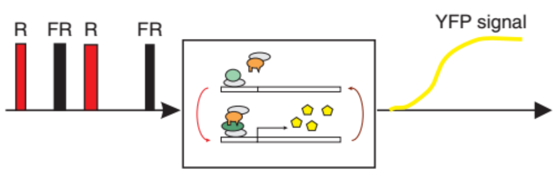
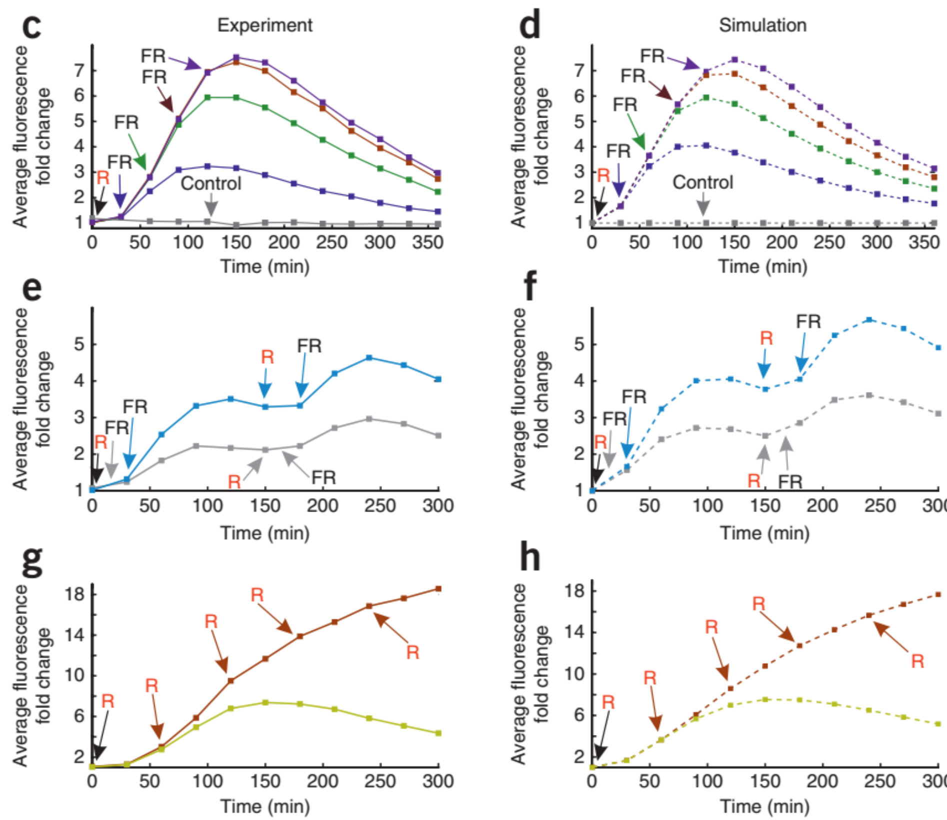


Characterization and model fits



Nat. Biotech. 29(12), 1114-1116, 2011

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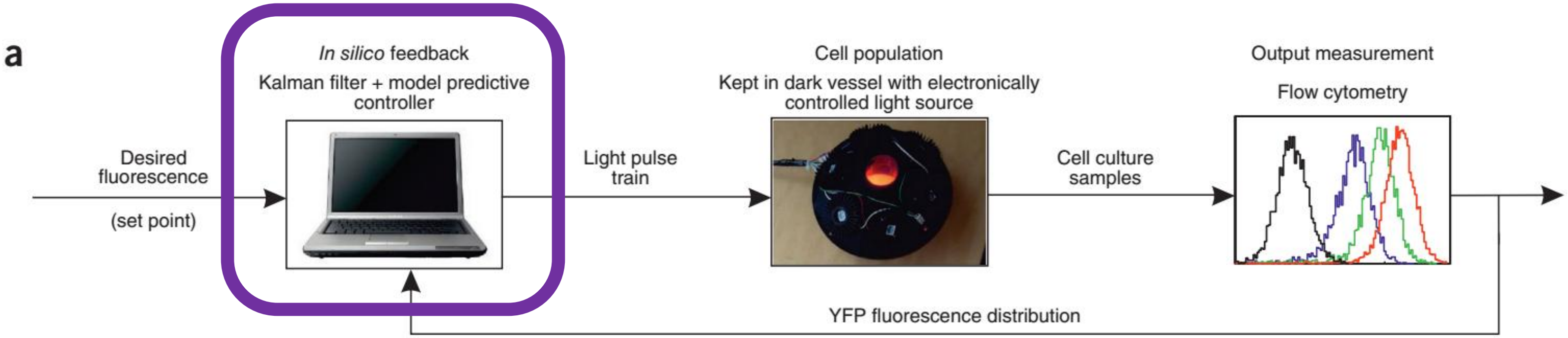
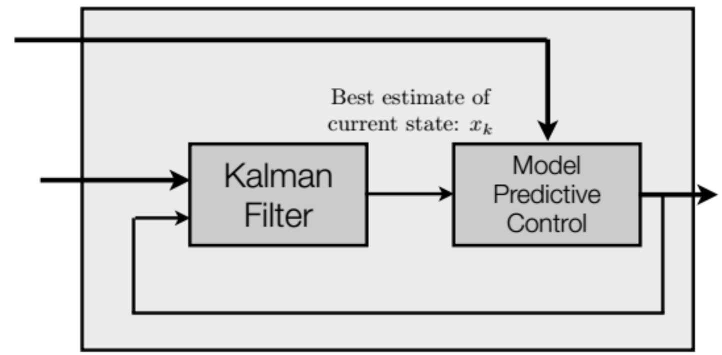
Closing the loop



Model Predictive Control



1. At each step, solve a finite horizon optimal control problem
2. Obtain optimal input sequence
3. Apply the first sample
4. At next step: measure output, update controller info and repeat

Kalman Filter:

At each measurement, estimate *all* (measured & unmeasured) system states, as required by MPC

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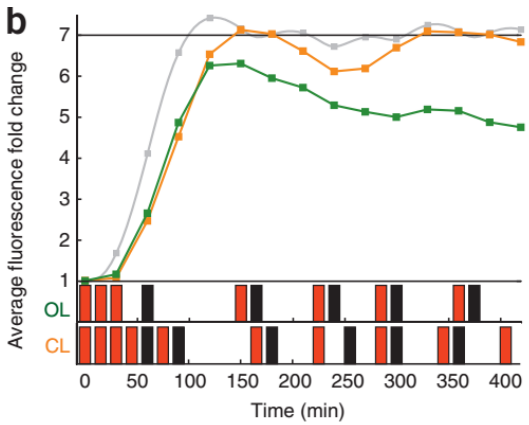
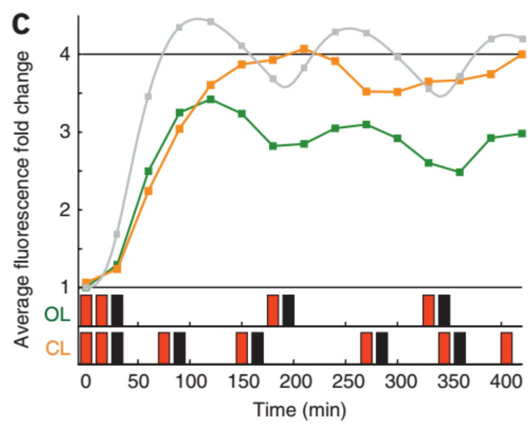
Tracking a constant YFP reference

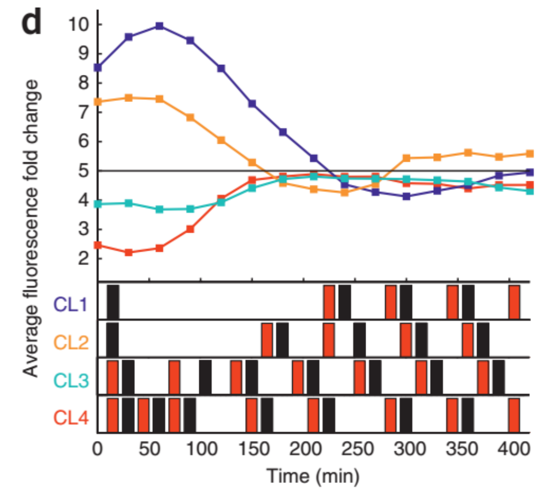
Setpoint = 7



Setpoint = 4



Setpoint = 5



Precomputed input (no feedback) — Model response (grey) — Experiment (green)

Feedback control — Experiment (orange)

Random pulses ($t<0$)

Feedback control ($t \geq 0$)

Shortcomings…

- Modest tracking accuracy
- Non-robust controller operation (no disturbance rejection, day-to-day variability of cells)
- **Completely manual closed-loop operation** (flow cytometry, light inputs, culture dilutions)

Limited timing accuracy, error-prone procedure, time-consuming, limited frequency of measurements and input changes

To make a more useful system for applications:

Completely **automatic**, **long-term**, **precise** and **robust** optical control of gene expression

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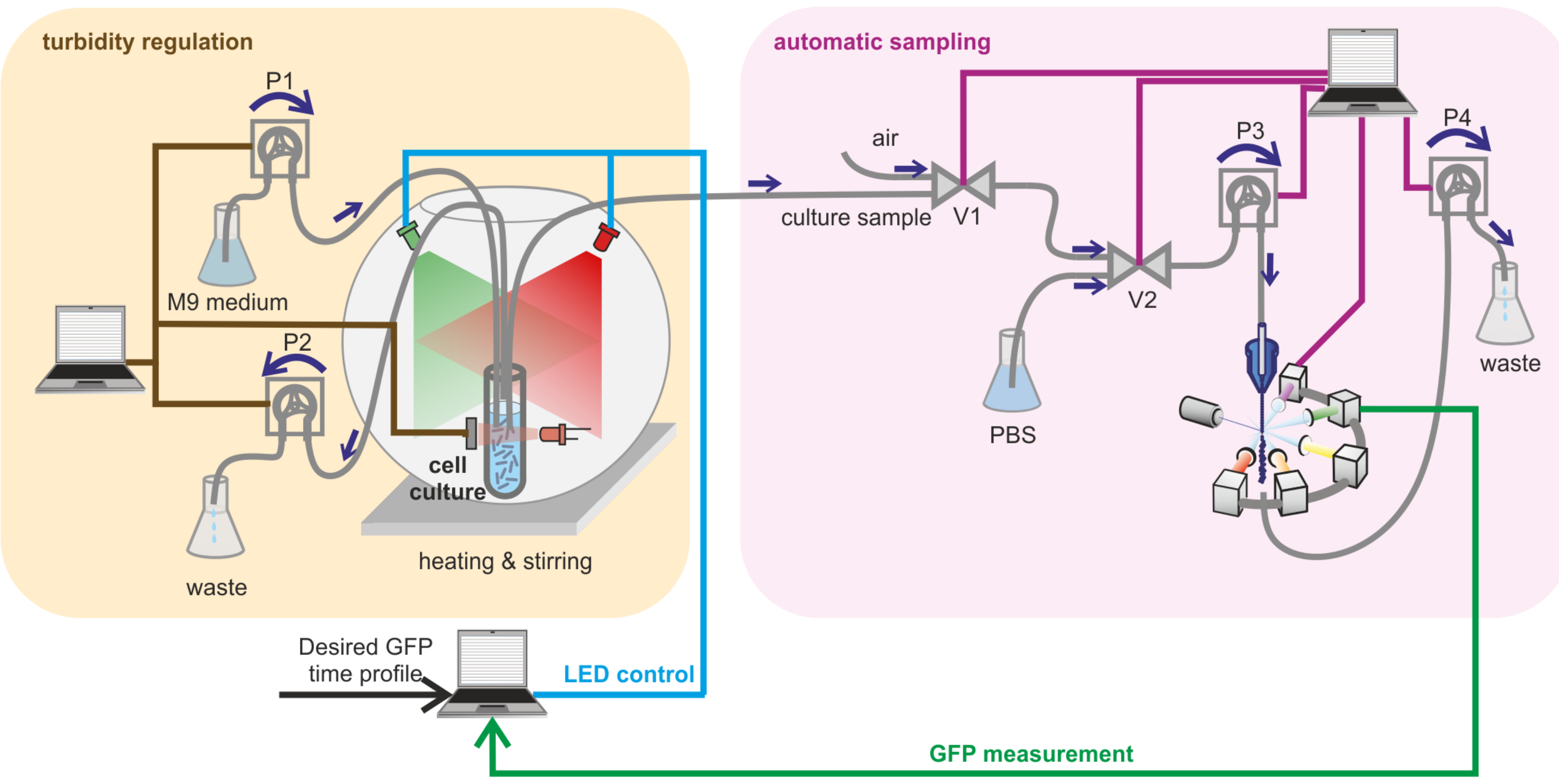
Moving beyond the proof-of-concept

Fully automatic experimental platform

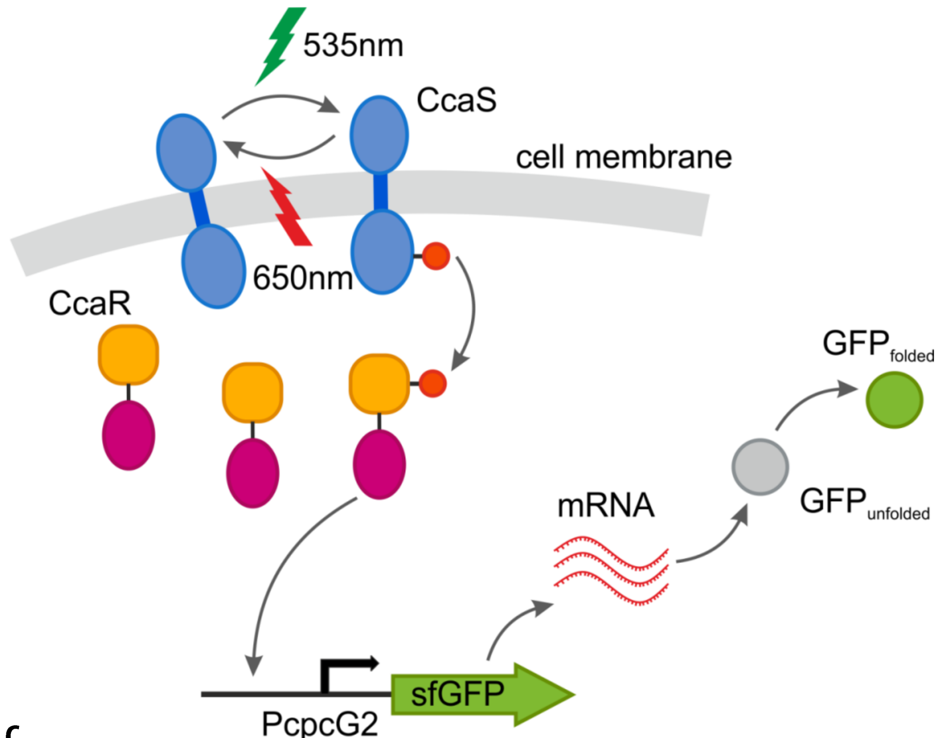


Nat. Comms. 7: 12456, 2016

The optogenetic system

The **CcaS-CcaR** cyanobacterial two-component system:

- Histidine kinase: CcaS
- Response regulator: CcaR
- Native target promoter (PcpcG2)
- Output monitoring: sfGFP/ gene of interest



Implemented in *E. coli*

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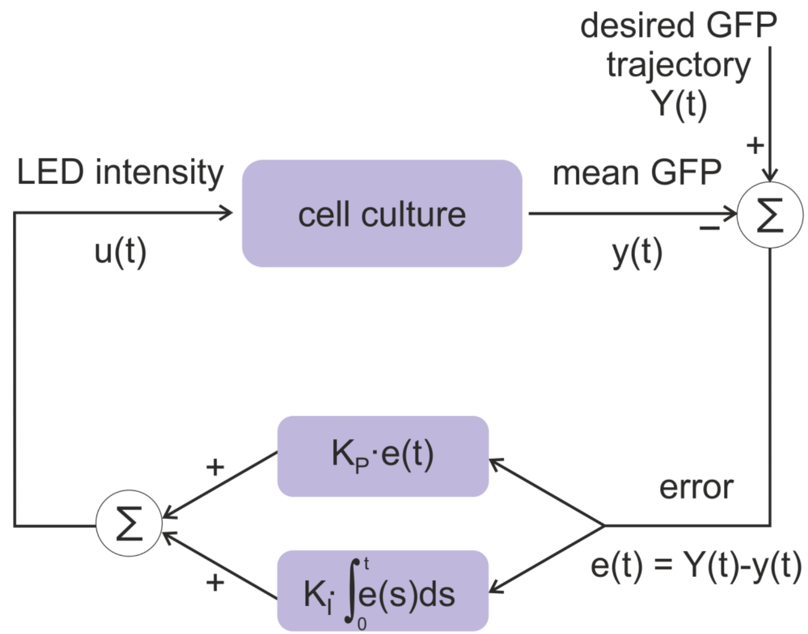
PI controller

Pros:

- Zero tracking error for constant references
- Rejection of constant disturbances

Cons:

- Careful tuning of two parameters (+ gain-scheduling for perturbation rejection)
- Poor non-constant reference tracking
- System nonlinear, tuning changes with setpoint



Discrete-time implementation (forward difference), sampling time T = 10 min.

Model Predictive Control

- Simple MPC is insufficient:
  - Simple linear model cannot capture nonlinear effects following the application of a light input,
  - Day-to-day variability in cell behavior
  - Non-additive perturbations

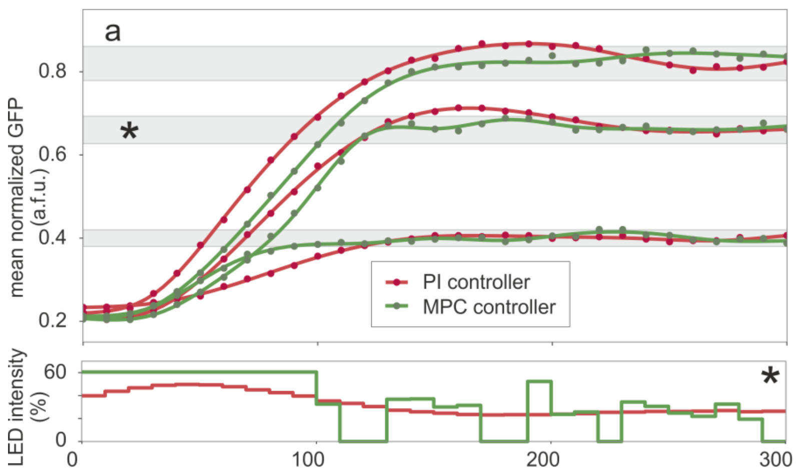
- **Adaptive MPC** with:
  - State estimation for MPC feedback
  - Parameter estimation for counteracting parameter shifts due to day-to-day variability
  - Disturbance estimation (e.g. medium or temperature shift)

Particle filtering

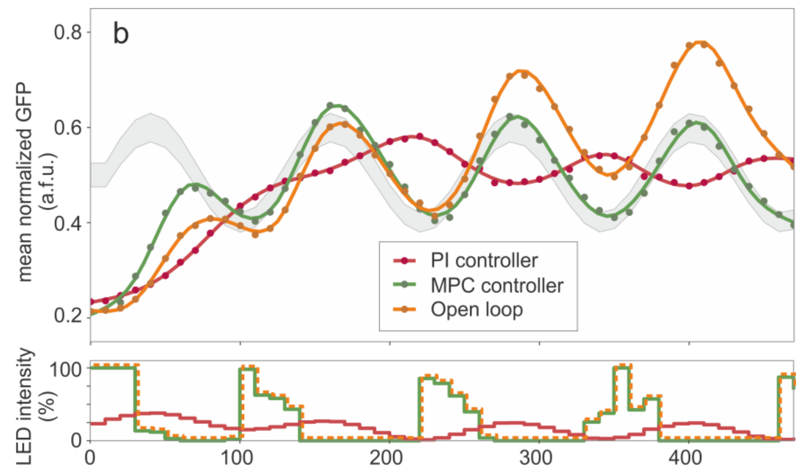
The model becomes *nonlinear* when parameters are considered as states.
Extended Kalman filtering failed…

GFP reference tracking

Constant reference tracking

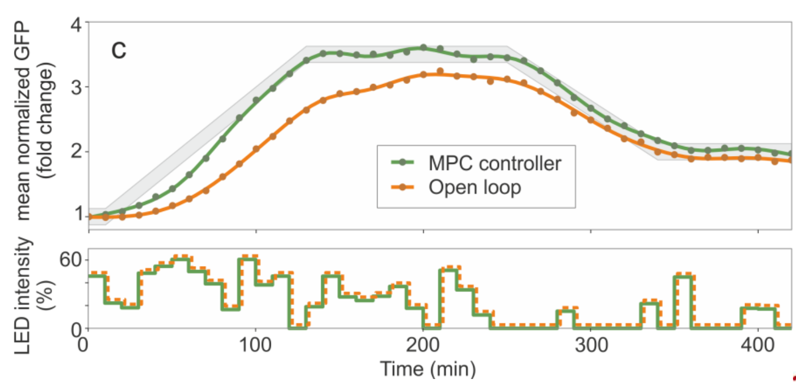


Sinusoid reference tracking



- Precise output control
- Compensation of day-to-day variability

Piecewise Linear reference tracking



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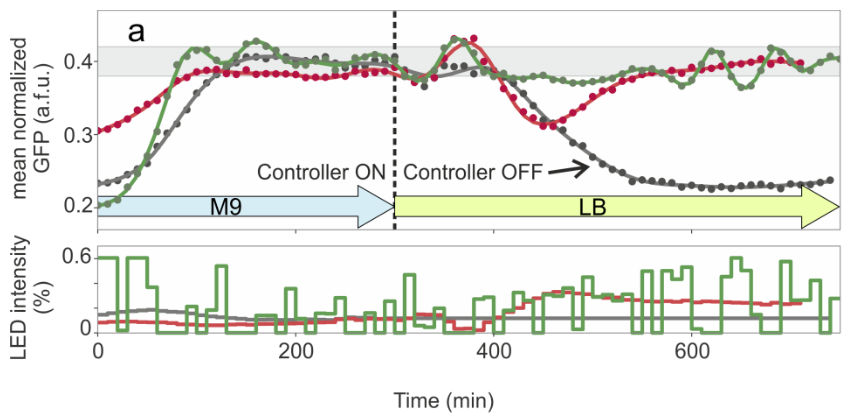
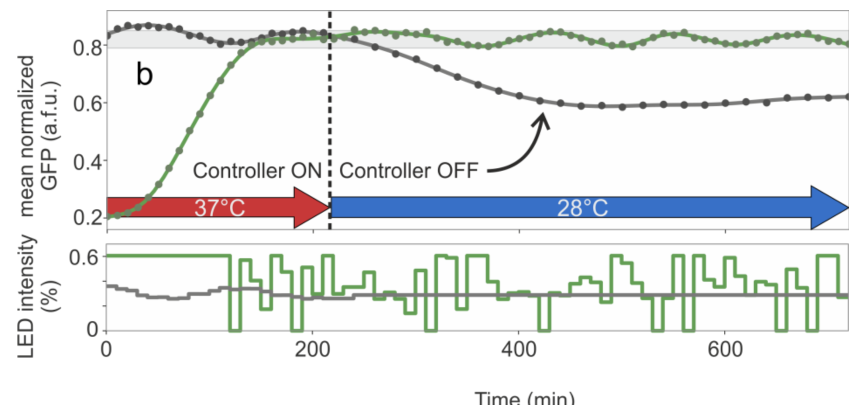
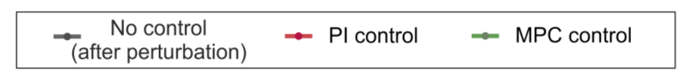
Disturbance rejection during GFP tracking

Change of growth medium

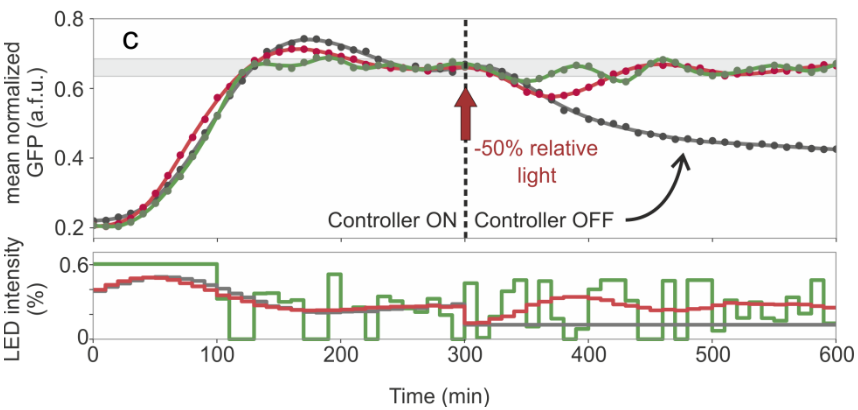


Change in temperature





Input perturbation



- MPC controller learns model parameters online, estimates and counteracts the applied perturbations

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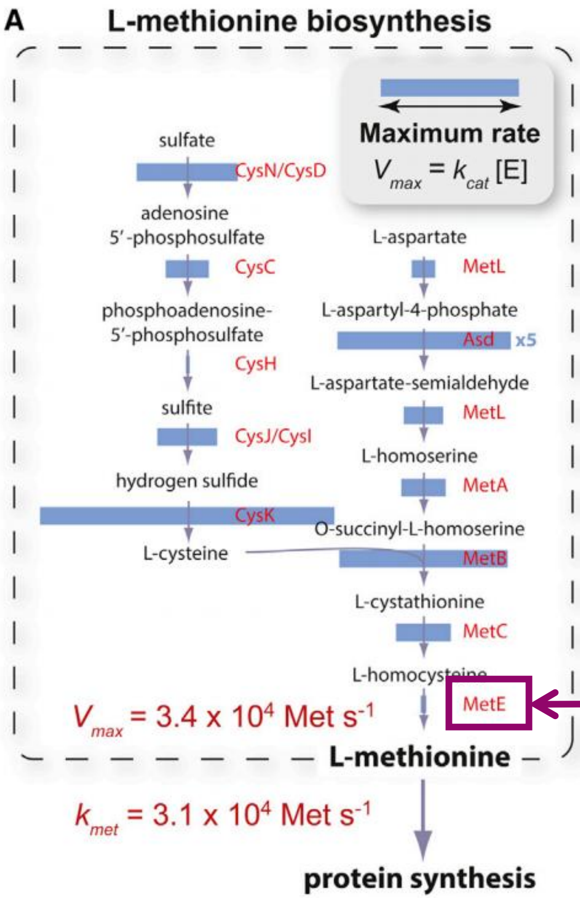
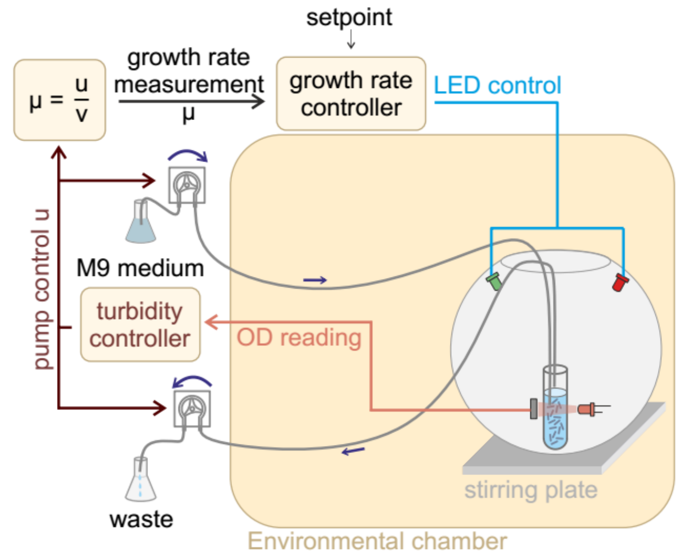
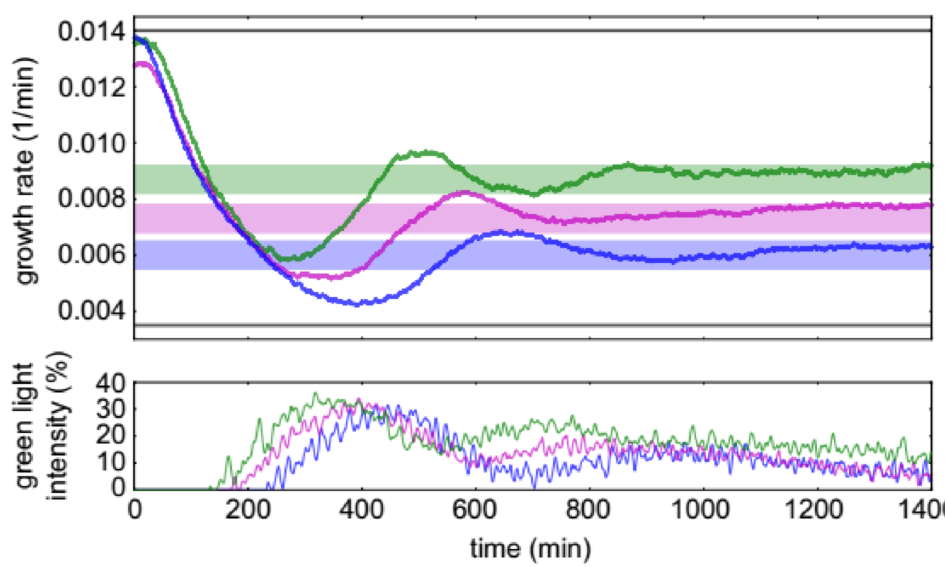
Application: dynamic growth rate control



controlled variable

Li, Burkhardt, Gross and Weissman, *Cell* 157, 2014





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Key advances

1. Automatic, high-frequency sampling (flow cytometry and growth rate)
2. Constant culture conditions over hours or days
3. Control approaches for robustness and precision
4. High-precision reference tracking
5. Adaptation to global perturbations of culture conditions
6. **Towards biotechnological applications: dynamic optogenetic control of the growth rate**

Possible expansions/applications

- Parallel continuous cell cultures (in progress)
- Multiplexed sampling (e.g. with robotic arm – in progress)
- Multivariable control with multiple optogenetic systems in the same cell
- Rich field of applications: bioprocess control, to improve productivity, robustness and batch-to-batch variability
- Online monitoring and optogenetic feedback control of cells inside bioreactors

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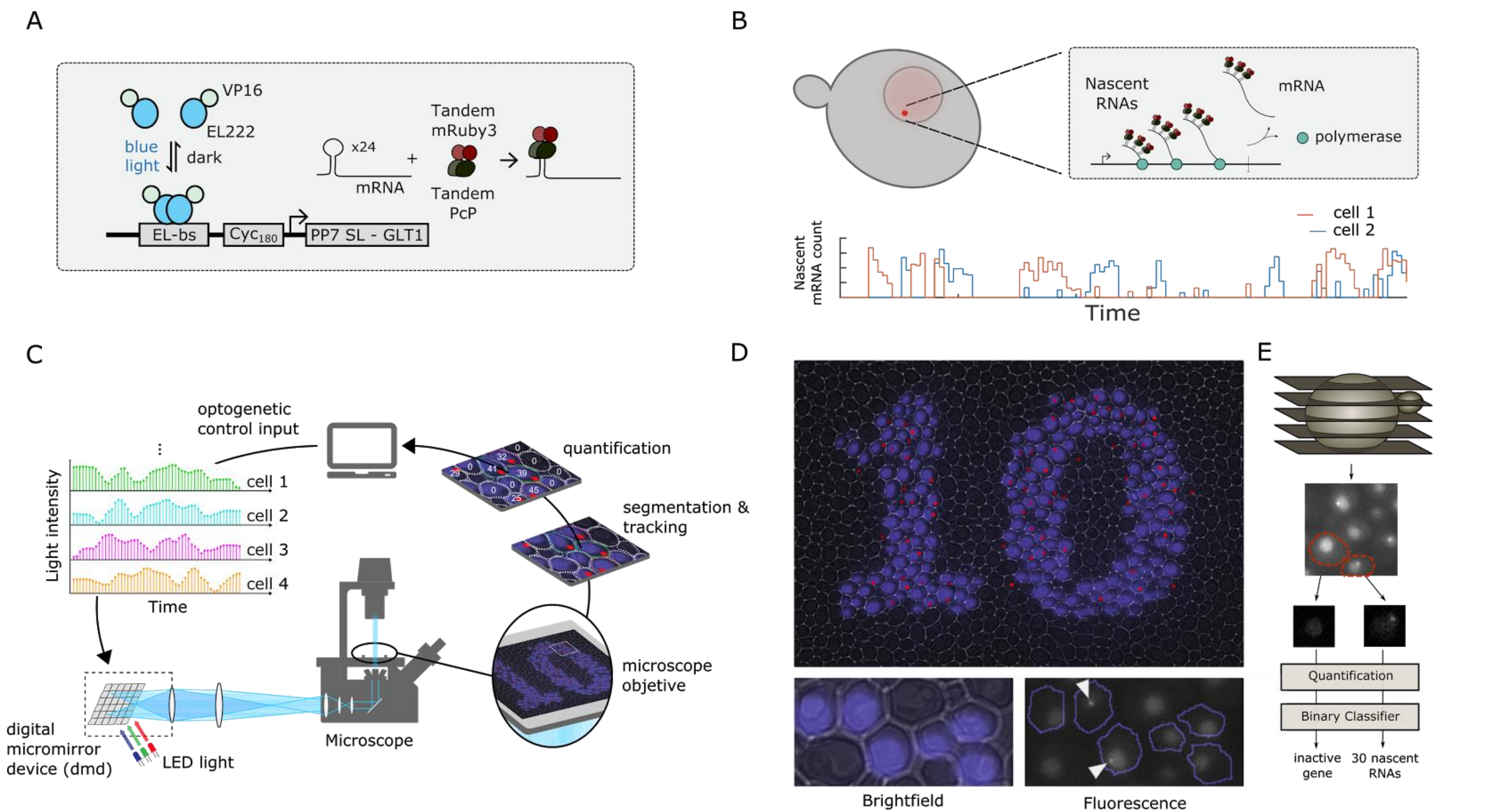
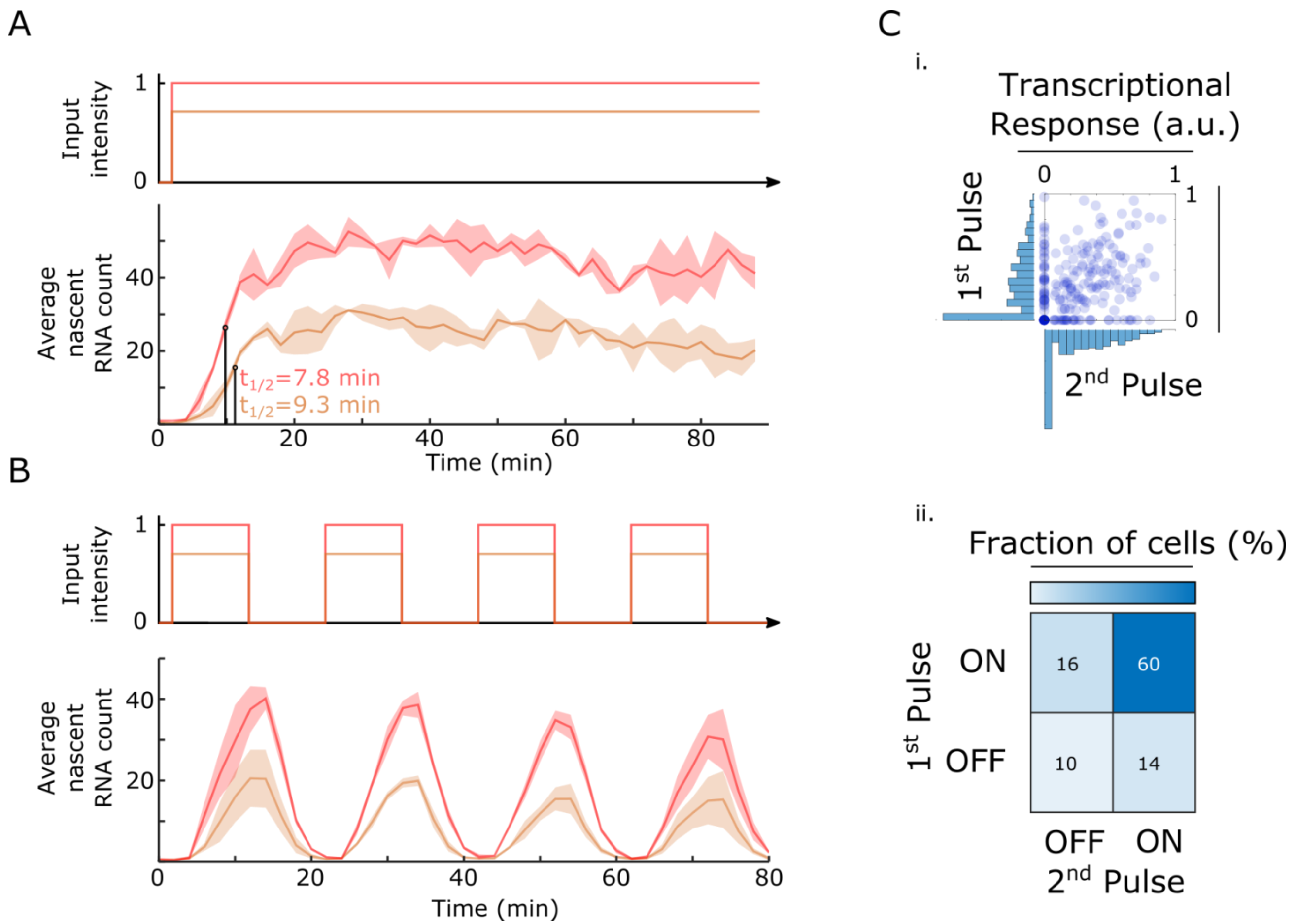
Single-cell optogentic interrogation of transcription regulation



Characterization of transcriptional dynamics



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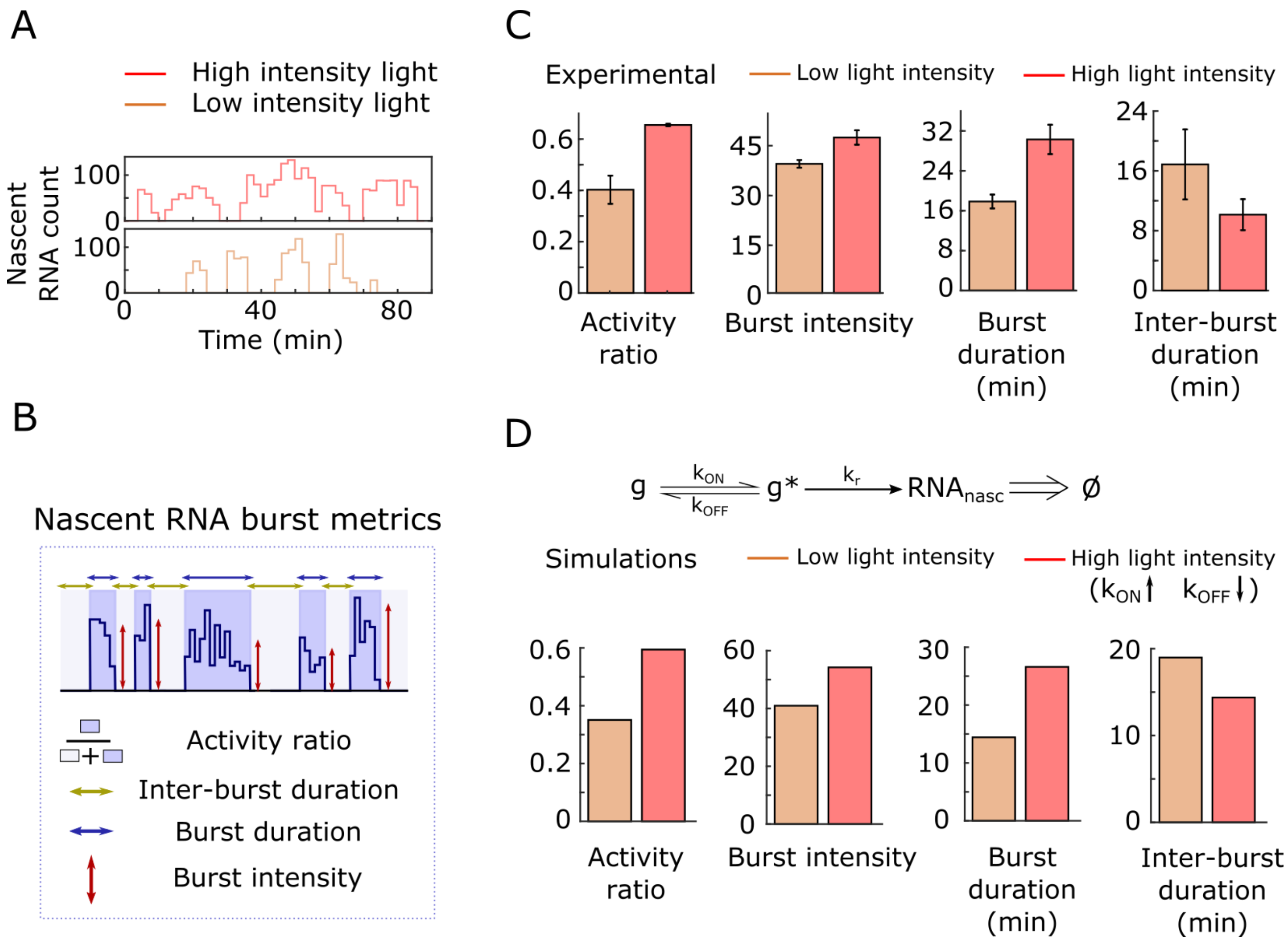

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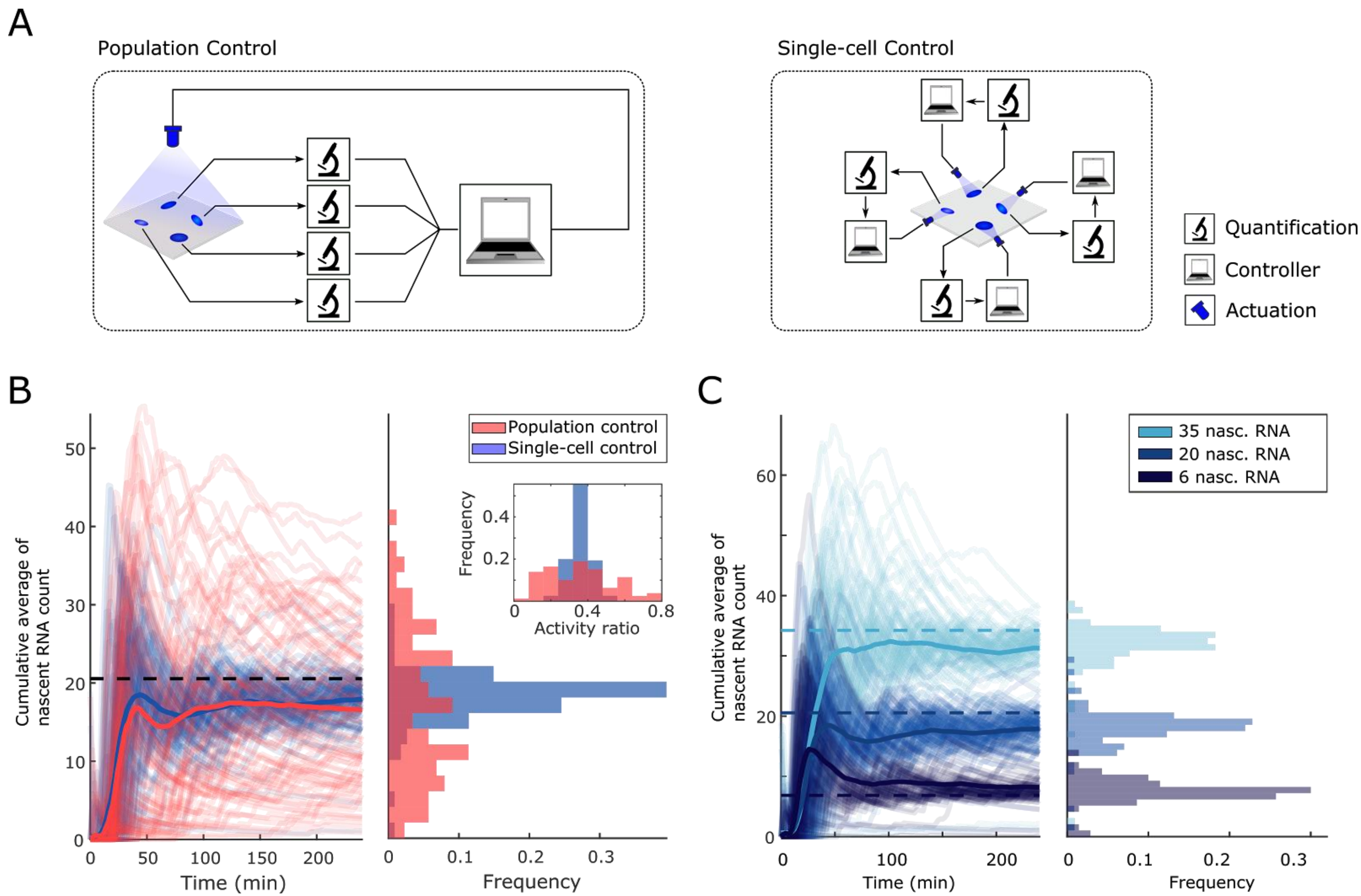
Single-cell vs. Population control



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