Challenges and opportunities in feedback control of living cells

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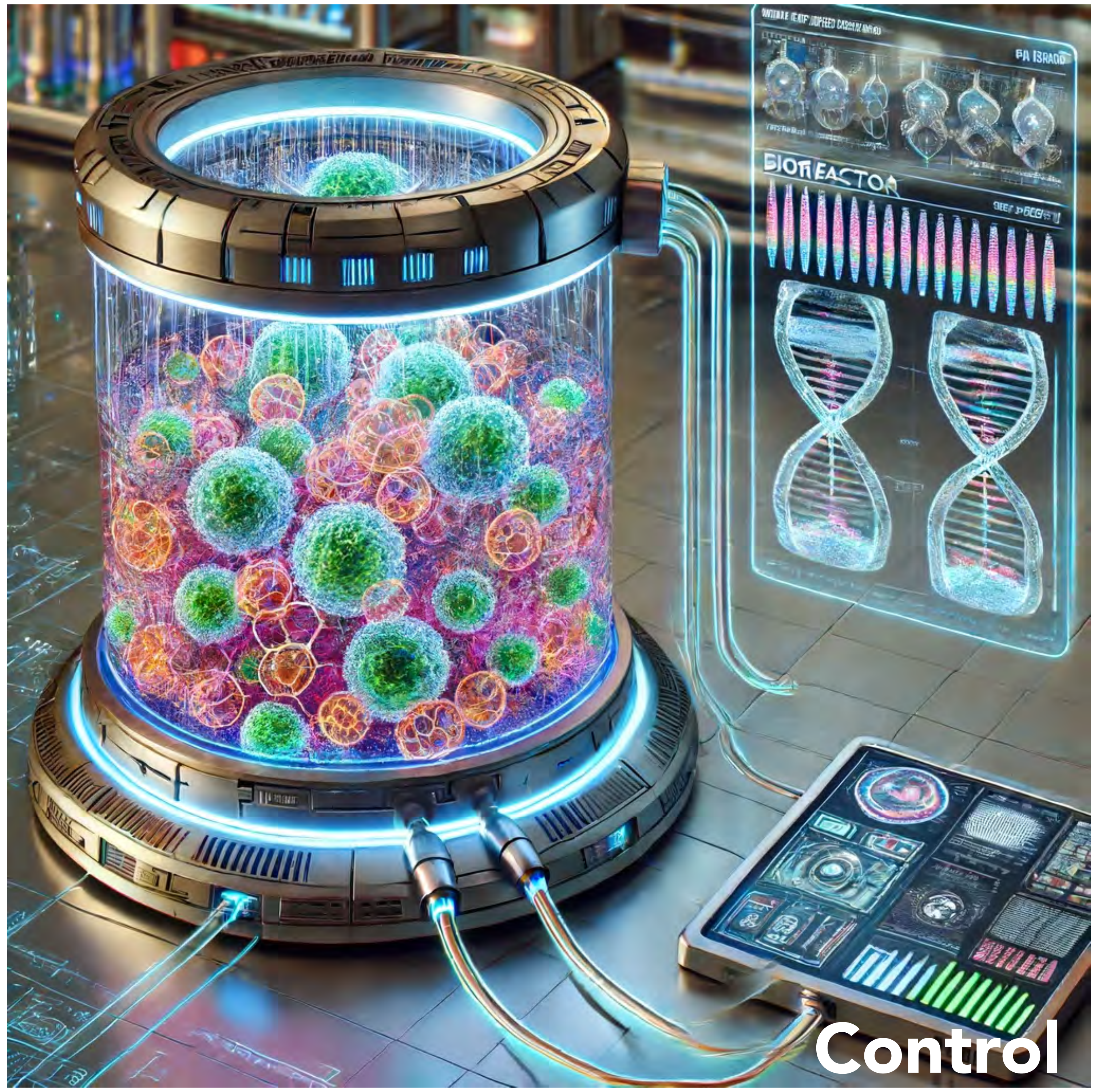
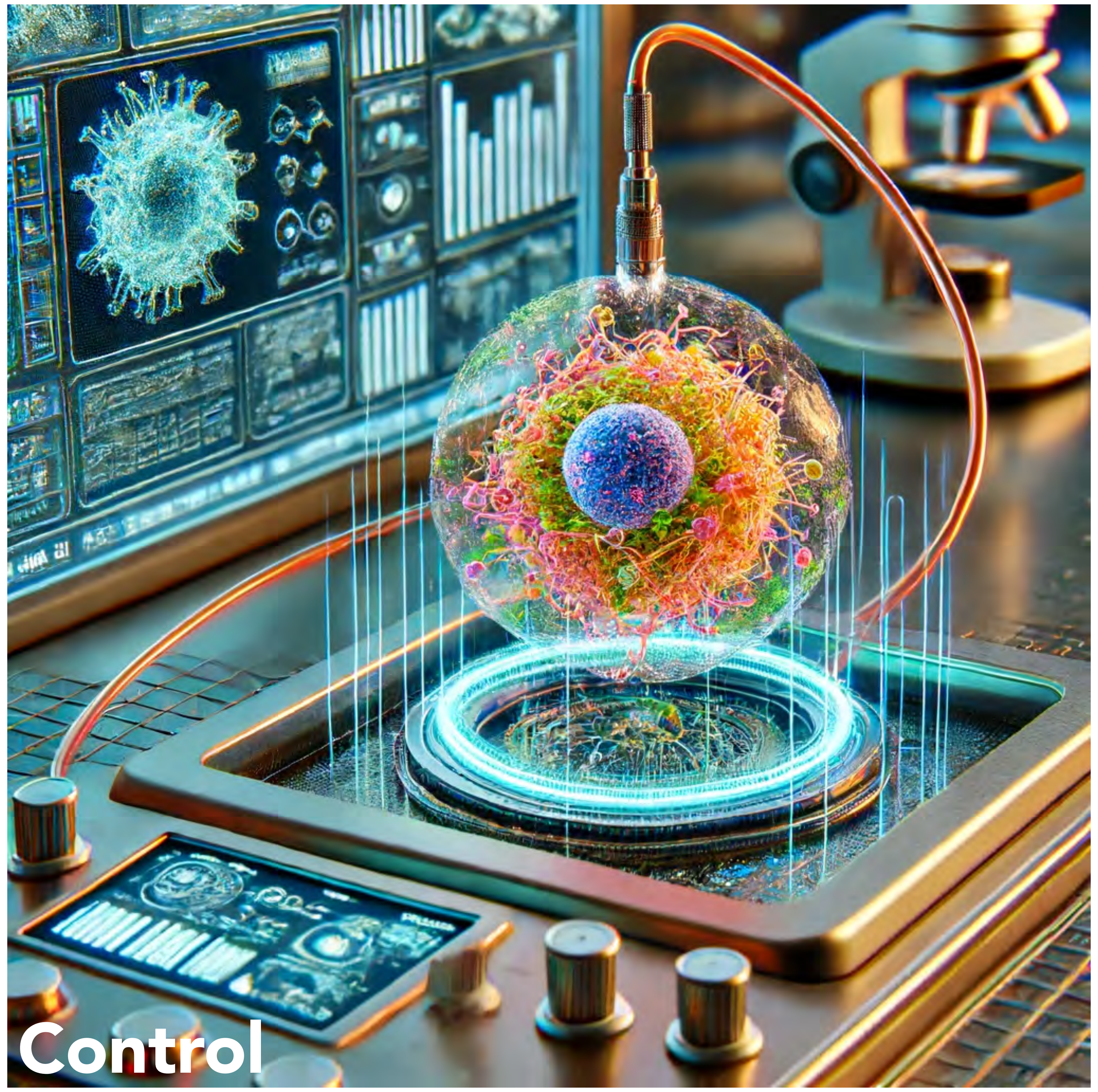
Control Modalities

Computer Control

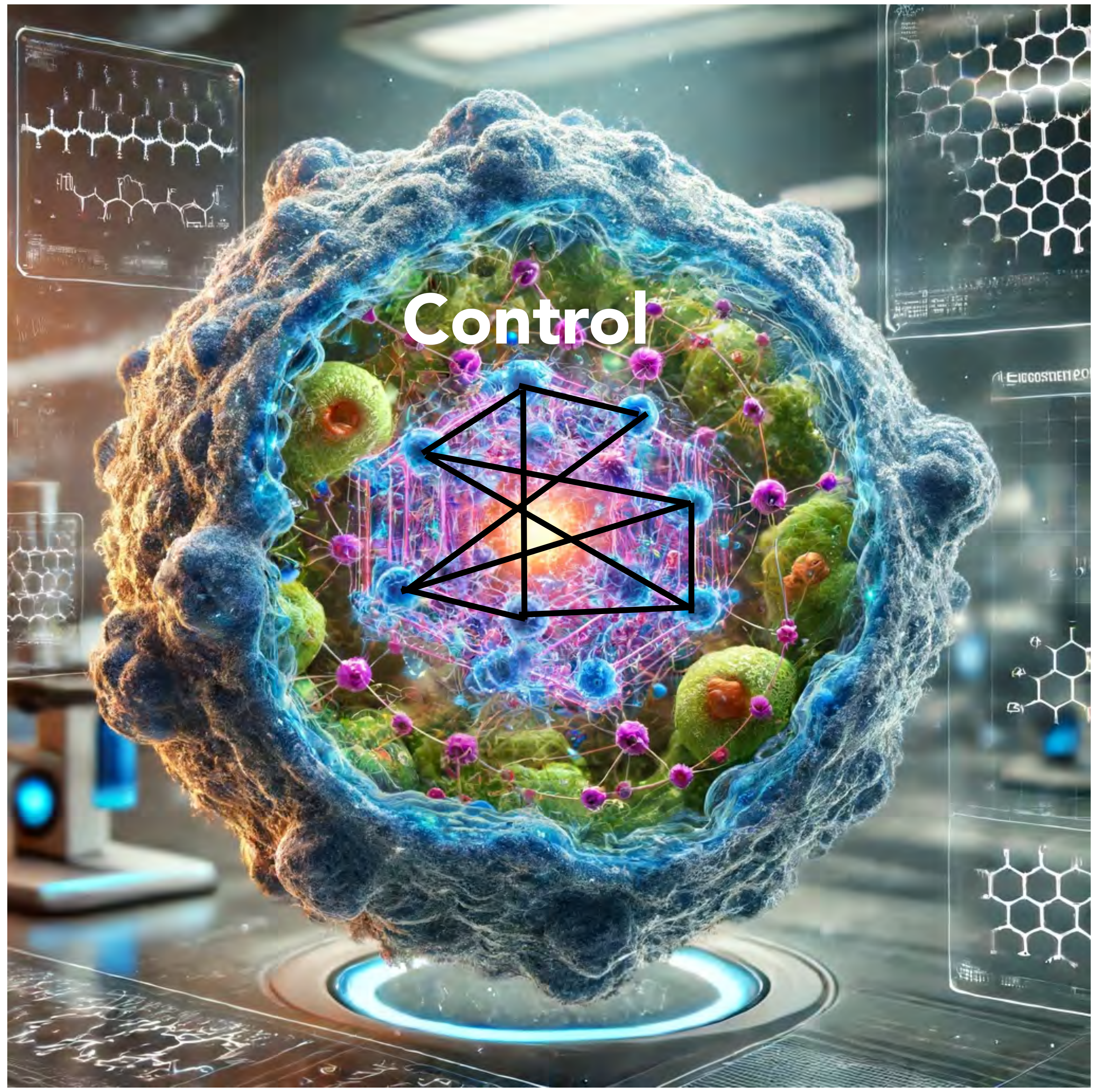
Biomolecular Control



control of cell populations



control of single cells

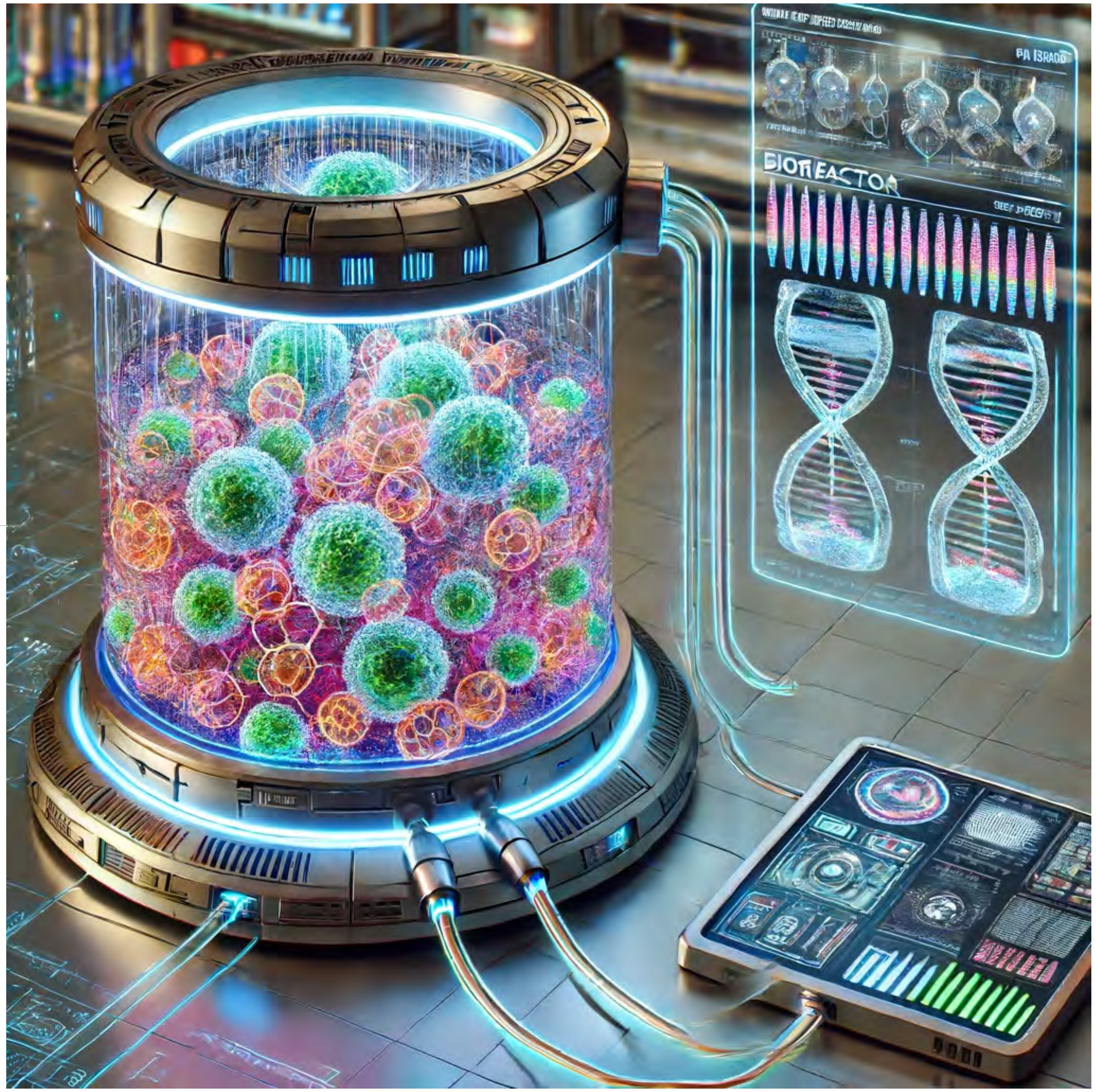


genetically engineered controller

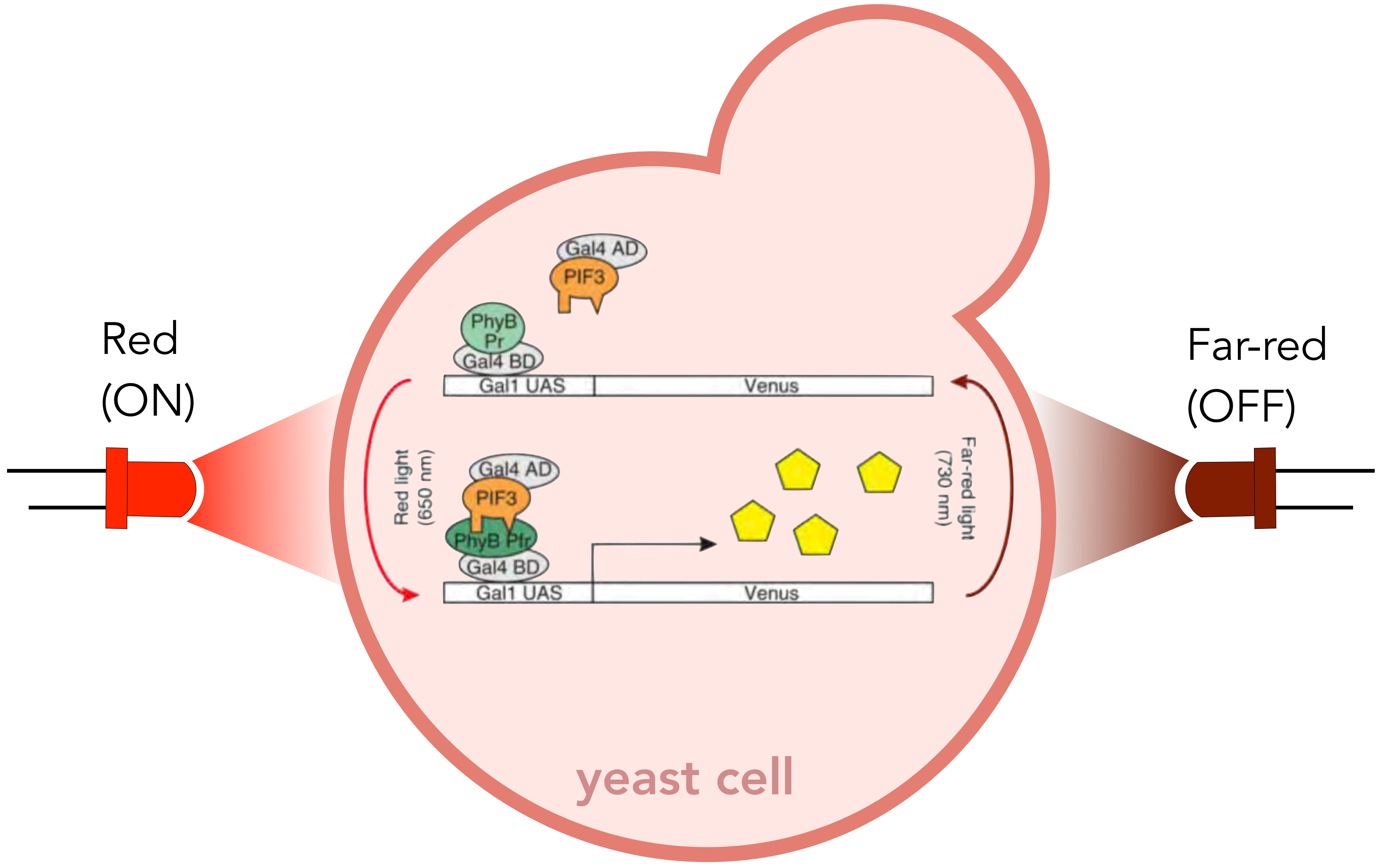
Images generated with *DALL.E 3*

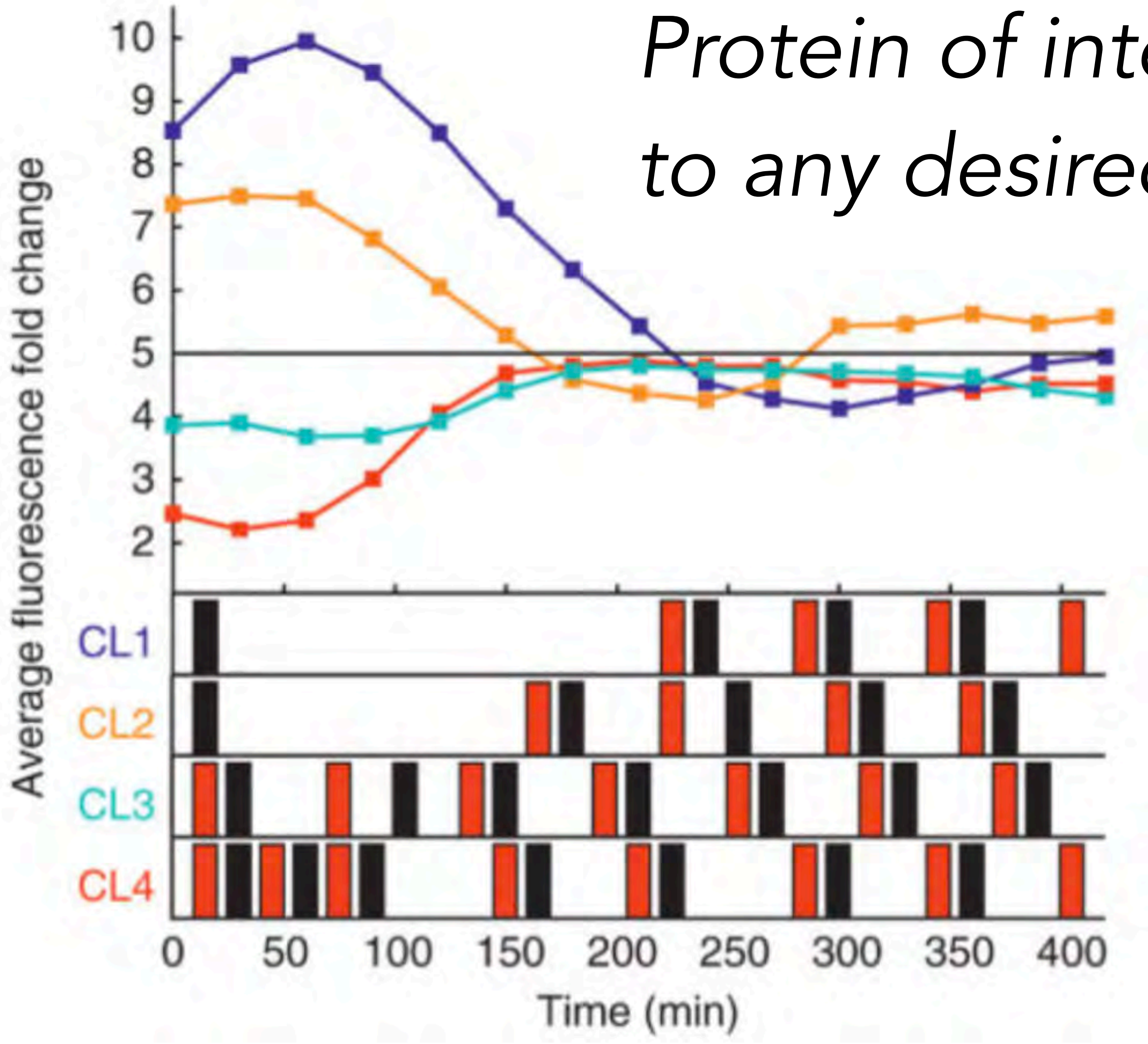
Computer Control

Feedback control of cell populations



Computer Control of Gene Expression in Yeast



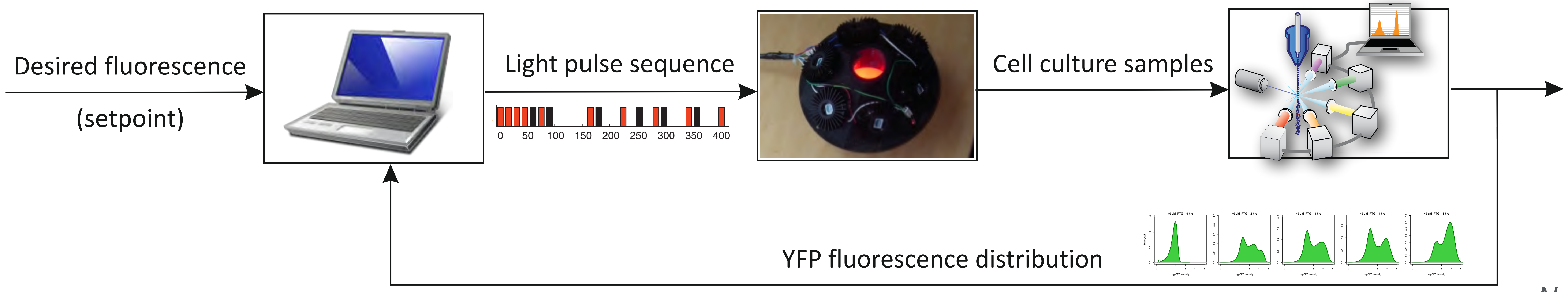


Protein of interest clamped to any desired value!

***in silico* feedback**
Kalman filter + Model Predictive Controller

cell population
Kept in dark vessel with electronically controlled light source

output measurement
Flow cytometry

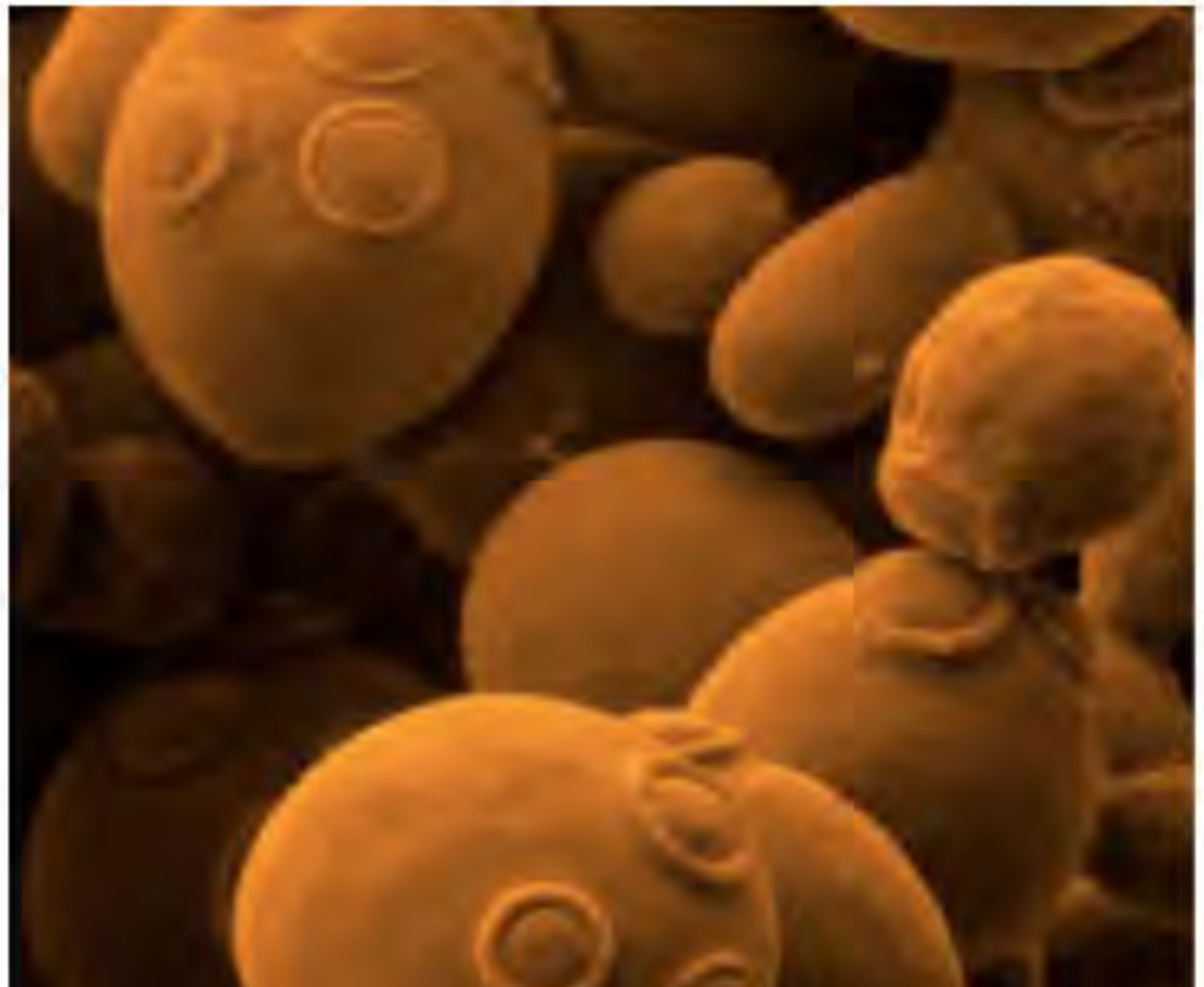


Nature Biotechnology, Nov. 2011

6 November 2011 Last updated at 19:02 GMT

'Cyborg' yeast genes run by computer

By Jason Palmer
Science and technology reporter, BBC News



Scientists have succeeded in forming a "feedback loop" between a computer and a common yeast to precisely control the switching on and off of specific genes.

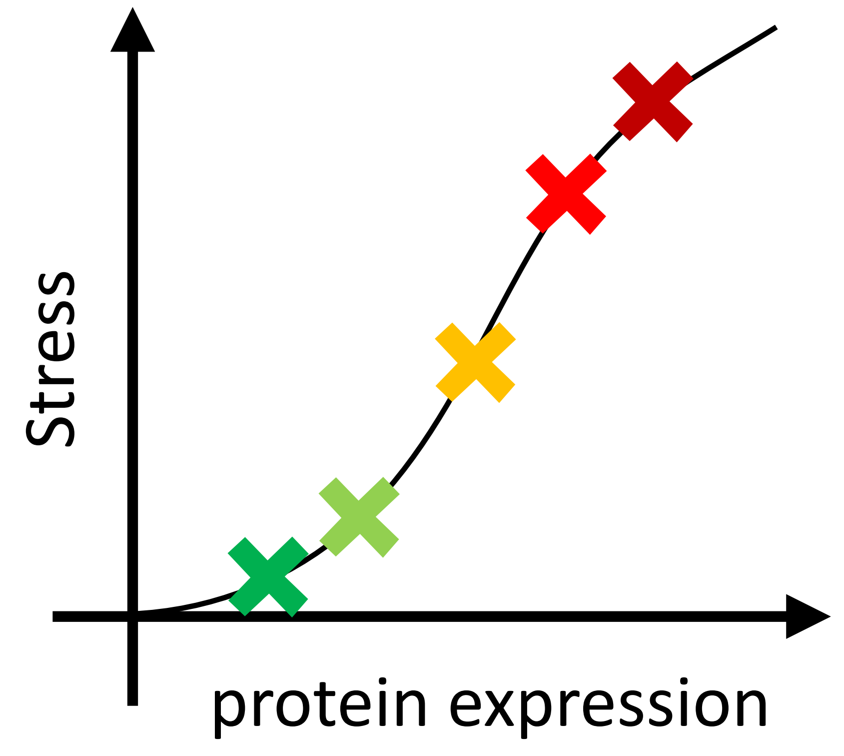
The computer controlled flashes of light to start and stop this gene expression, "learning" how to reach and maintain a set value.

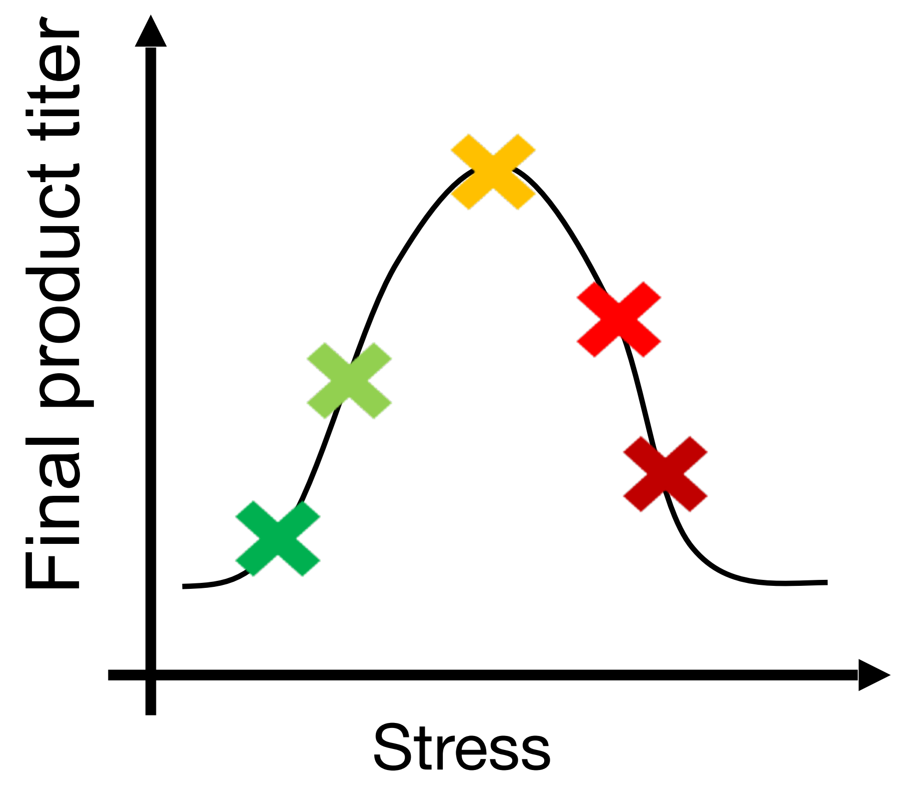
The groundbreaking approach could find use in future efforts to control biological processes, such as the production of biofuel from microbes.

It appears in Nature Biotechnology.

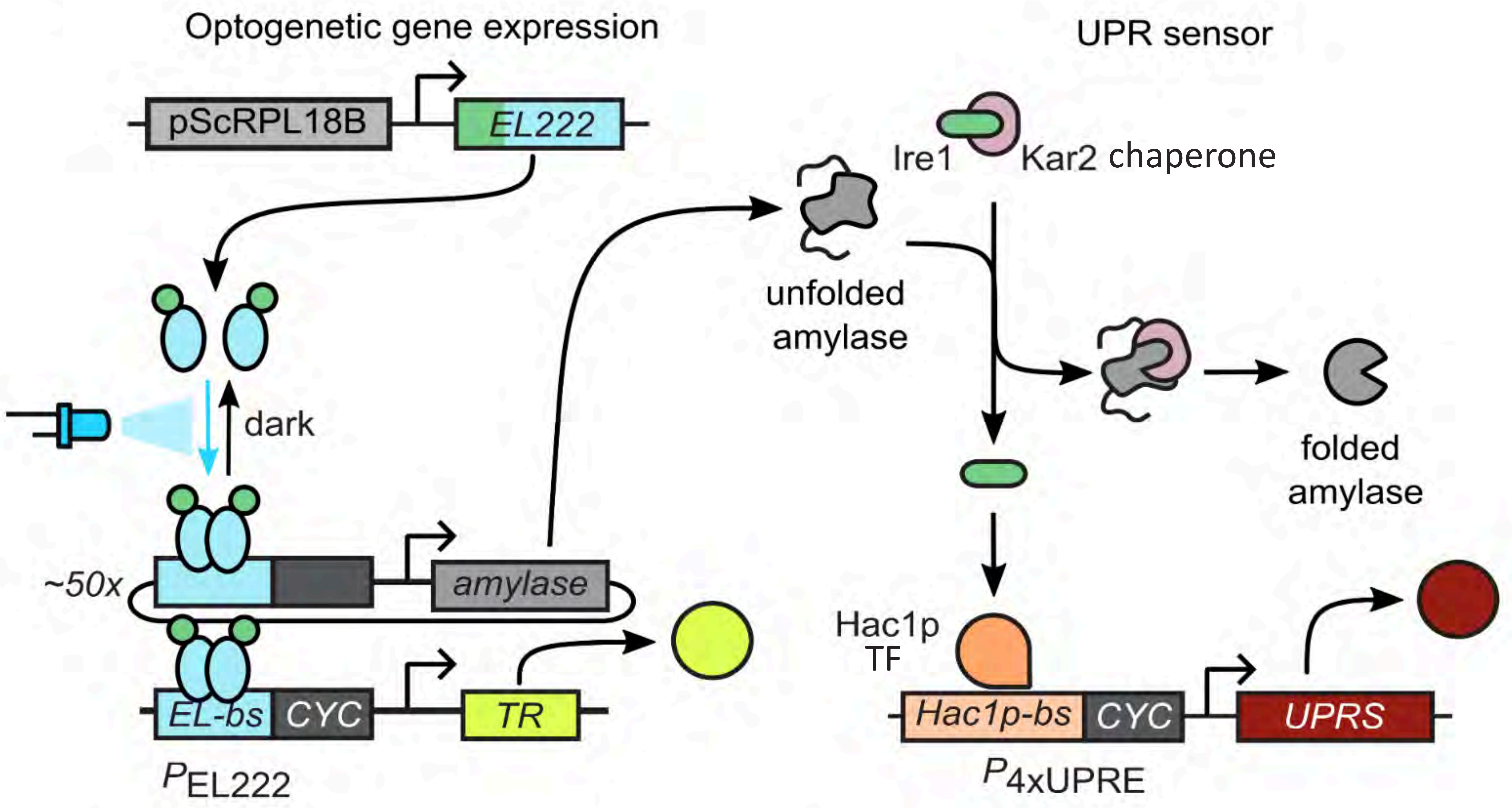
Feedback control of recombinant protein production

Protein Expression vs Stress



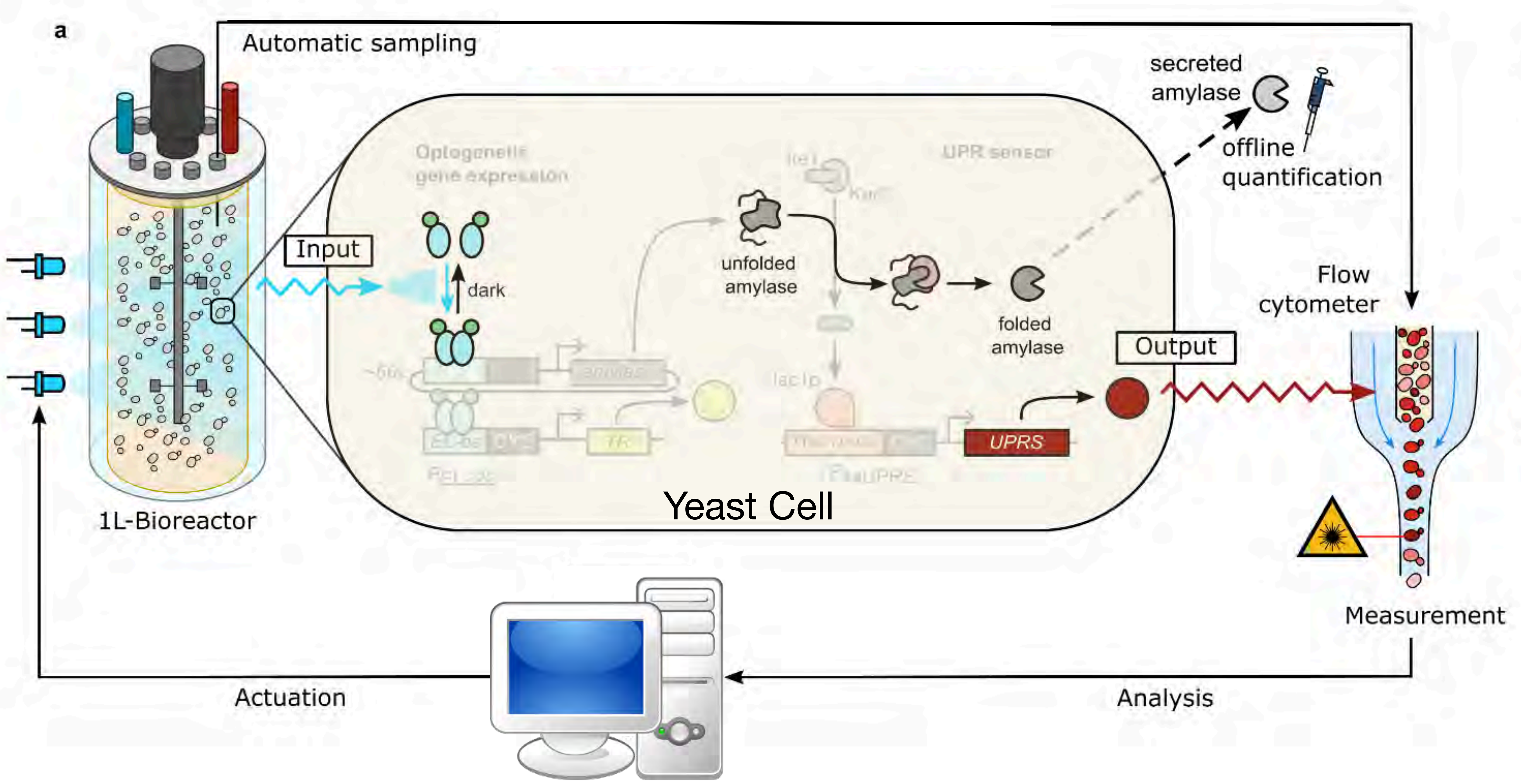


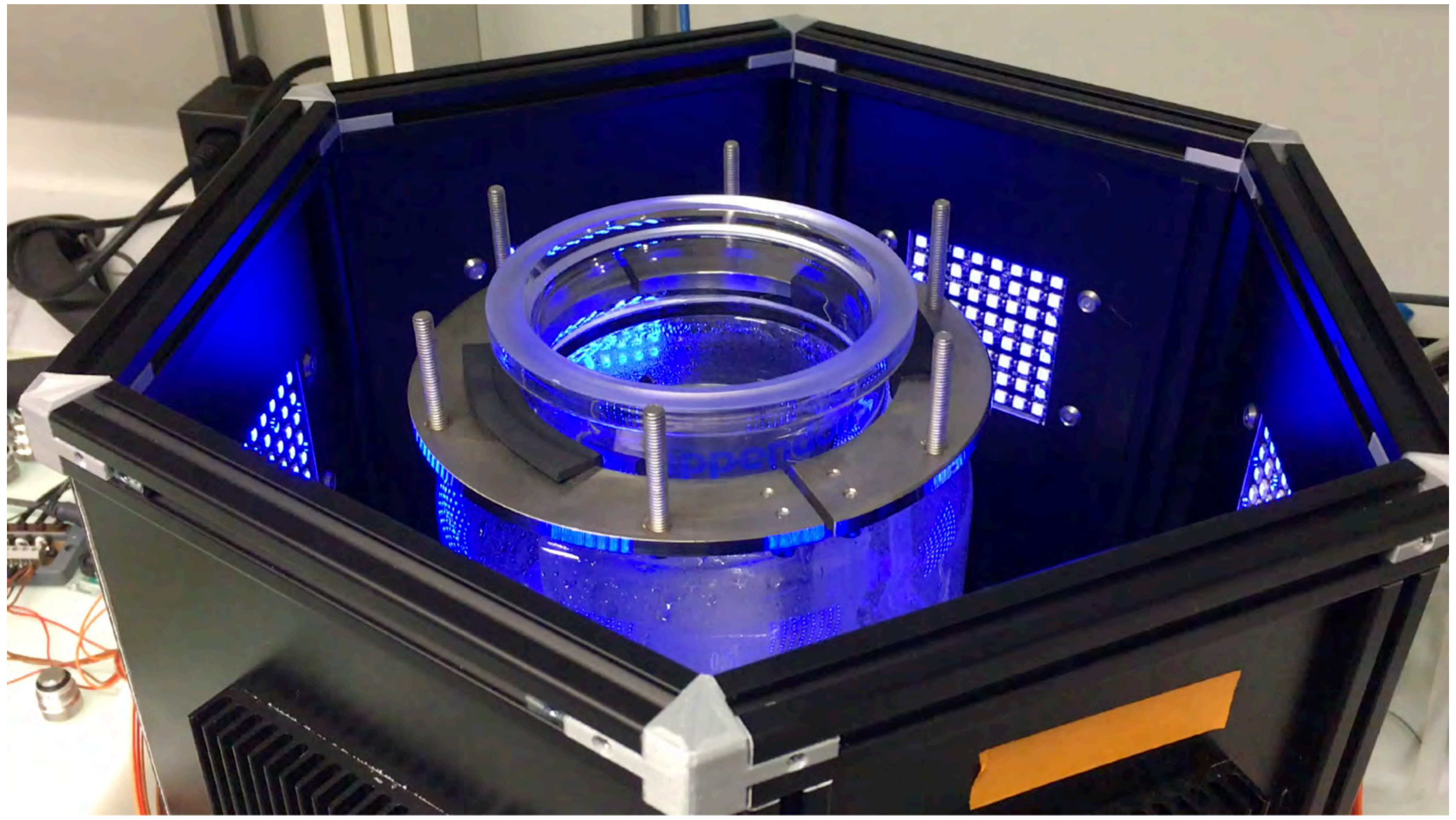
Sensing Gene-Expression-Induced Stress



Benisch et al, *Metabolic Engineering* (2023)

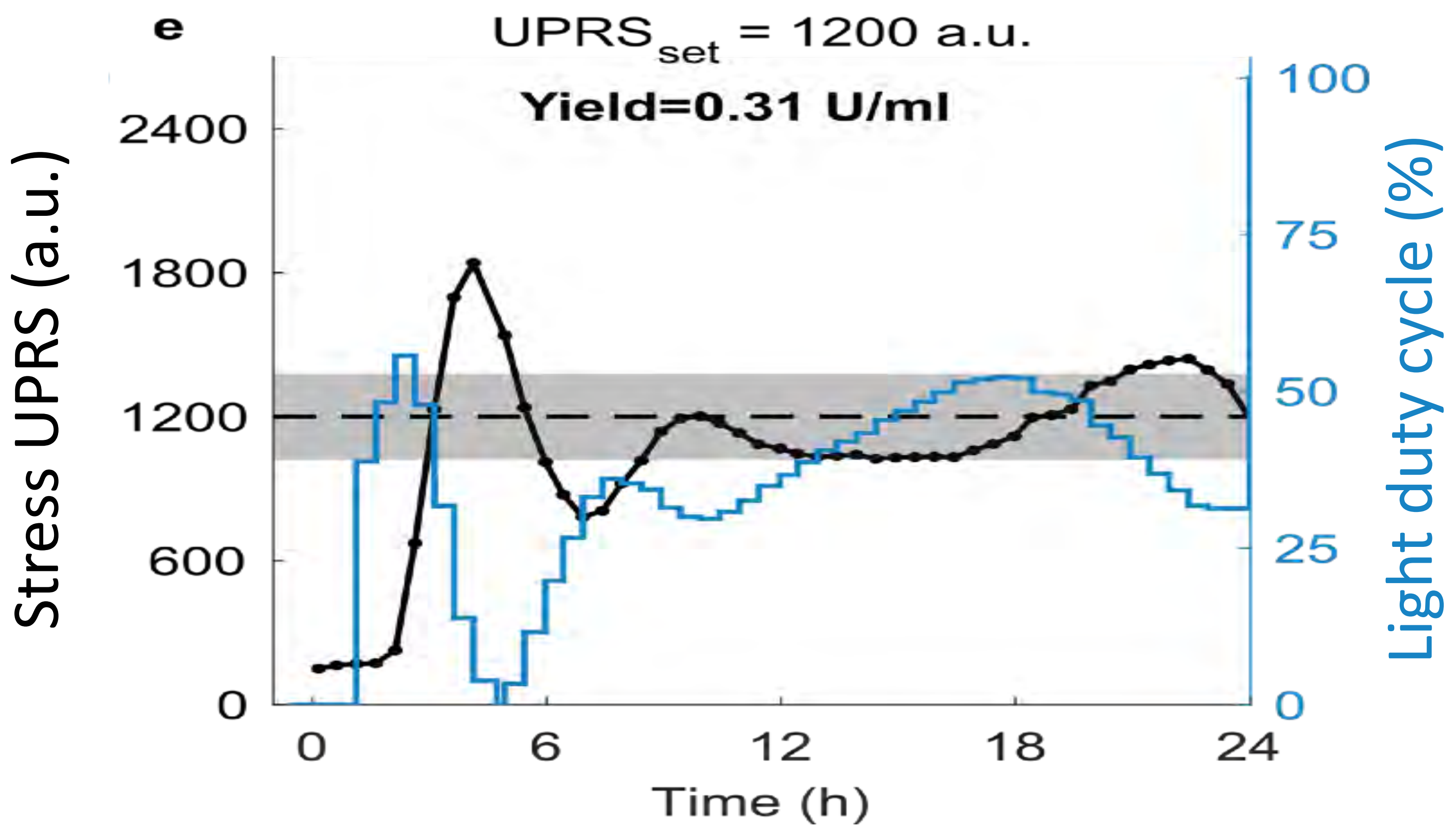
Cybergenetic control of recombinant protein production

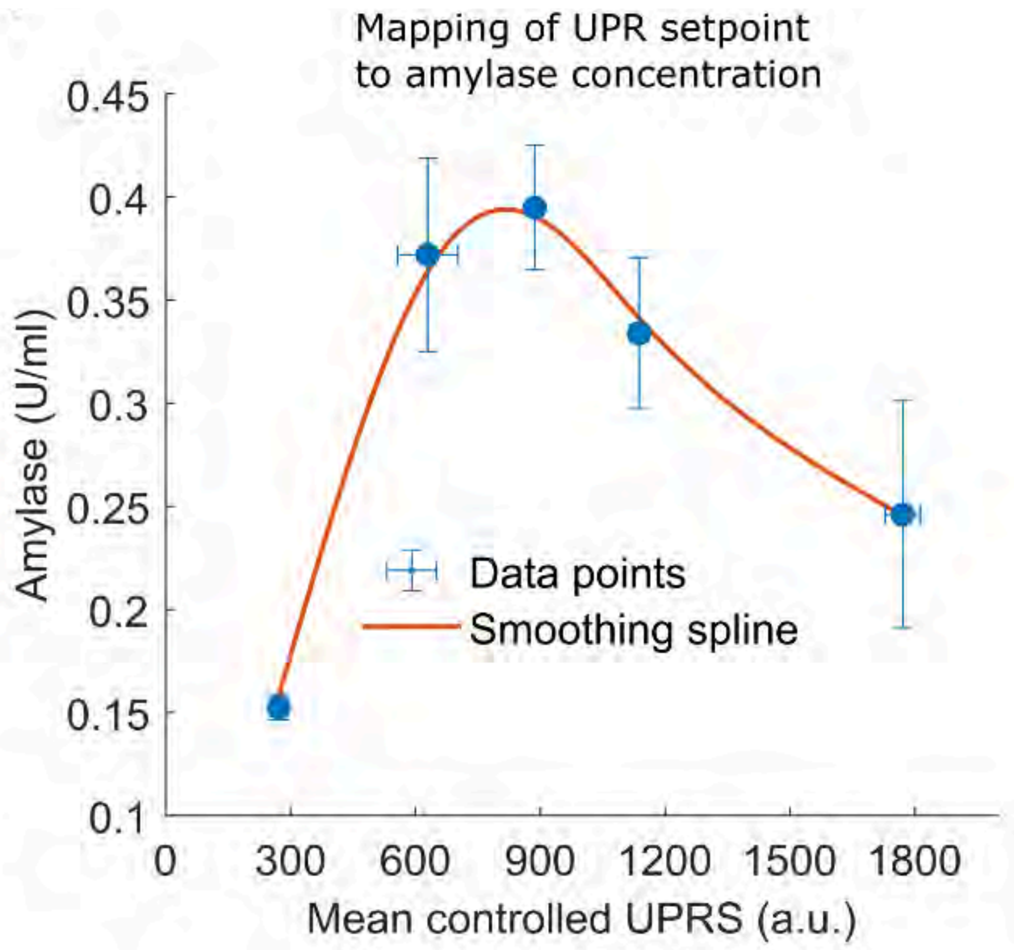




Model-Based PID Control Systems

A typical 24h run



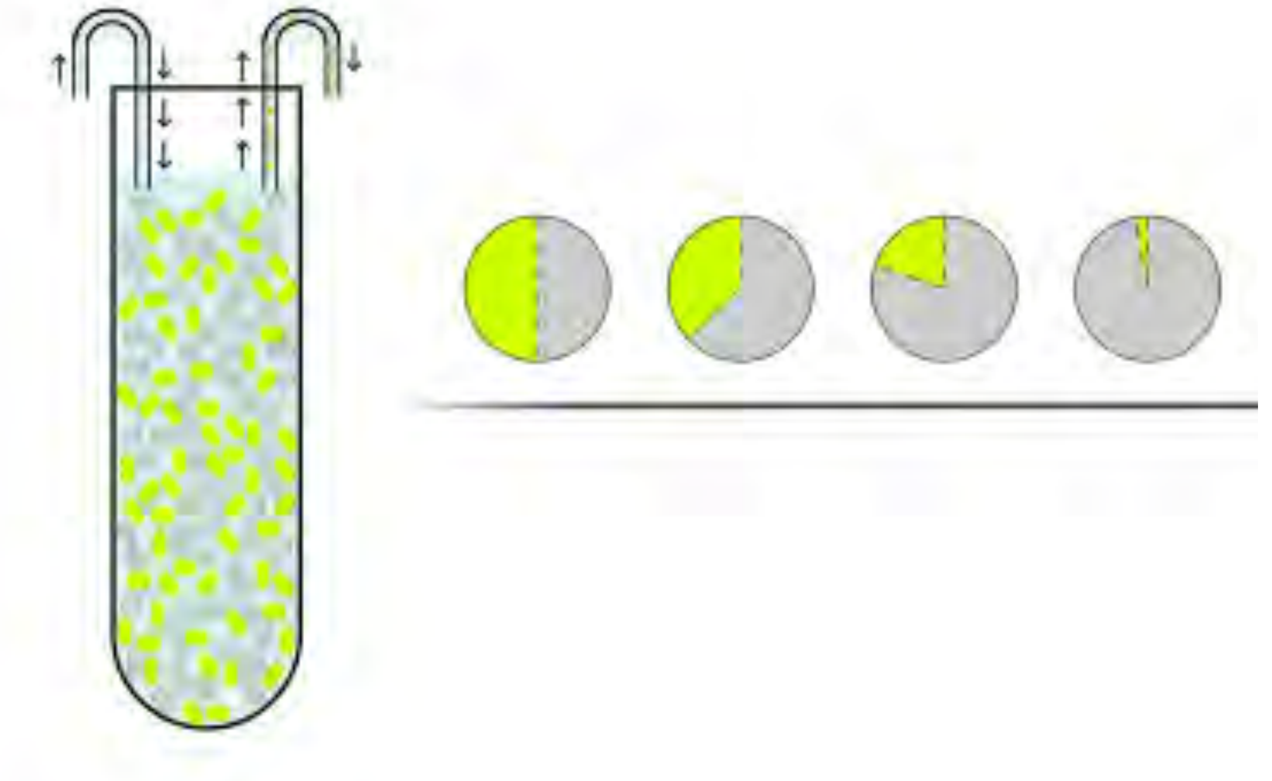


60% increase in yield!

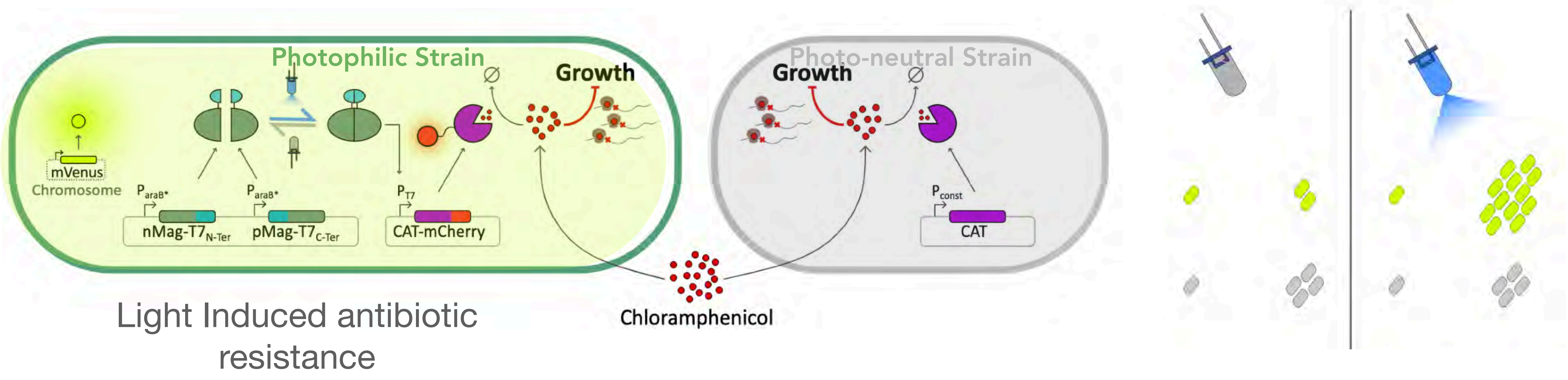
Benisch et al, *Metabolic Engineering* (2023)

Feedback Stabilization of Microbial Co-Cultures

2 strains with different growth rates

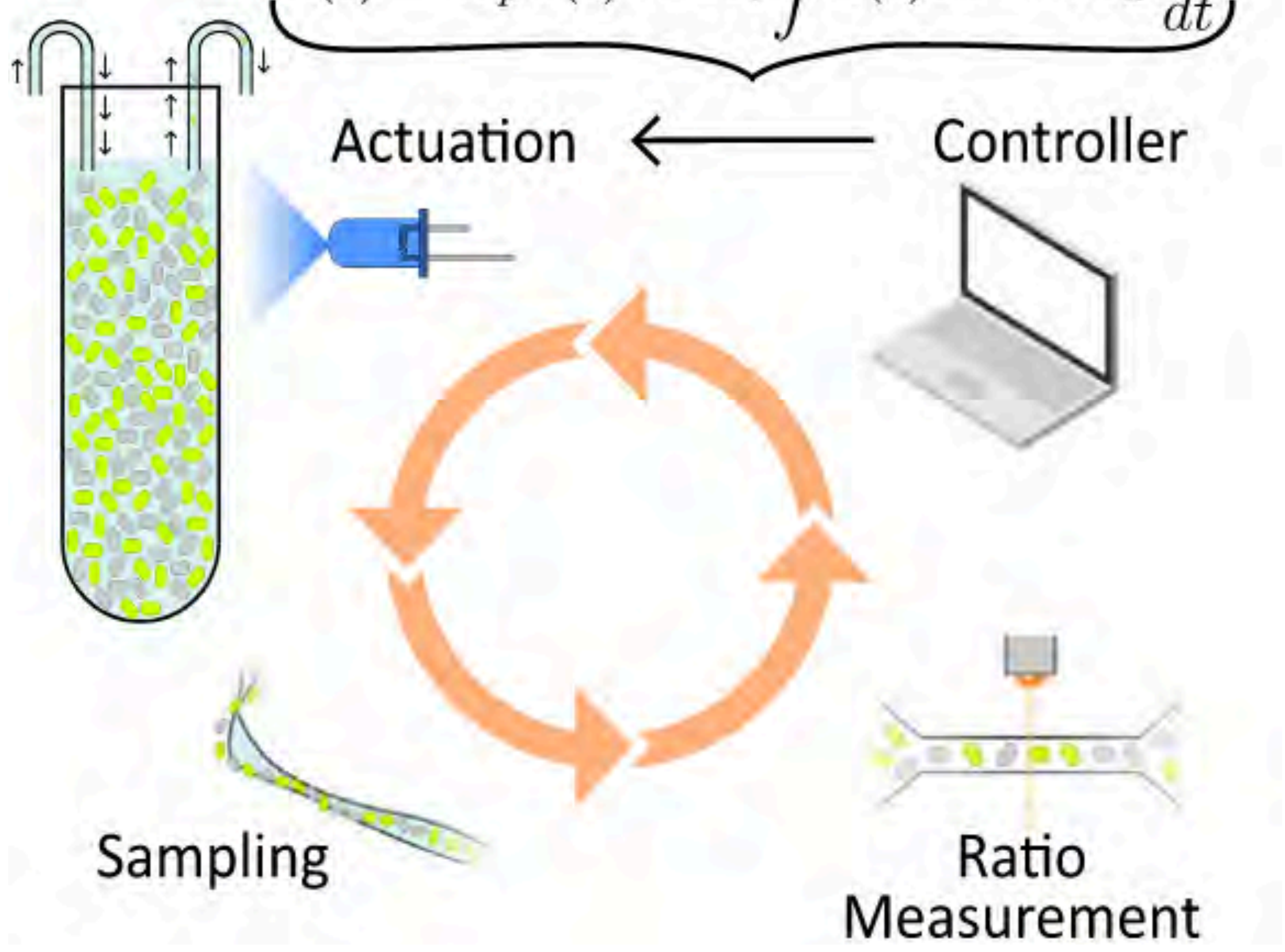


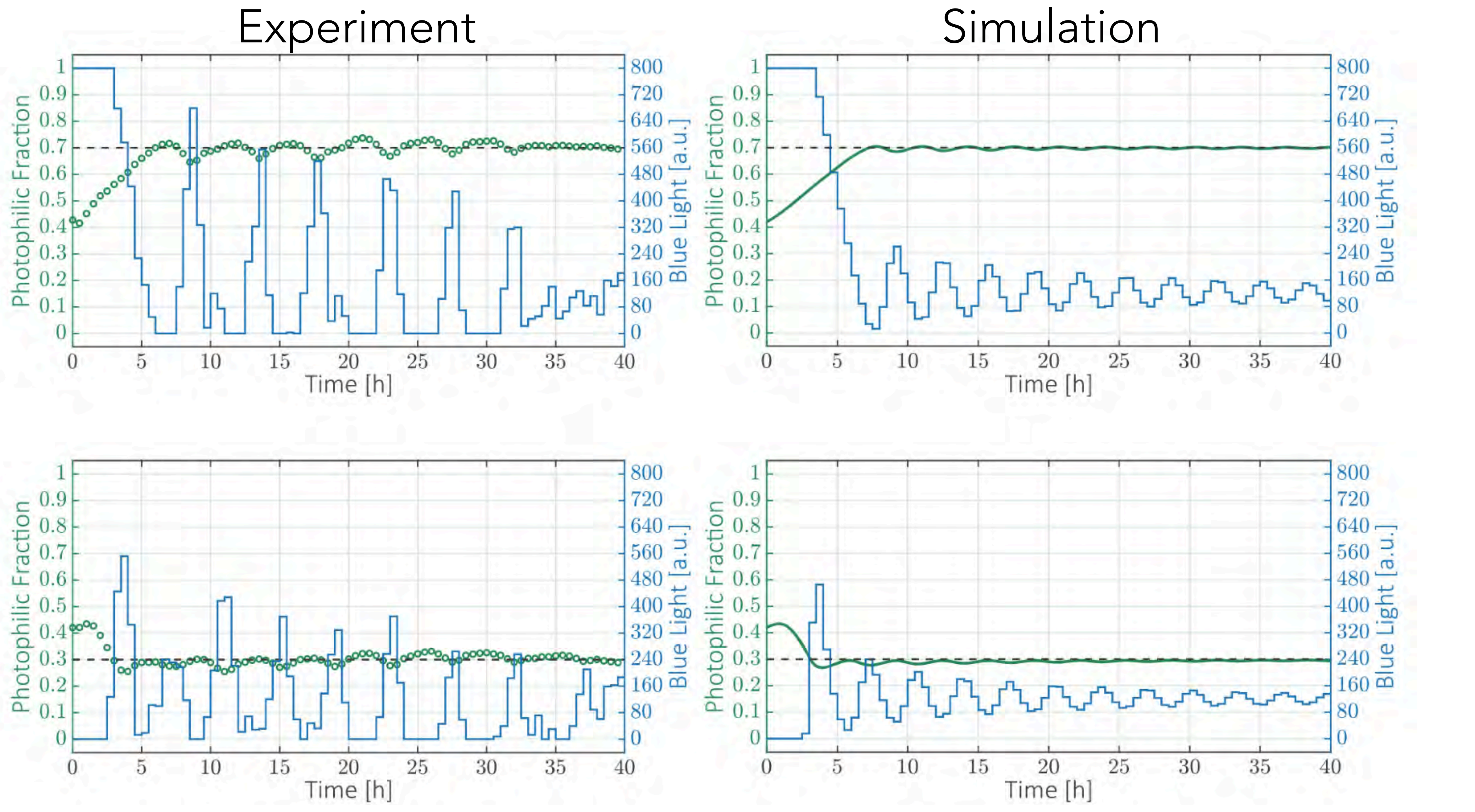
Engineered light-controlled cell growth



Feedback Control

$$u(t) = K_p e(t) + K_i \int e(t)\,dt + K_d \frac{de}{dt}$$



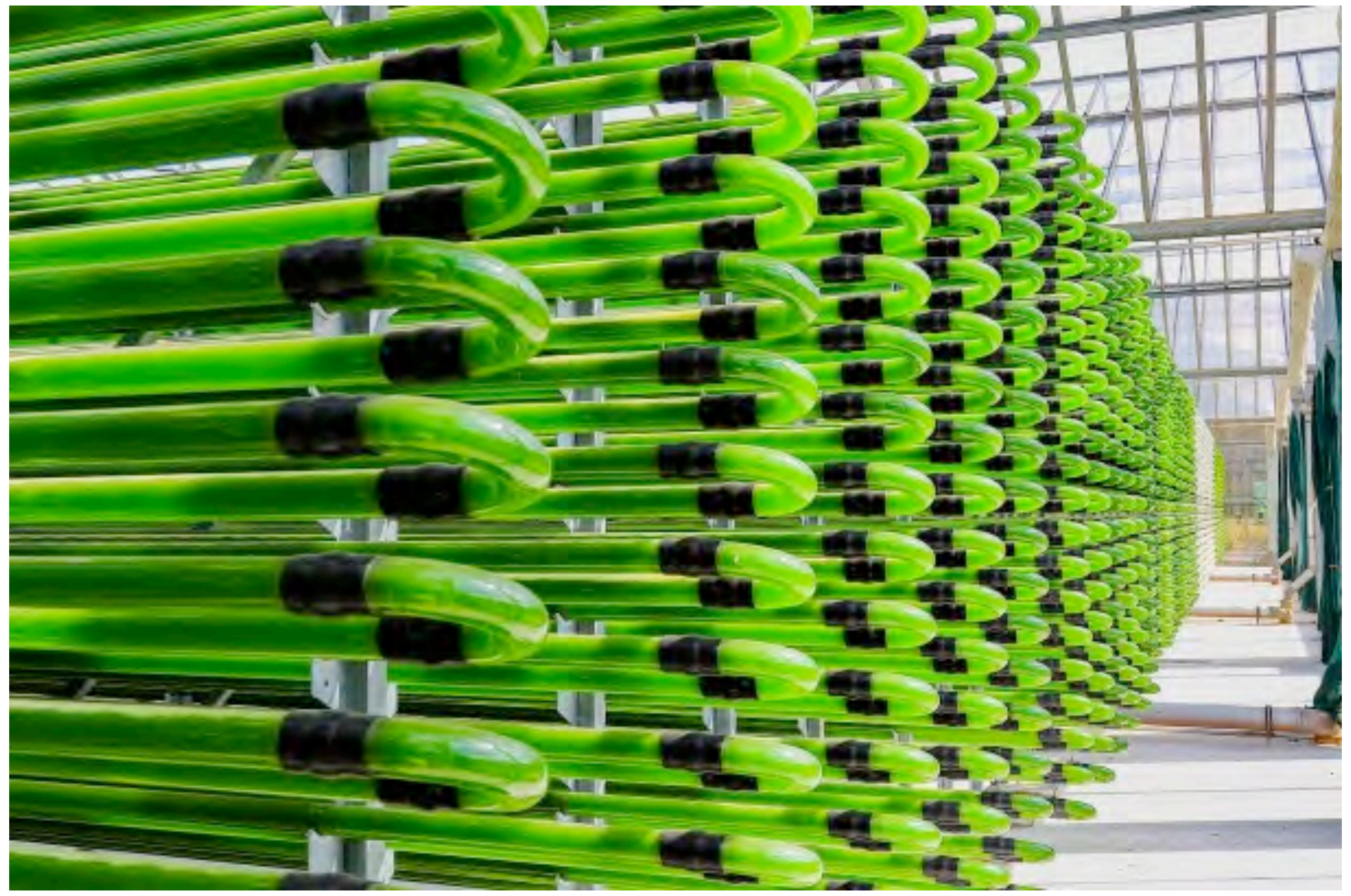


Gutierrez, Kumar, Khammash, *Nature Communications* (2022)

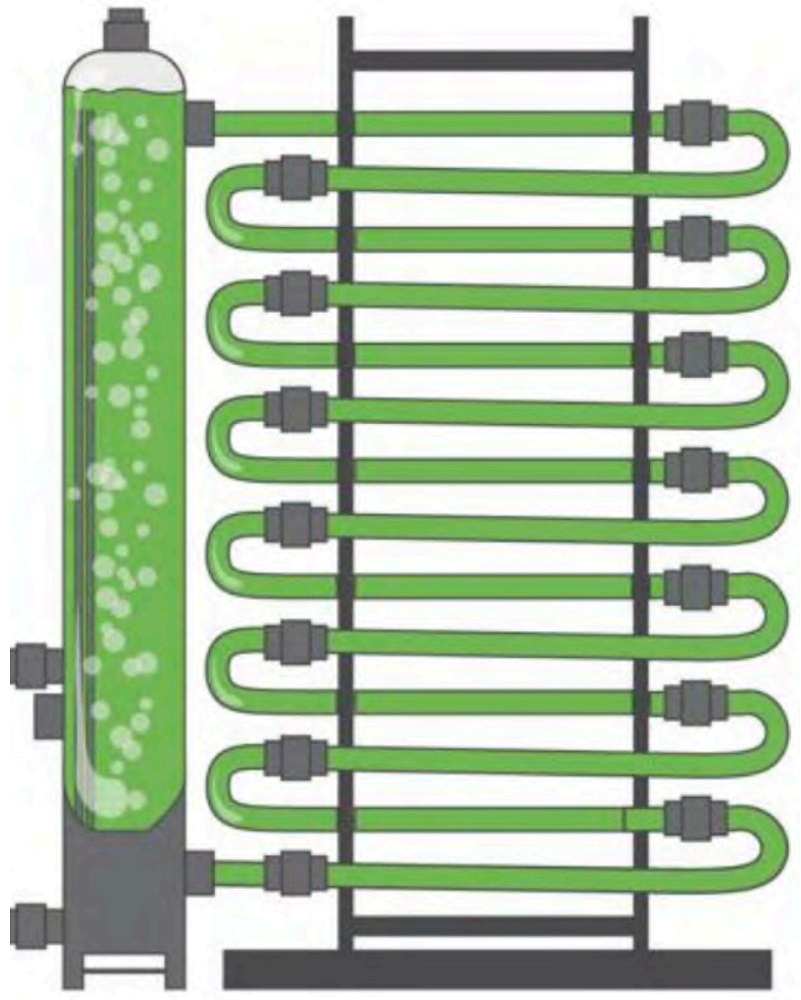
Key Challenges

- Genetic engineering of production strains (cost, burden, …)
- Suitable sensors (key enzymes, metabolites, intermediates, products, cell state, …)
- Light penetration in bioreactors

500 L to 200,000 L



Photobioreactor

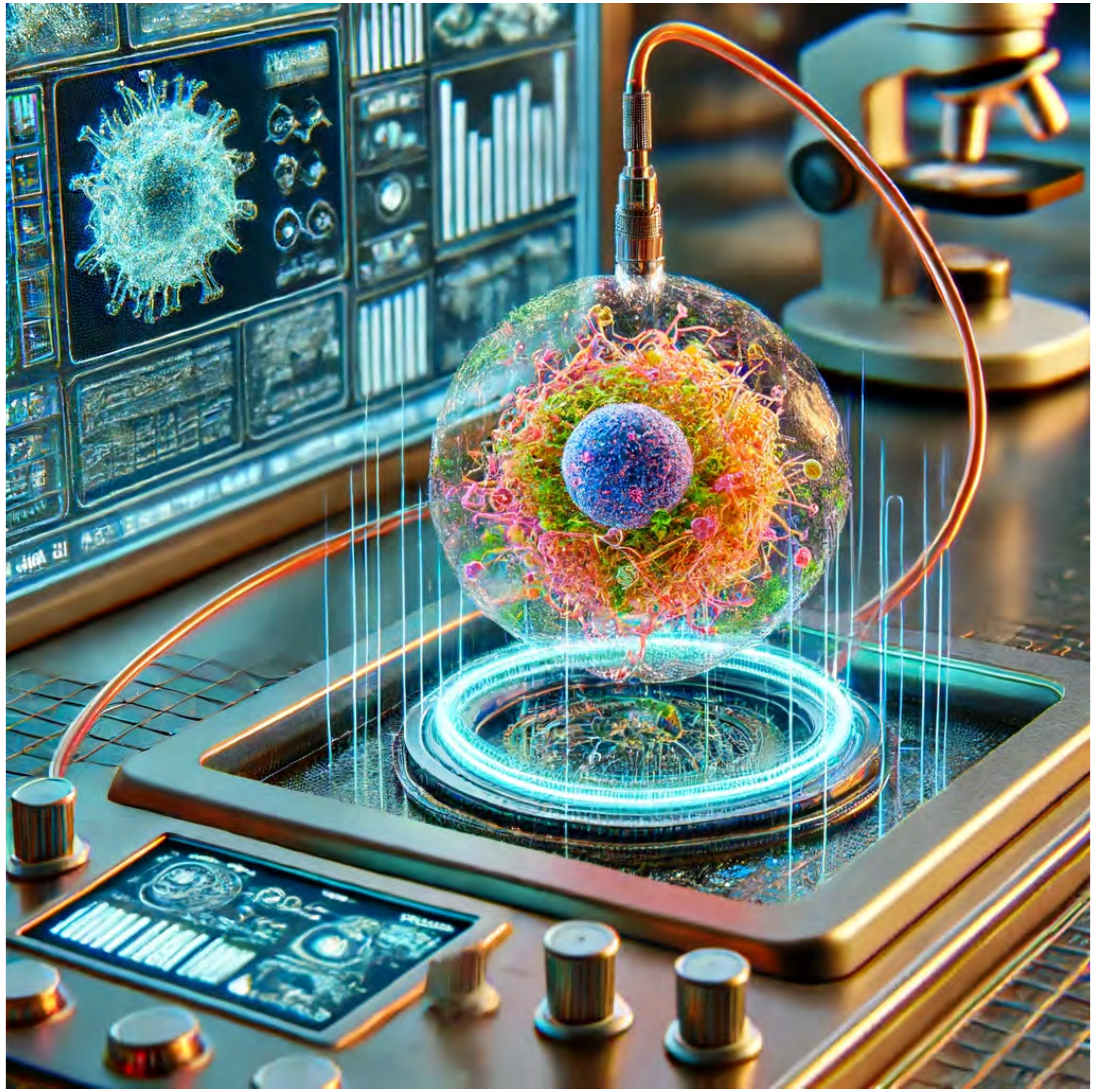


Photobioreactor

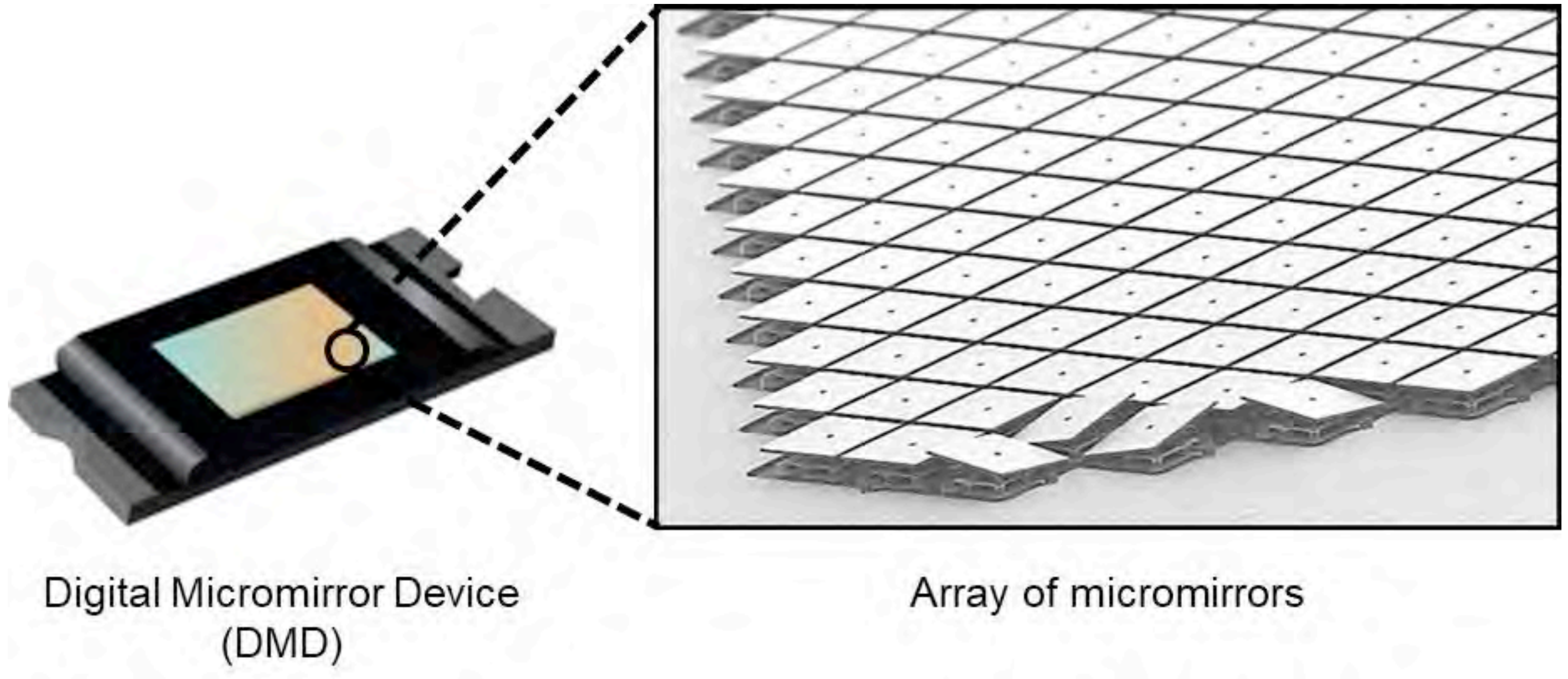
- May be limited to low-volume high-value products
- Insights from lab scale bioreactor controllers will guide the design of genetic controllers (no light needed)

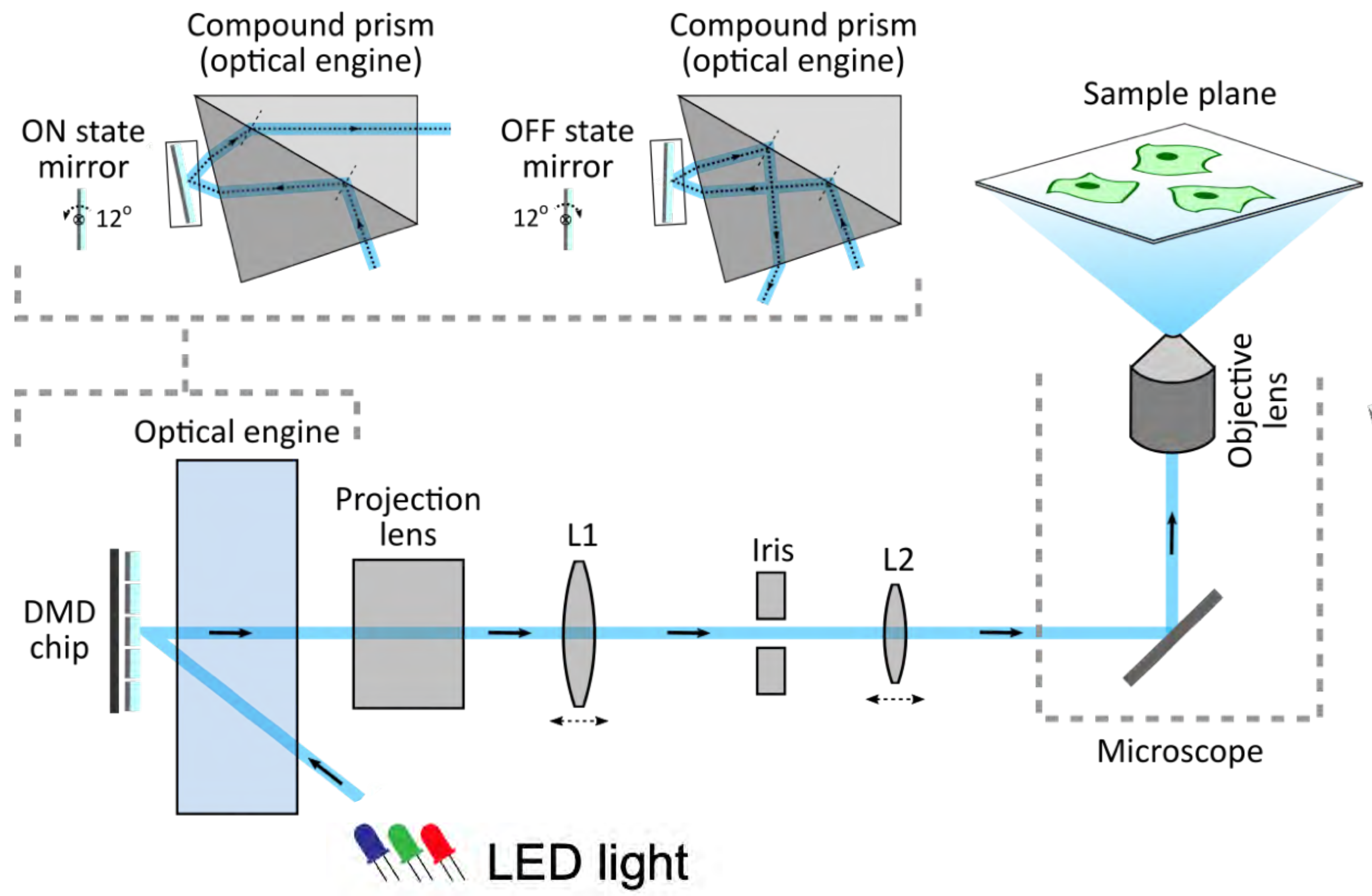
Computer Control

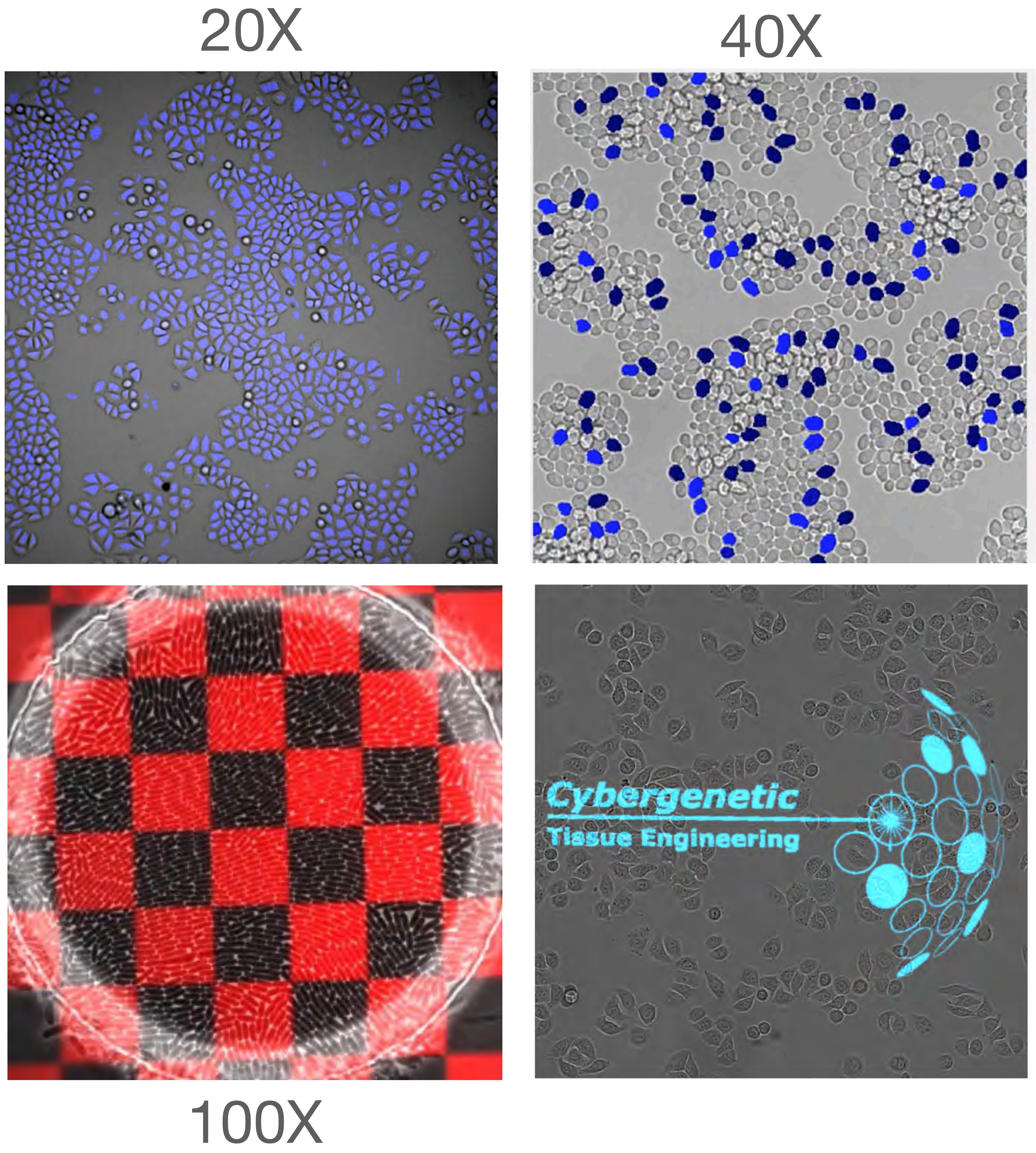
Feedback control of single cells



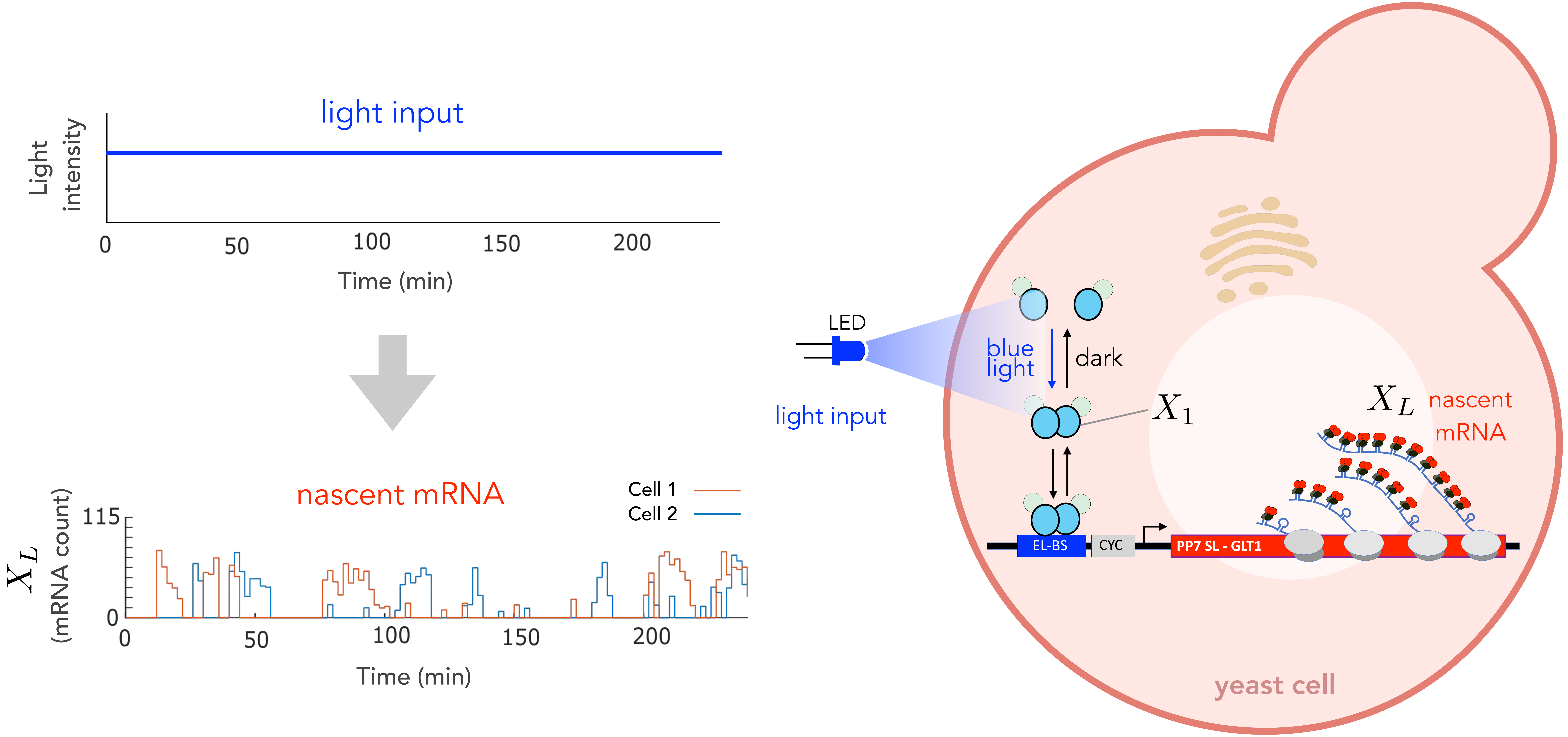
Underlying Technology: A Device for Patterned Illumination



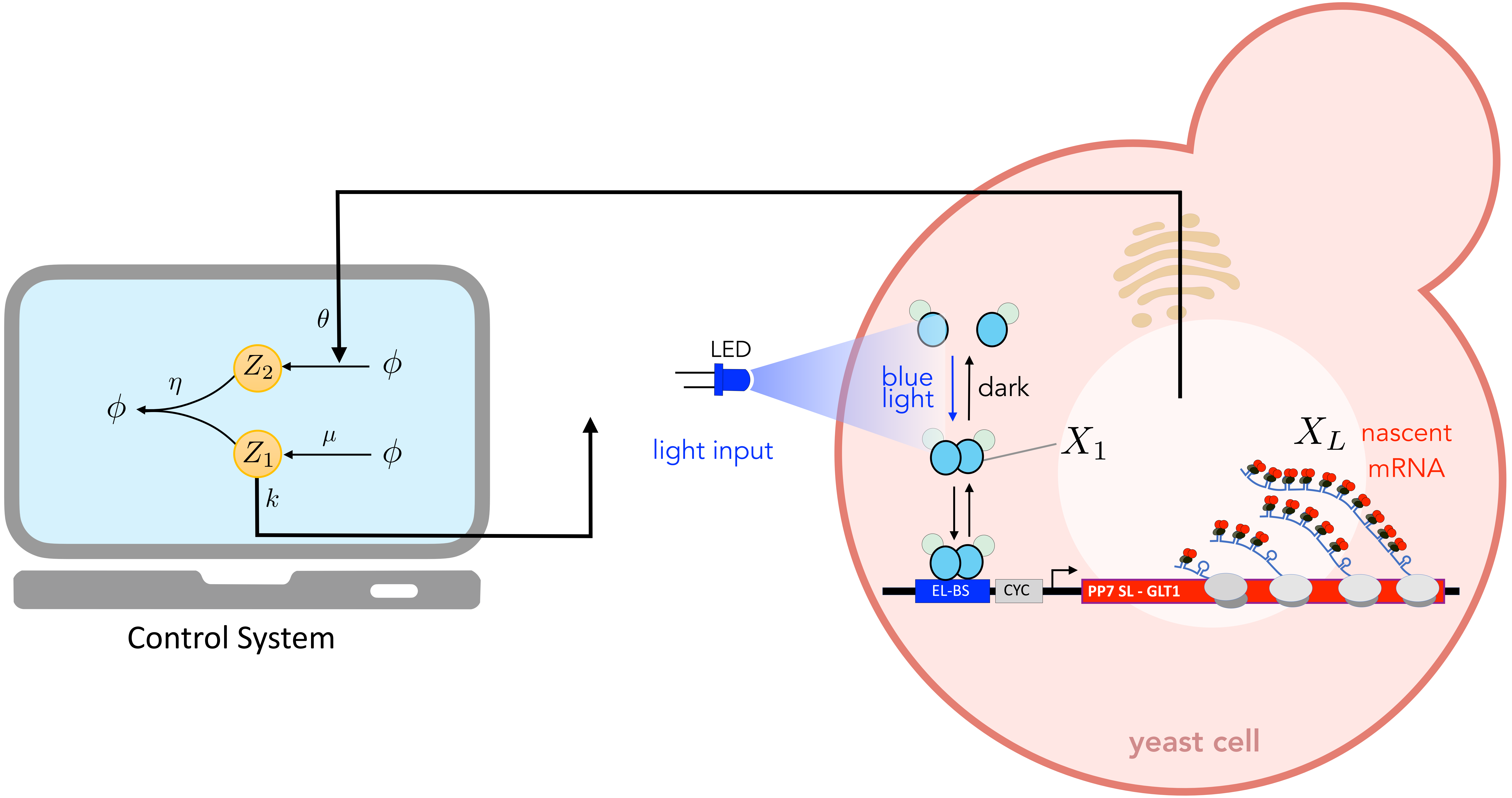




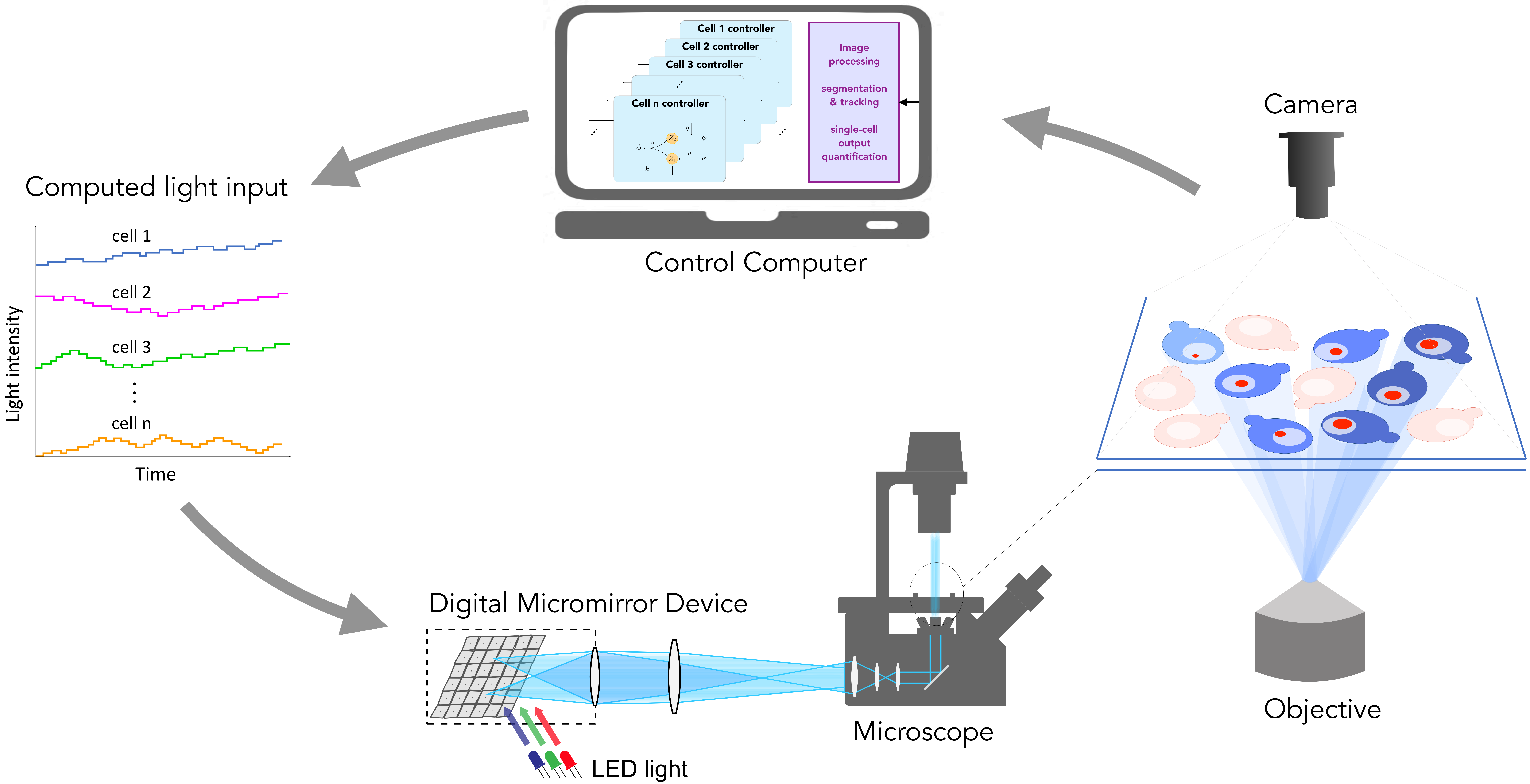
Computer Control of Single Cells



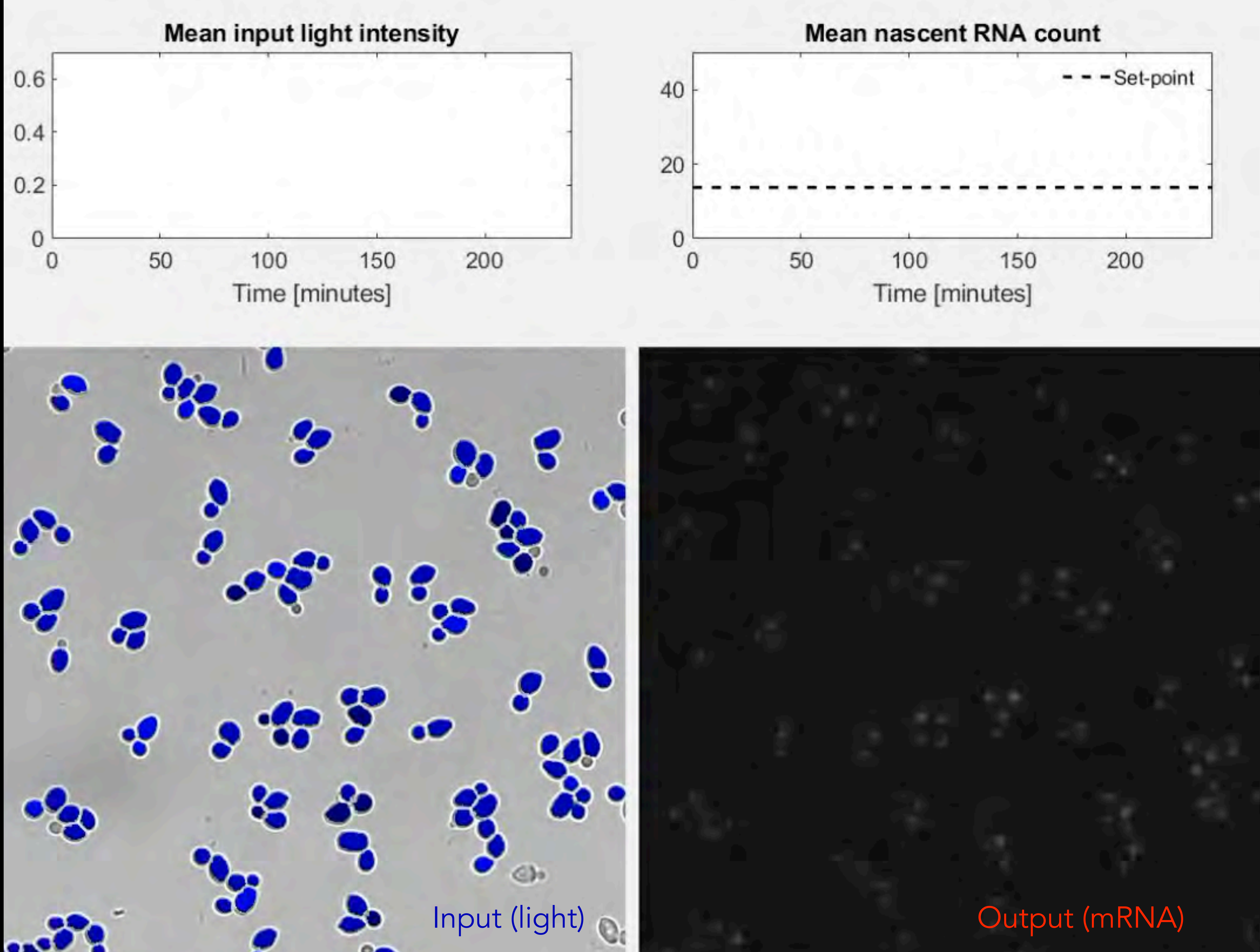
Computer Control of Single Cells



The Cyberloop

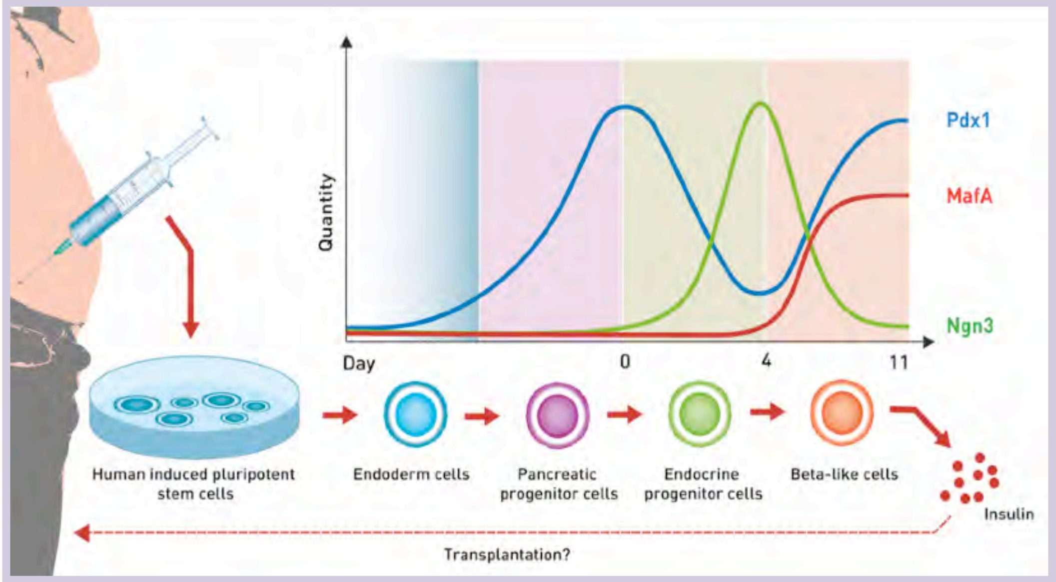


Closed-Loop Control
of Single Yeast Cells

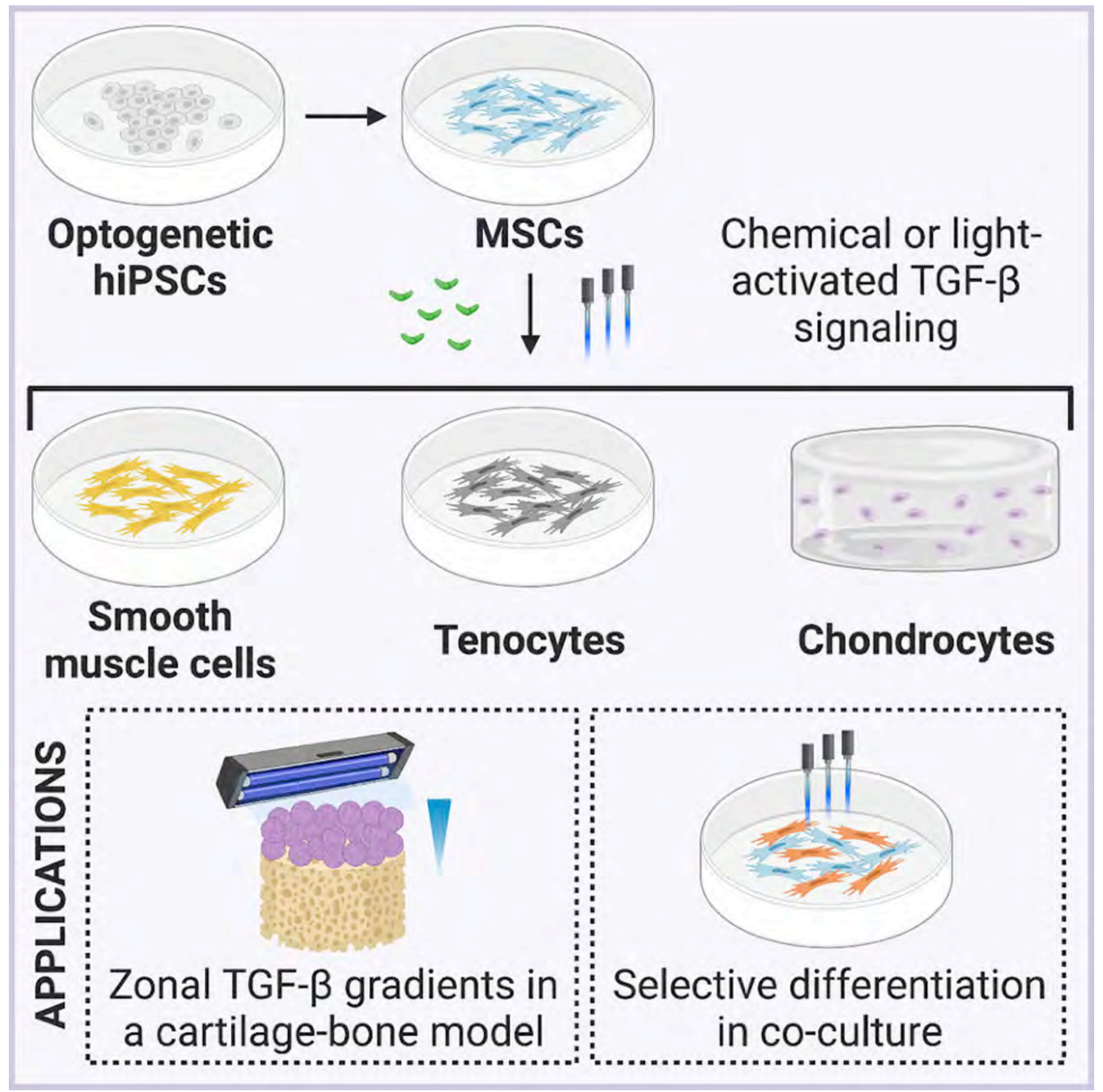


Application Opportunities: Tissue and Organ Engineering

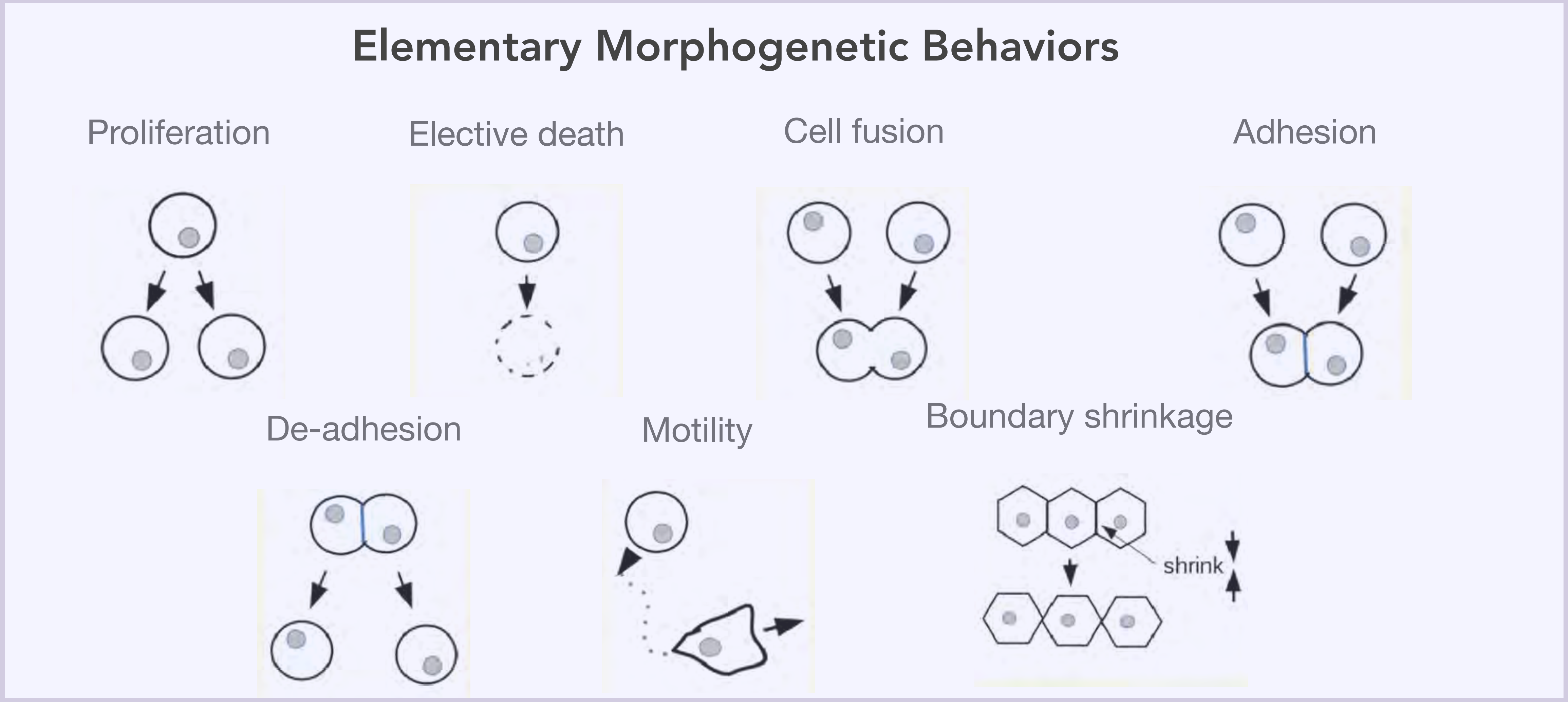
Stem cell differentiation



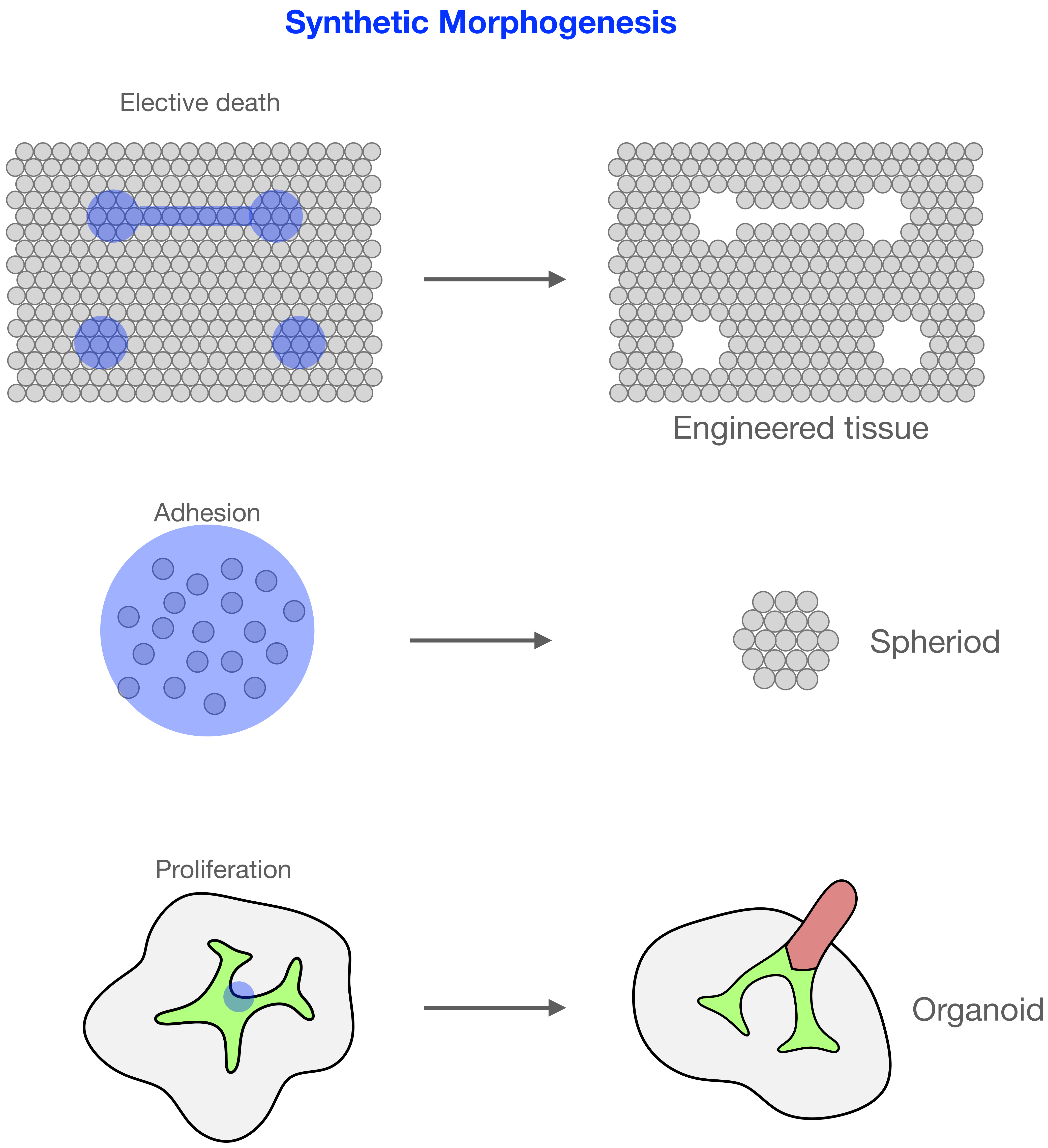
Saxena et al (2016), *Nat. Comms*



Wu et al. (2023), *Cell Reports*

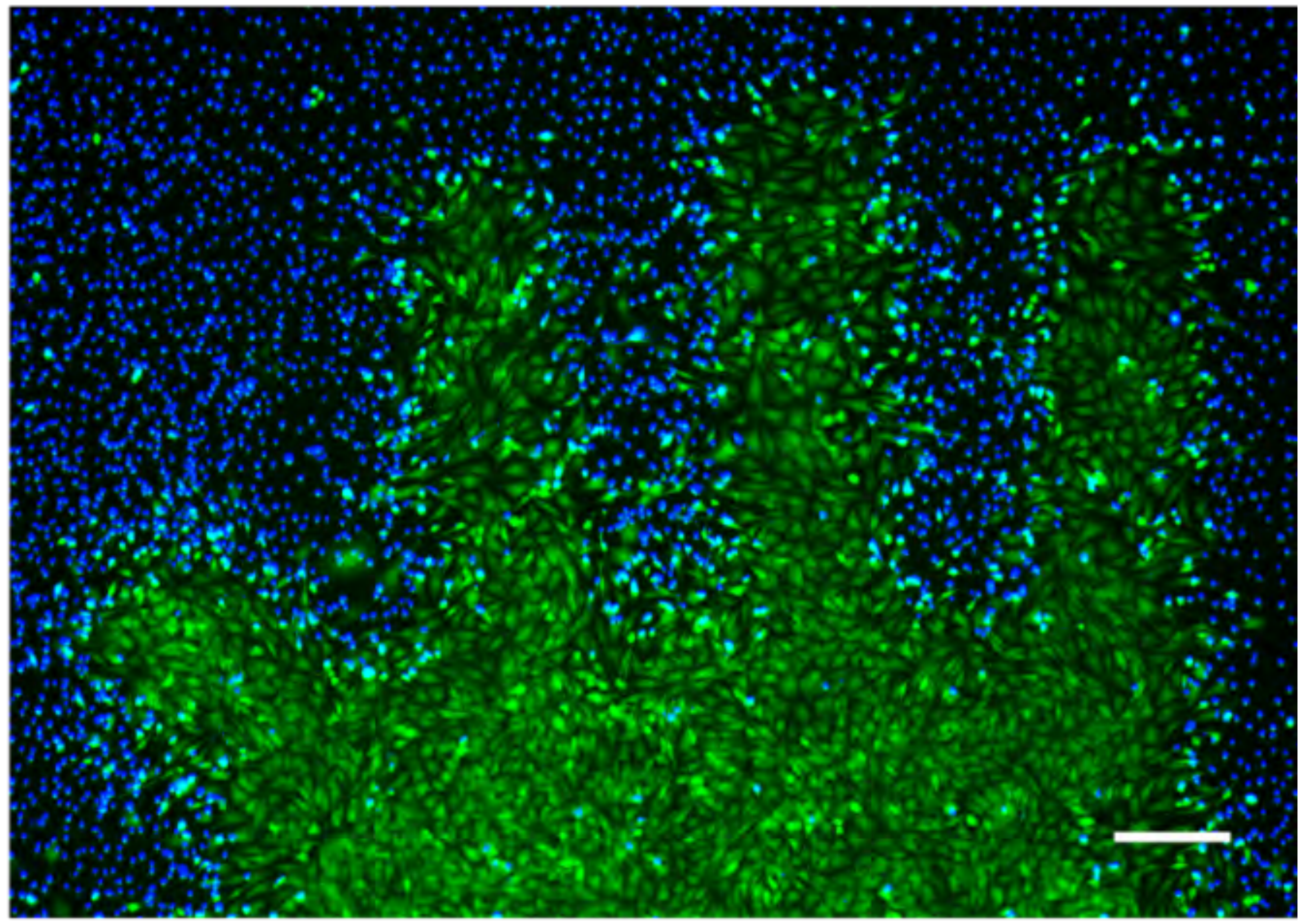


Davies (2023), Proc. IEEE



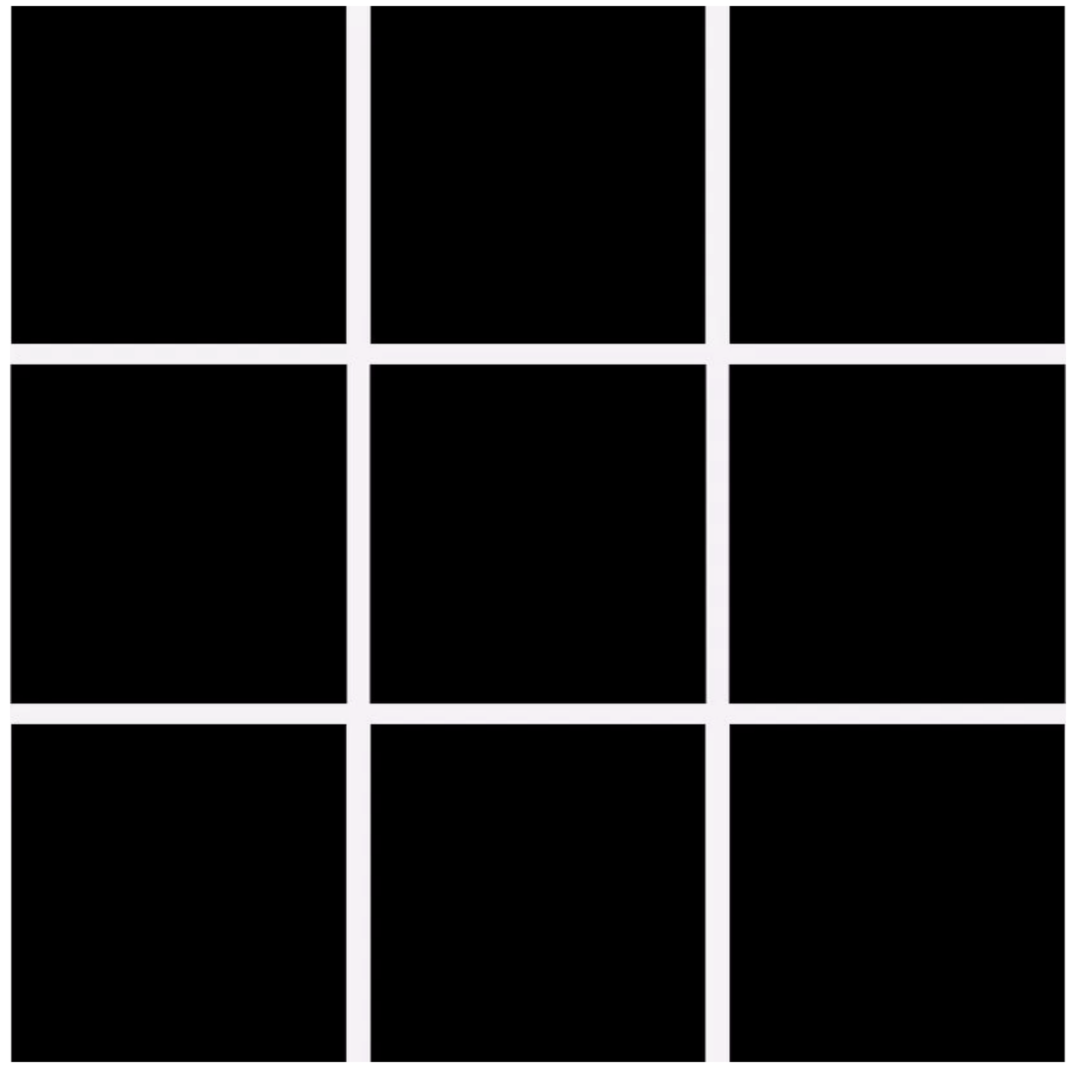
Opportunities: Tissue and Organ Engineering

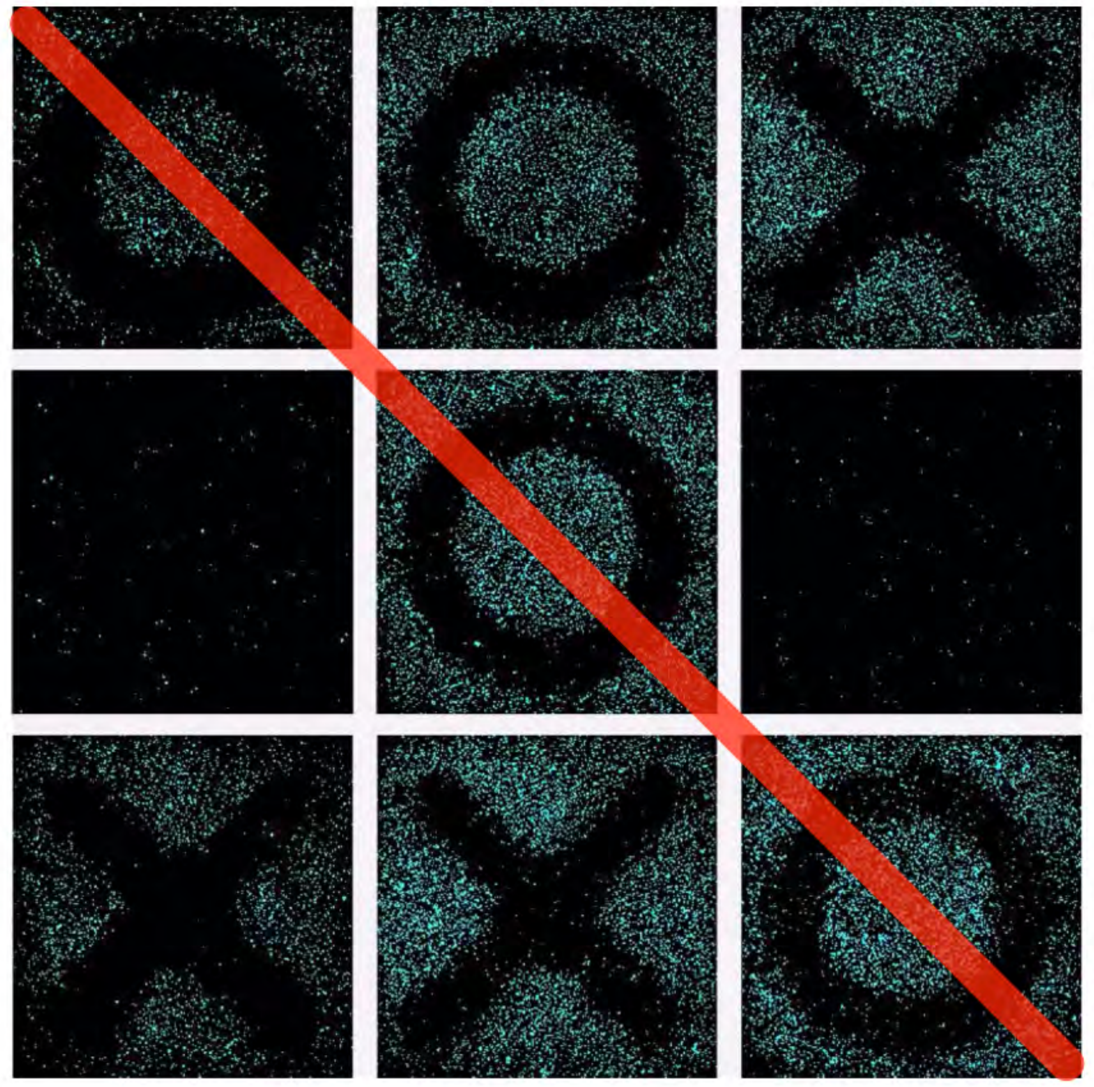
Scale bar, 200 μm



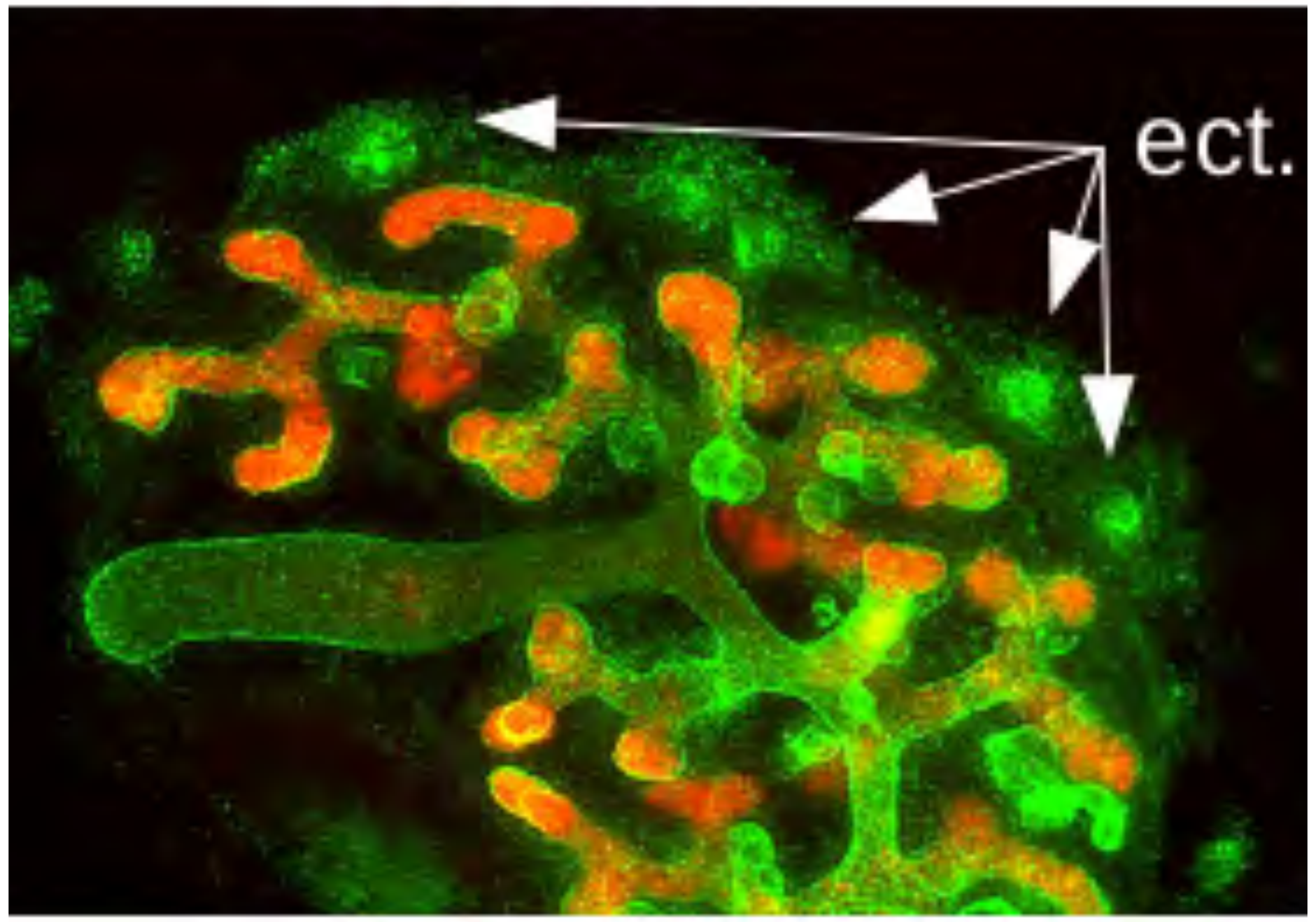
(blue stain indicates dead cells)

Kumar *et al*. (2024) *Nature Comms.,* in revision





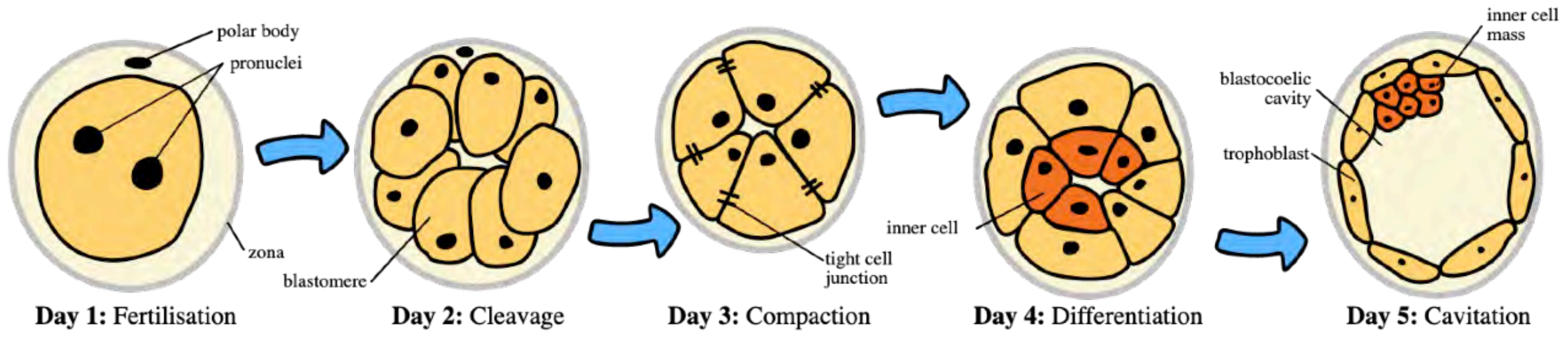
Kumar *et al*. (2024) *Nature Comms.,* in revision



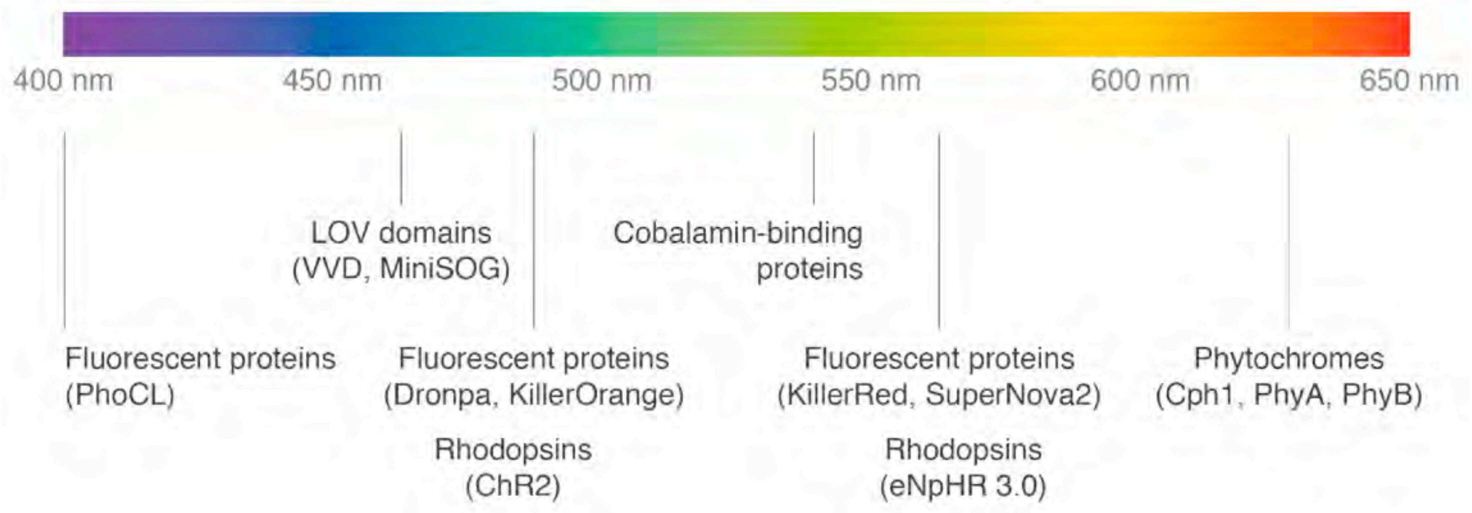
Collab. w J. Davies and M. Zurbriggen (unpublished)

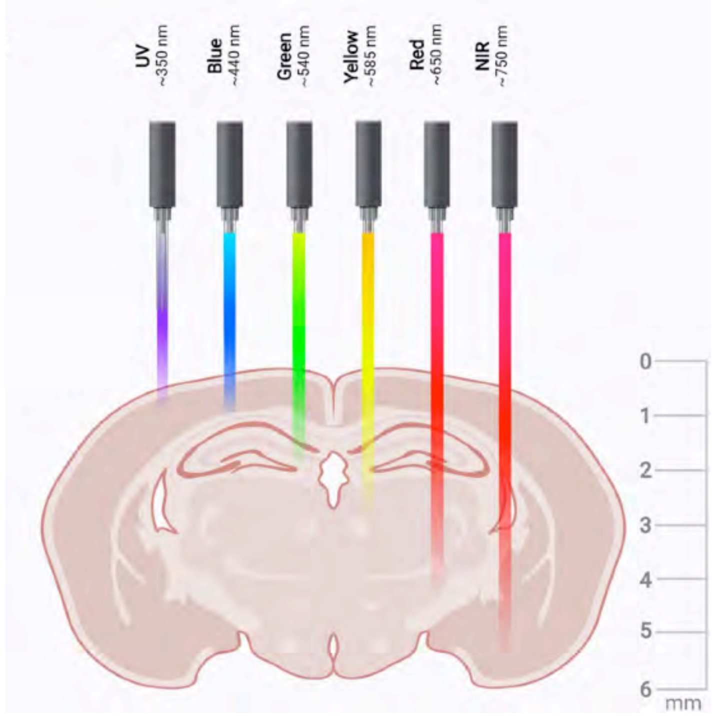
Single-Cell Control: Challenges

- Poor understanding of developmental processes

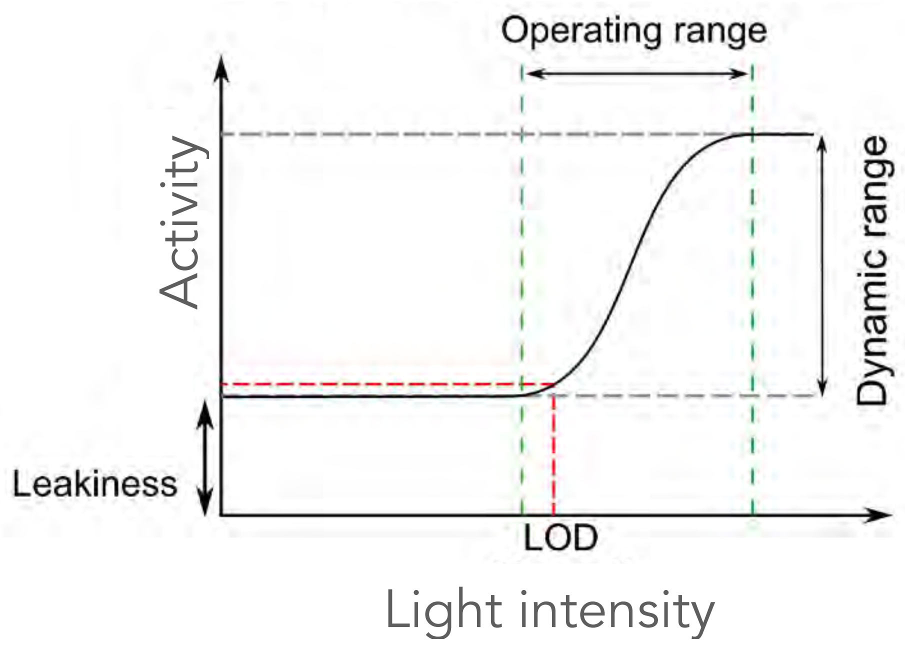


- Usable wavelengths and penetration

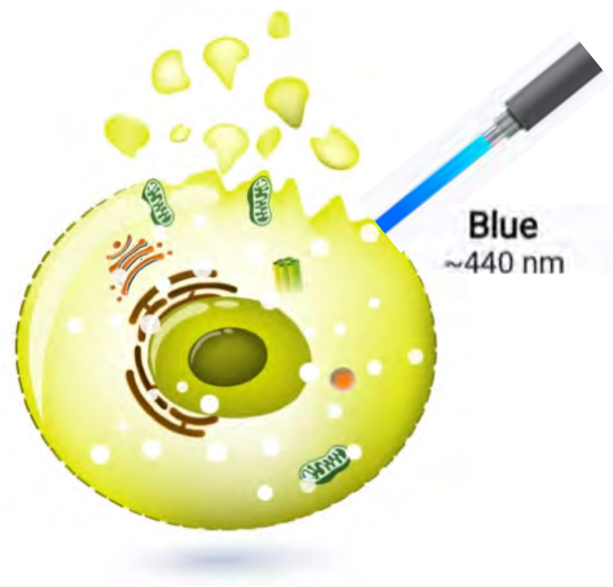




- Performance of optogenetics in mammalian cells



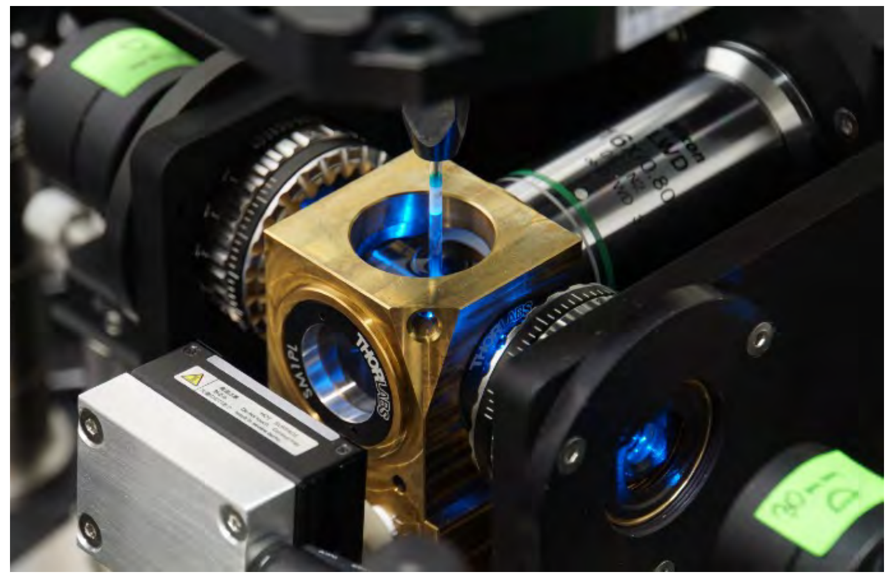
Dynamic performance



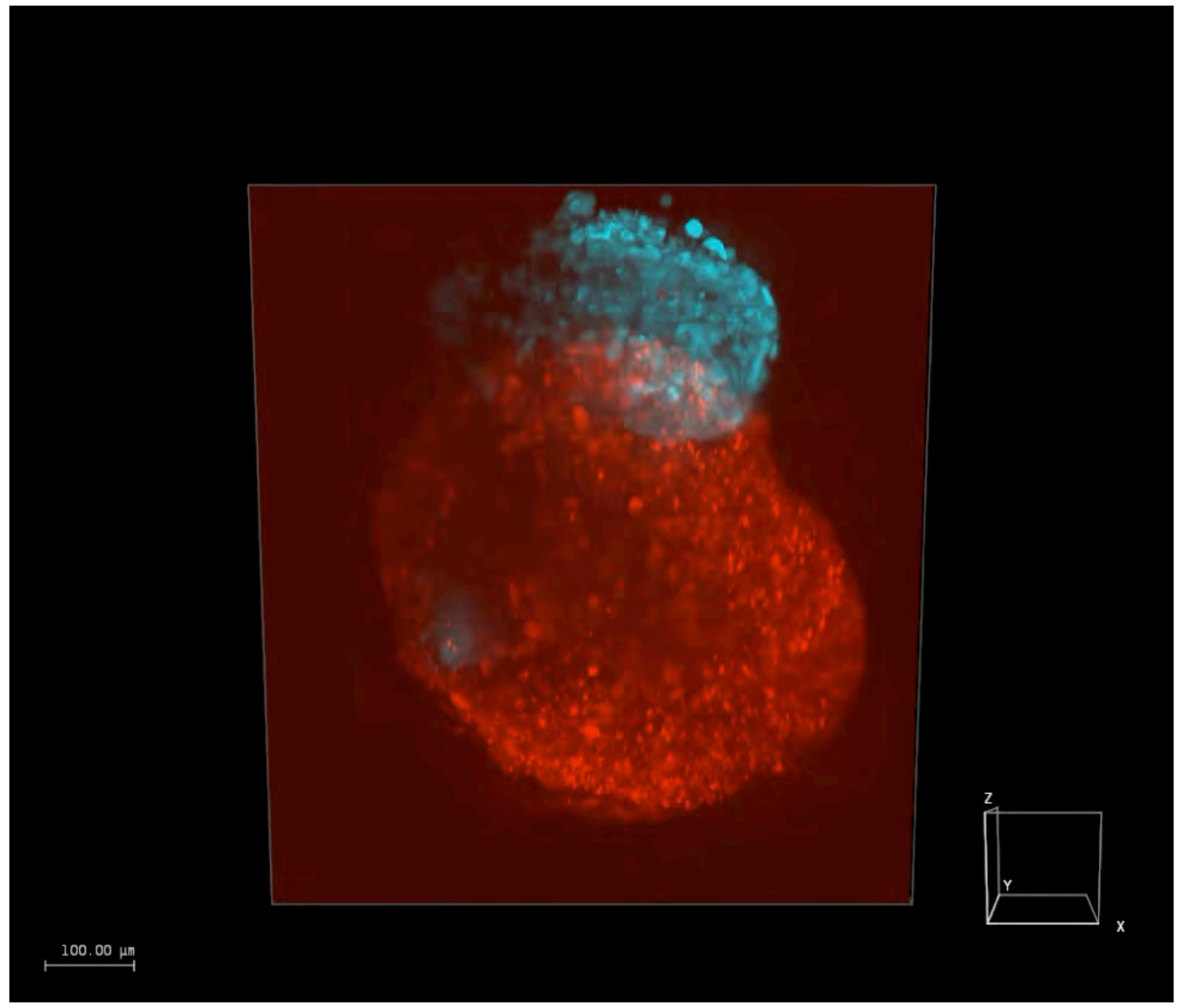
Phototoxicity

Gene Silencing

- 3D localized light delivery



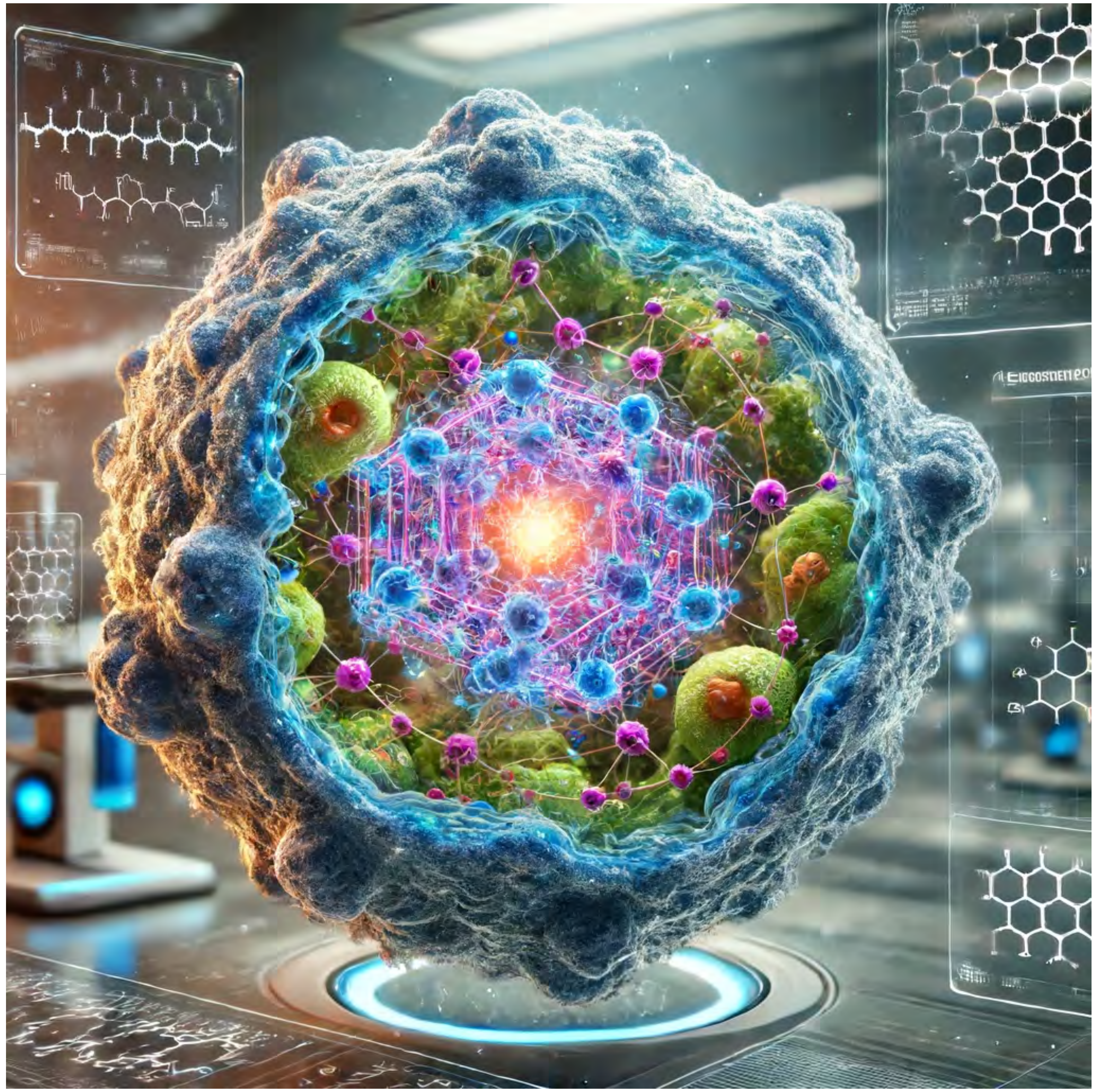
Custom Lightsheet microscope



Decker & Khammash (unpublished)

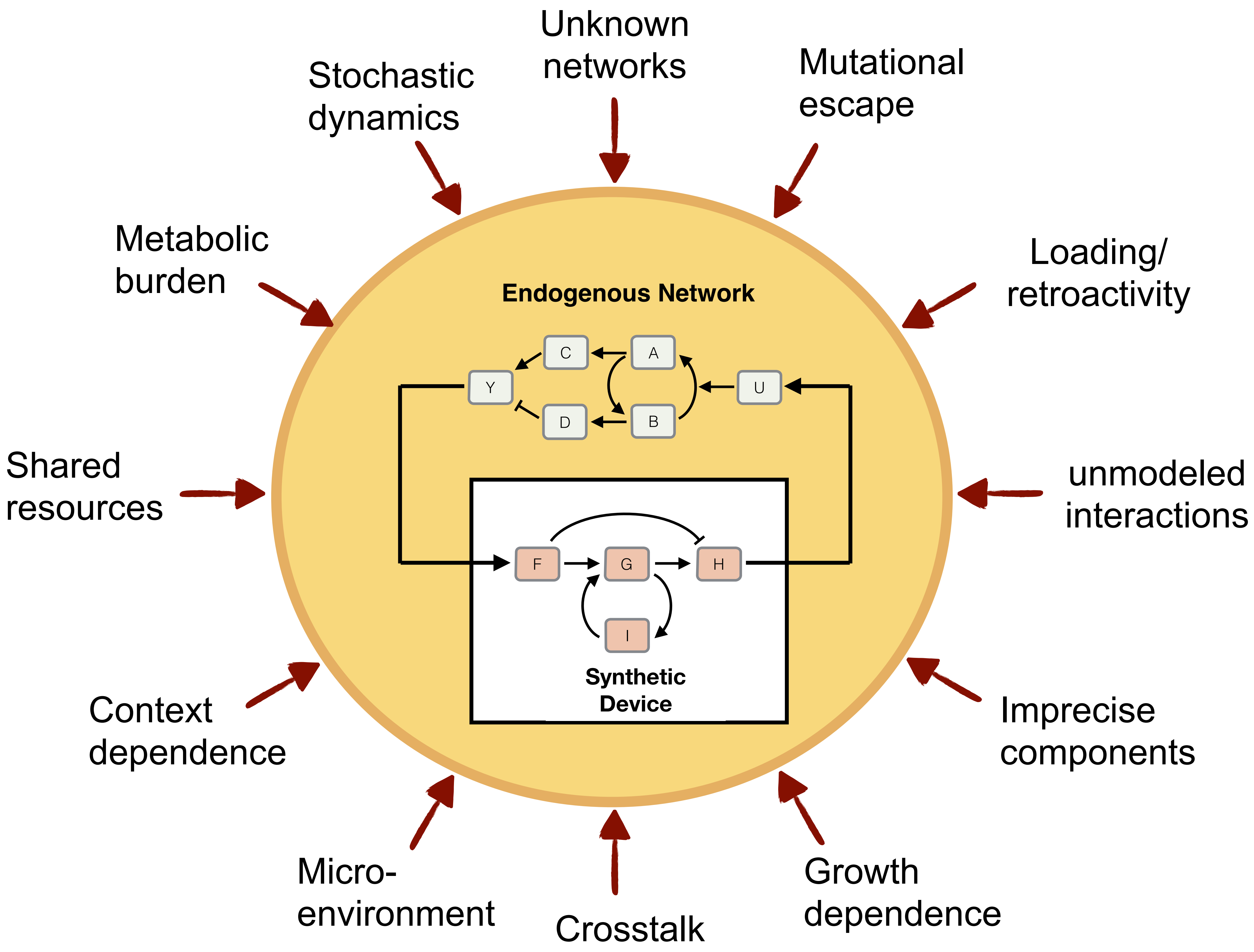
Biomolecular Control

Genetically engineered control systems



Challenges for Genetic Engineering of Functional Circuits

Challenges

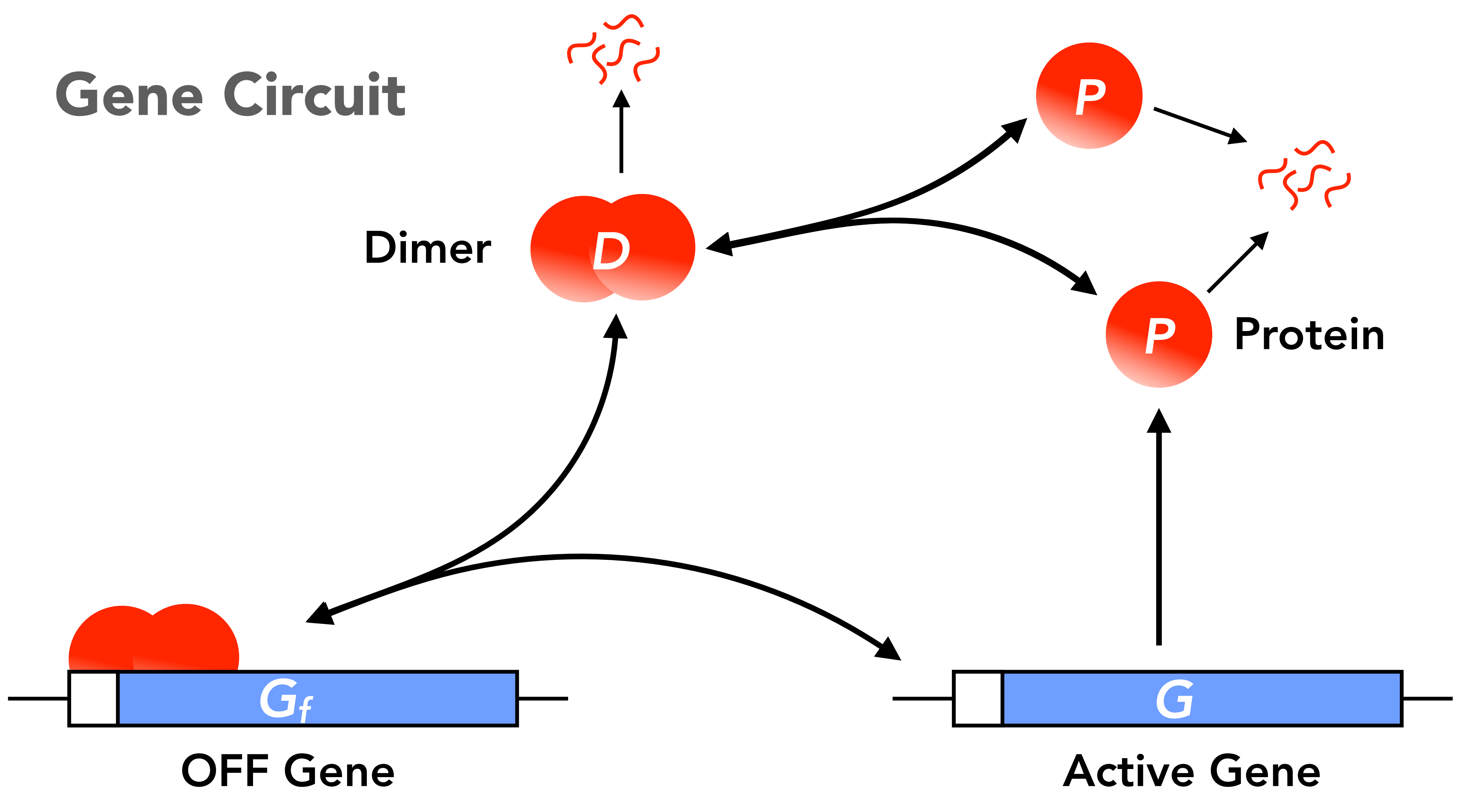


Opportunities (methodology)

Rationally design circuits that are:

- robust (uncertainty and noise)
- modular and composable
- context aware
- predictable and reliable
- have small metabolic footprint

Deterministic Models of Chemical Reactions



System Dynamics

Deterministic: $\dot{X}(t) = S\ \lambda(X(t))$

Chemical Reactions

$$\mathbf{G} \xrightarrow{\theta_1} \mathbf{G} + \mathbf{P}$$

$$\mathbf{P} \xrightarrow{\theta_2} \emptyset$$

$$2\mathbf{P} \underset{\theta_4}{\overset{\theta_3}{\rightleftarrows}} \mathbf{D}$$

$$\mathbf{D} \xrightarrow{\theta_5} \emptyset$$

$$\mathbf{D} + \mathbf{G} \underset{\theta_7}{\overset{\theta_6}{\rightleftarrows}} \mathbf{G_f}$$

Concentrations

$$X(t) = \begin{bmatrix} P(t) \\ D(t) \\ G(t) \\ G_f(t) \end{bmatrix} \in \mathbb{R}^4_{\geq 0}$$

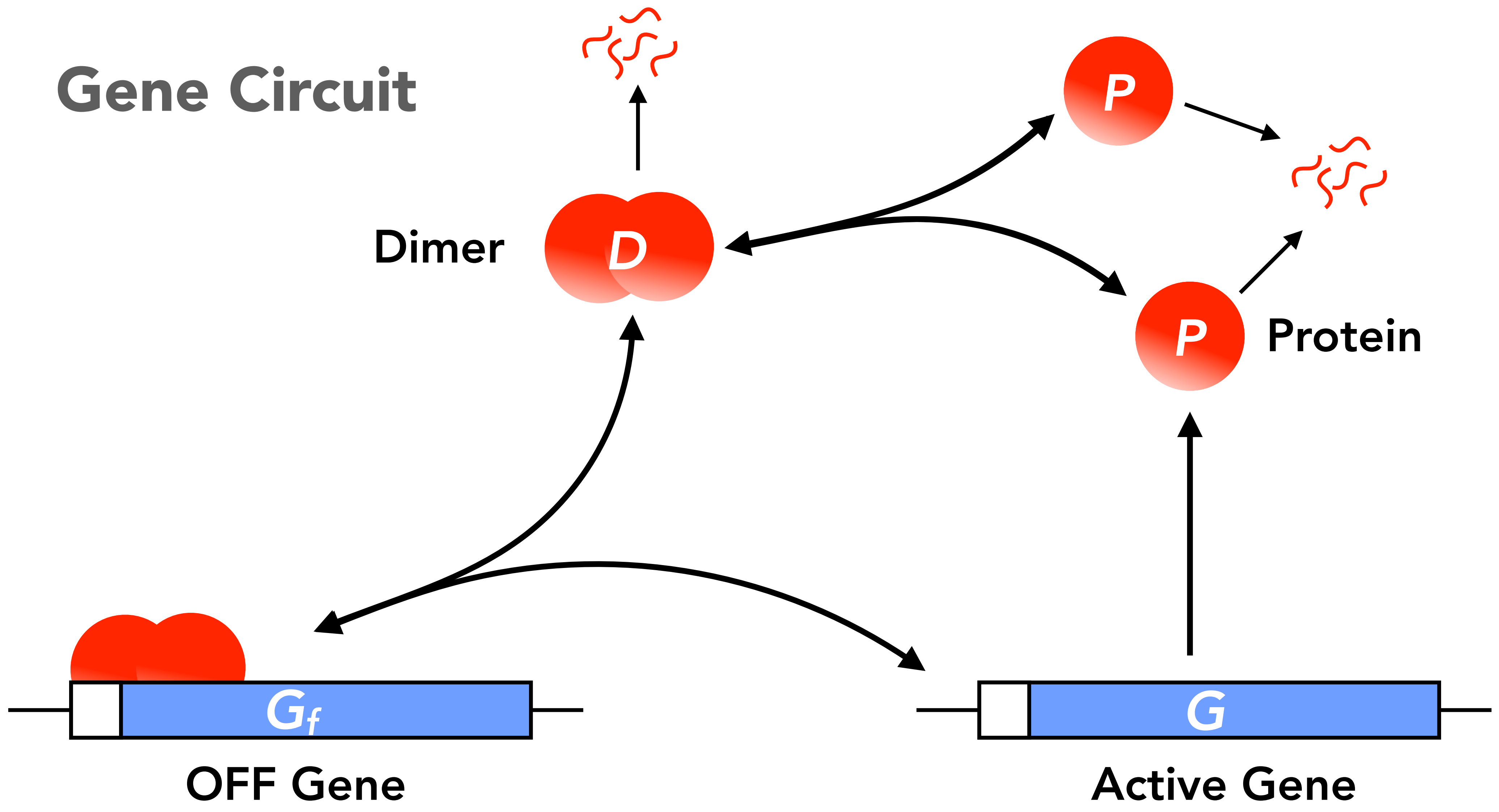
Stoichiometry

$$S = \begin{bmatrix} 1 & -1 & -2 & 2 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}$$

Reaction Rates

$$\lambda(X(t)) = \begin{bmatrix} \theta_1 G \\ \theta_2 P \\ \theta_3 P^2 \\ \theta_4 D \\ \theta_5 D \\ \theta_6 DG \\ \theta_7 G_f \end{bmatrix}$$

Stochastic Models of Chemical Reactions



System Dynamics

$$X(t) = X(0) + \sum_{k=1}^{7} s_k \, Y_k \left(\int_0^t \lambda_k(X(\tau) d\tau) \right)$$

$Y_k(\cdot)$ independent unit Poisson processes

$X(t)$ is a continuous-time discrete-state Markov process

Chemical Reactions

$$\mathbf{G} \xrightarrow{\theta_1} \mathbf{G} + \mathbf{P}$$

$$\mathbf{P} \xrightarrow{\theta_2} \emptyset$$

$$2\mathbf{P} \underset{\theta_4}{\overset{\theta_3}{\rightleftarrows}} \mathbf{D}$$

$$\mathbf{D} \xrightarrow{\theta_5} \emptyset$$

$$\mathbf{D} + \mathbf{G} \underset{\theta_7}{\overset{\theta_6}{\rightleftarrows}} \mathbf{G_f}$$

Concentrations

$$X(t) = \begin{bmatrix} P(t) \\ D(t) \\ G(t) \\ G_f(t) \end{bmatrix} \in \mathbb{Z}^4_{\geq 0}$$

Discrete random variable

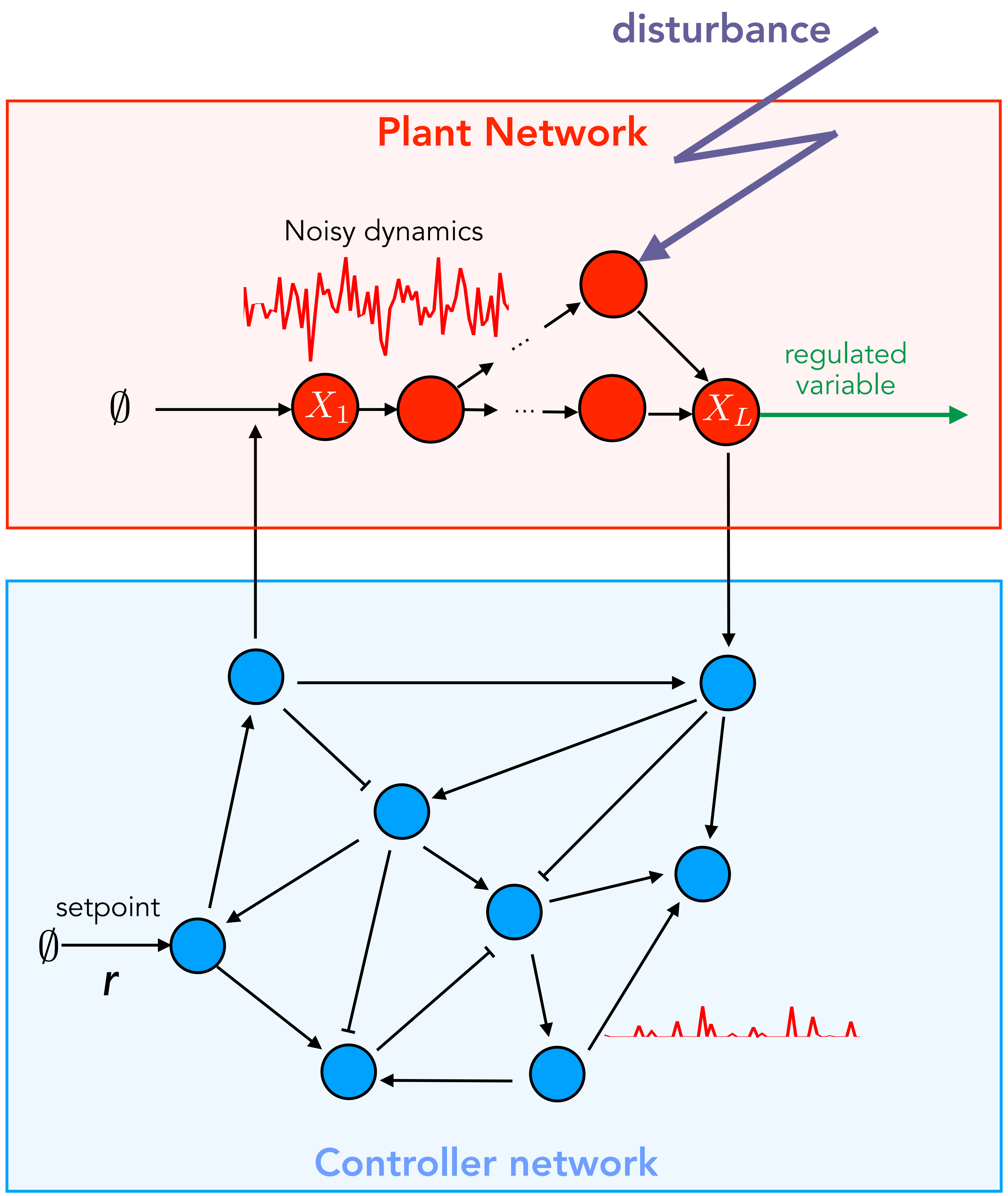
Reaction Propensities

$$\lambda(X(t)) = \begin{bmatrix} \theta_1 G \\ \theta_2 P \\ \theta_3 P(P-1)/2 \\ \theta_4 D \\ \theta_5 D \\ \theta_6 DG \\ \theta_7 G_f \end{bmatrix}$$

Stoichiometry

$$S = \begin{bmatrix} 1 & -1 & -2 & 2 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}$$

The Biomolecular Regulation Problem



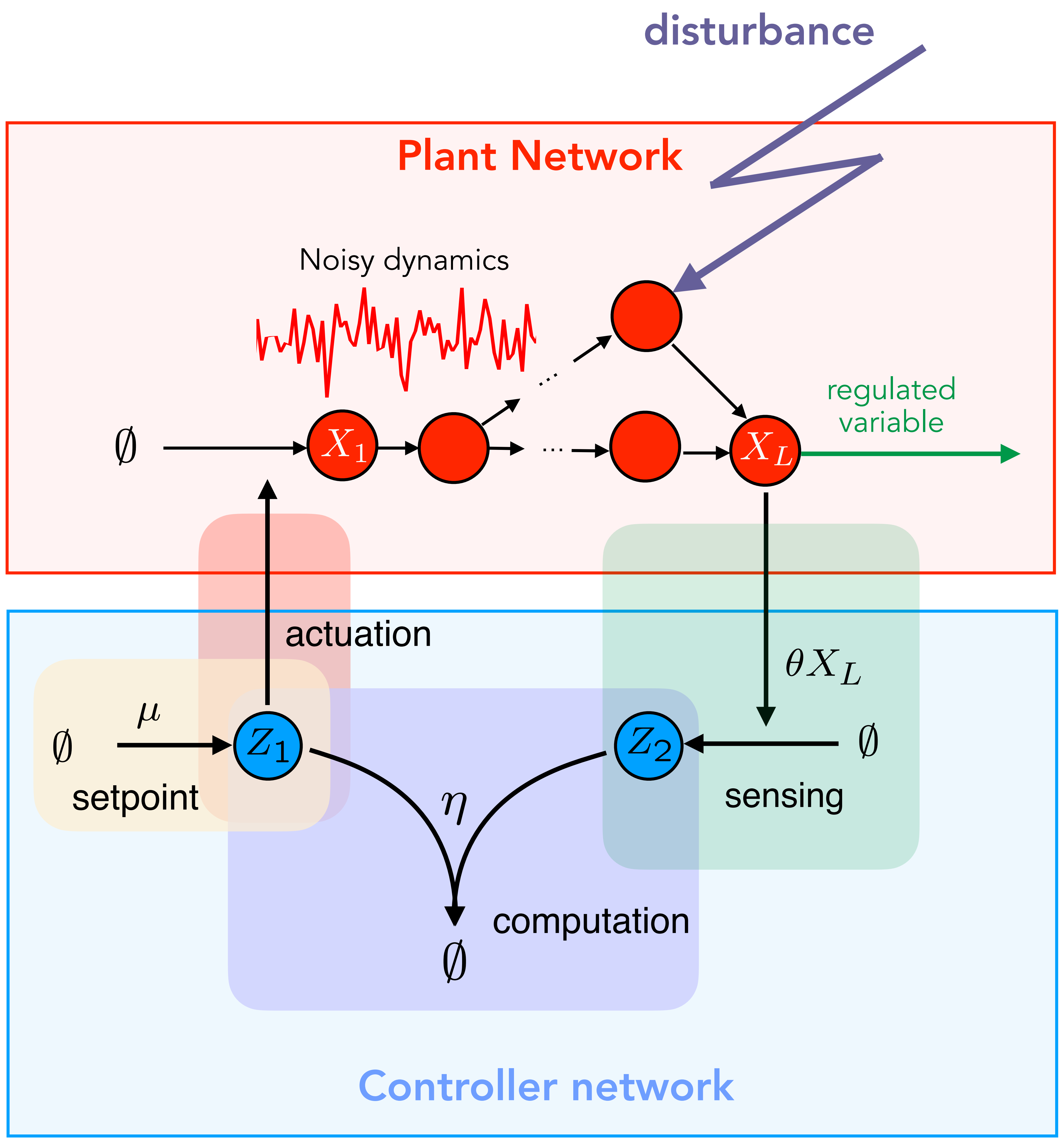
Problem Statement

Given a stochastic biochemical reaction network (plant).

Augment it with a biochemical reaction network (controller) such that the composite network (closed-loop) achieves:

- Stability: A unique attracting stationary distribution (ergodicity)
- Set point tracking: a variable of interest, $\mathbf{X}_L$, is steered to a set-point r
- Robust perfect adaptation (RPA): $\mathbf{X}_L$ is maintained at r in spite of
  - constant disturbances
  - changes in parameters
  - changes in plant network topology

A Molecular Motif for RPA: Antithetic Integral Feedback



Antithetic integral control architecture

Controller Implements Integral Control

$$\mathbb{E}(Z_1(t) - Z_2(t)) = \int_0^t (\mu/\theta - \mathbb{E}[X_L(s)])ds$$

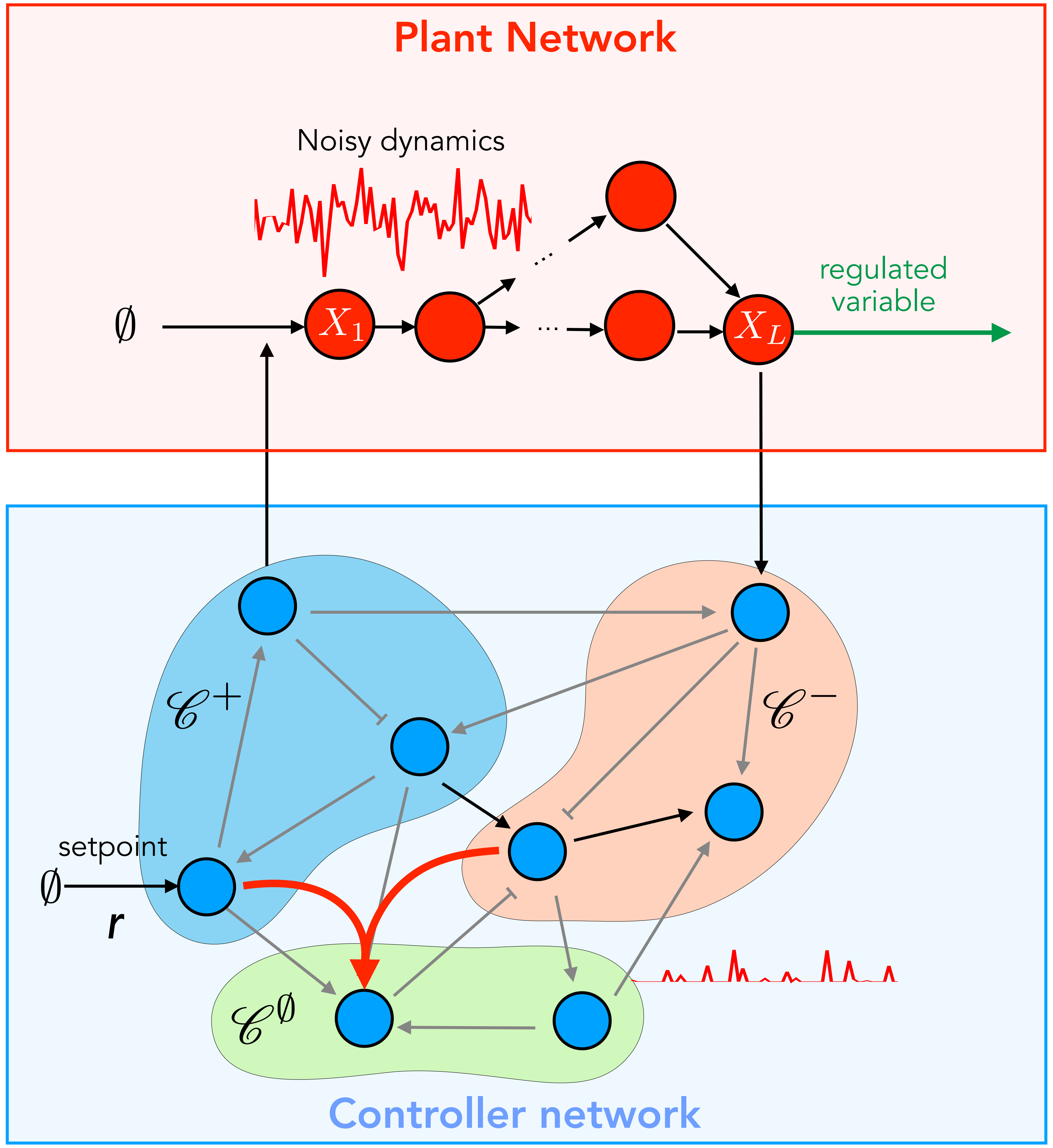
$$\mathbb{E}(X_L(t)) \xrightarrow{t\to\infty} \frac{\mu}{\theta}$$

The set-point is determined by $r = \frac{\mu}{\theta}$

Moreover, $\lim_{T\to\infty} \frac{1}{T} \int_0^T X_L(t)dt = \frac{\mu}{\theta}$

Briat, Gupta, Khammash, Cell Systems (2016)

Universality of the Antithetic Integral Controller



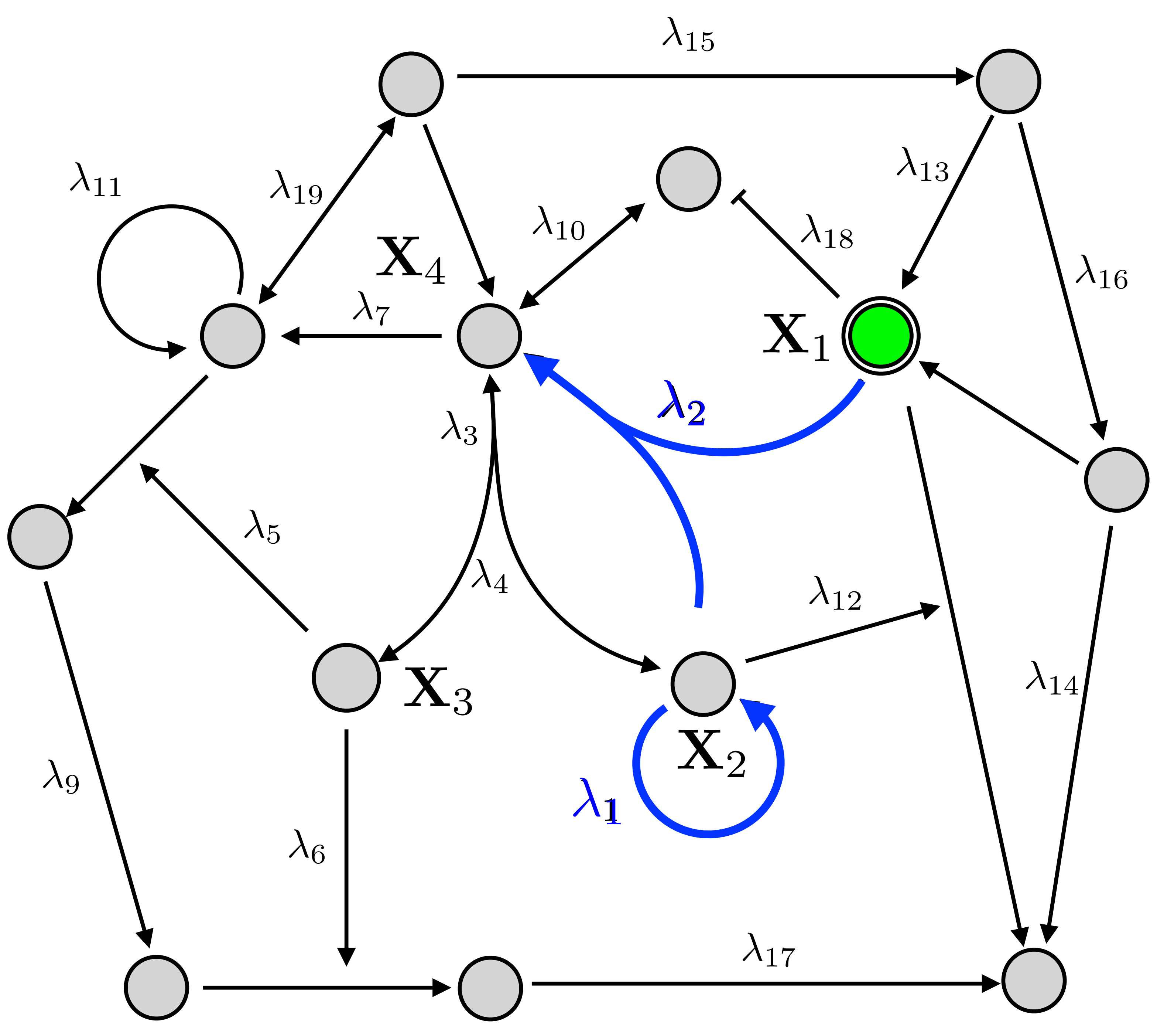
Theorem (Universality):

Every controller that achieves RPA must imbed an antithetic controller.

Aoki, Lillacci, Gupta, Baumschlager, Schweingruber, & Khammash, *Nature*, 2019.

Characterizing maxRPA Networks: Deterministic Setting

Example Network



$$\mathbf{X}_2 \xrightarrow{\theta_1} 2\mathbf{X}_2 \qquad \nu_{11} = 0$$

$$\mathbf{X}_1 + \mathbf{X}_2 \xrightarrow{\theta_2} \mathbf{X}_4 \qquad \nu_{12} = 1$$

$$\left.\begin{aligned} q^T &= (0,1,-1,0,\ldots,0) \\ q^T S &= (1,-1,0,\ldots,0). \end{aligned}\right\} \Rightarrow \text{RPA} \quad \left(\mathbf{r} = \frac{\theta_1}{\theta_2}\right)$$

The deterministic RPA problem:

Given a *deterministic* reaction network with stable dynamics, find conditions for an output, $\mathbf{X}_1$, to achieve **maxRPA** (robust to *almost* all rates).

Setpoint encoding: **r** is encoded by a subset of the chemical reactions. It can be shown that *at least two* such reactions must be involved:

$$\begin{aligned} \nu_{11}\mathbf{X}_1 + \cdots + \nu_{N1}\mathbf{X}_N &\xrightarrow{\theta_1} \nu'_{11}\mathbf{X}_1 + \cdots + \nu'_{N1}\mathbf{X}_N \\ \nu_{12}\mathbf{X}_1 + \cdots + \nu_{N2}\mathbf{X}_N &\xrightarrow{\theta_2} \nu'_{12}\mathbf{X}_1 + \cdots + \nu'_{N2}\mathbf{X}_N \end{aligned}$$

($\nu_{11} \neq \nu_{12}$, $\nu_{N1} = \nu_{N2}$)

Theorem (RPA Characterization): The network achieves maxRPA if and only if

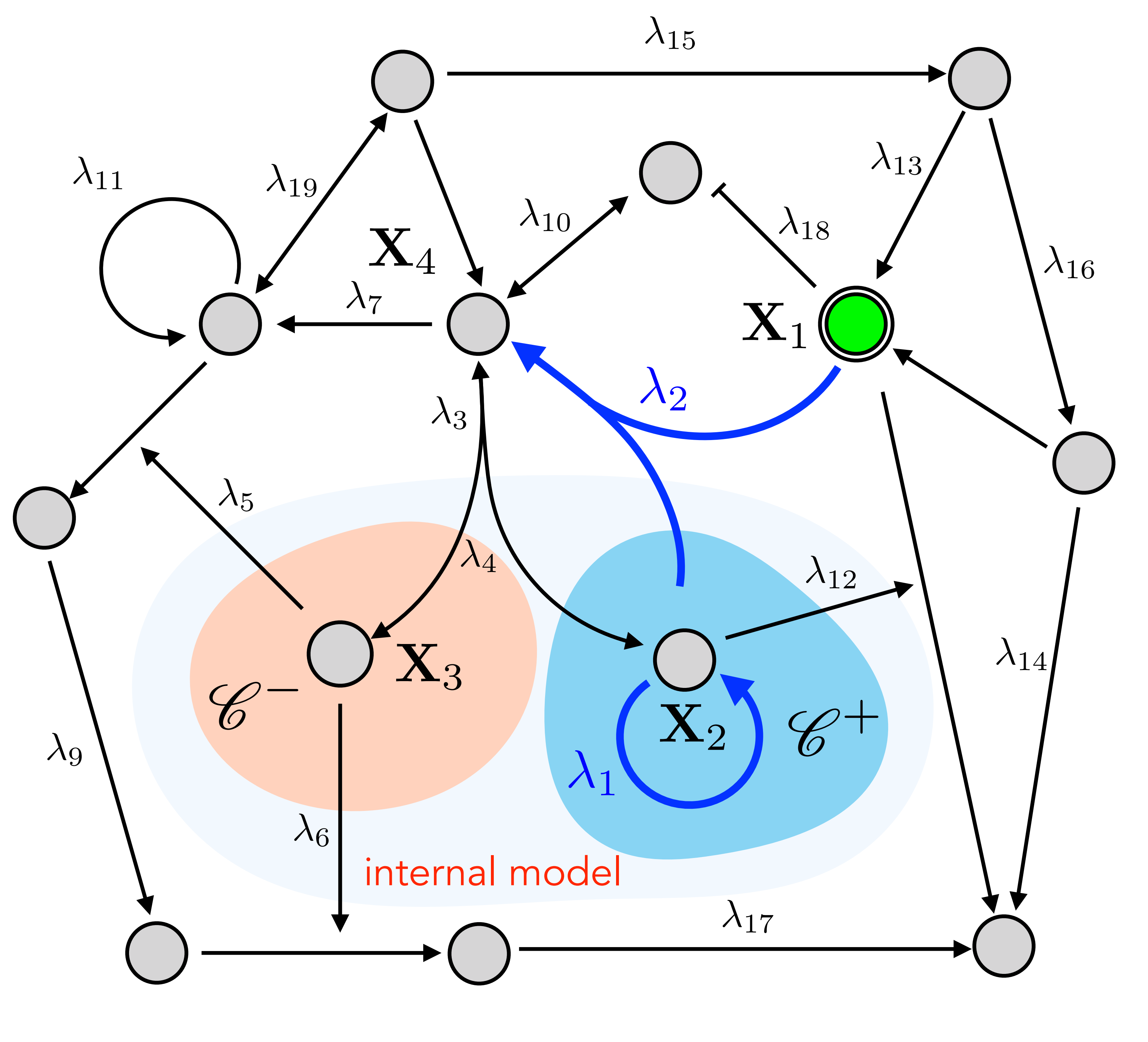
1. $\nu_{11} \neq \nu_{12}$, and $\nu_{i1} = \nu_{i2}$ for $i \neq 1$.
2. There exists a vector q and a scalar κ satisfying $q^T S = (\kappa, -1, 0, \ldots, 0)$.

In this case, $\mathbf{r} = \left(\kappa \frac{\theta_1}{\theta_2}\right)^{\frac{1}{\nu_{12} - \nu_{11}}}$.

Gupta & Khammash, *Proc. Nat. Acad. Sci.* (2022)

An Internal Model Principle for Chemical Reaction Networks

Example Network



$$q^T = (0, 1, -1, 0, \ldots, 0)$$

$$F(x) = x_2 - x_3 = \int_0^t \theta_2 x_2(\tau)\left(\frac{\theta_1}{\theta_2} - x_1(\tau)\right) d\tau$$

Classification of species in RPA networks:

Consider the vector q satisfying $q^T S = (\kappa, -1, 0, \ldots, 0)$, for $\kappa > 0$.

We can think of the *sign* of q_i as the 'charge' of species $\mathbf{X}_i$.

Positive	Negative	Neutral
$\mathscr{C}^+ = \{\mathbf{X}_i : q_i > 0\}$,	$\mathscr{C}^- = \{\mathbf{X}_i : q_i < 0\}$,	$\mathscr{C}^\emptyset = \{\mathbf{X}_i : q_i = 0\}$.

Molecular Internal Model Principle

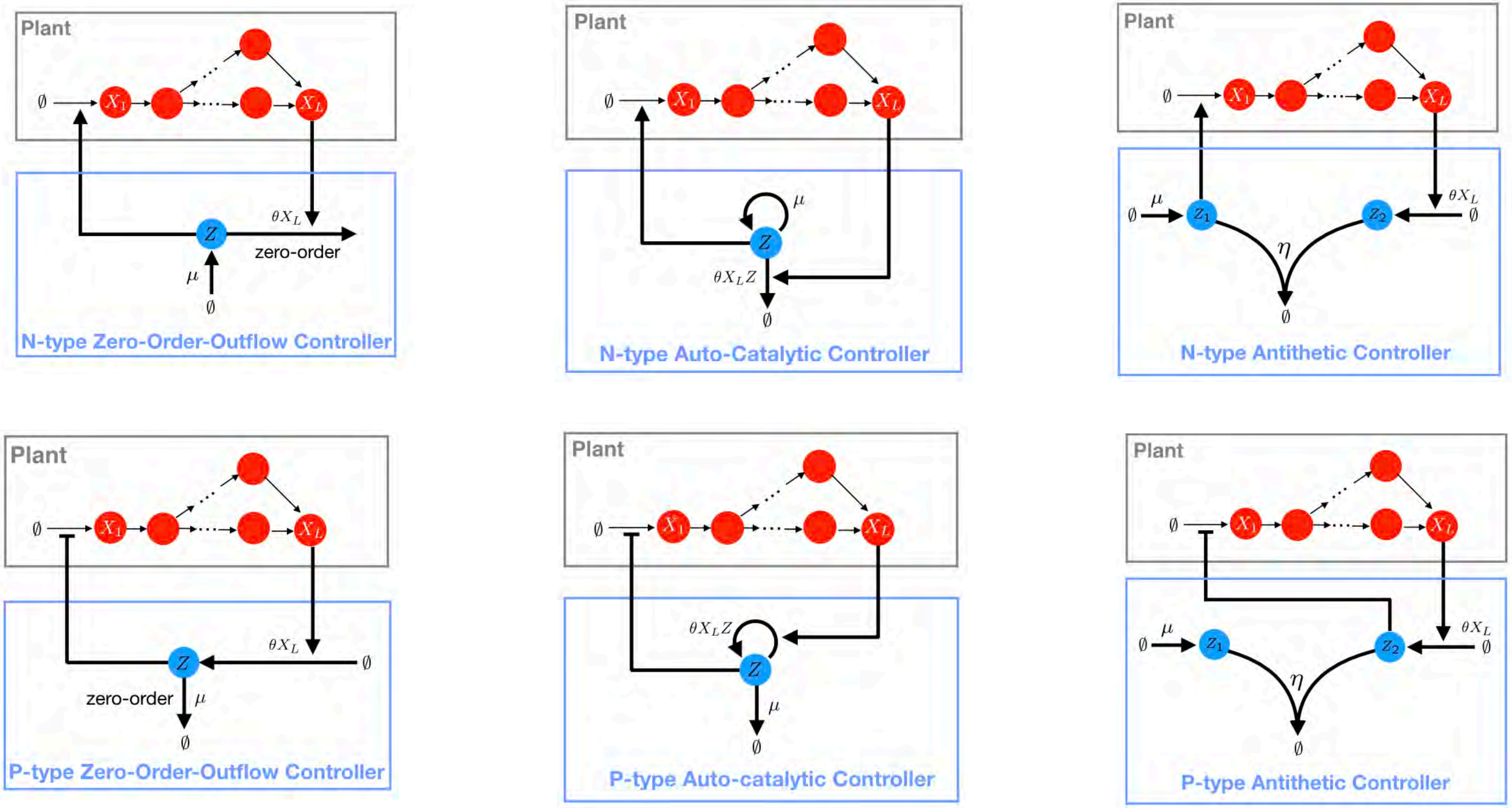
The 'charged' species $\mathscr{C}^- \cup \mathscr{C}^+$ define an integrator $F(x) := \sum_{i=1}^{N} q_i x_i$,

such that
$$F(x) = \int_0^t m_1(x)\left(\kappa\frac{\theta_1}{\theta_2} - x_1^{\nu_{12}-\nu_{11}}(\tau)\right) d\tau.$$

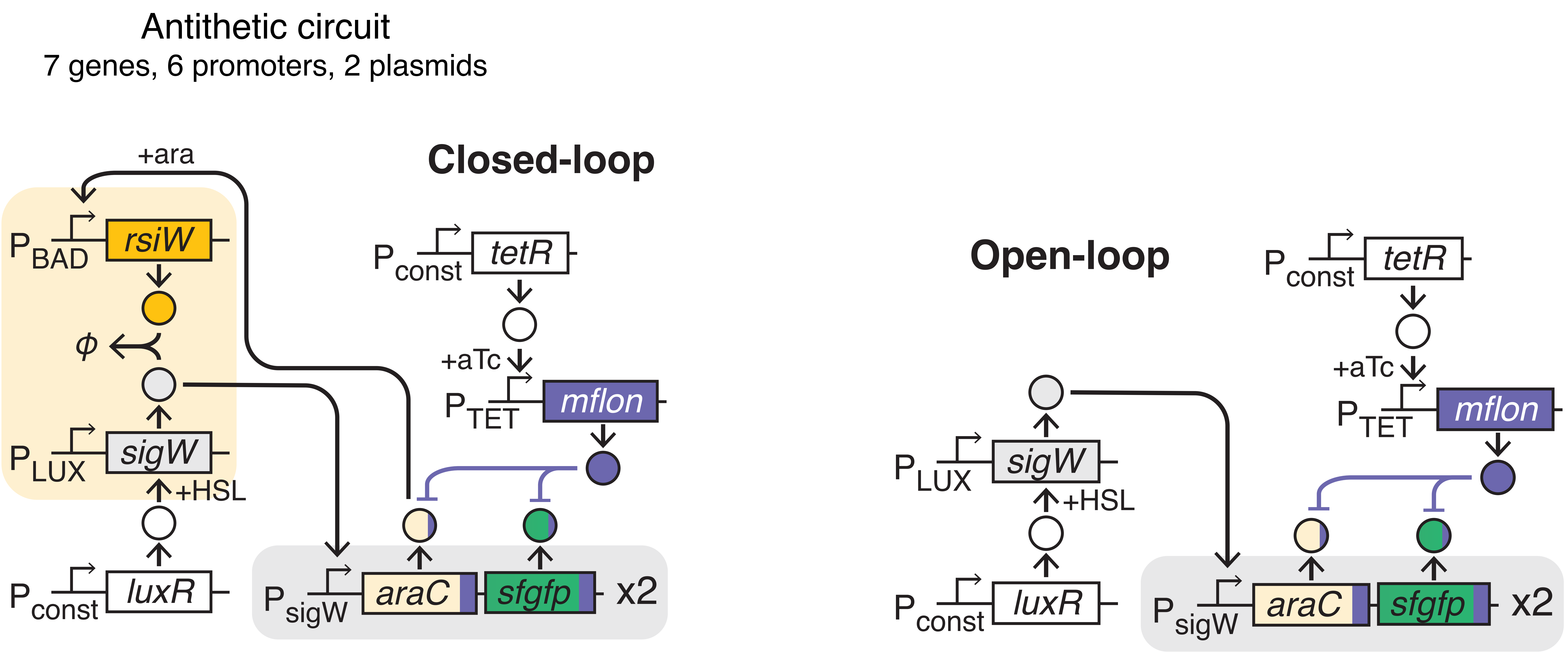
Note that $x_1(t) \to \left(\kappa\frac{\theta_1}{\theta_2}\right)^{\frac{1}{\nu_{12}-\nu_{11}}}$.

Gupta & Khammash, *Proc. Nat. Acad. Sci.* (2022)

Integral Feedback Motifs

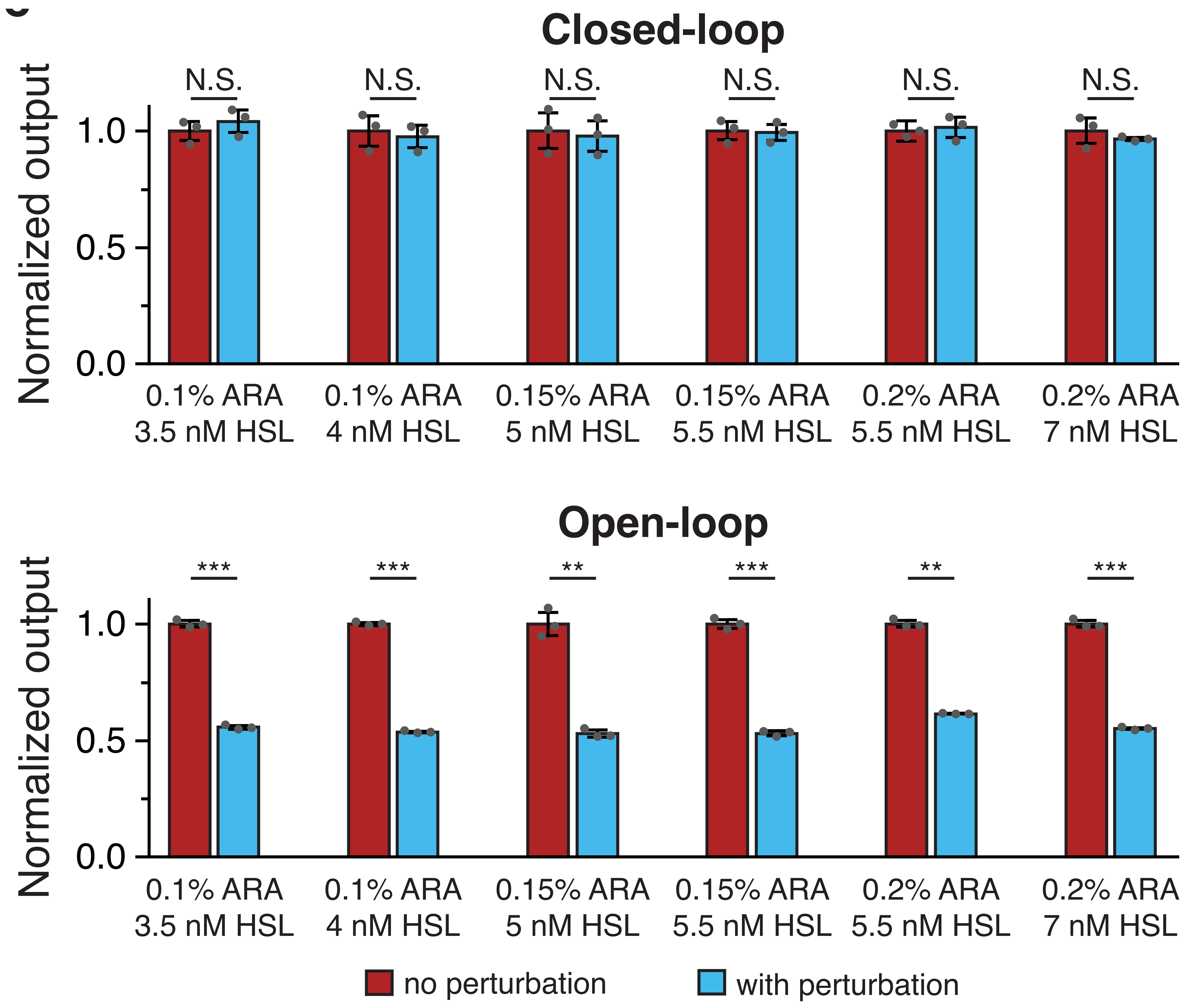


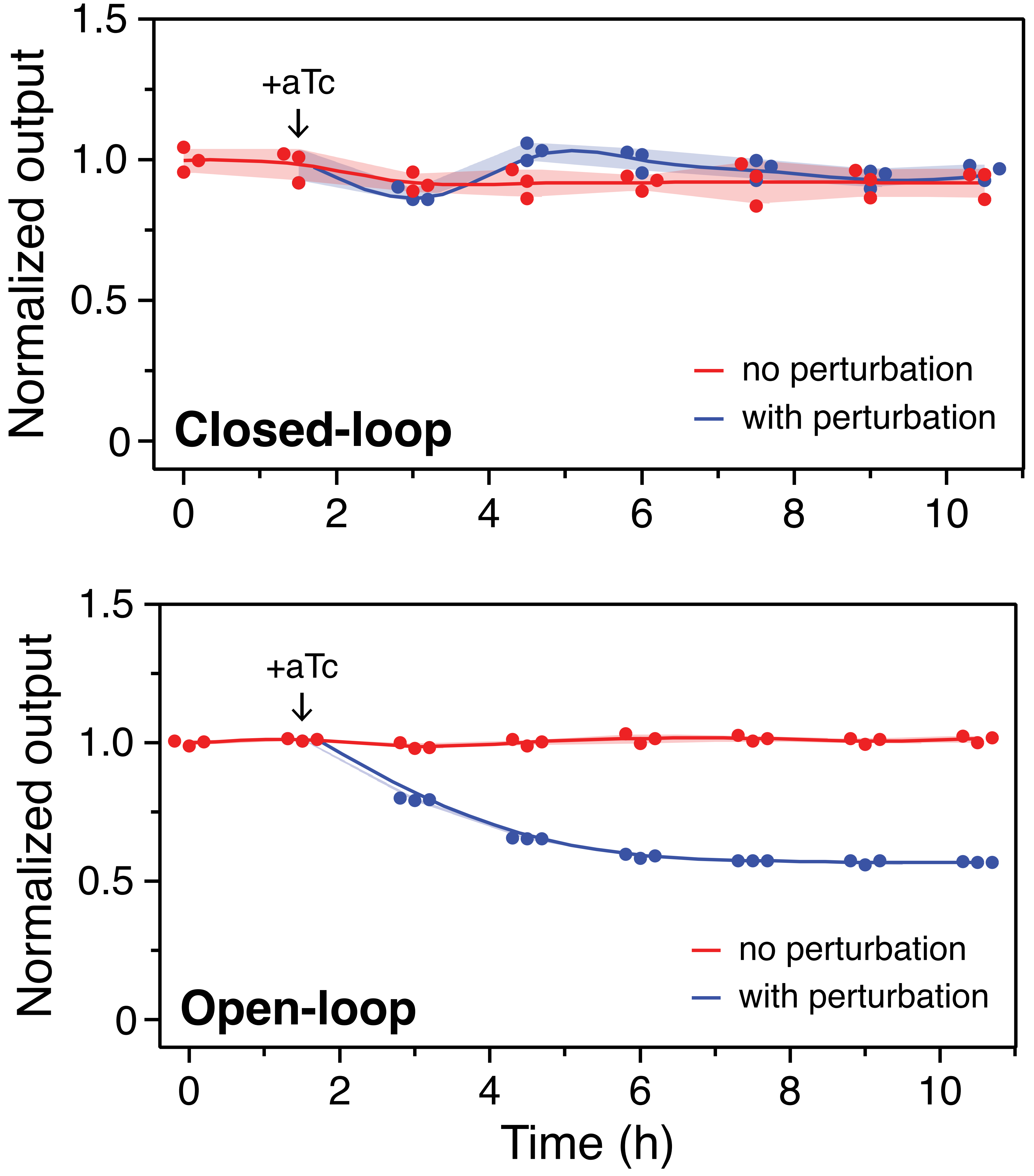
Building the *First* Synthetic Integral Controller in a Living Cell



Aoki, Lillacci, Gupta, Baumschlager, Schweingruber, & Khammash, *Nature* (2019).

Integral Circuit Achieves Perfect Adaptation

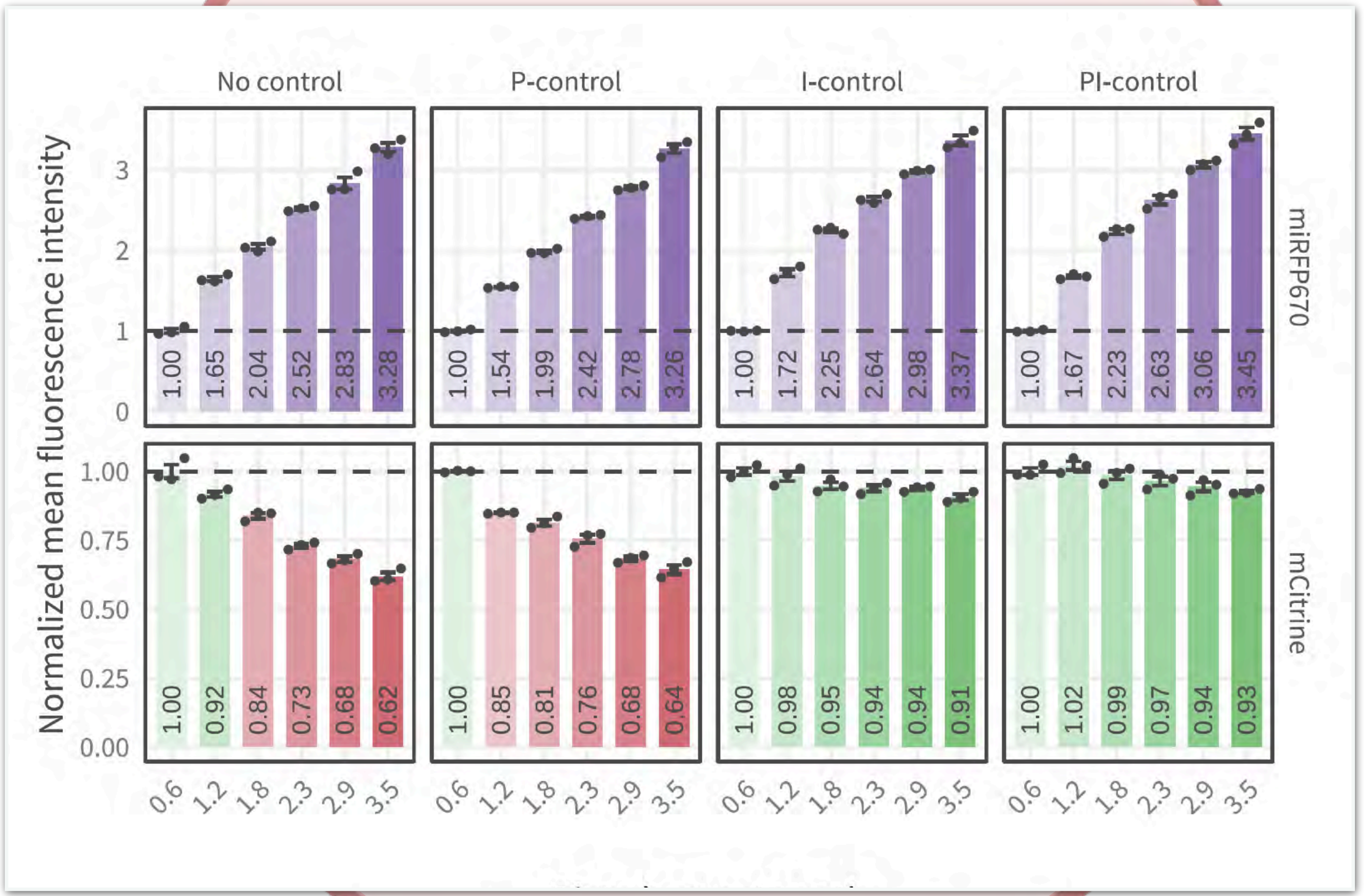




Aoki, Lillacci, Gupta, Baumschlager, Schweingruber, & Khammash, *Nature* (2019).

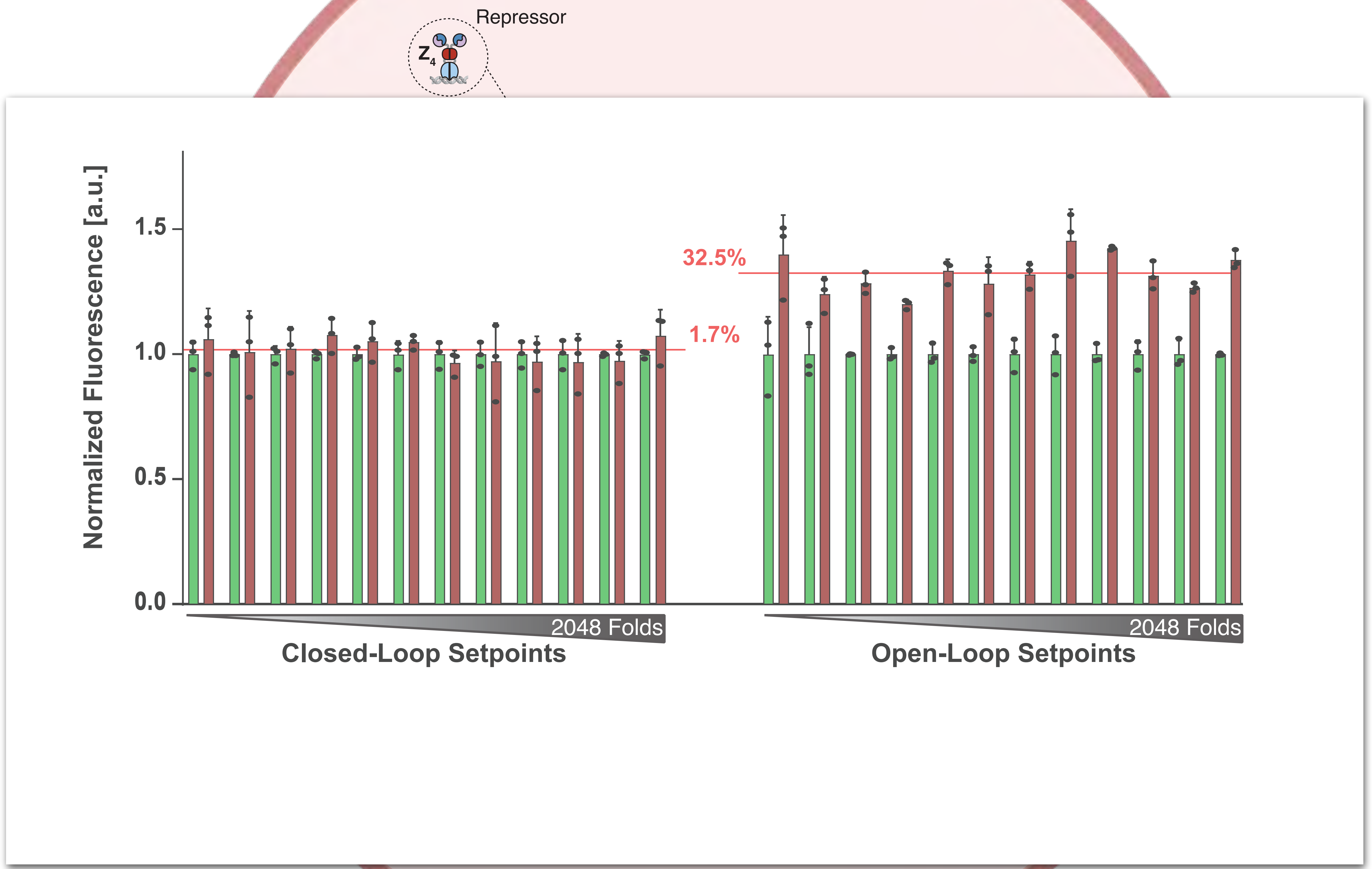
Engineering Controllers in Mammalian Cells

RNA-based PI Controller



HEK293T cells

Protein-based PI Controller



HEK293T cells

Frei, Chang, Filo, Arampatzis, Khammash, *Proc. Nat. Acad. Sci.* (2022)

Anastassov, Filo, Chang, Khammash, *Nature Comms.* (2023)

Summary

- Feedback is a recurring theme in natural systems (robustness)
- Feedback control in synthetic biology as a means to achieve robust and reliable designs
- A new field at the interface of control engineering and synthetic biology (*Cybergenetics*)
  - Deeper understanding of cellular regulation
  - Novel circuits for robust and precise cellular control
  - Applications: industrial biotechnology, synthetic biology, tissue/organ engineering, personalised medicine, living materials

Challenges

- Populations computer control:
  - Practical: light penetration, cost of modifying production strains, sensing key variables
  - Theoretical/computational: better models, multivariable control of bilinear systems
- Single-cell computer control:
  - Practical: specialized hardware, 3D localization of light, silencing, toxicity
  - Theoretical/computational: better models, stochastic control, image processing
- Genetic control:
  - Practical: dilution, saturation, burden, precision parts, post-translational circuits, anti-windup
  - Theoretical/computational: a control theory for chemical reactions, system ID methods