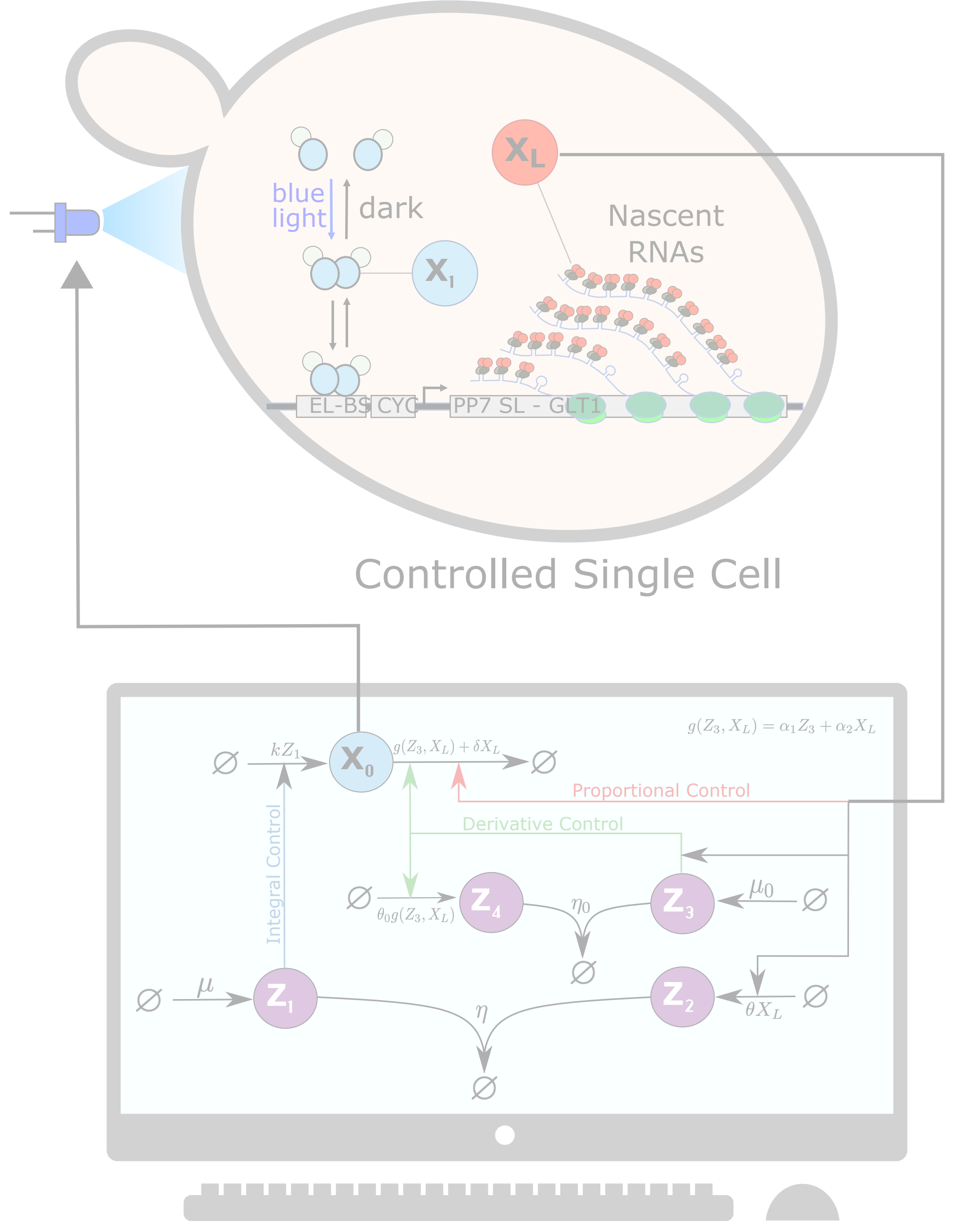
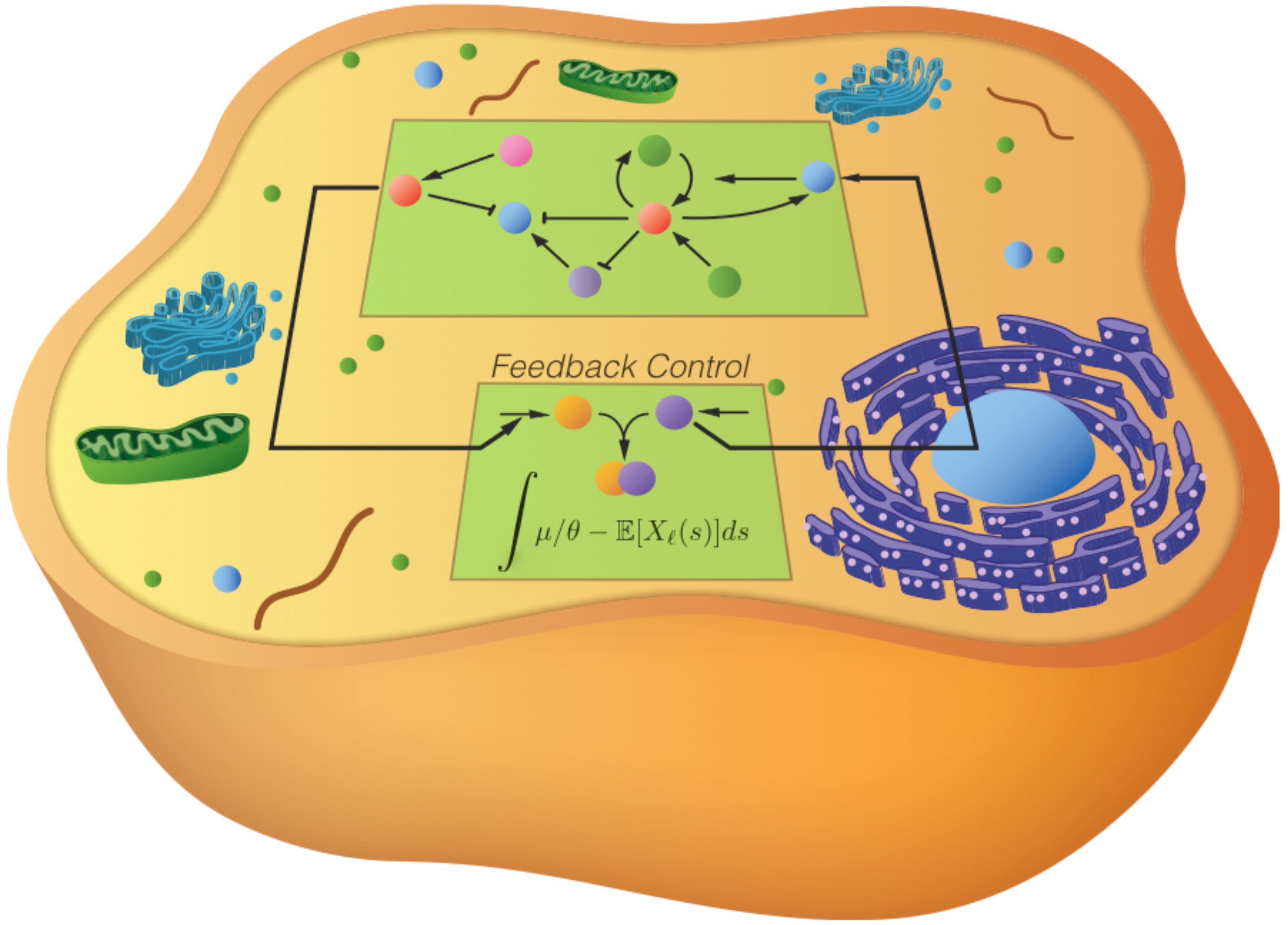
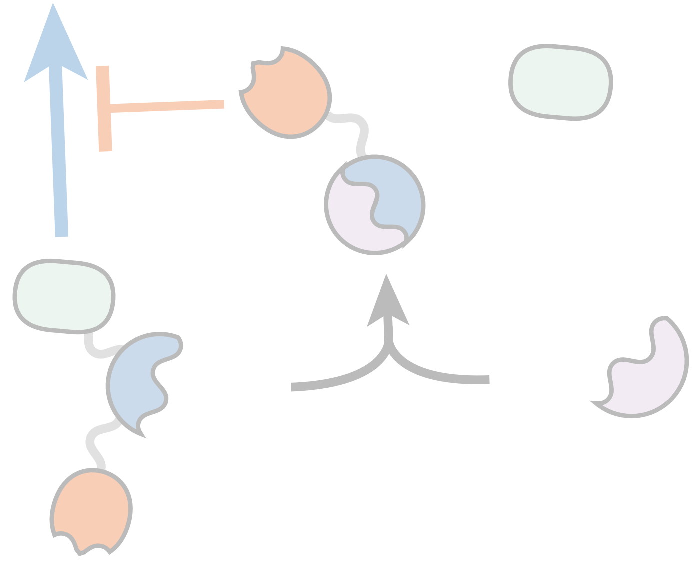
Engineering Genetic Controllers to Accelerate Adaptation and Attenuate Cellular Noise

Maurice Filo







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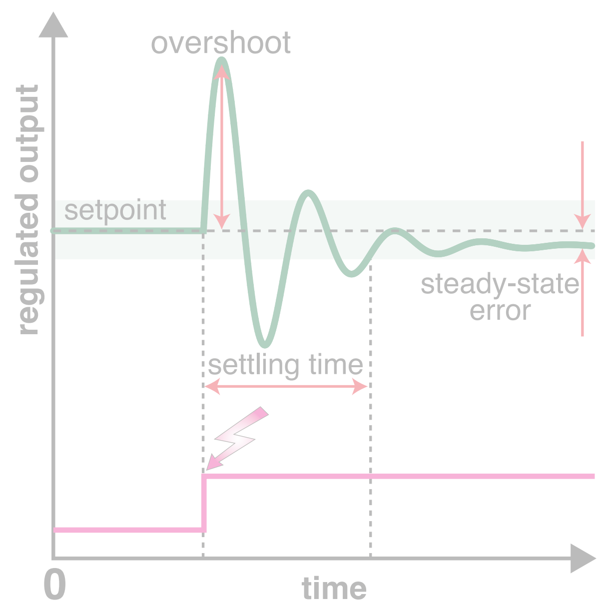

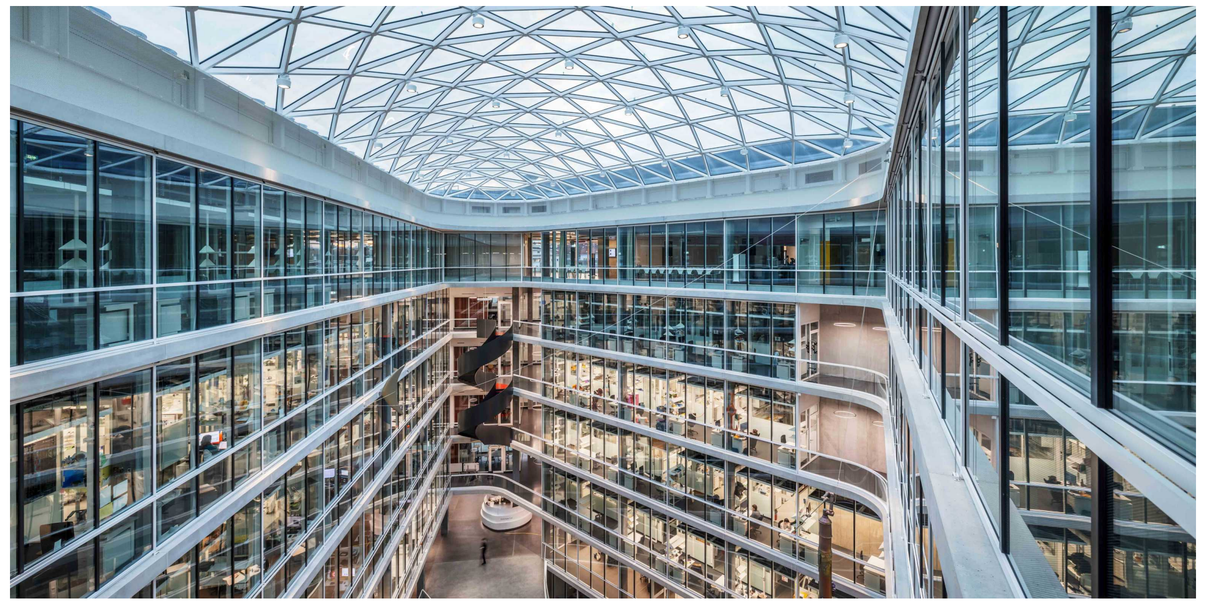

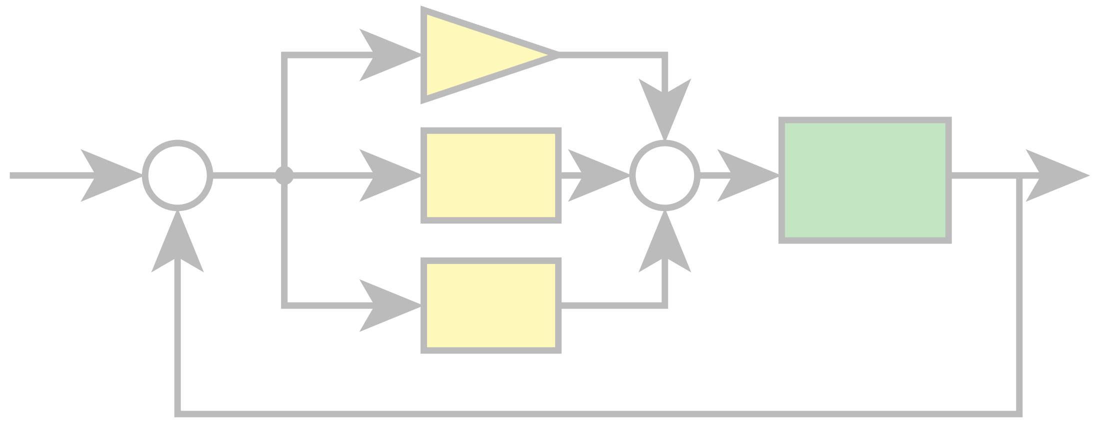

Circuit Design in Synthetic Biology

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Goal:

Build an **electric** device to perform a function

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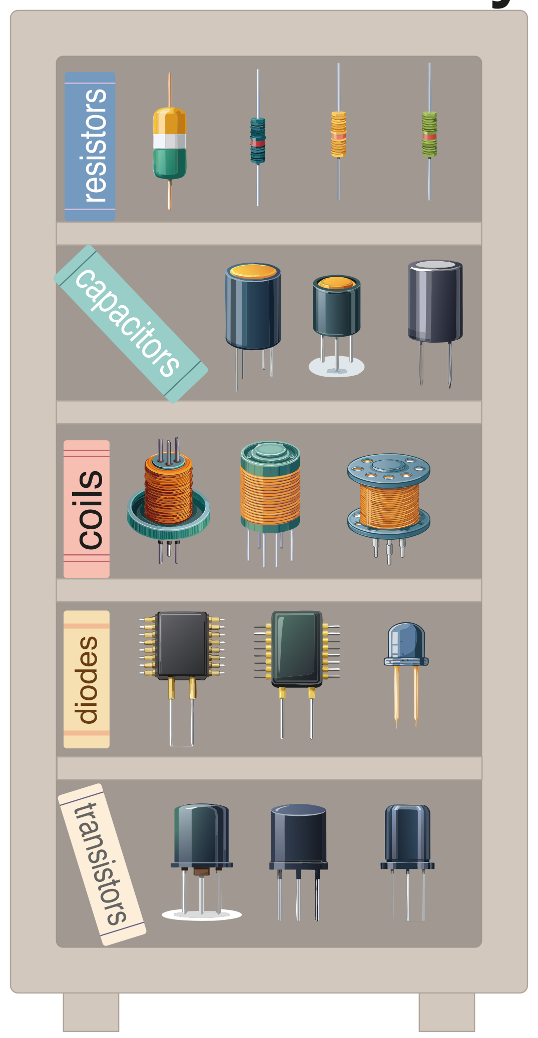
Parts Library



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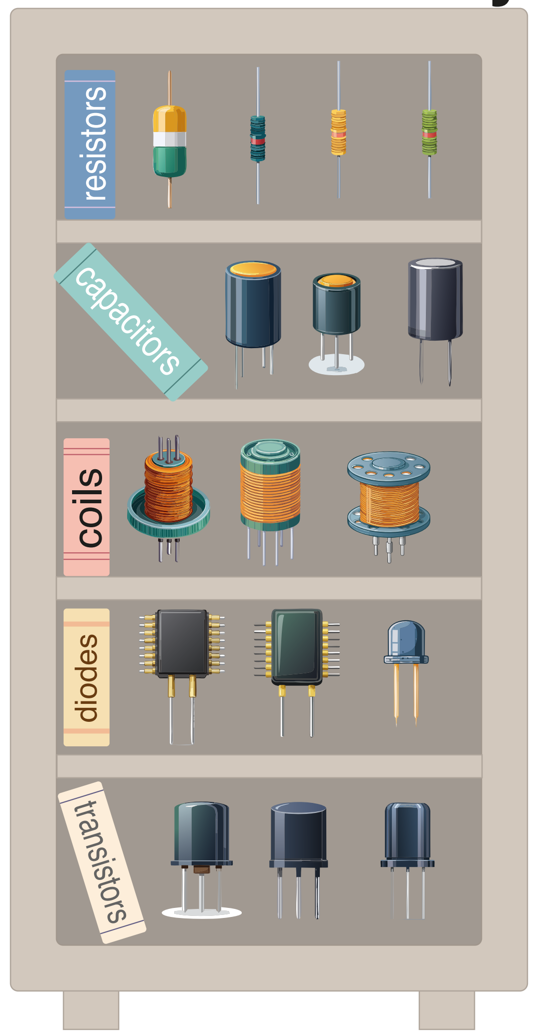
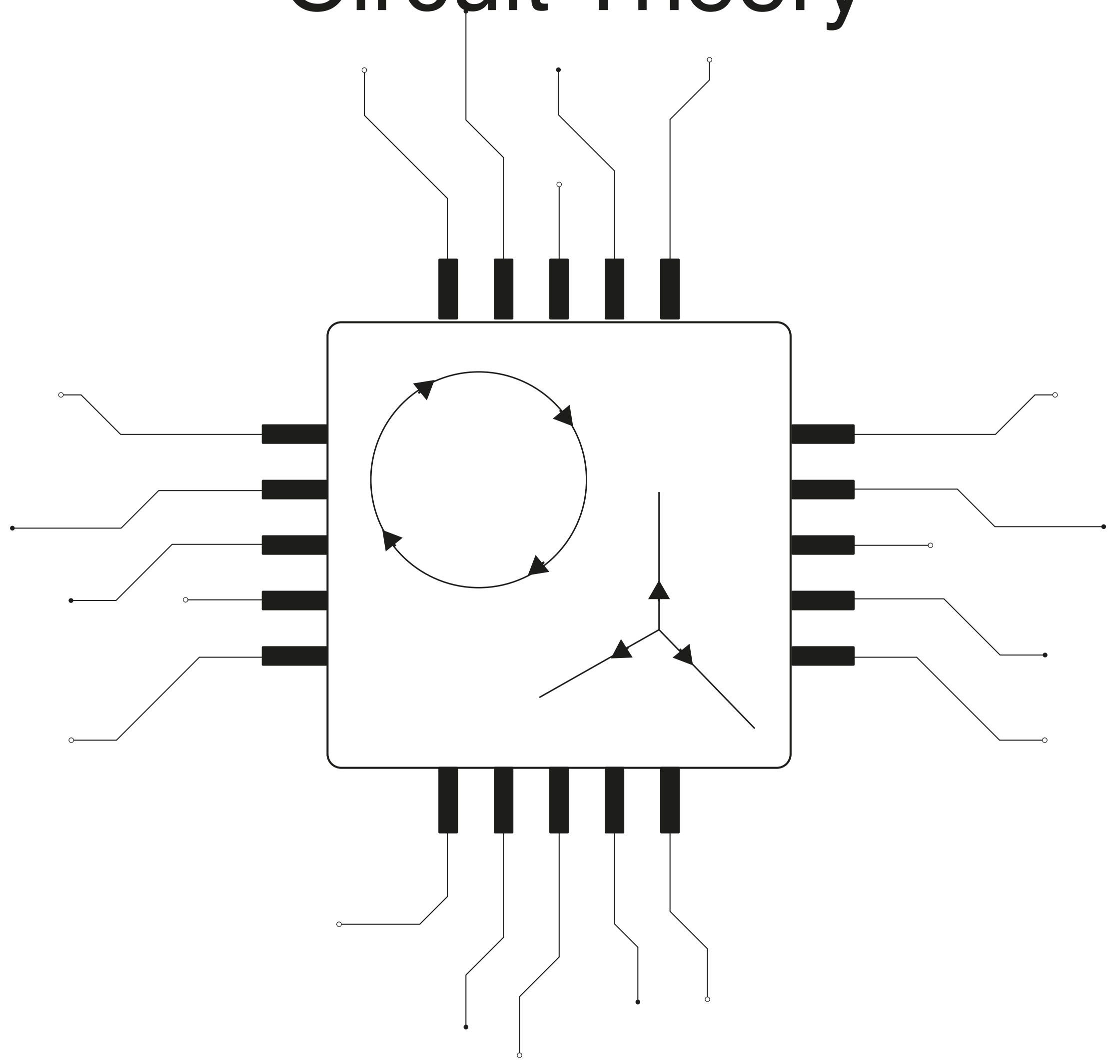


Circuit Theory



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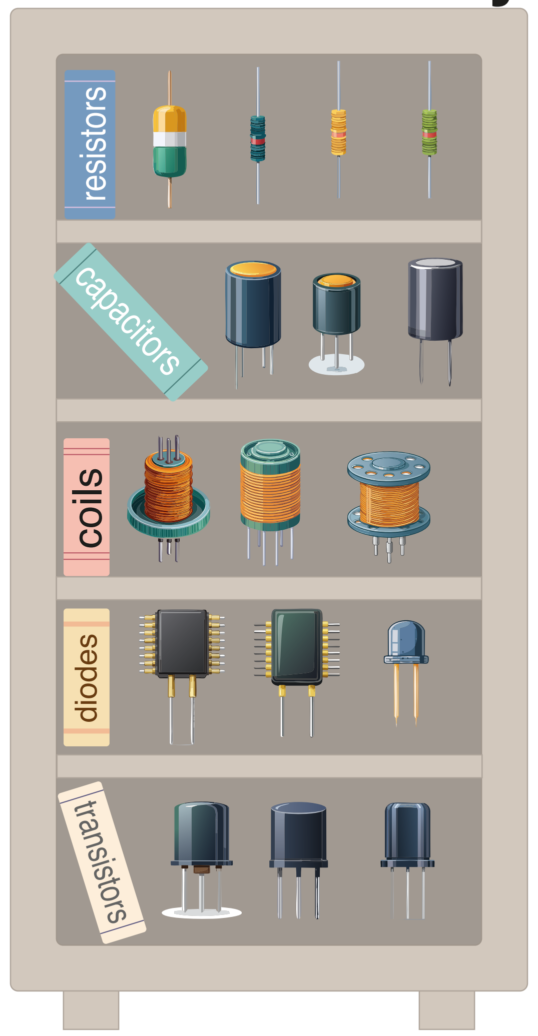

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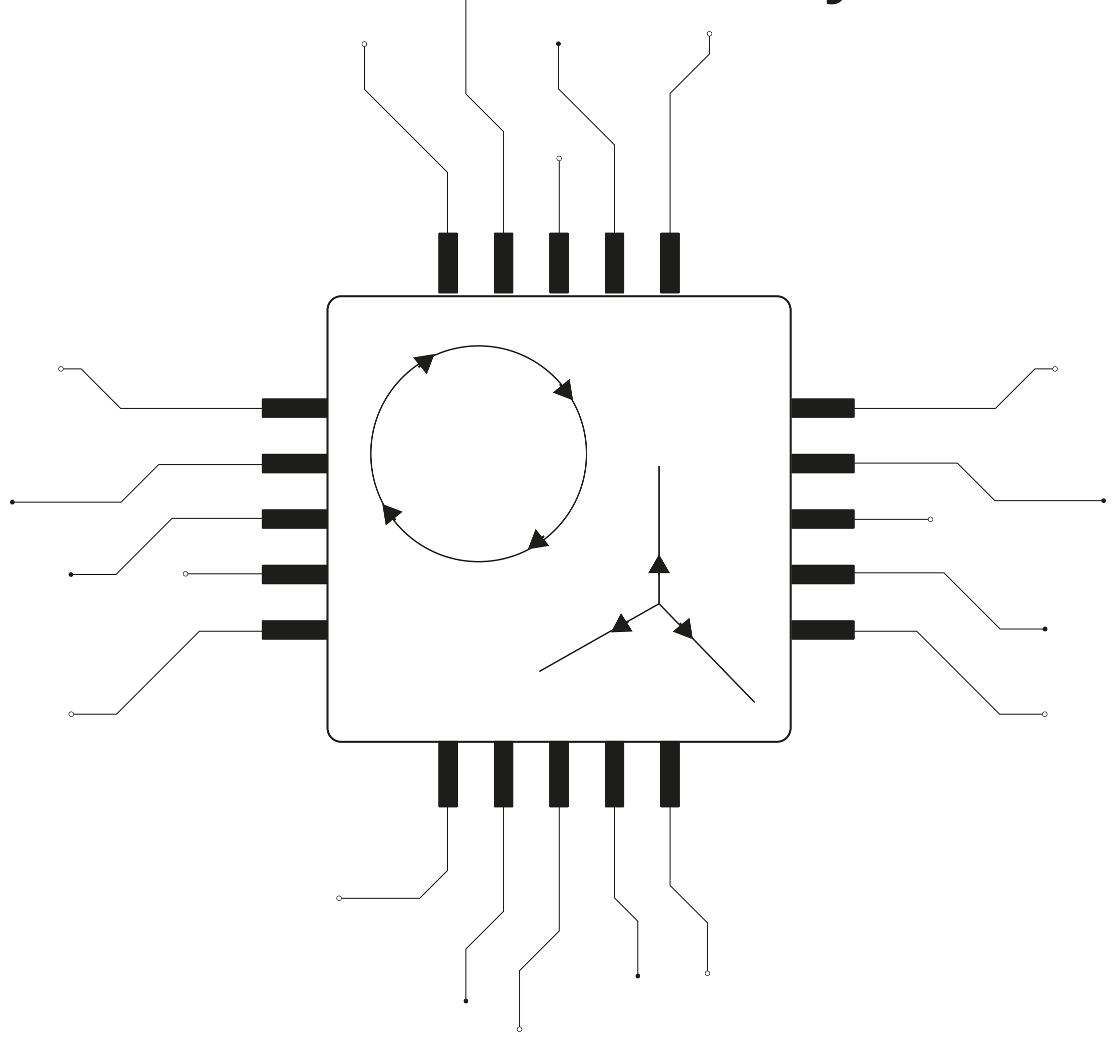

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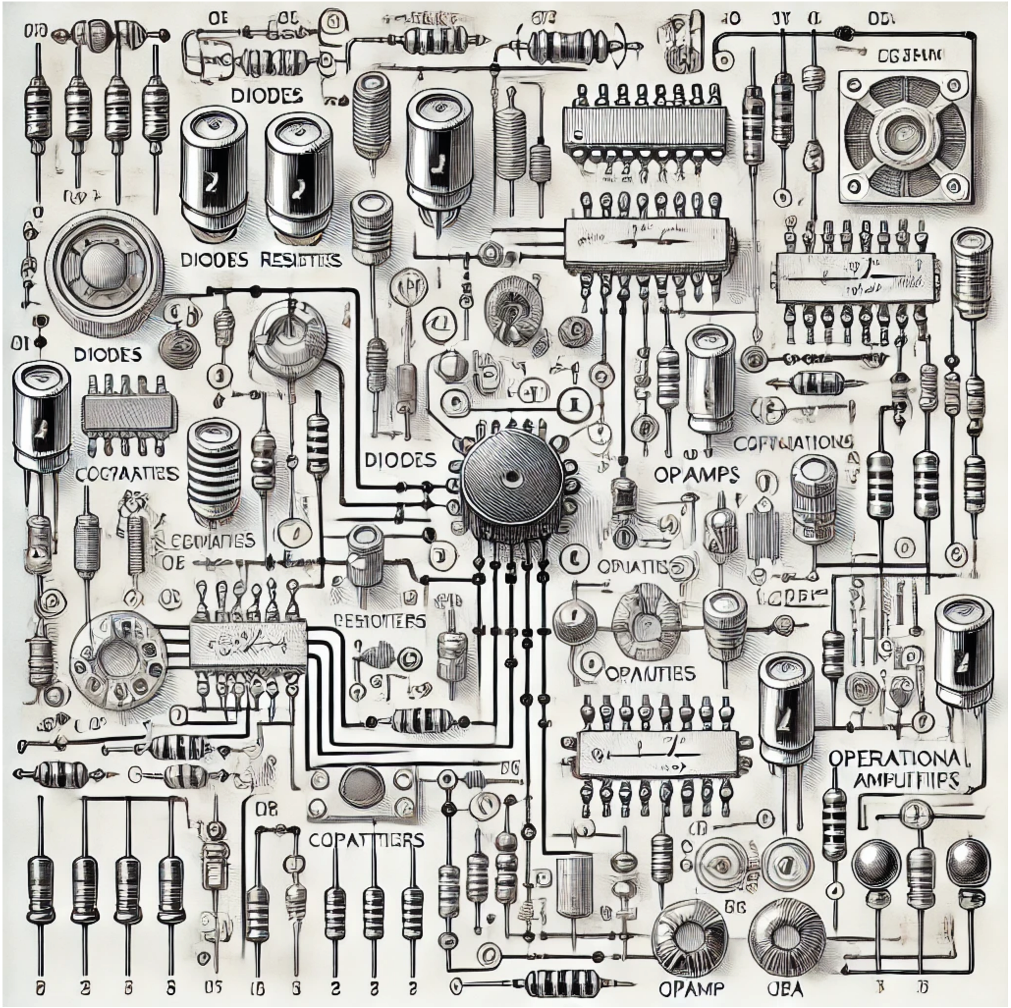

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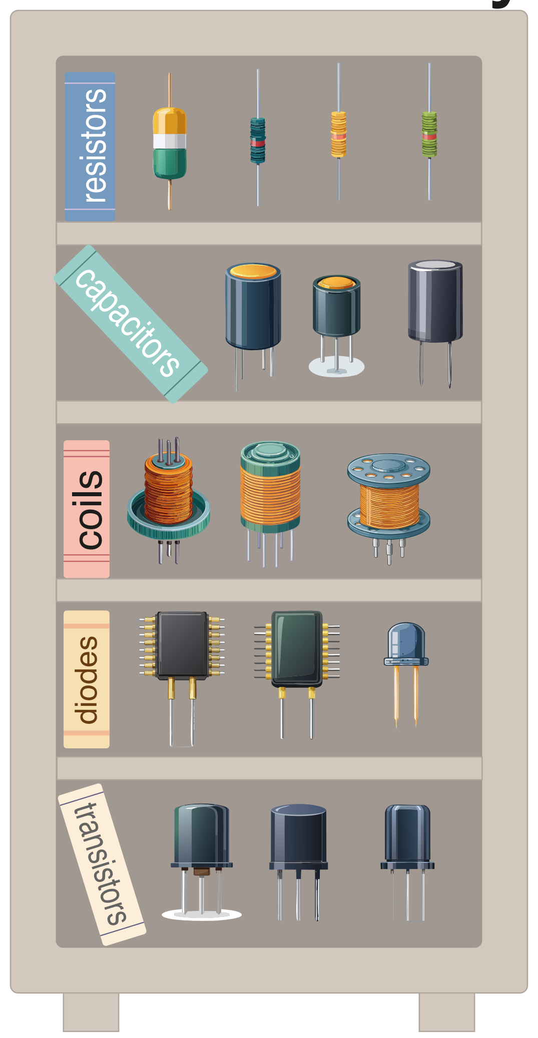

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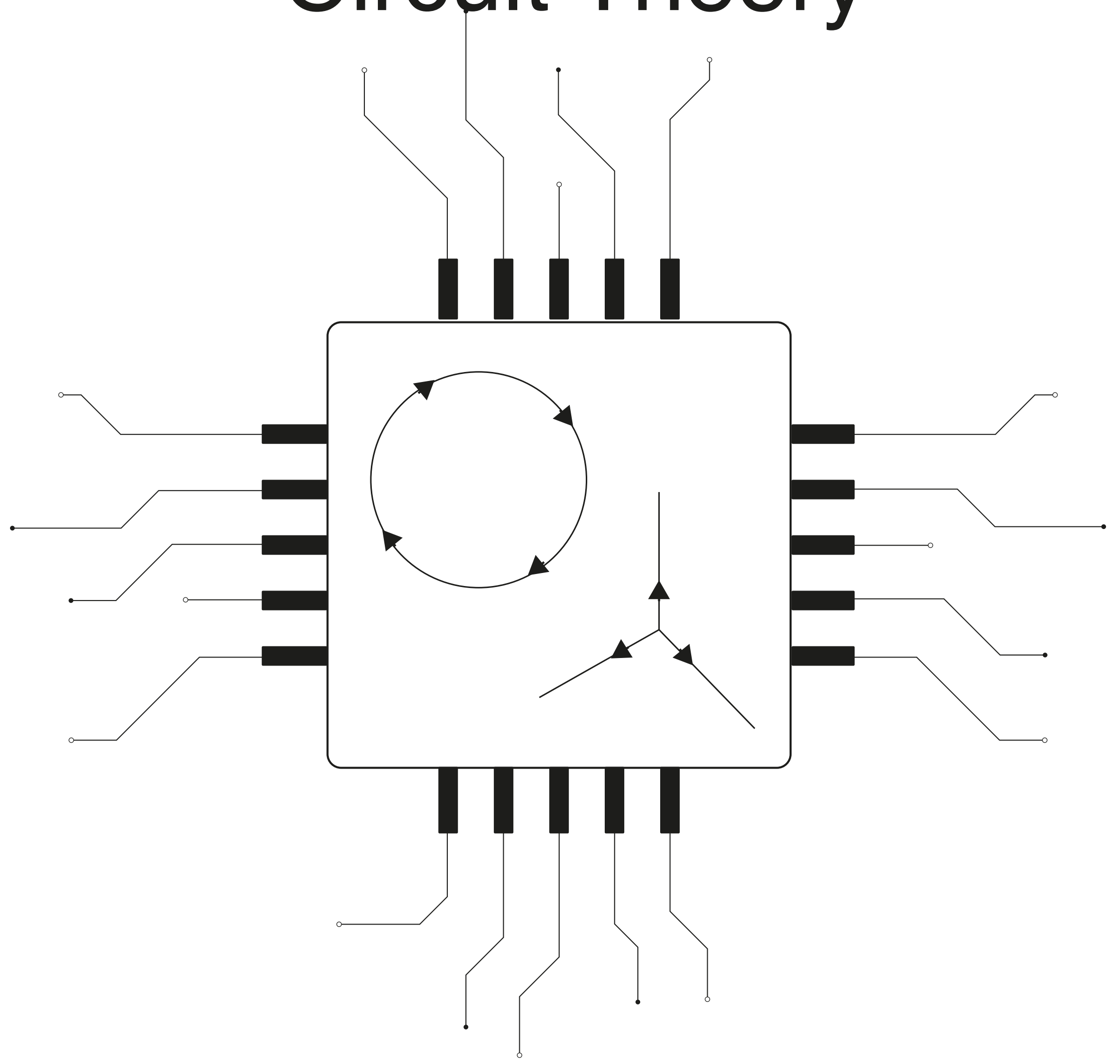

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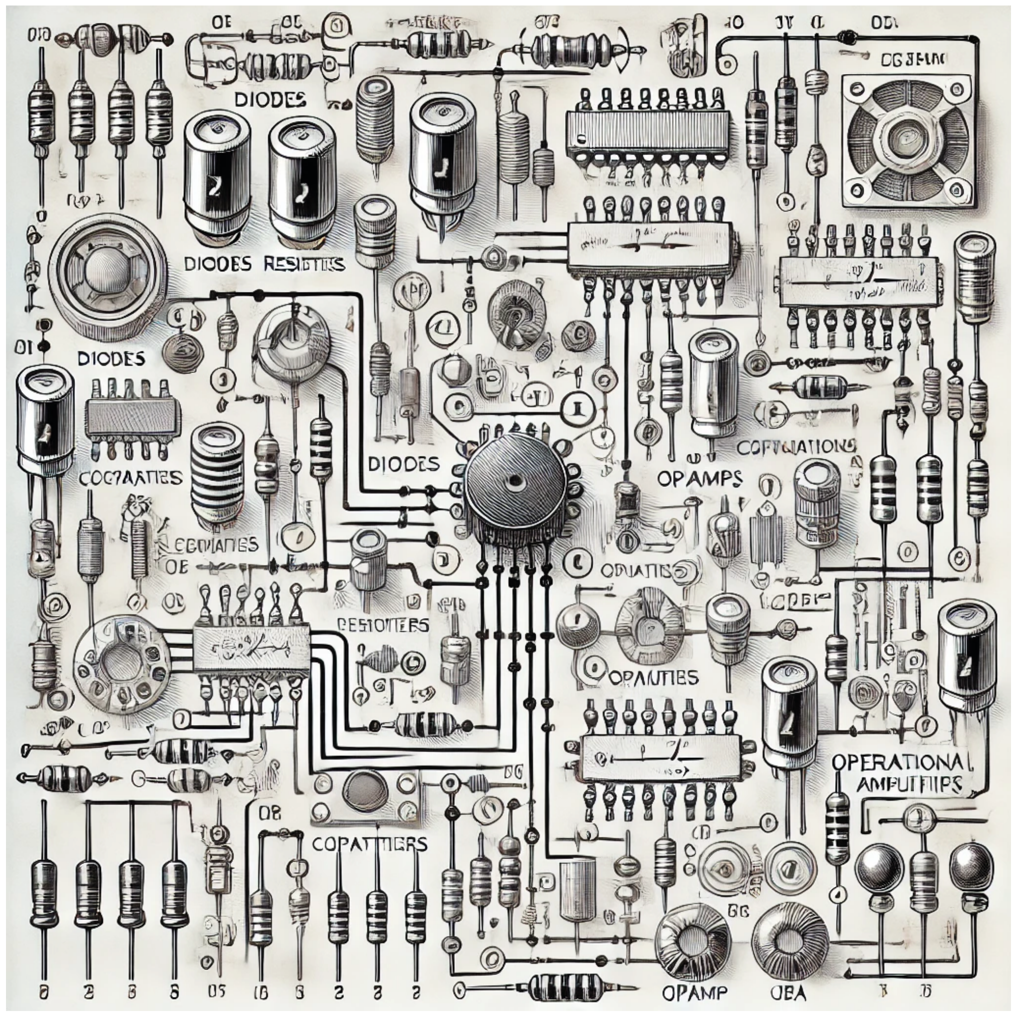

Goal:

Build a **biological** device inside the **cell** to perform a function

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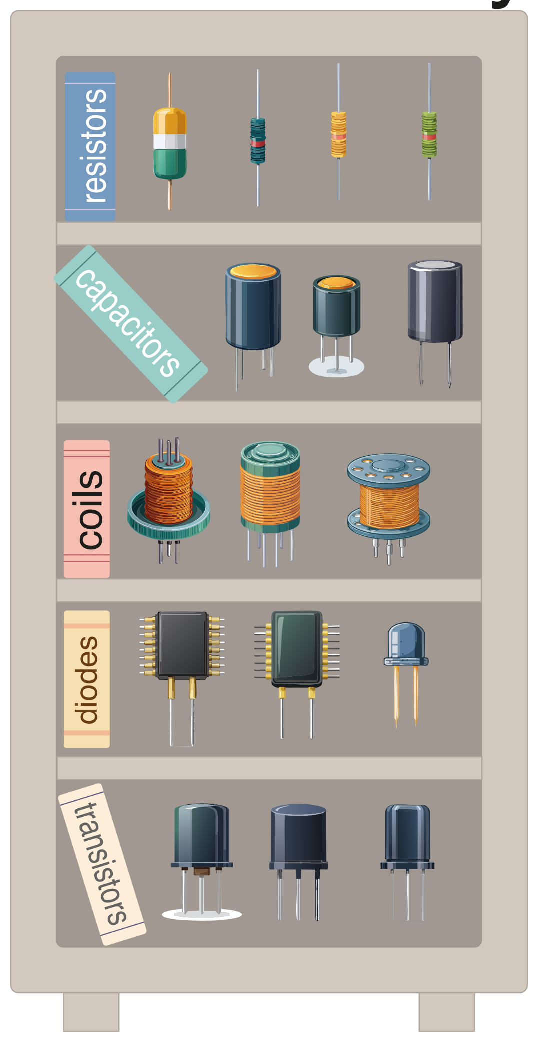

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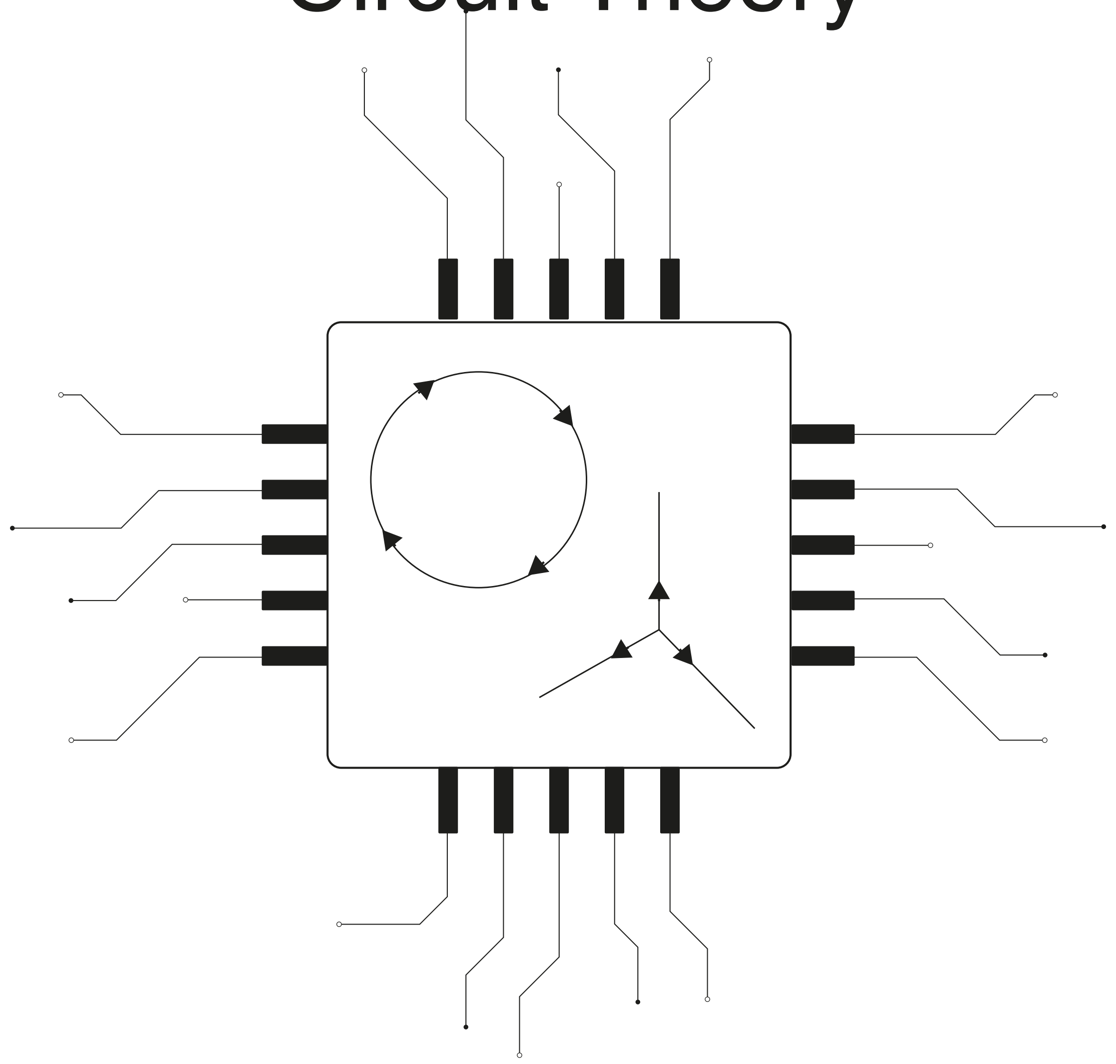

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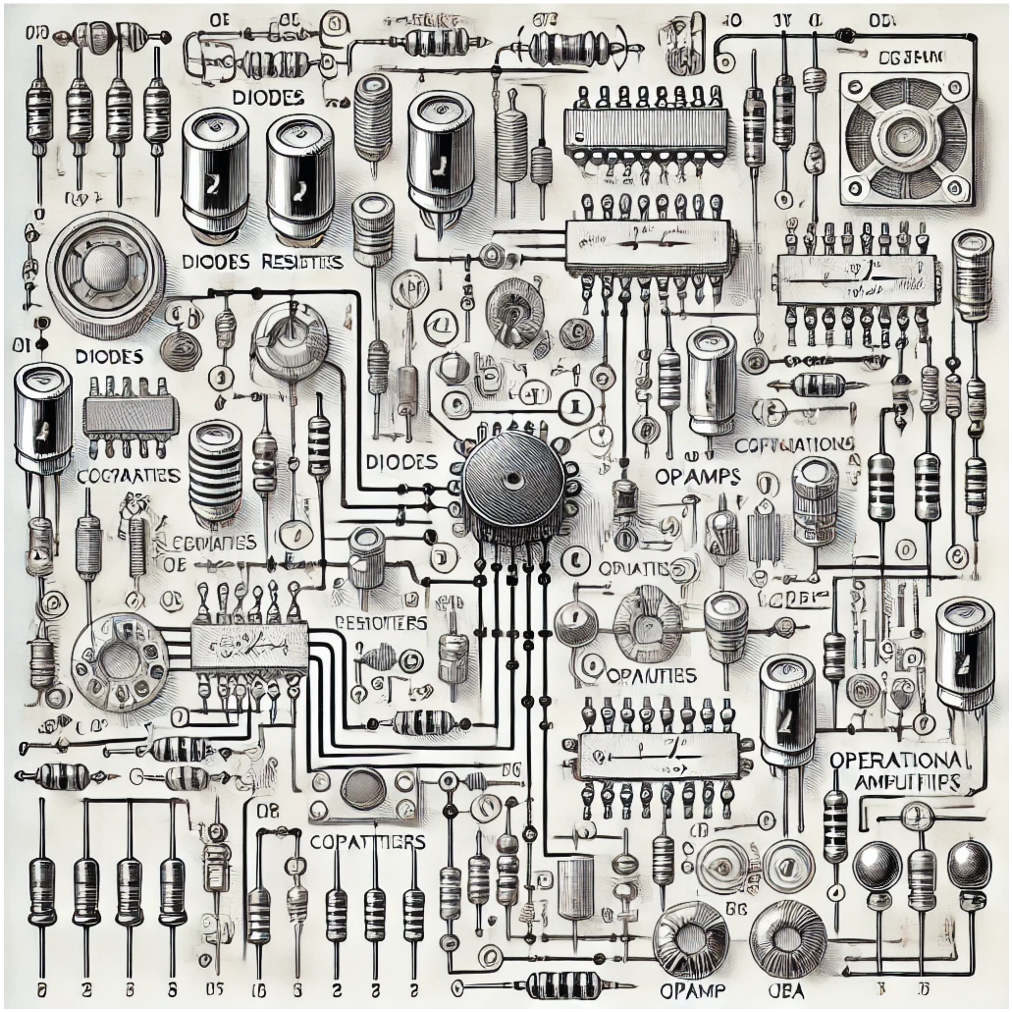

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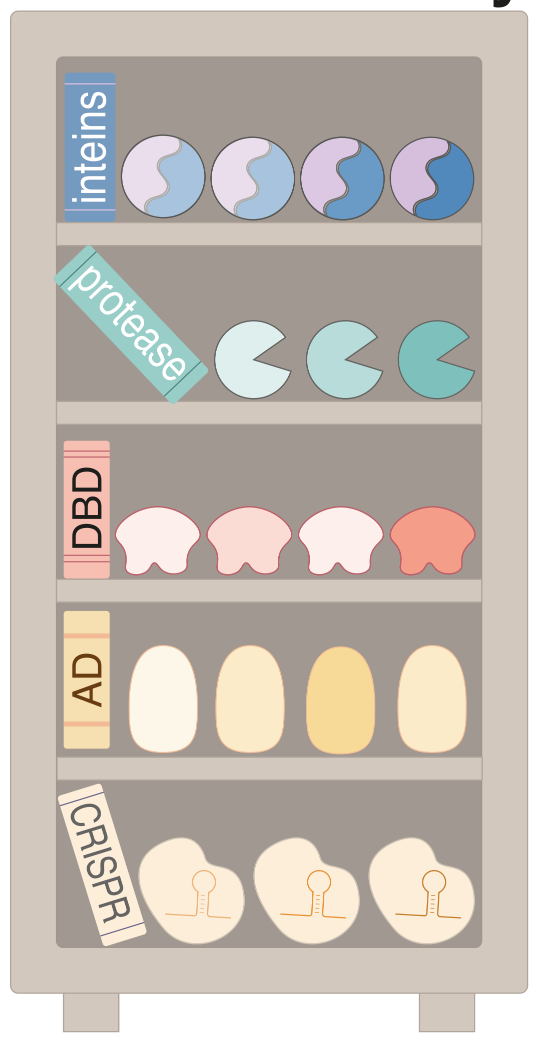

Circuit Design in Synthetic Biology

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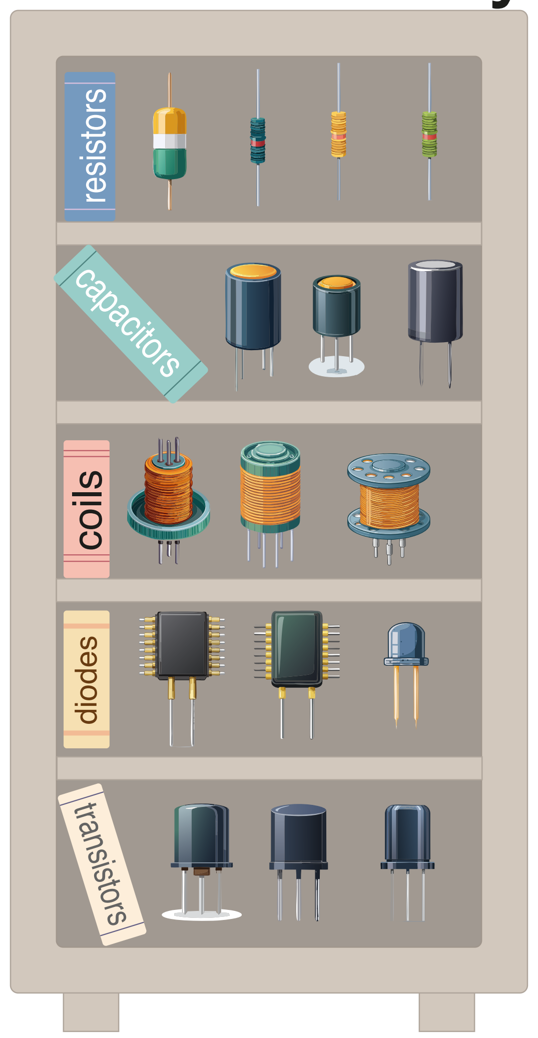

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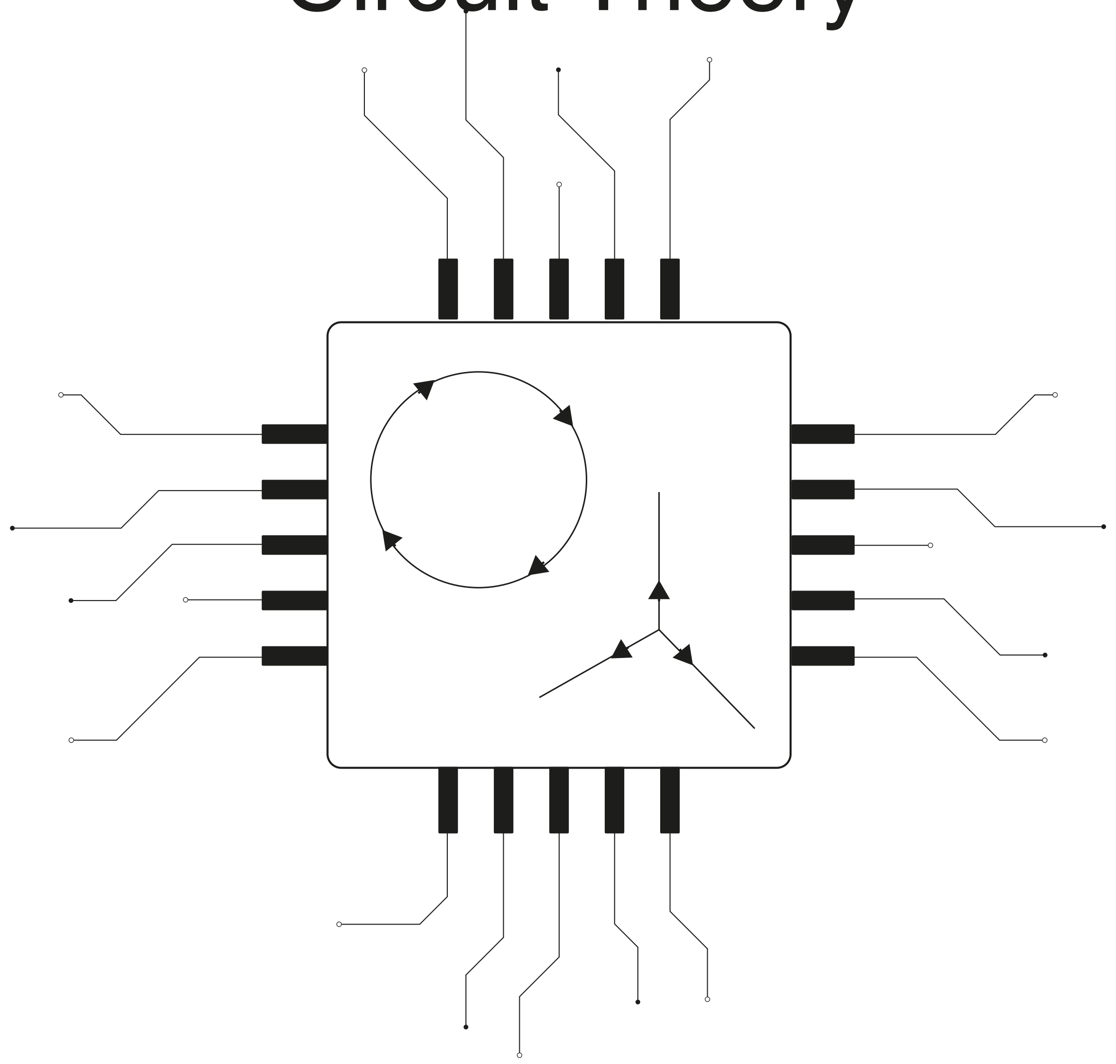

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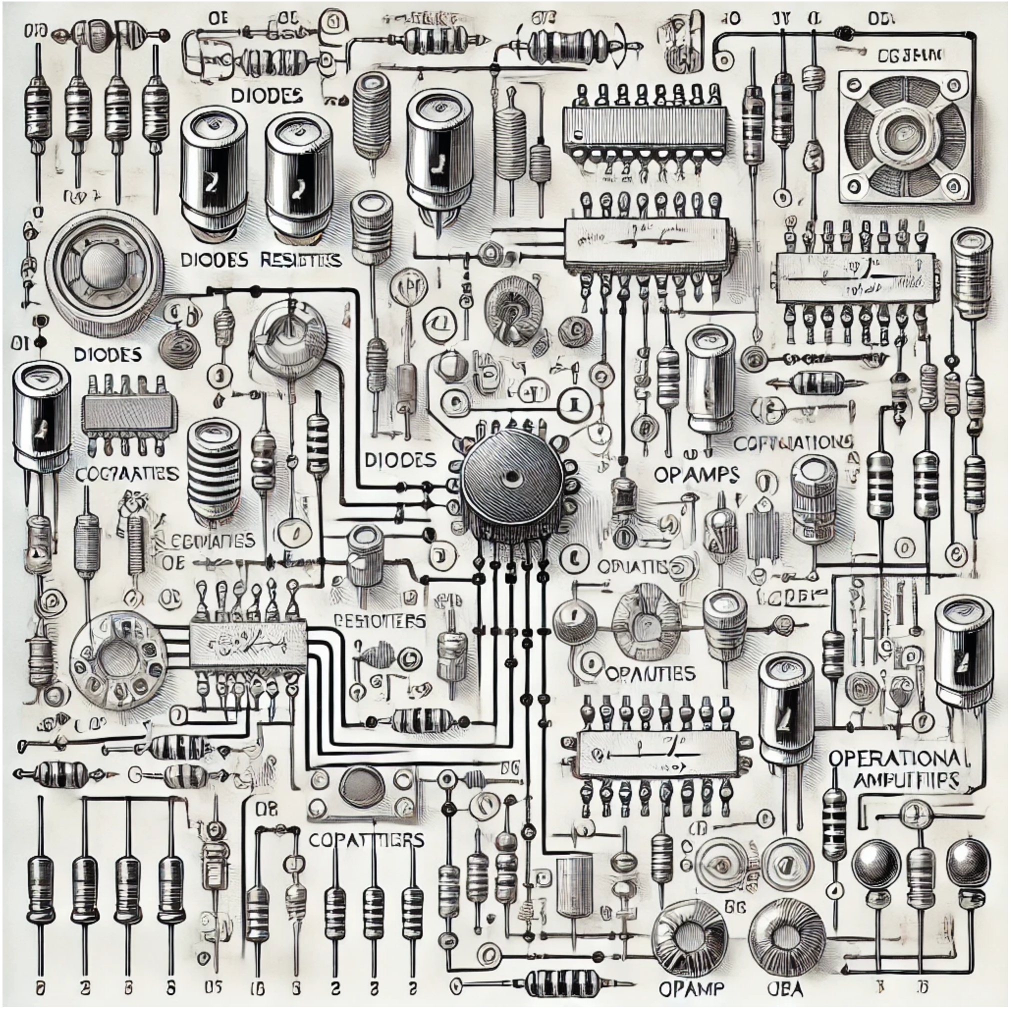

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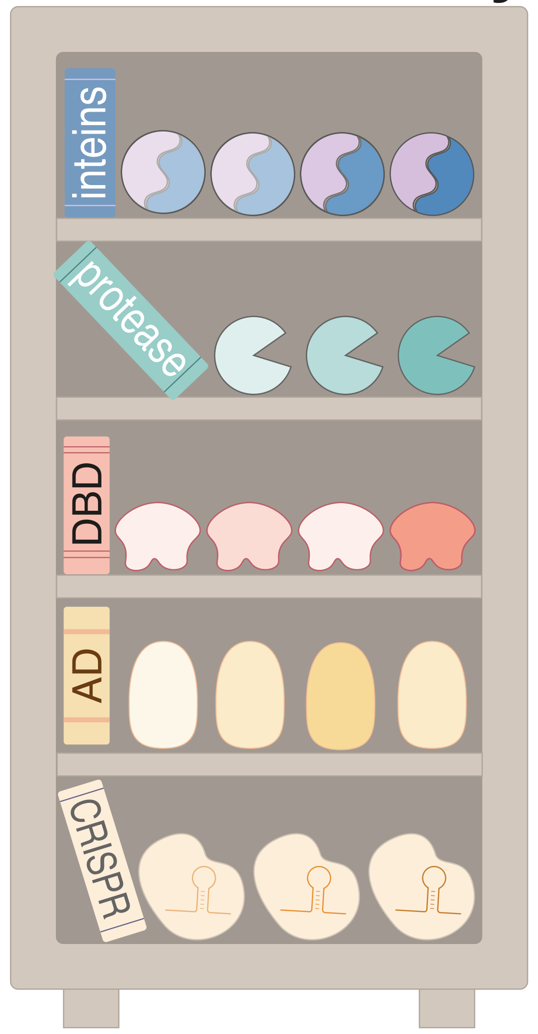

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Chemical Reaction Network Theory

$$X_1 \xrightarrow{k_1} X_1 + X_2$$

$$X_2 \xrightarrow{k_2} X_2 + X_3$$

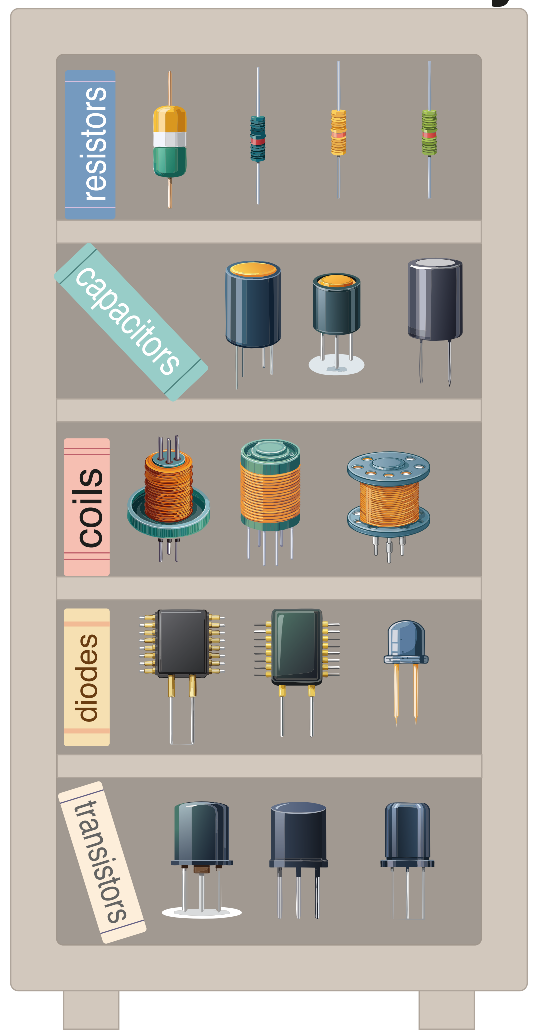
$$X_3 + X_4 \xrightarrow{k_3} \varnothing$$

$$X_1 + X_4 \xrightarrow{k_4} X_5 + X_6$$

$$\varnothing \xrightarrow{k_5} X_2$$

Circuit Design in Synthetic Biology

Parts Library



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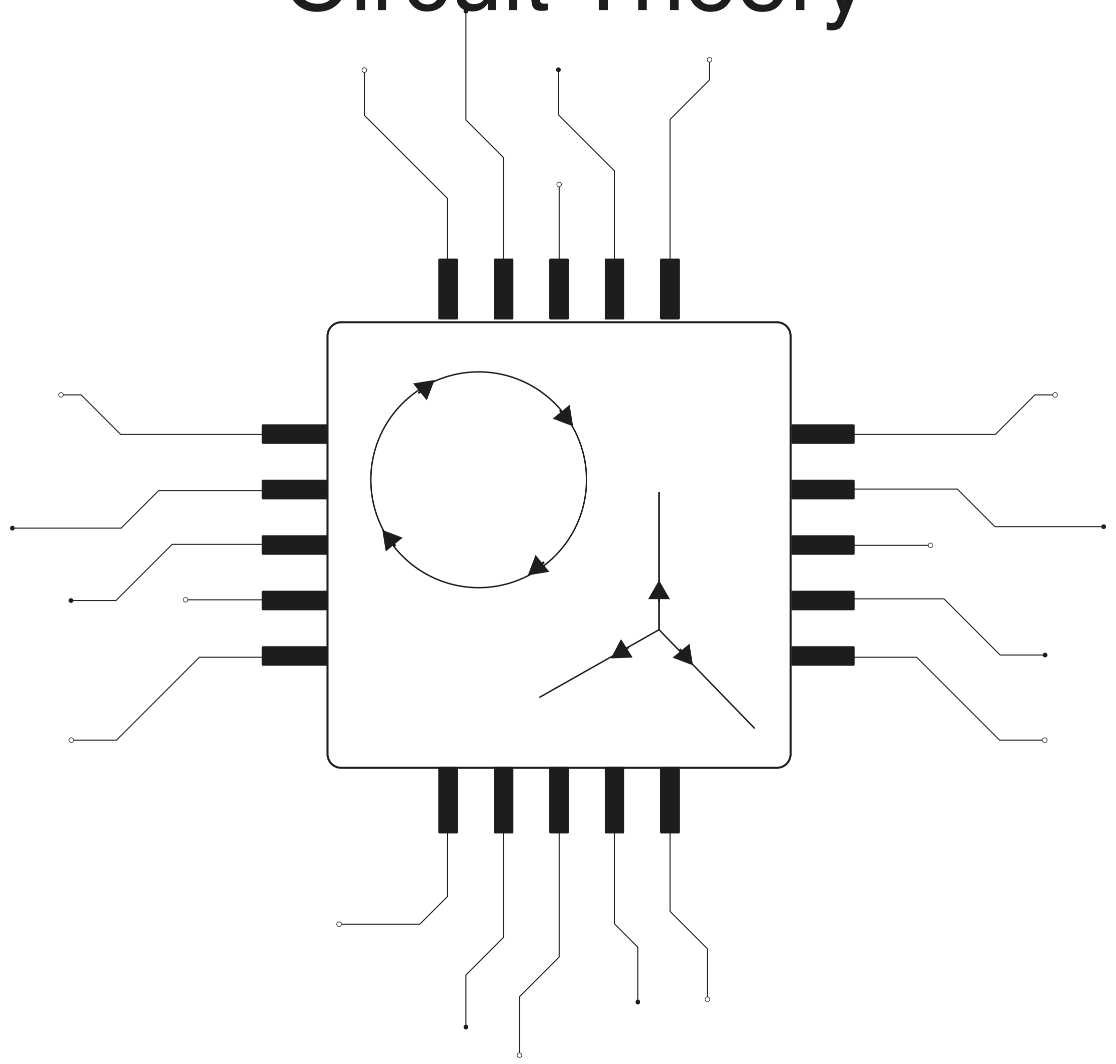

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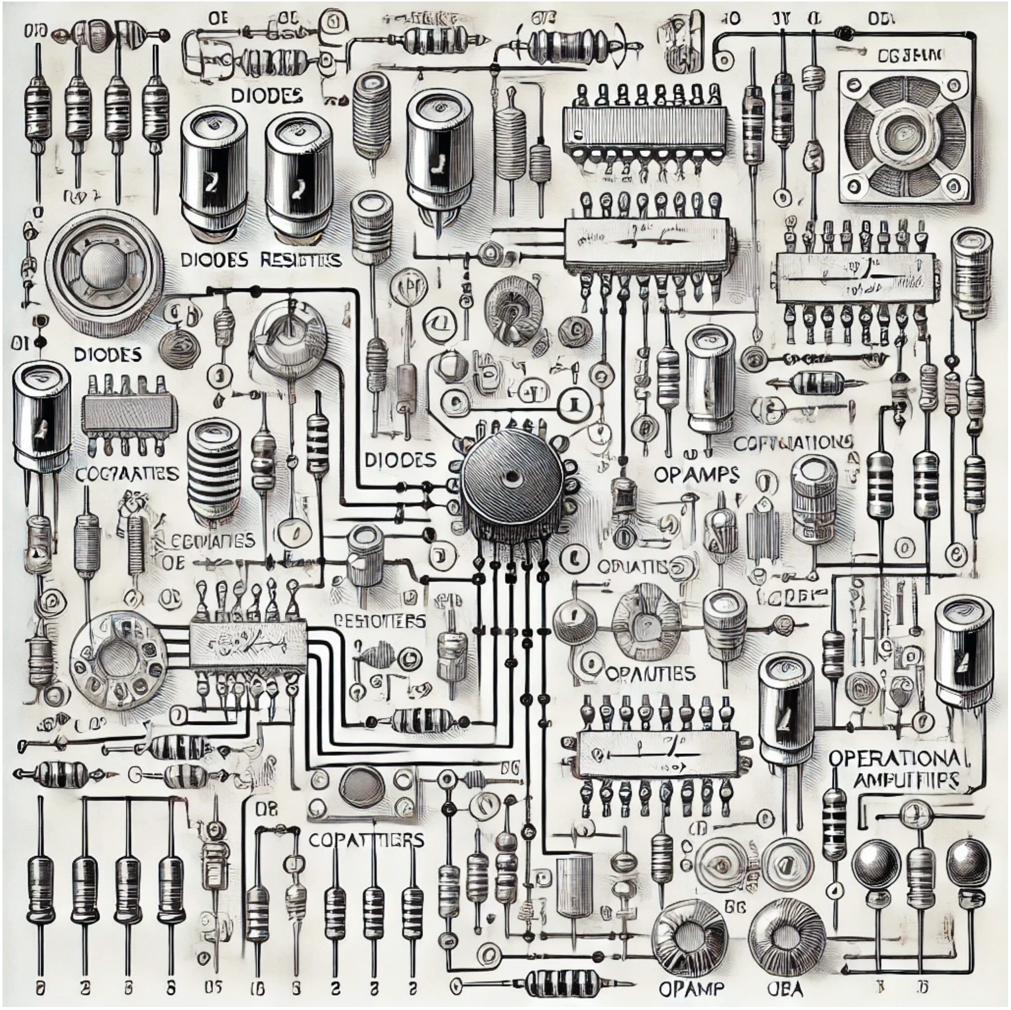

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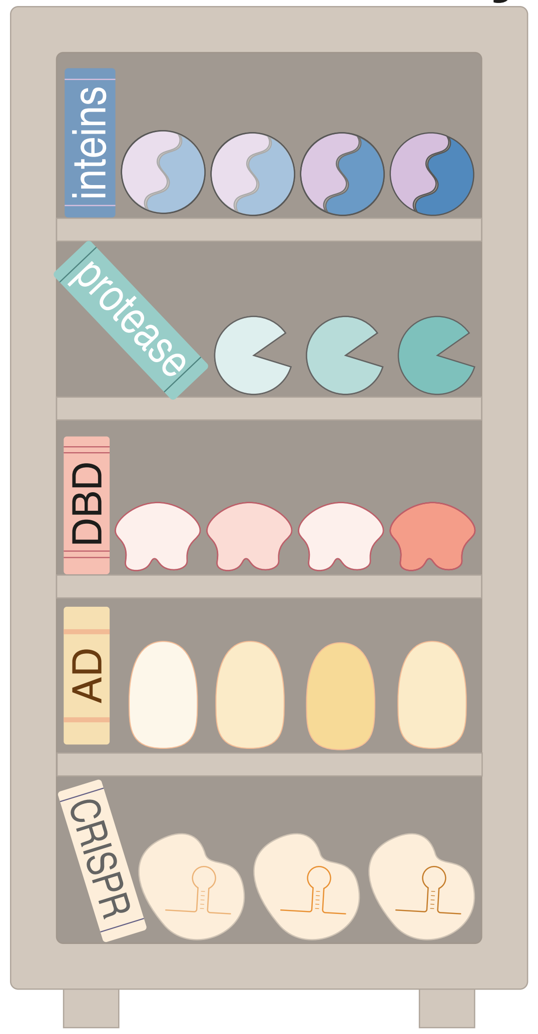

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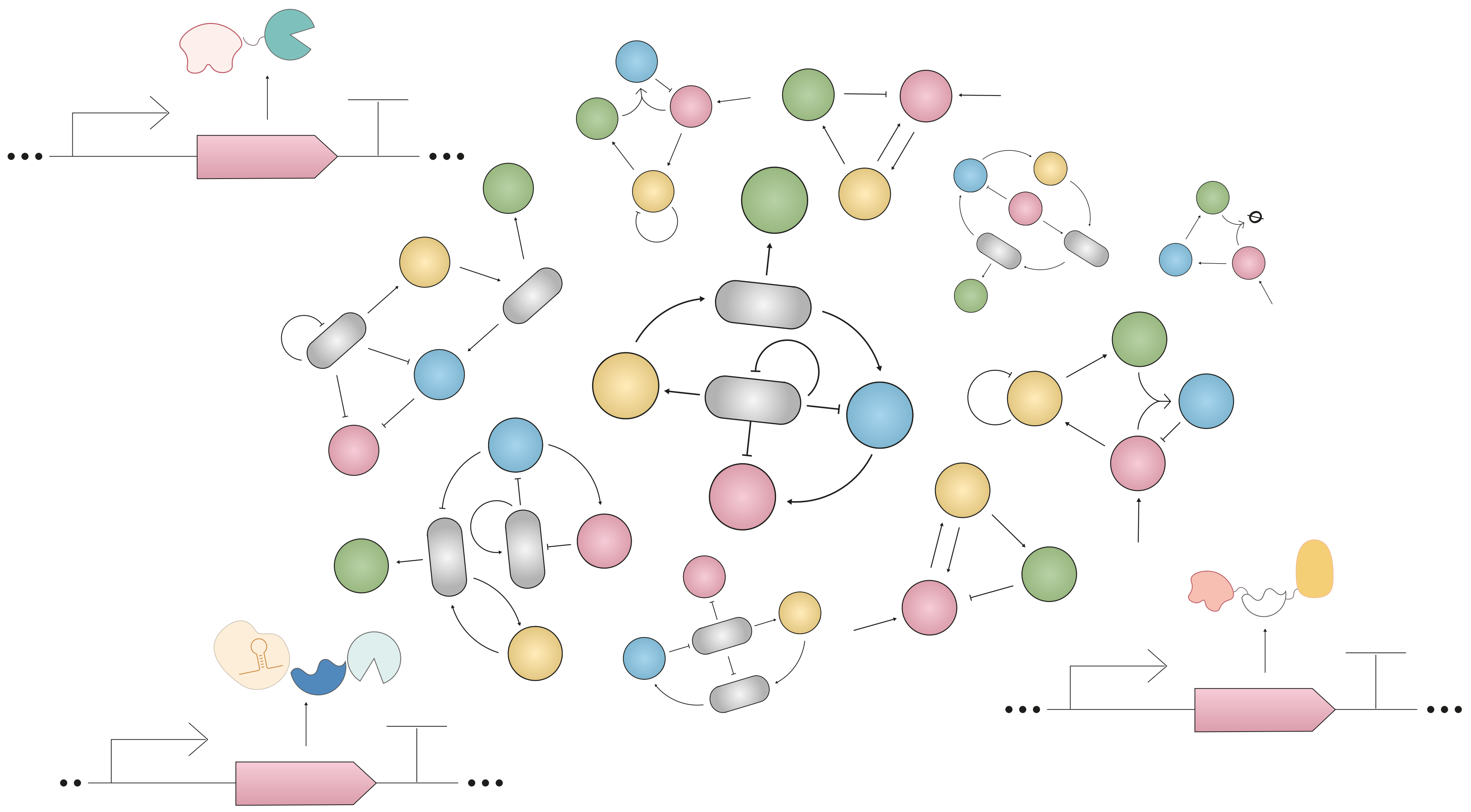
Genetic Circuit



Control Theory in Synthetic Biology

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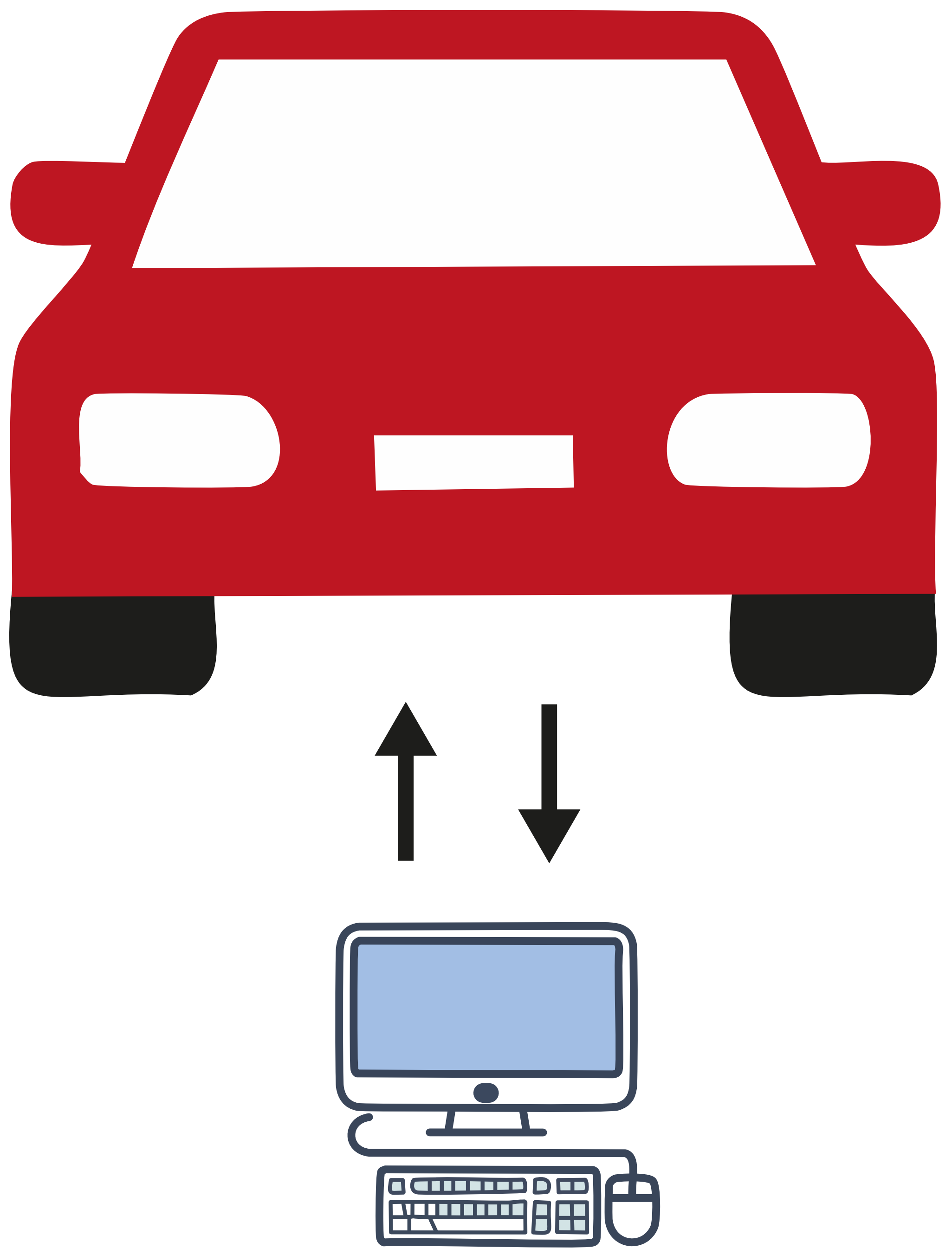
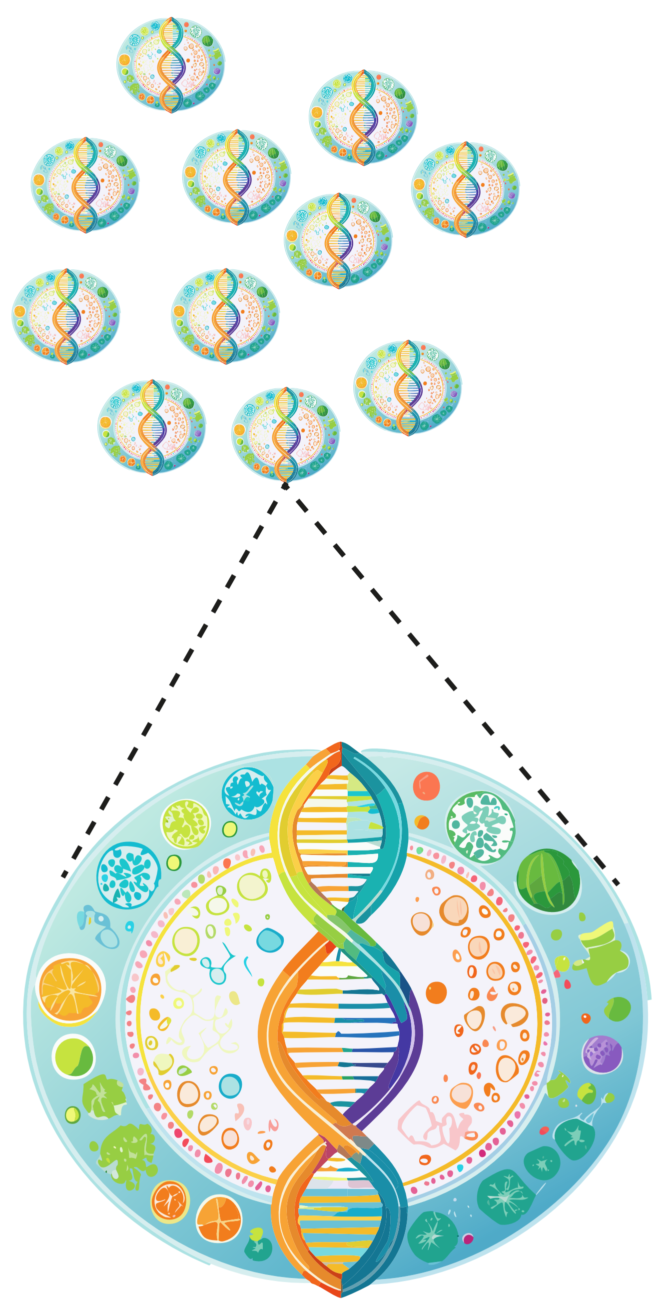


Program a *computer*
to control a car



Program the *DNA*
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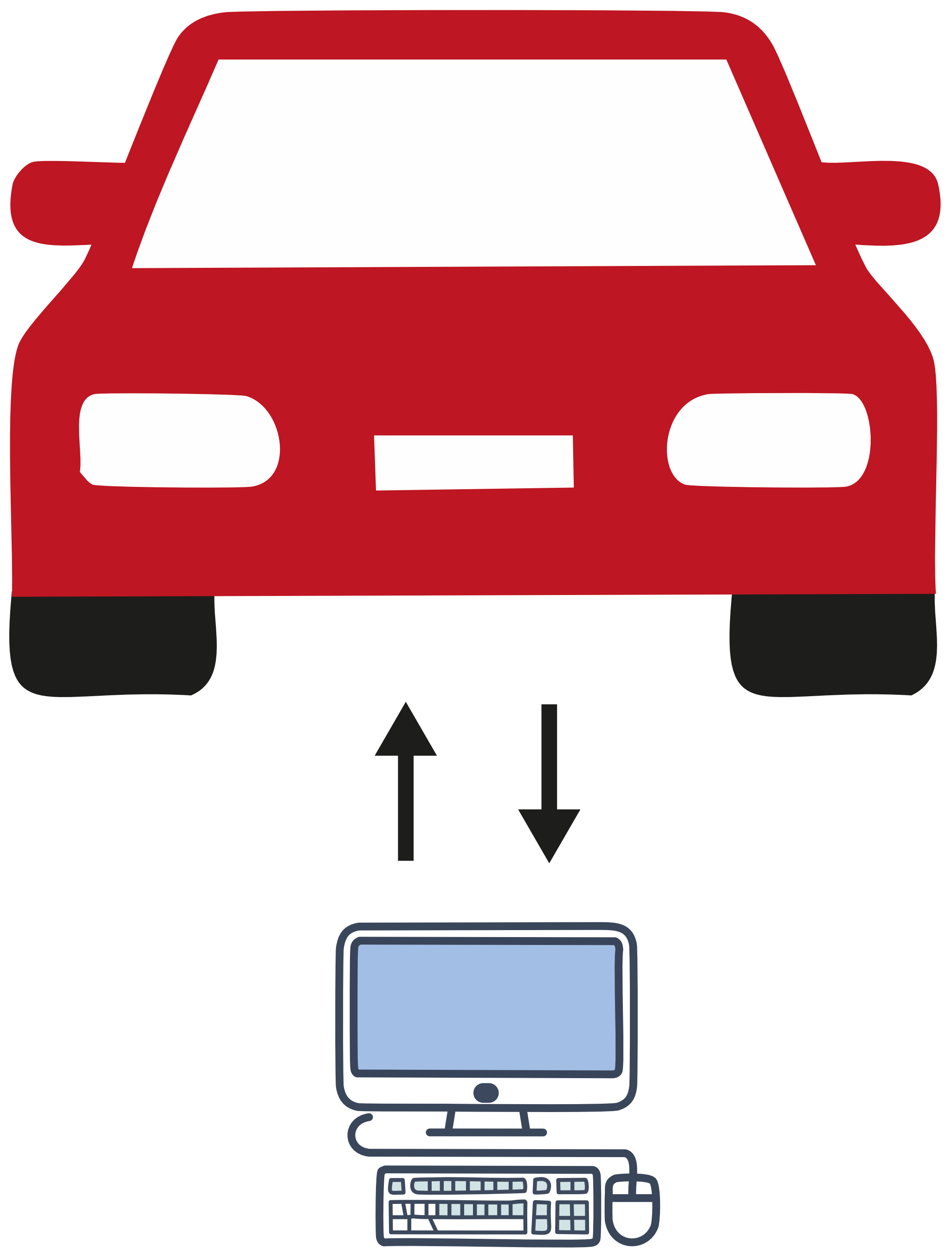
Control Theory in Synthetic Biology



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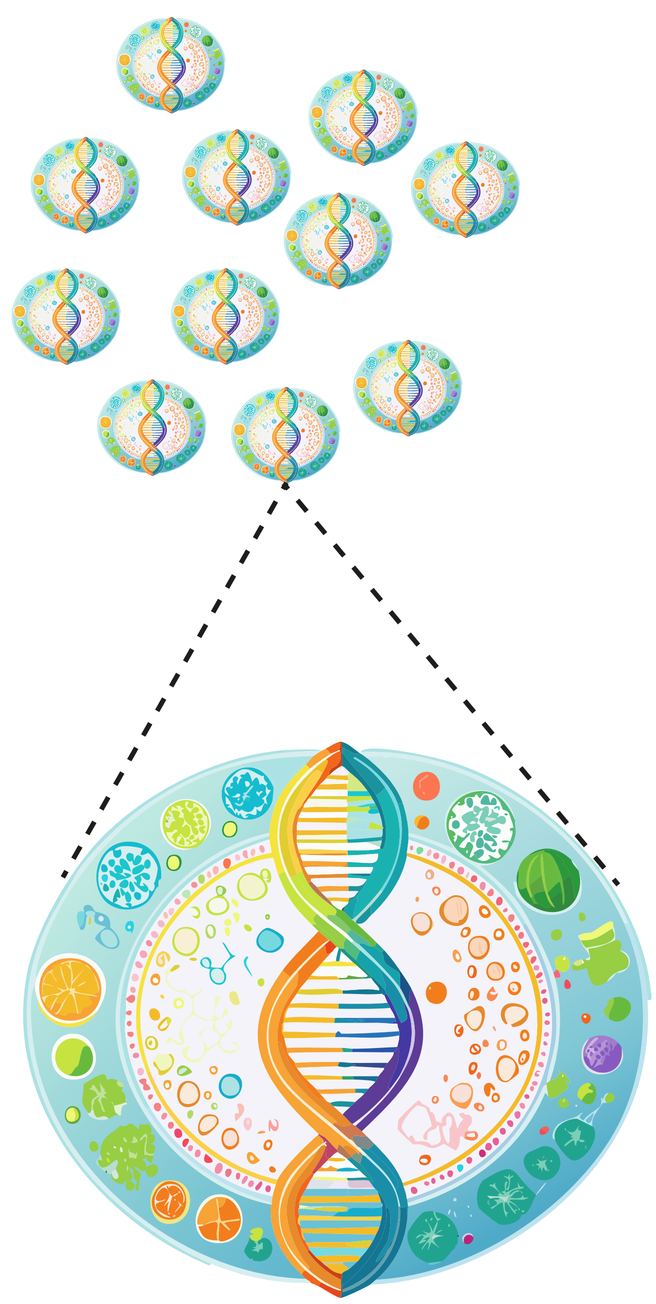

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Formidable challenges in biocontrol

- Stochasticity
- Intrinsic nonlinearities
- Positivity
- Finding the right genetic parts

Control Theory in Synthetic Biology

Applications

- **Bio production**: smart cells that optimize and robustify bioproduction

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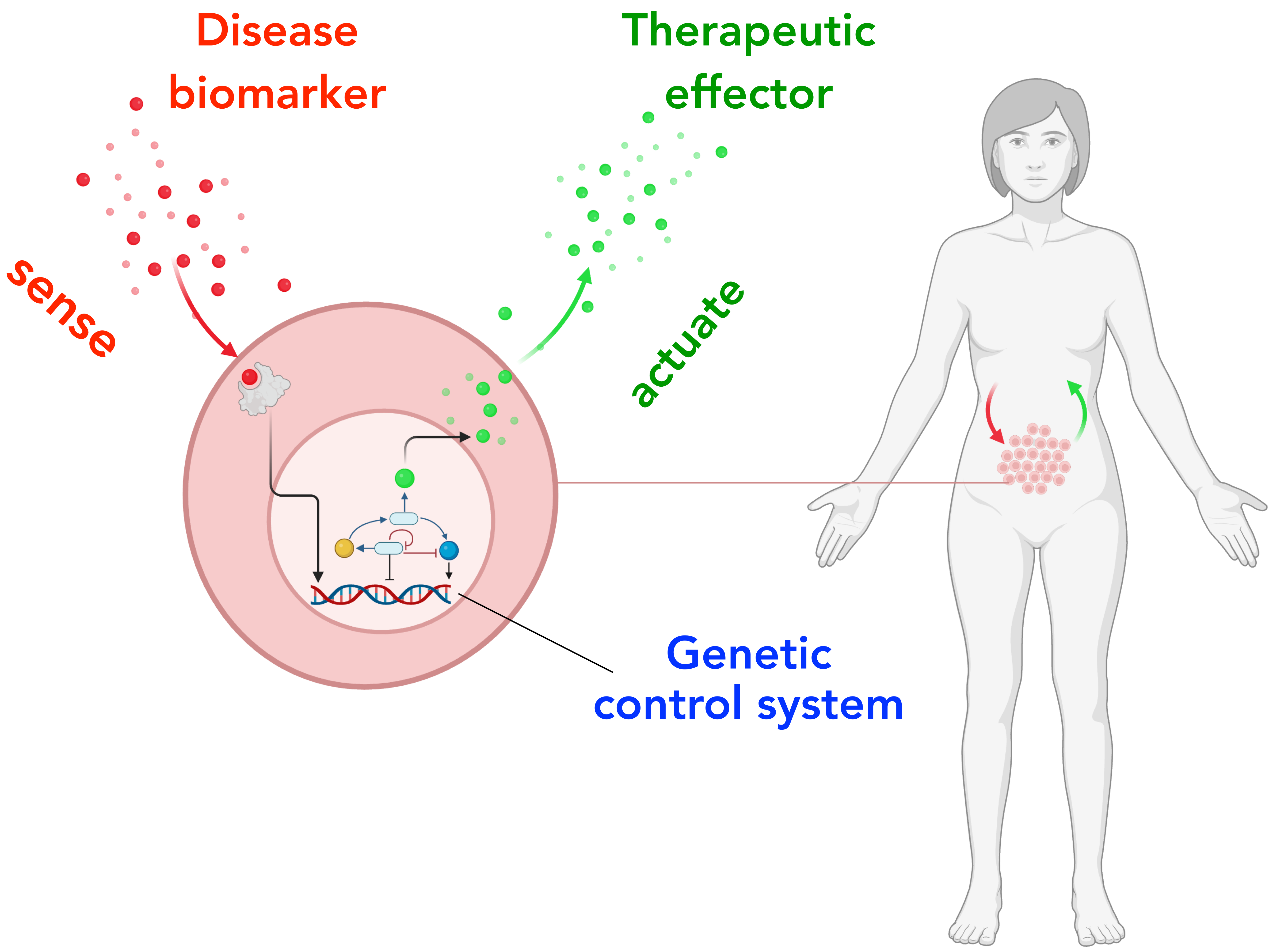

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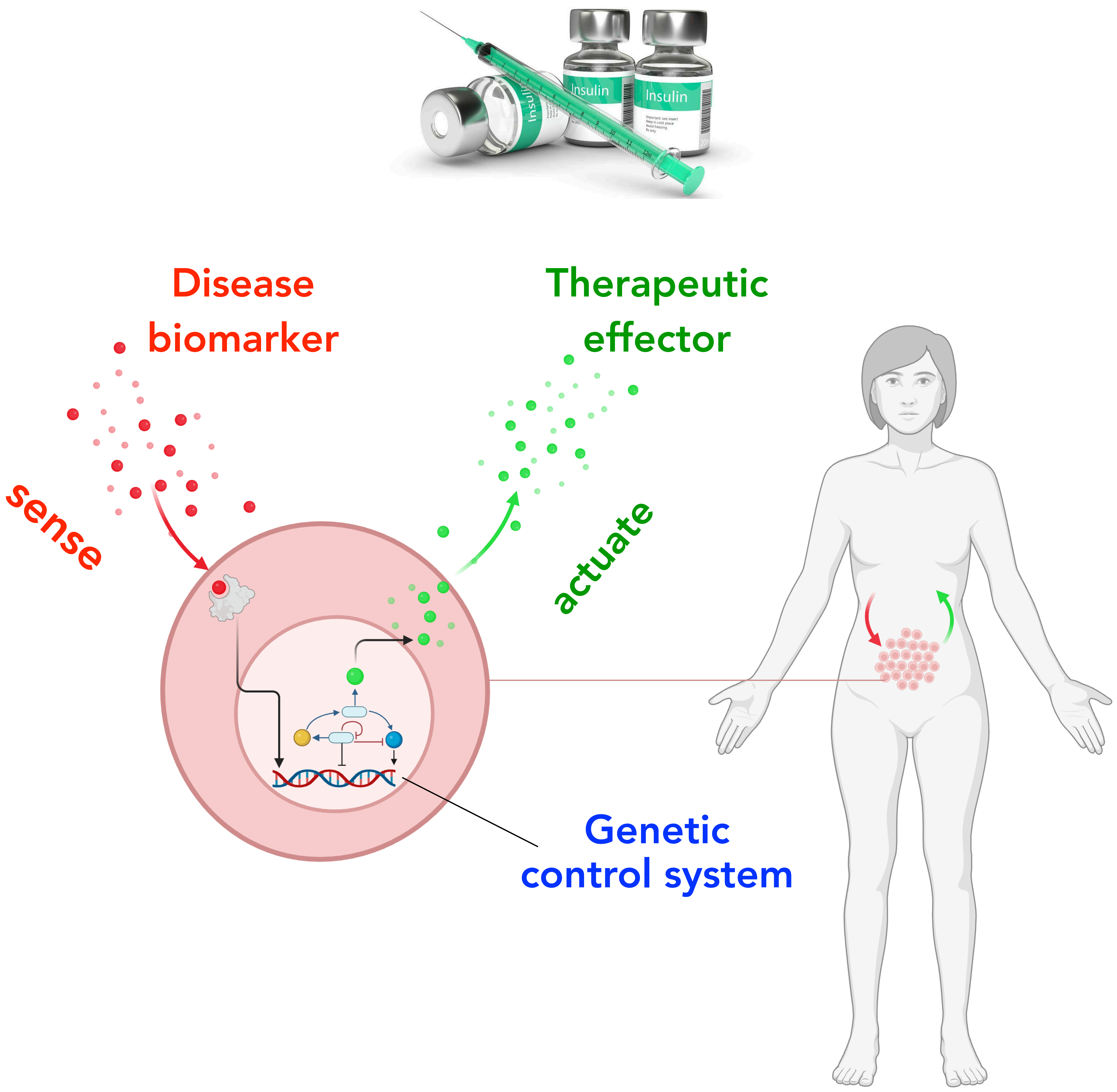

Applications

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- **Cell therapy**: smart cells that autonomously combat diseases
- **Bio computing**: analog biological computers

⋮

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Chemical Reaction Networks: the Mathematical Language of Biochemistry

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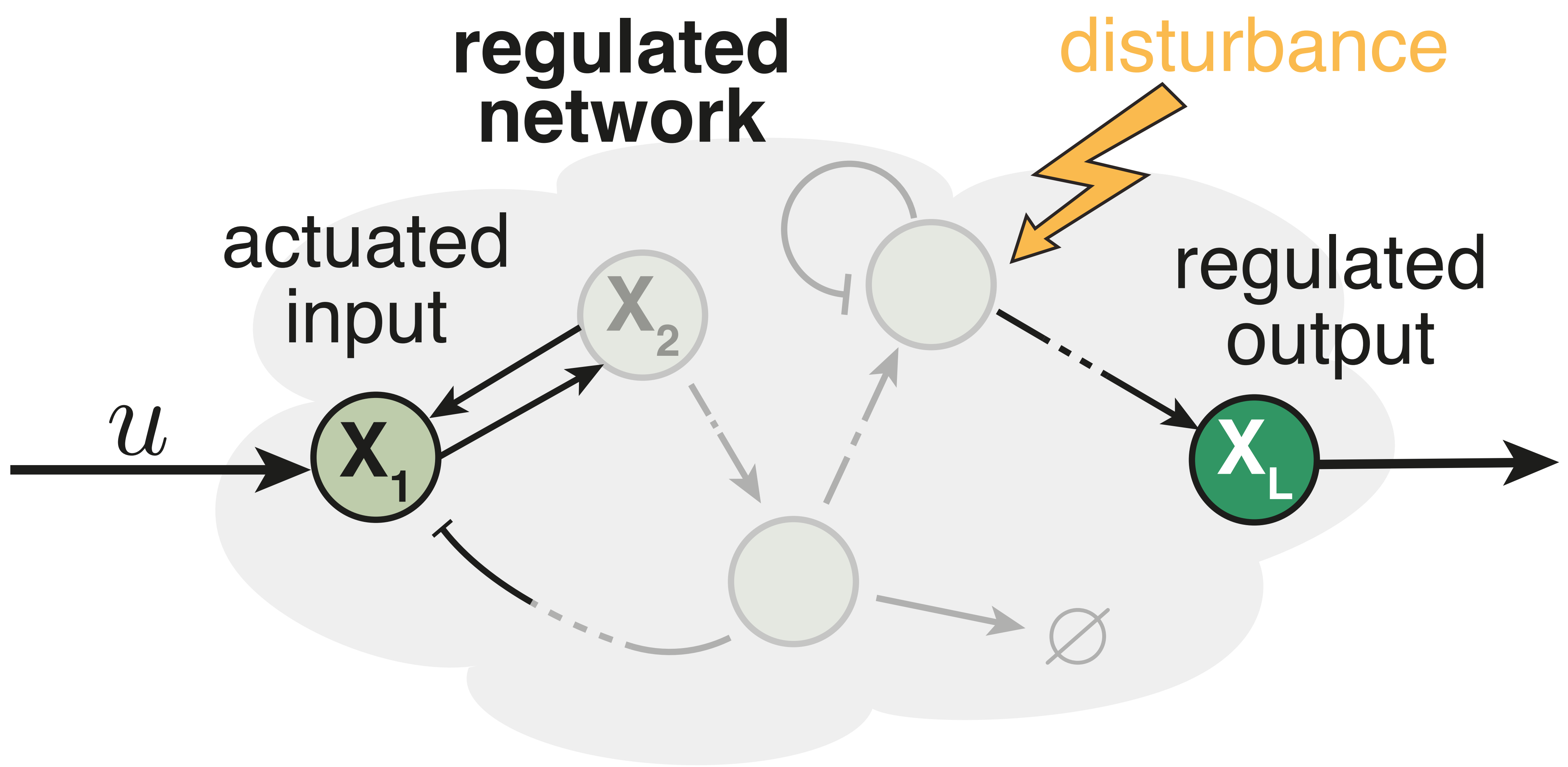
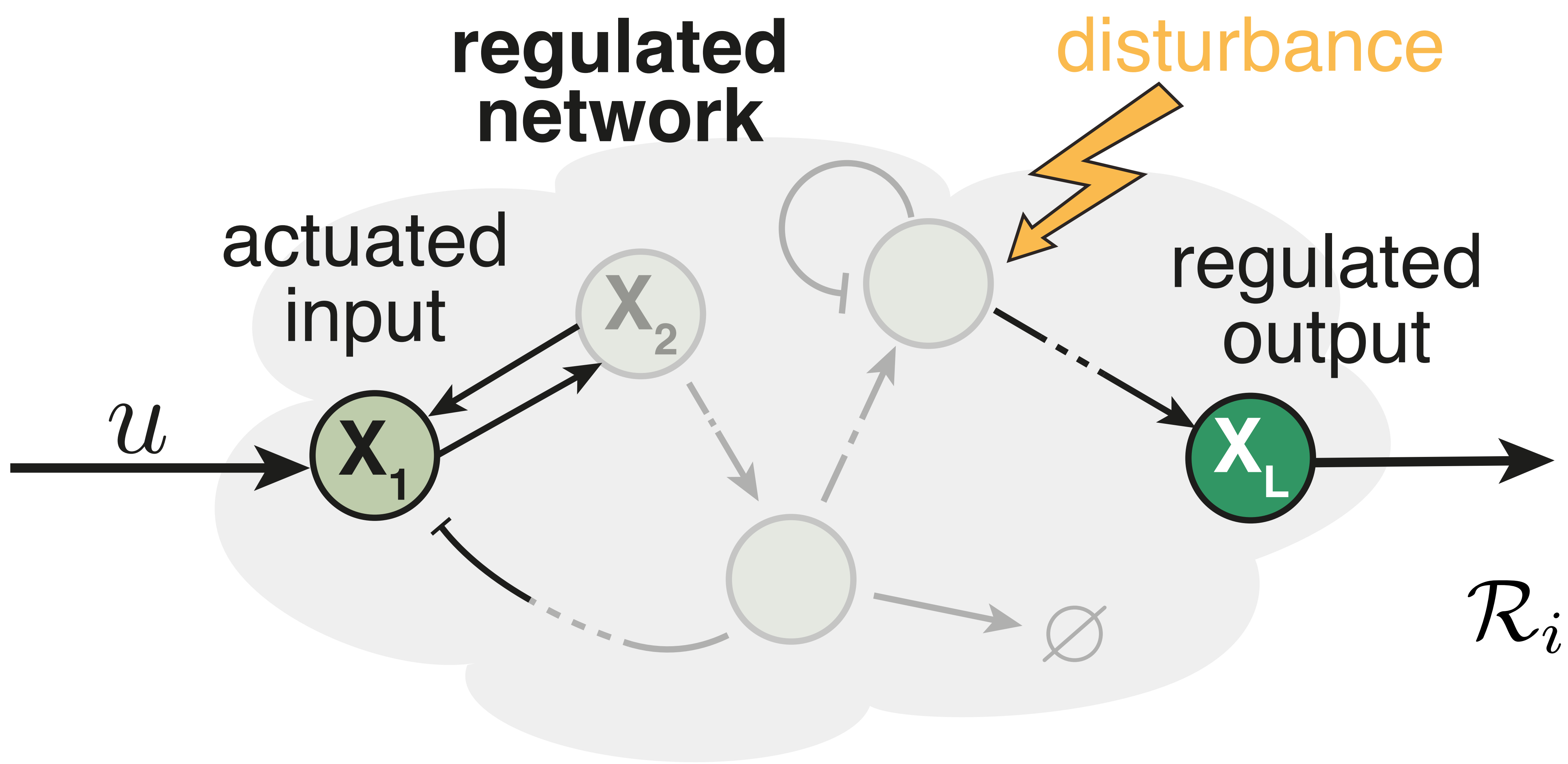


Species: $\mathbf{X} \triangleq \{\mathbf{X}_1, \mathbf{X}_2, \cdots, \mathbf{X}_L\}$

Reactions: $\mathcal{R} \triangleq \{\mathcal{R}_1, \mathcal{R}_2, \cdots, \mathcal{R}_n\}$

Chemical Reaction Networks: the Mathematical Language of Biochemistry



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$$\mathcal{R}_i : a_{i1}\mathbf{X}_1 + \cdots + a_{in}\mathbf{X}_L \xrightarrow{\lambda_i(\mathrm{x}, \mathrm{u})} b_{i1}\mathbf{X}_1 + \cdots + b_{in}\mathbf{X}_L$$

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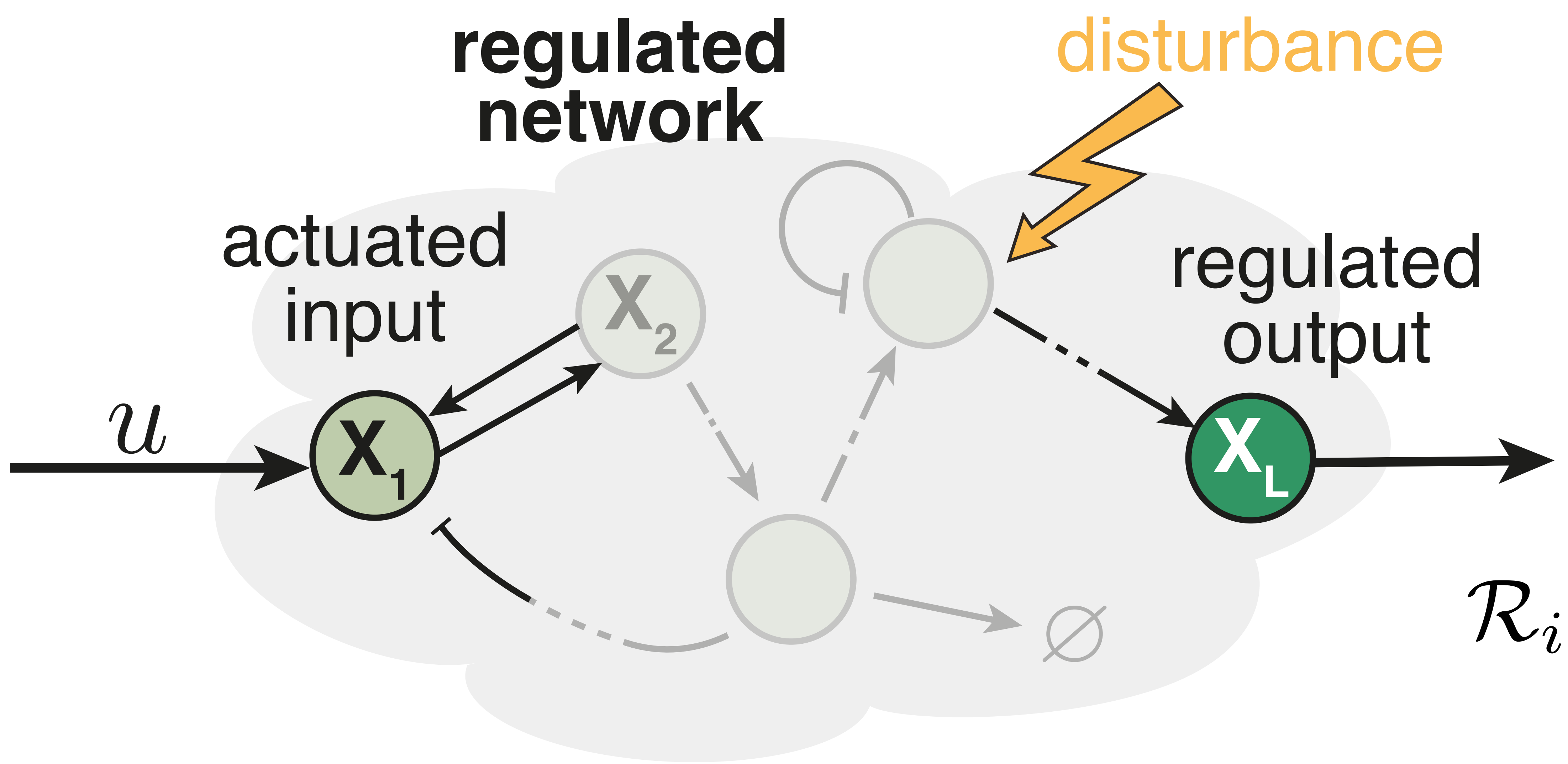

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Vector form: $a_i\mathbf{X} \xrightarrow{\lambda_i(\mathrm{x}, \mathrm{u})} b_i\mathbf{X}$

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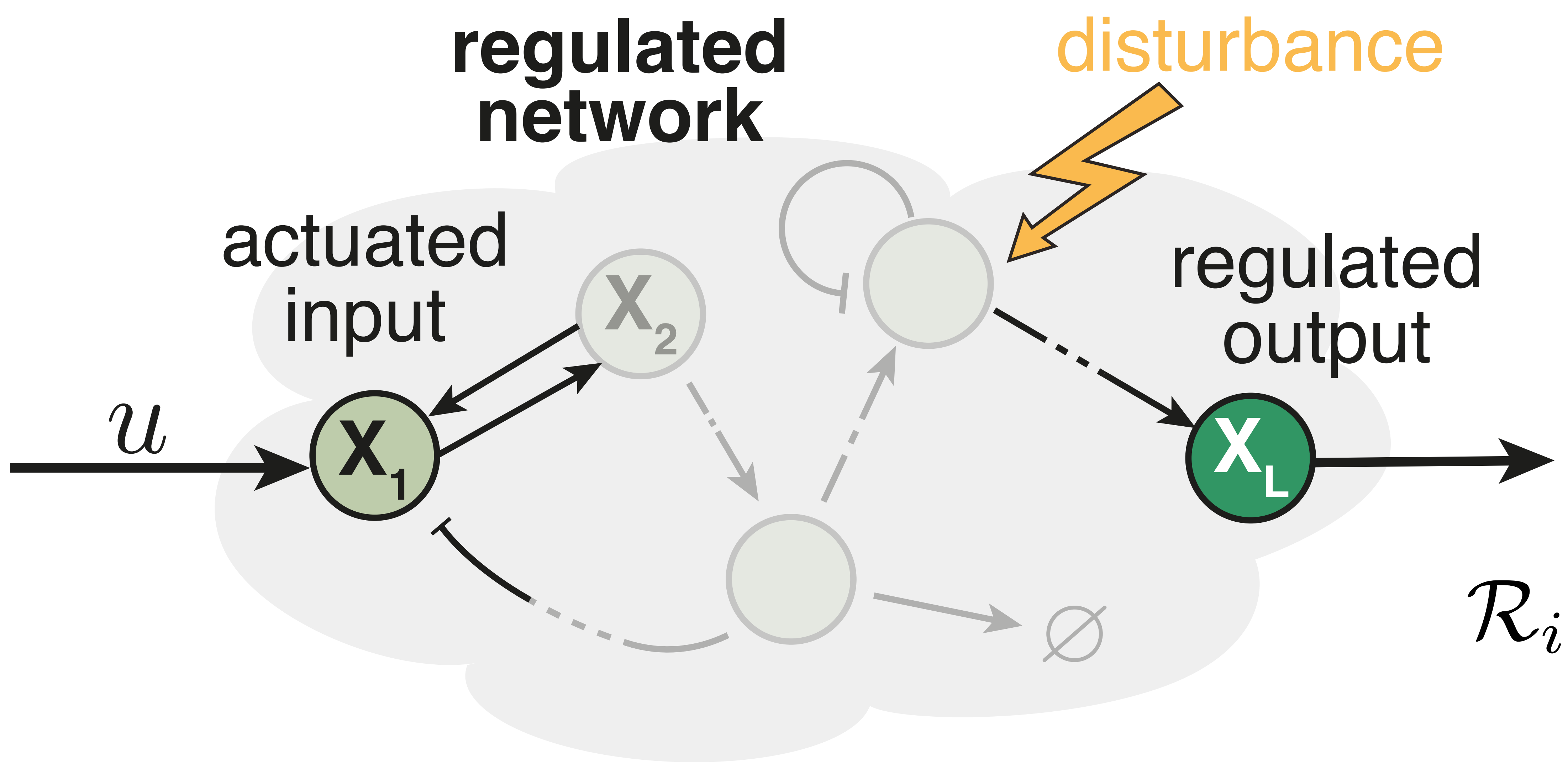

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Stoichiometry vector:

Propensity function:

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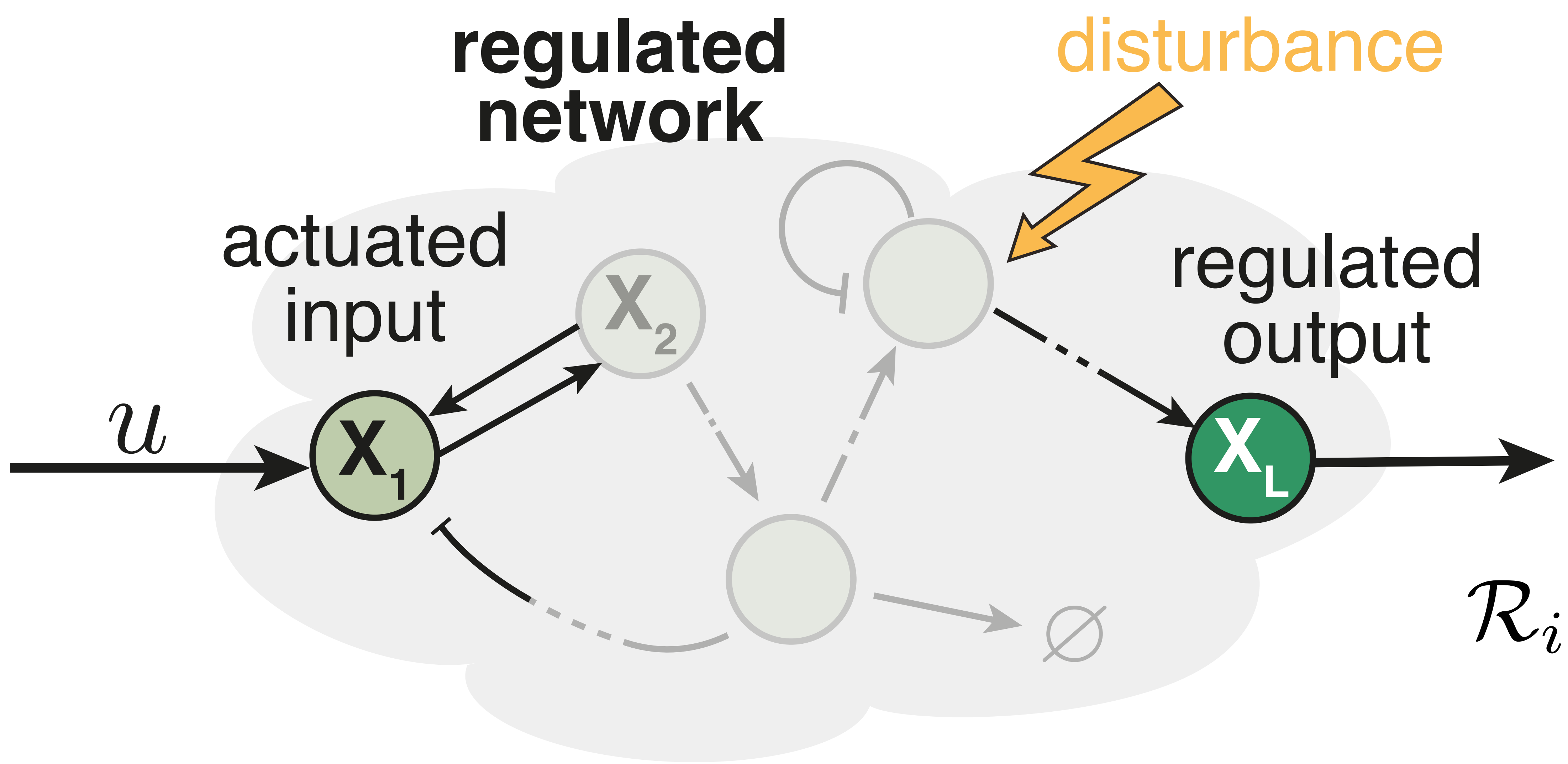

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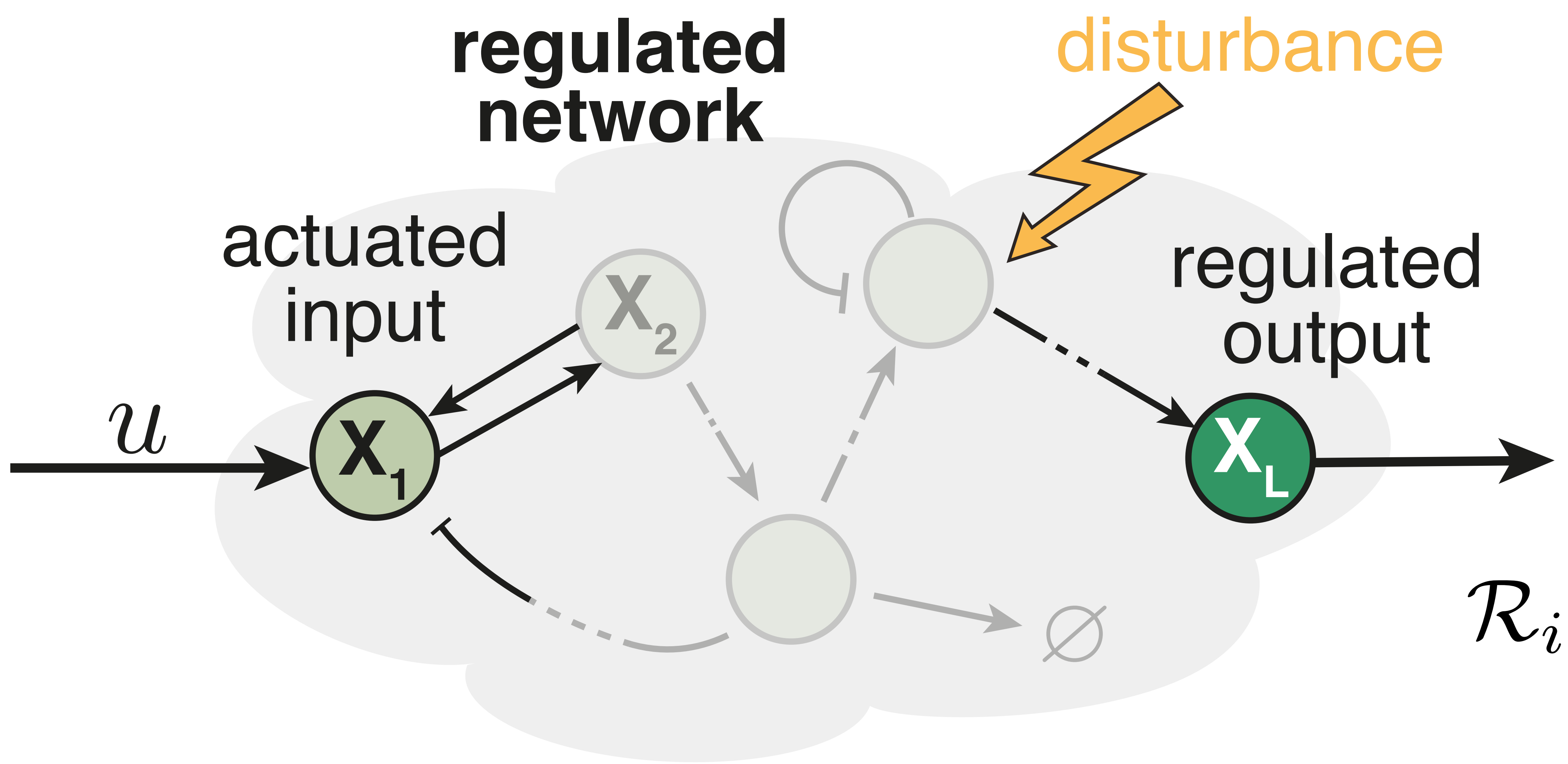

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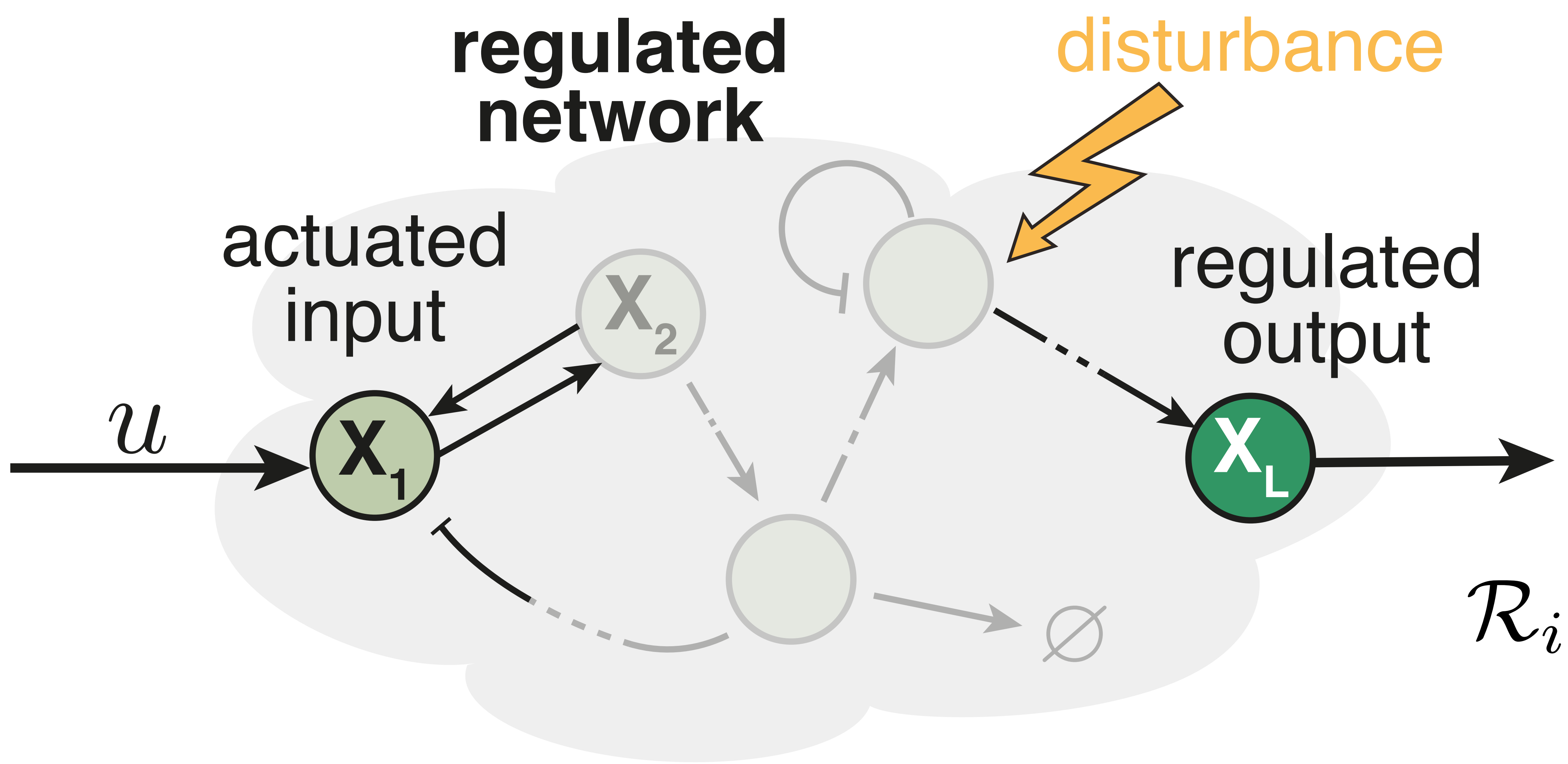

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Propensity function: $\lambda_i : \mathbb{R}_+^L \times \mathbb{R}_+ \to \mathbb{R}_+$

Deterministic: $\dot{x} = \sum_{i=1}^{n} s_i \lambda_i(x, u)$ $(x \in \mathbb{R}_+)$ Nonlinear ODEs

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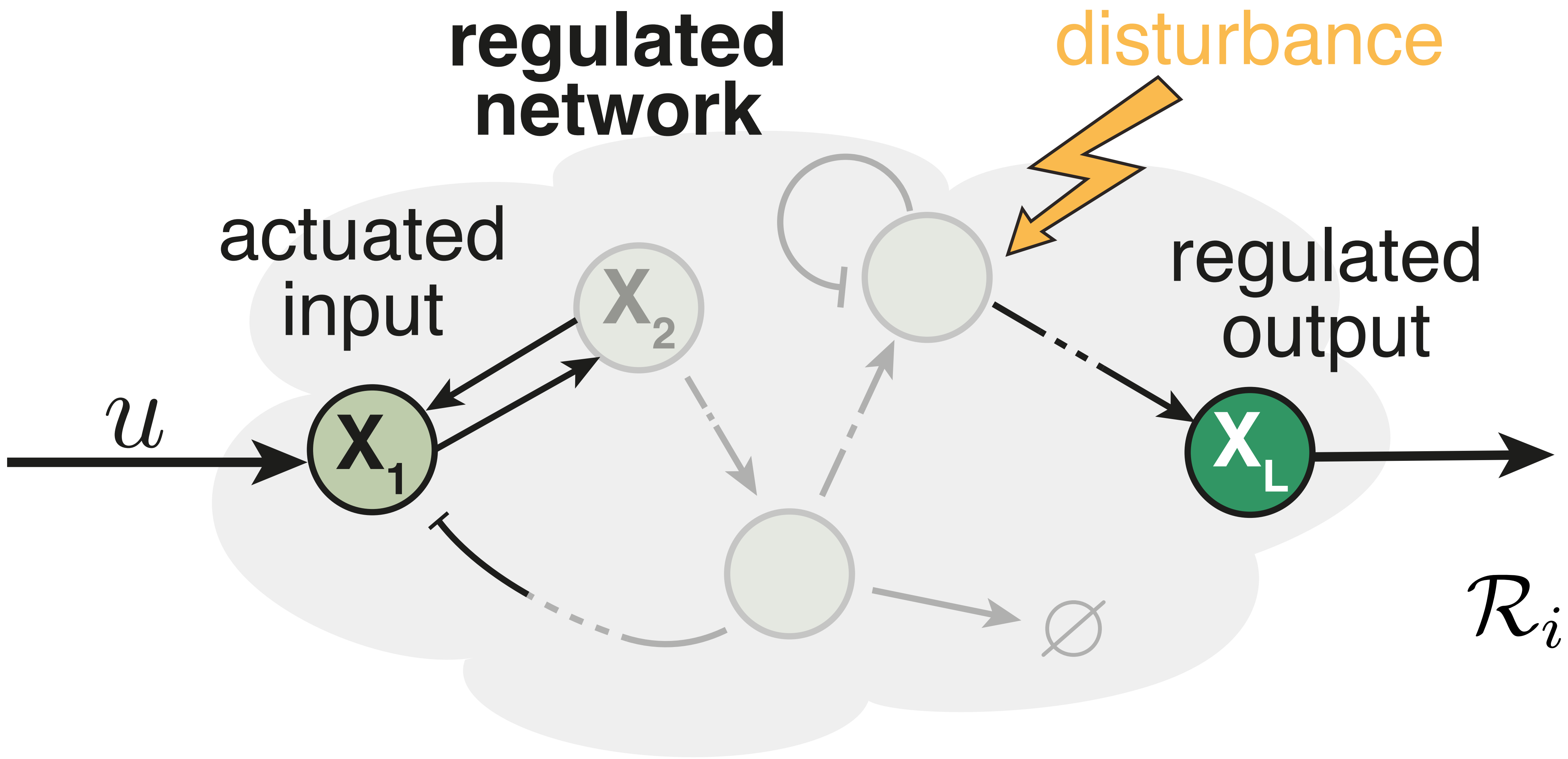

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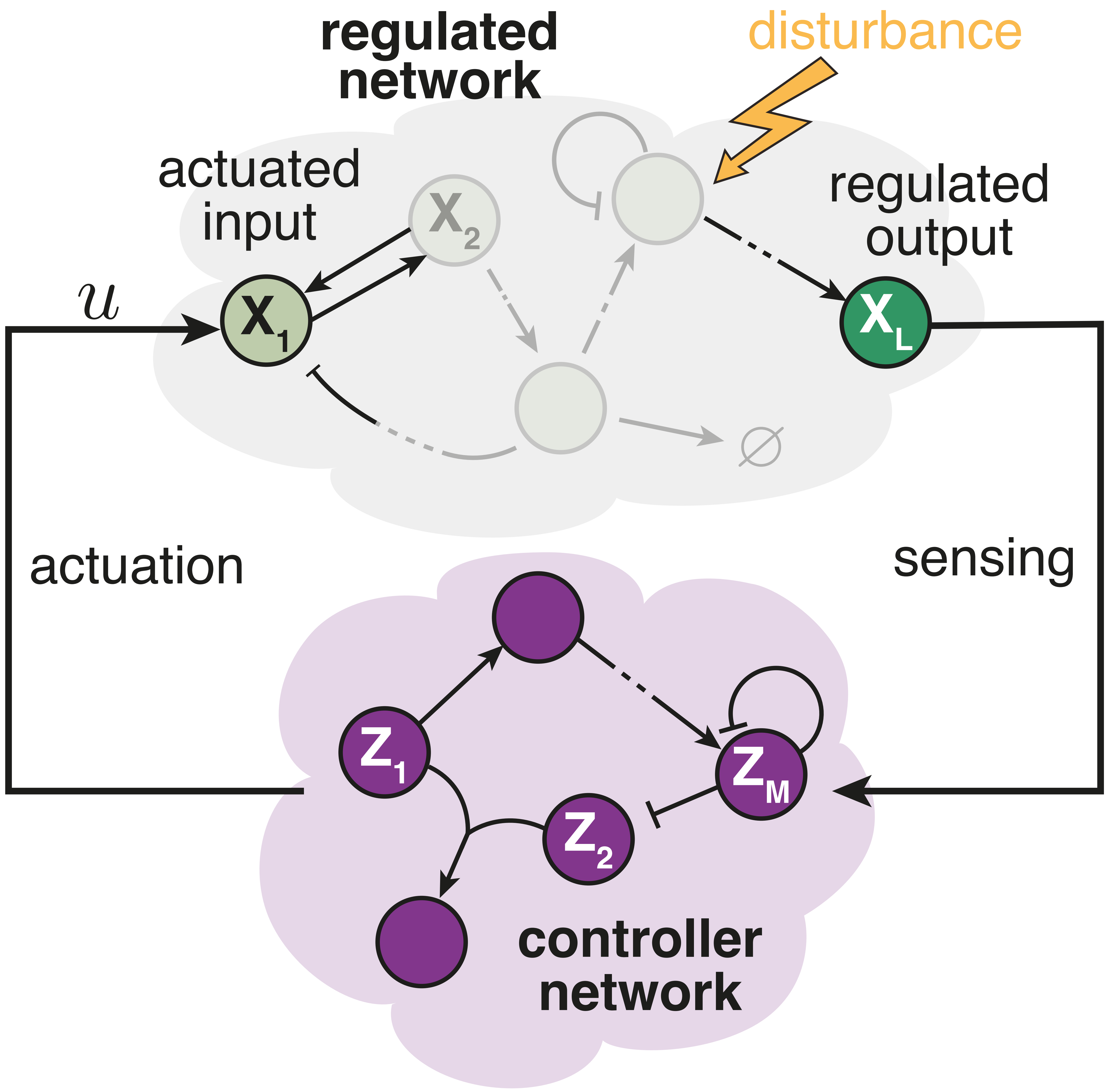
Stochastic: $\dot{p}_x(t) = \sum_{i=1}^{n} \lambda_i(x - s_i, u) p_{x-s_i}(t) - \lambda_i(x, u) p_x(t)$ $\quad (x \in \mathbb{N}_+)$ Chemical Master Equation (for a CTMC)

Biomolecular Feedback Controllers: Framework & Goals



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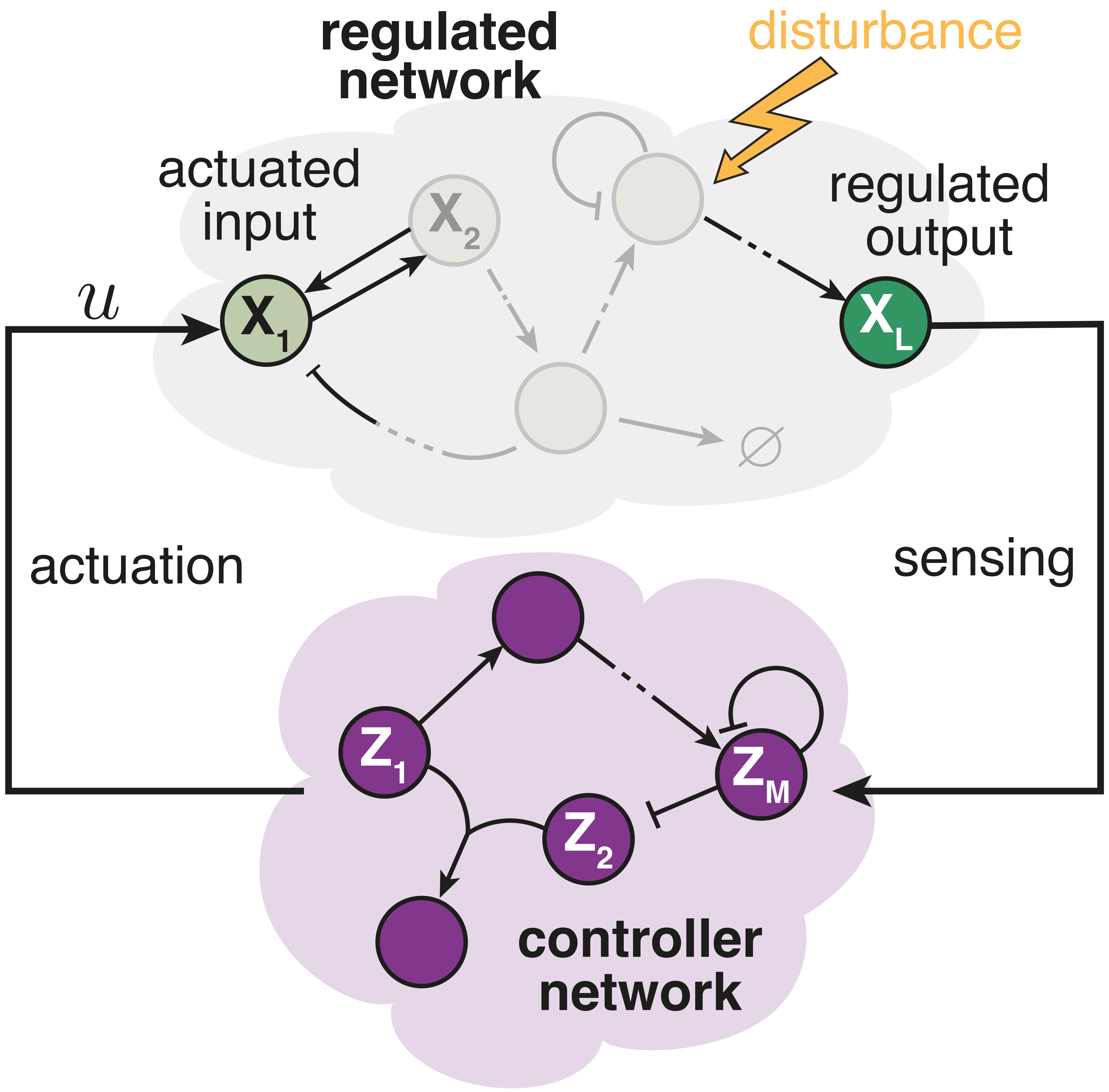

Goal: Design controller reaction networks that

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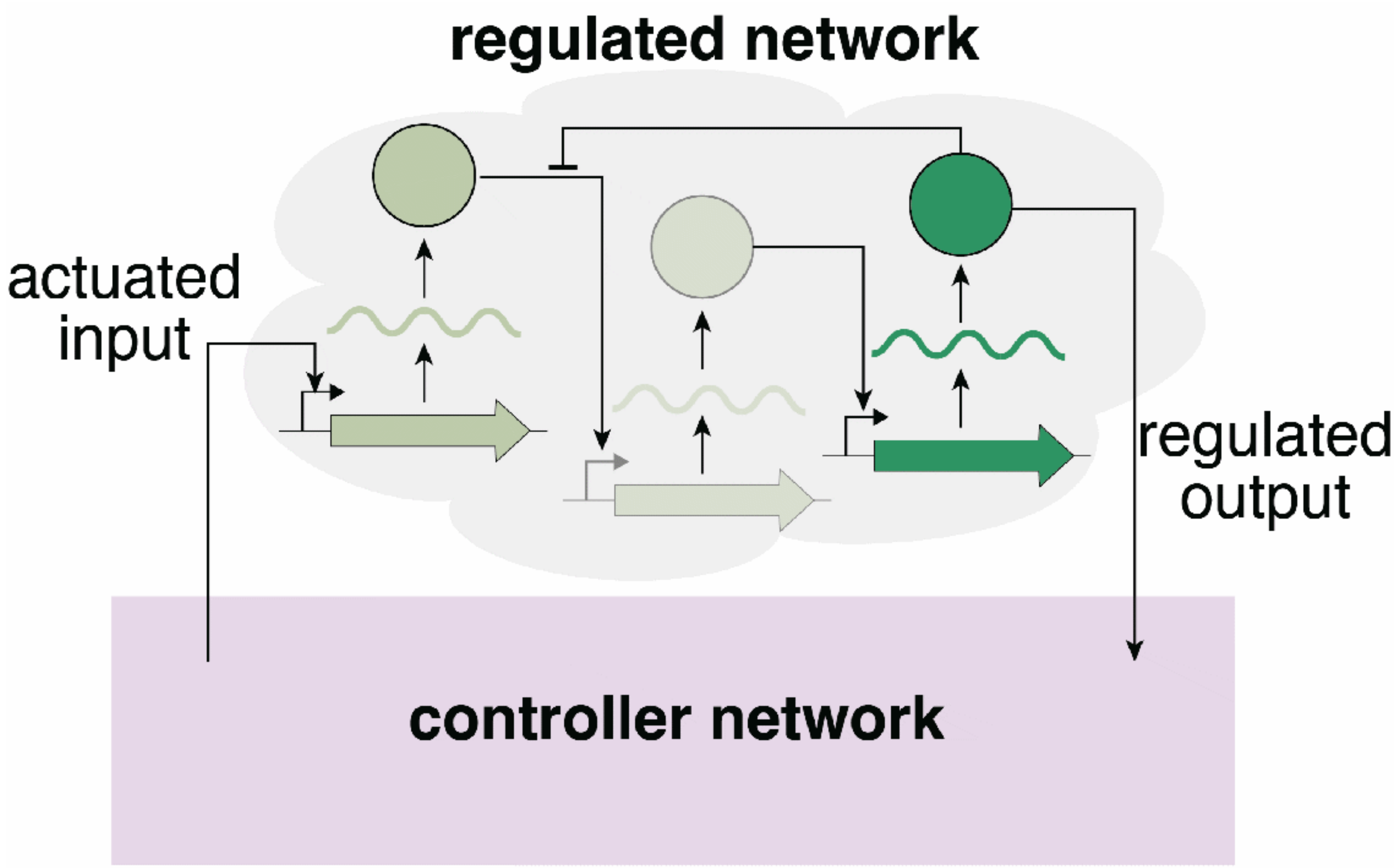

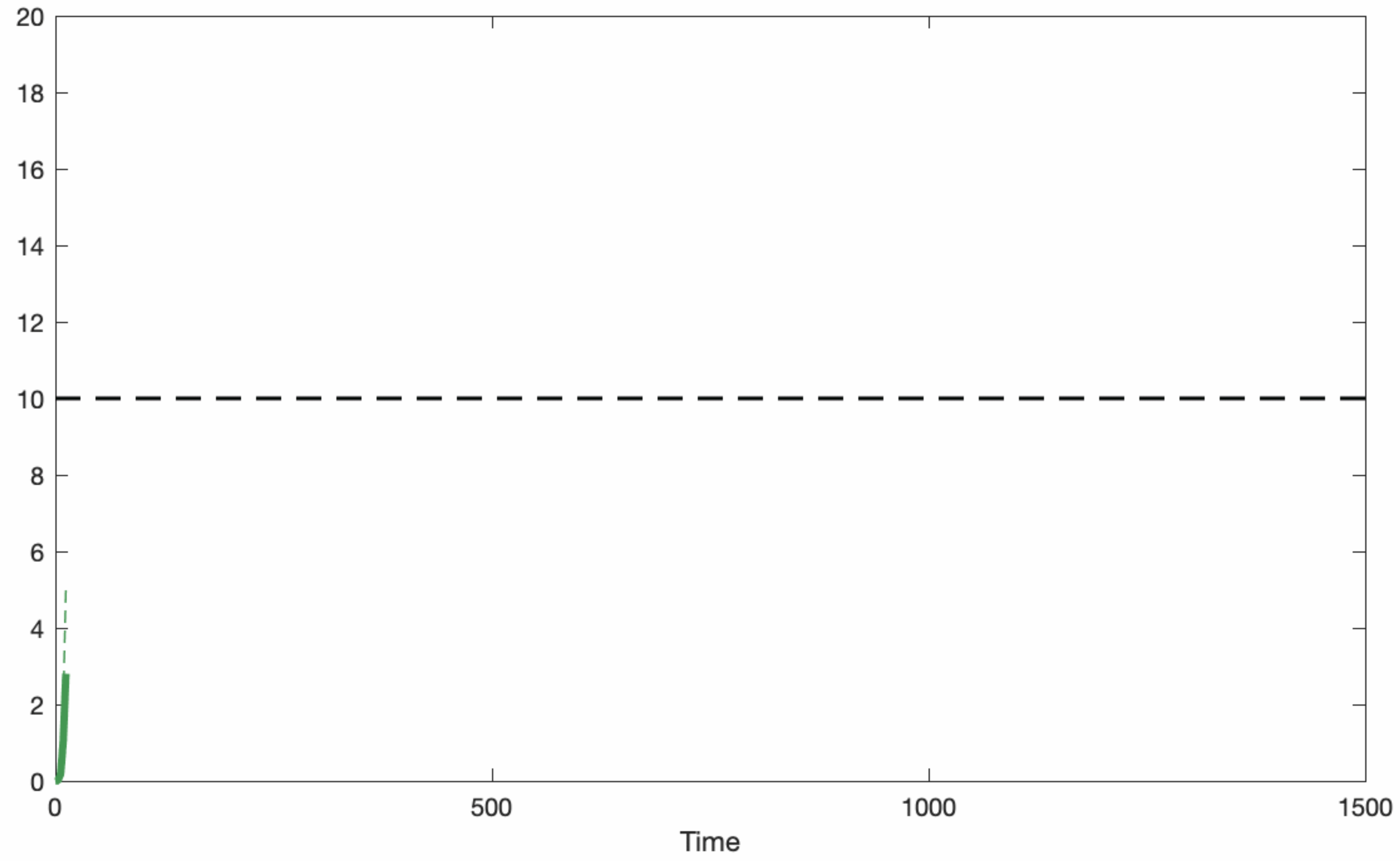

Goal: Design controller reaction networks that

(1) achieve robust steady-state tracking despite:

- ✦ disturbances
- ✦ unknown initial conditions
- ✦ uncertain regulated network

a.k.a. Robust Perfect Adaptation (RPA)

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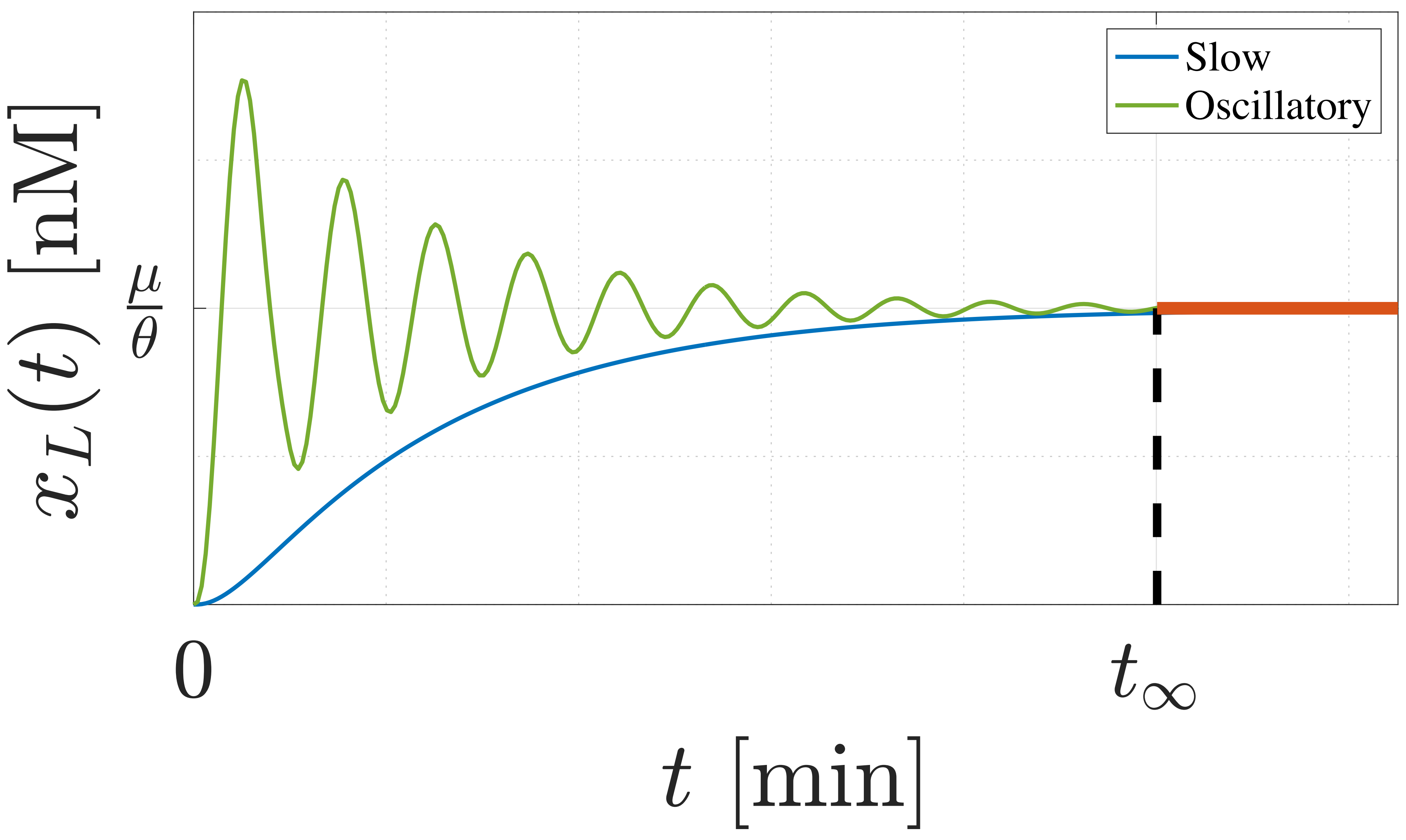

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a.k.a. Robust Perfect Adaptation (RPA)

(2) achieve high dynamic performance

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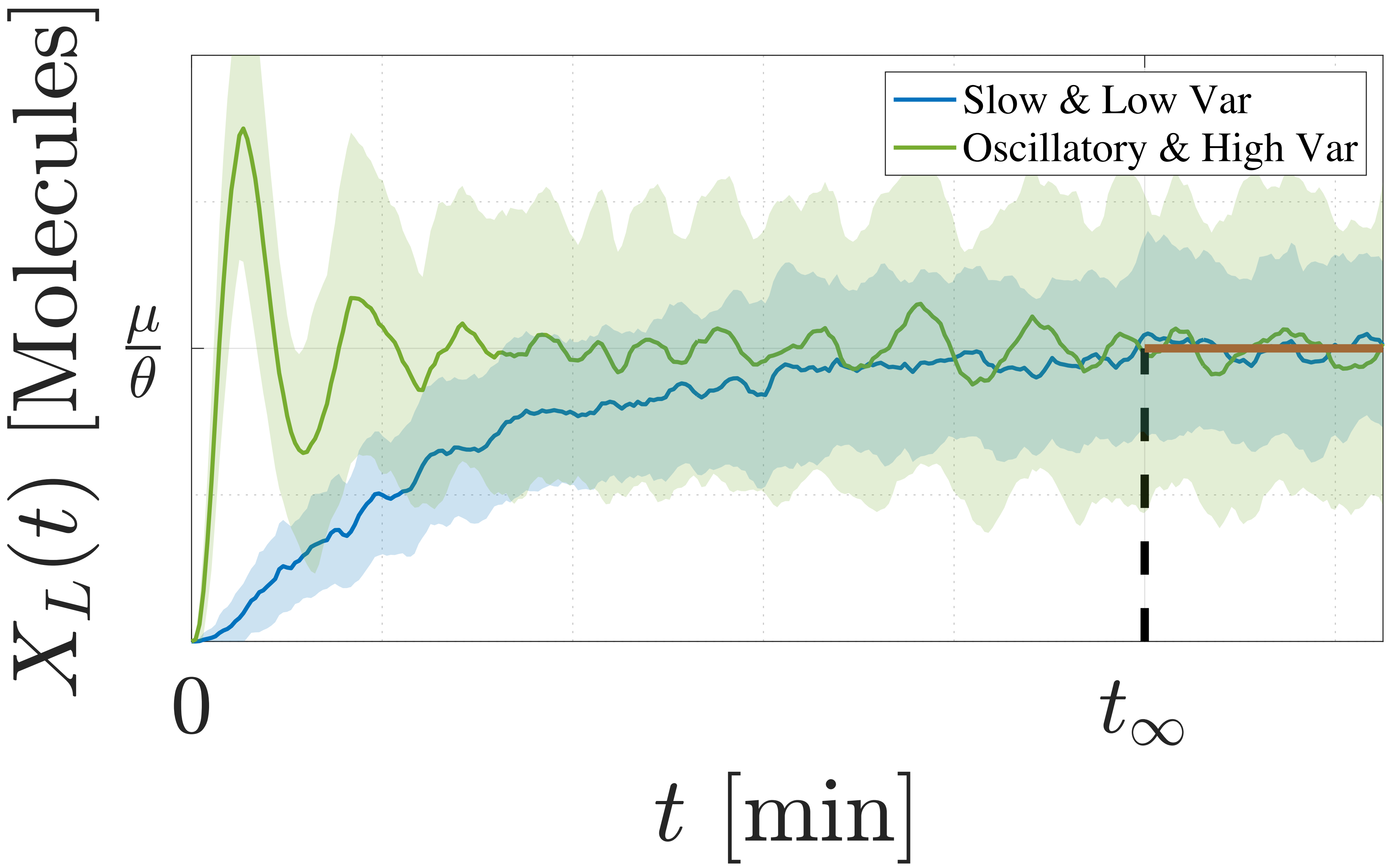

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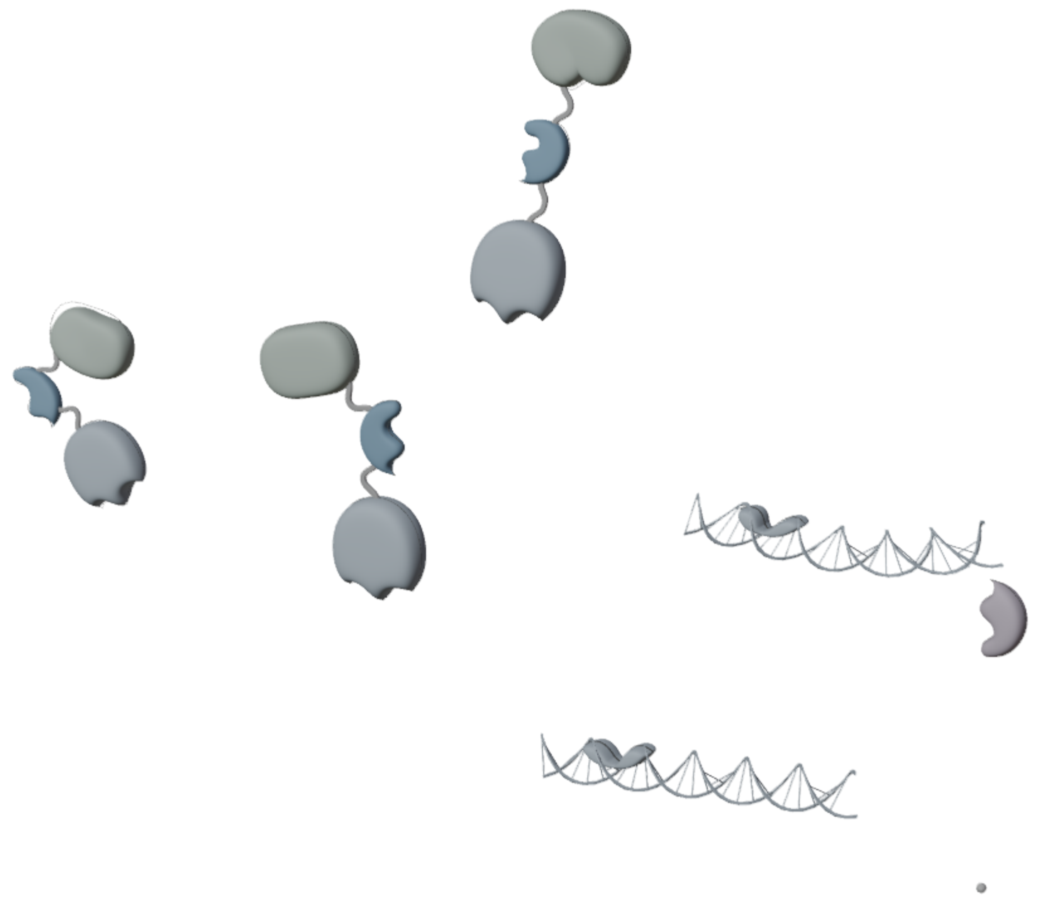
(2) achieve high dynamic performance

(3) attenuate cellular intrinsic noise

Biomolecular Feedback Controllers: Framework & Goals



Goal: Design controller reaction networks that

(1) achieve robust steady-state tracking
despite:
- ✦ disturbances
- ✦ unknown initial conditions
- ✦ uncertain regulated network

a.k.a. Robust Perfect Adaptation (RPA)

(2) achieve high dynamic performance

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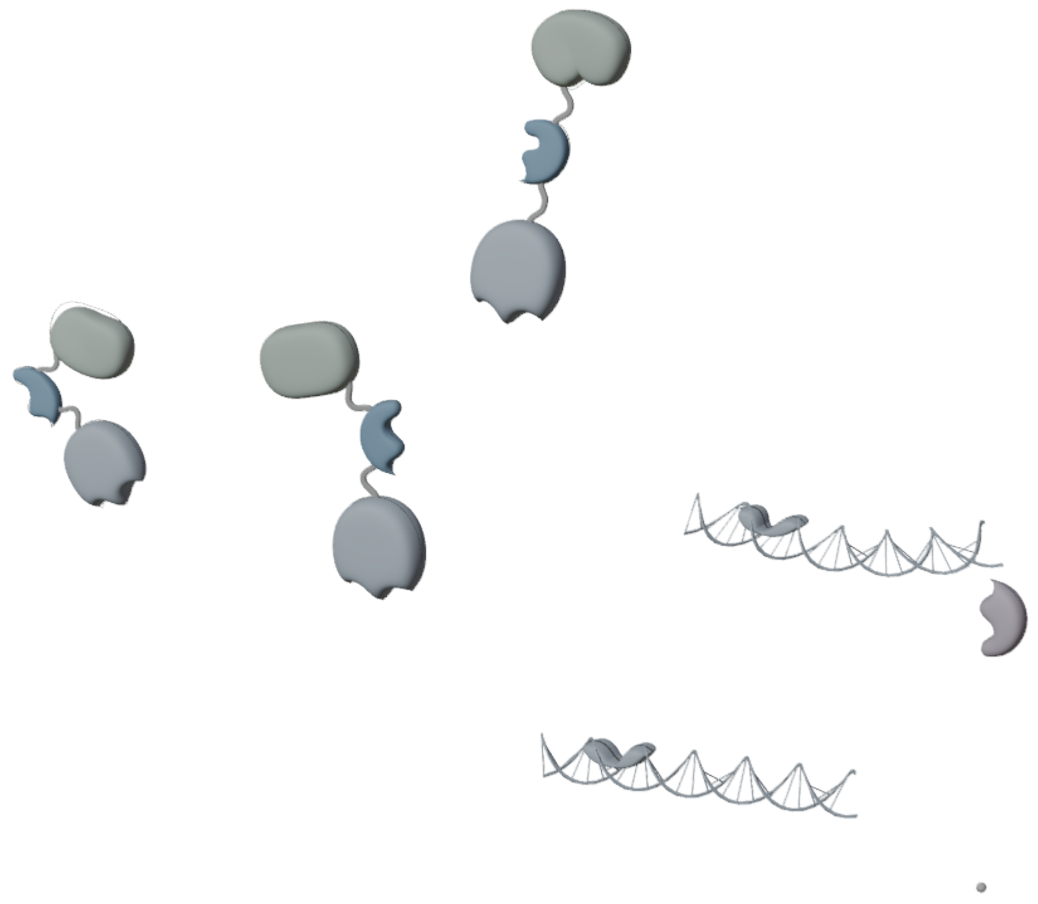

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(2) achieve high dynamic performance

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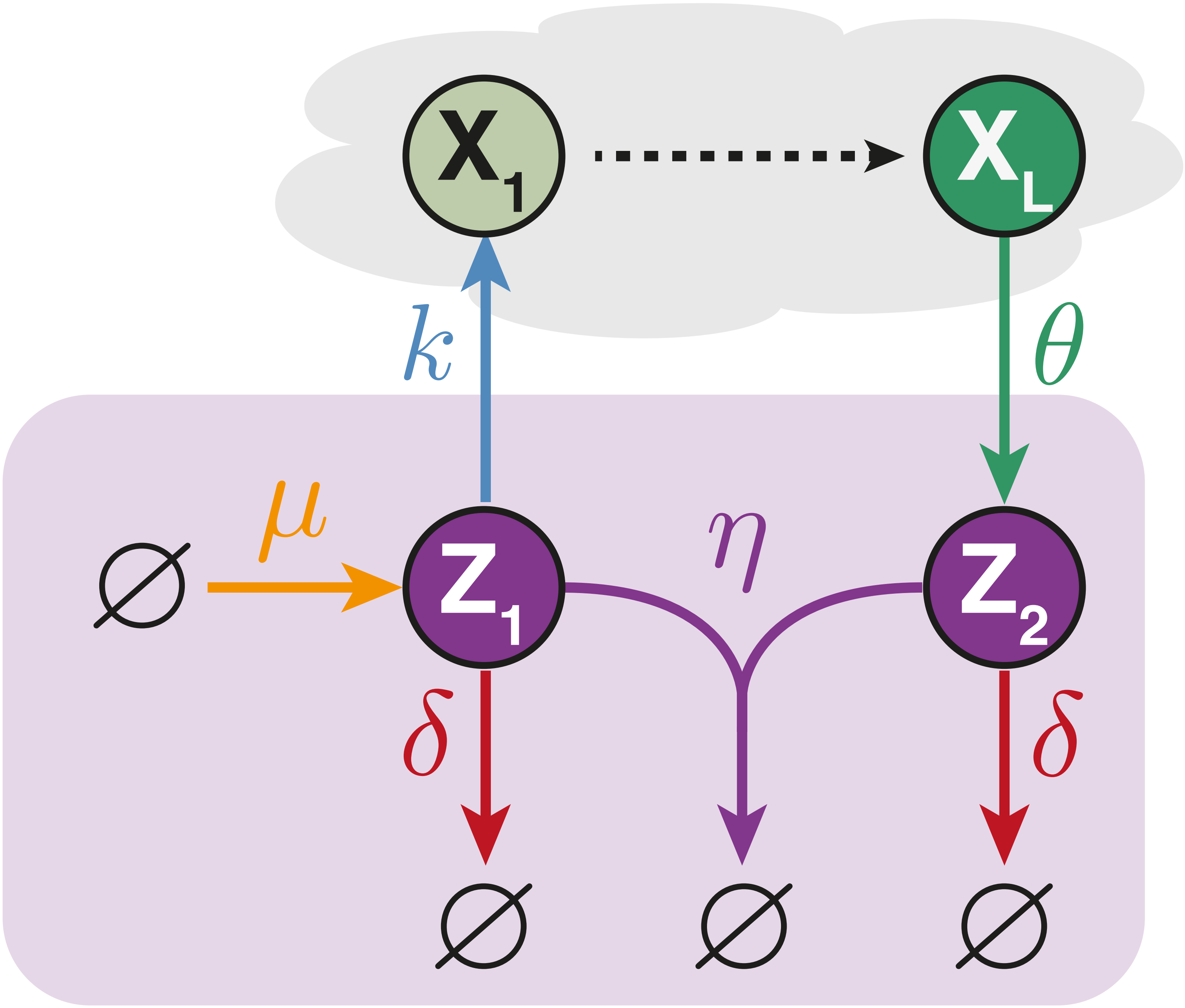
(4) are implementable with genetic parts

(5) are minimal (if possible)

Antithetic Integral Feedback (AIF) Control for RPA



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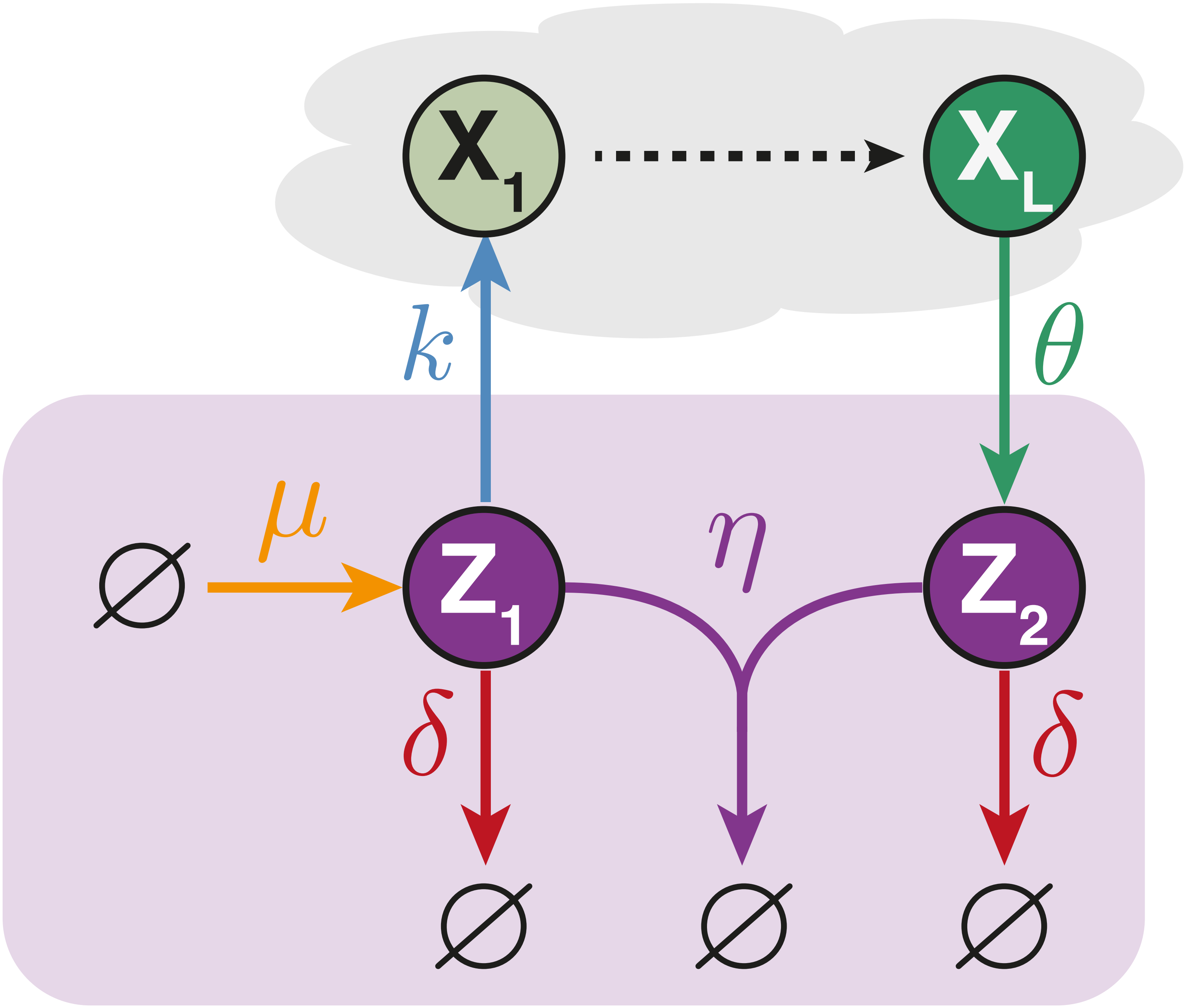

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Actuation	$\mathbf{Z}_1 \xrightarrow{k} \mathbf{Z}_1 + \mathbf{X}_1$
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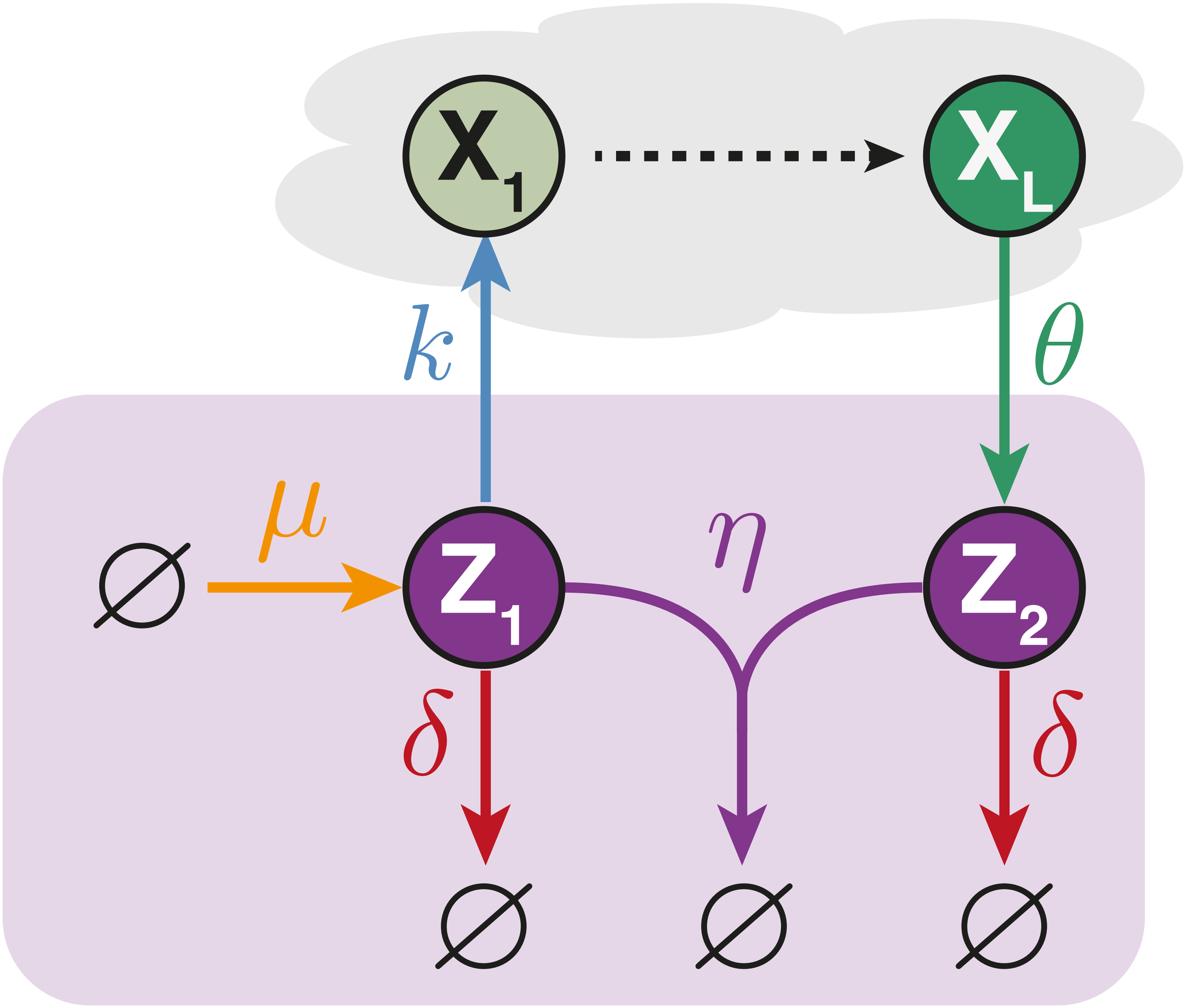

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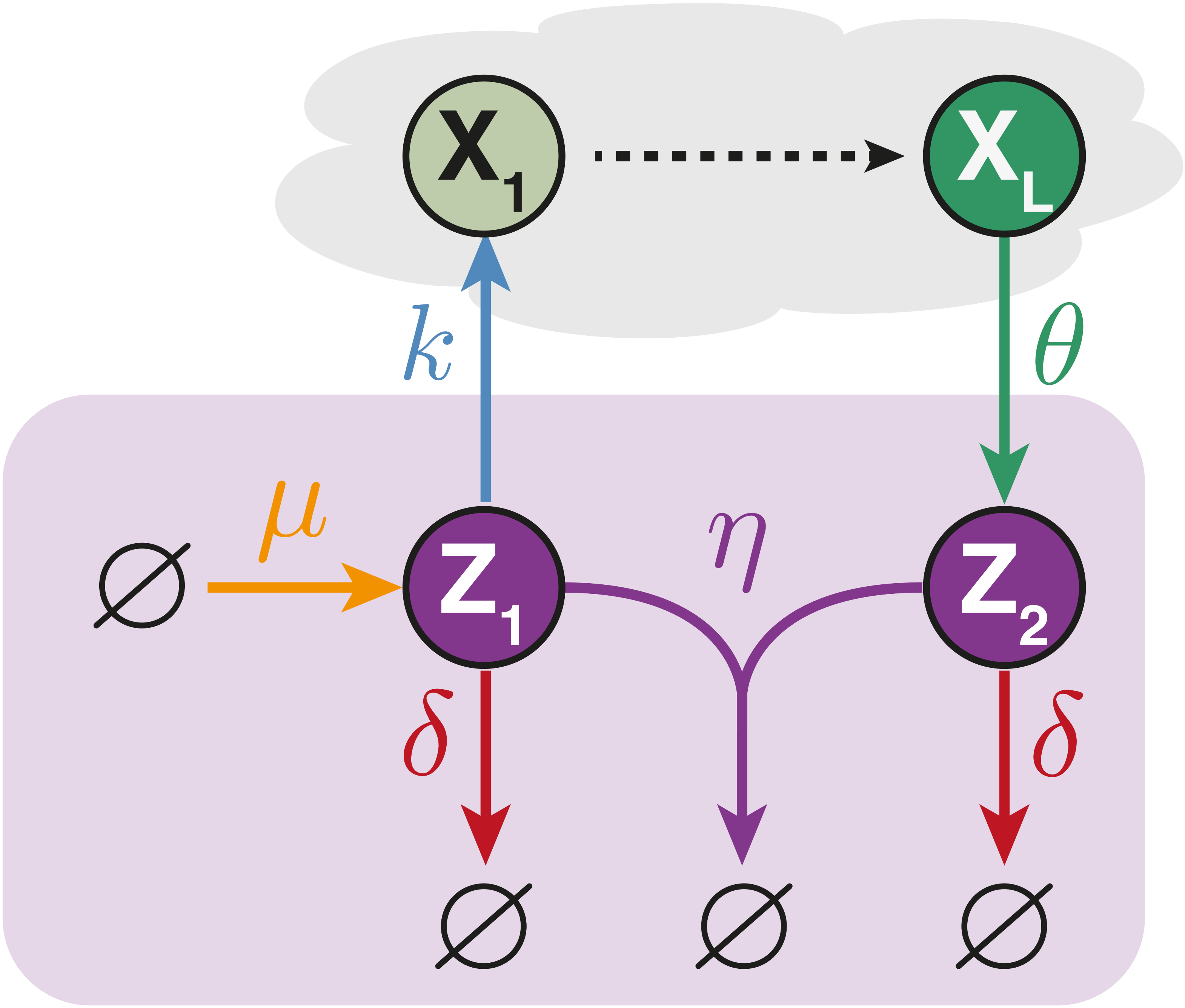

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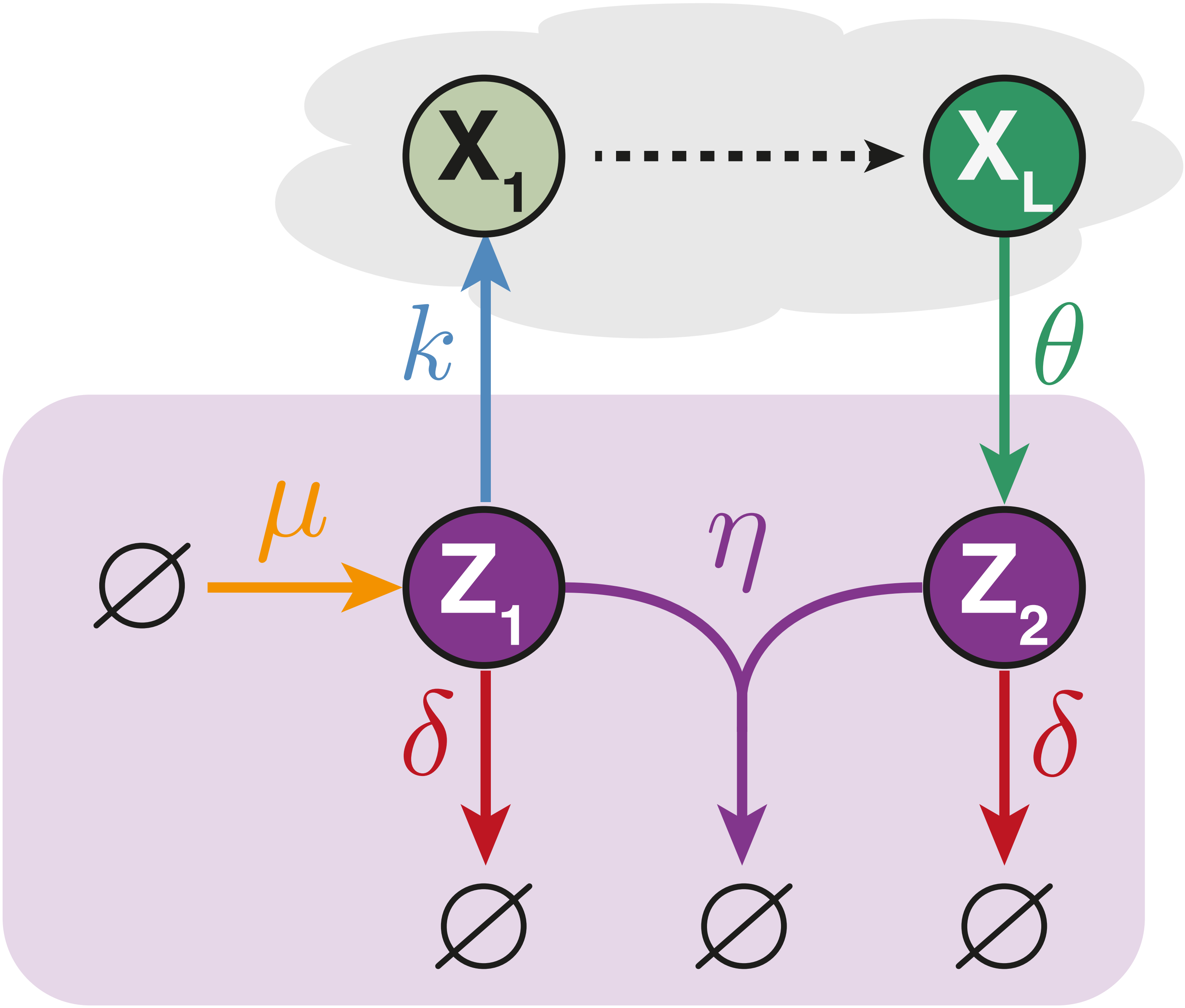

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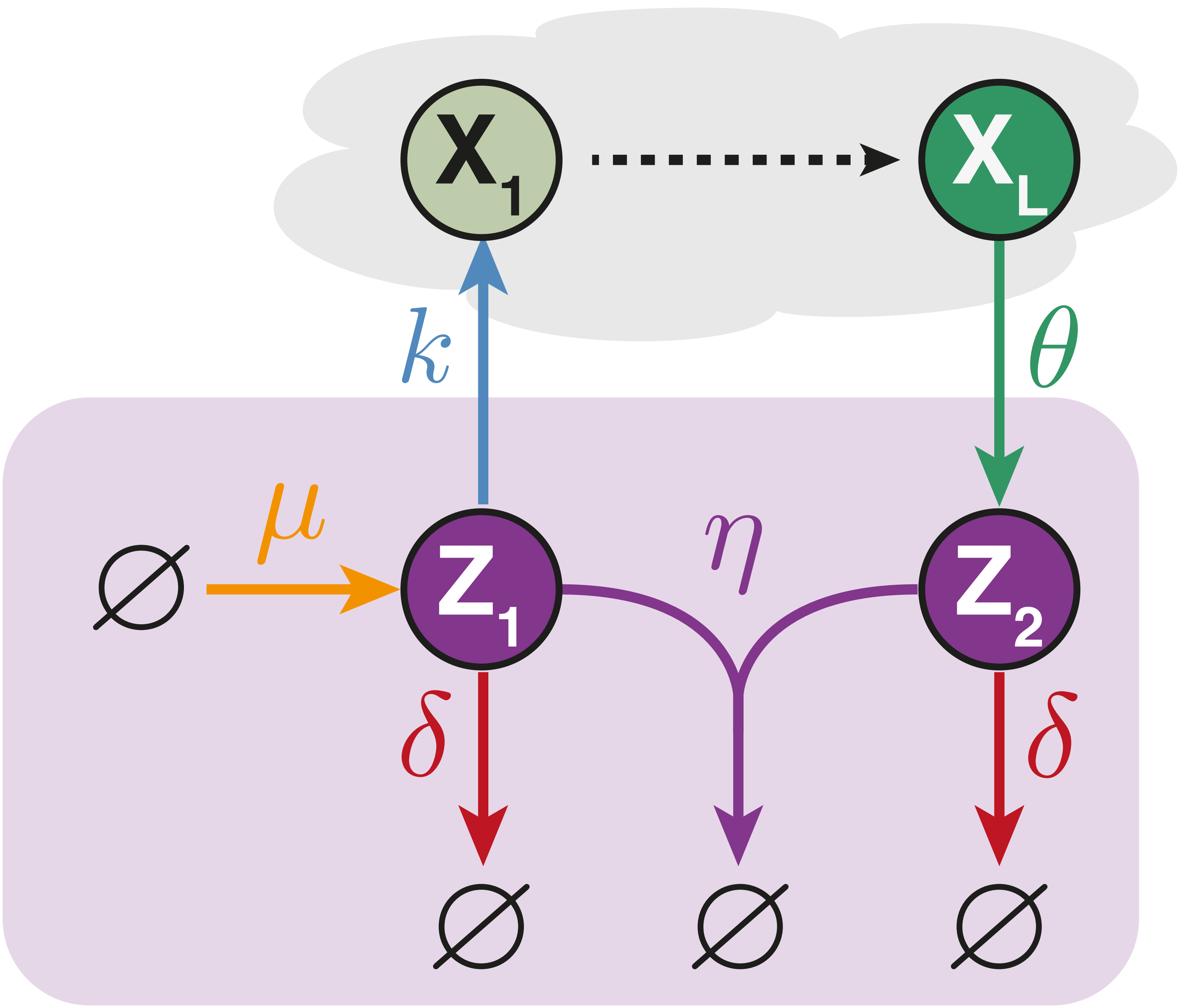

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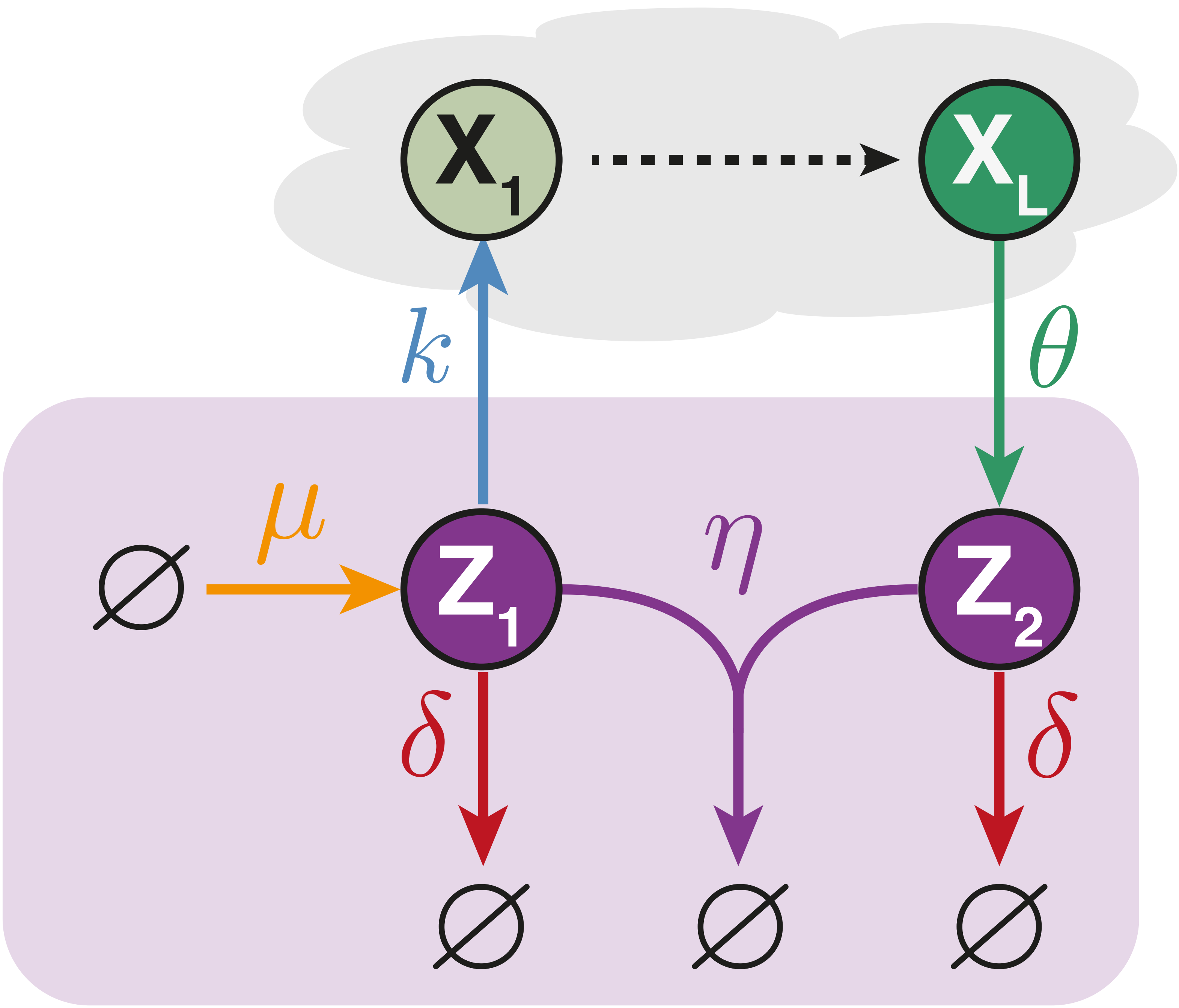

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RPA!

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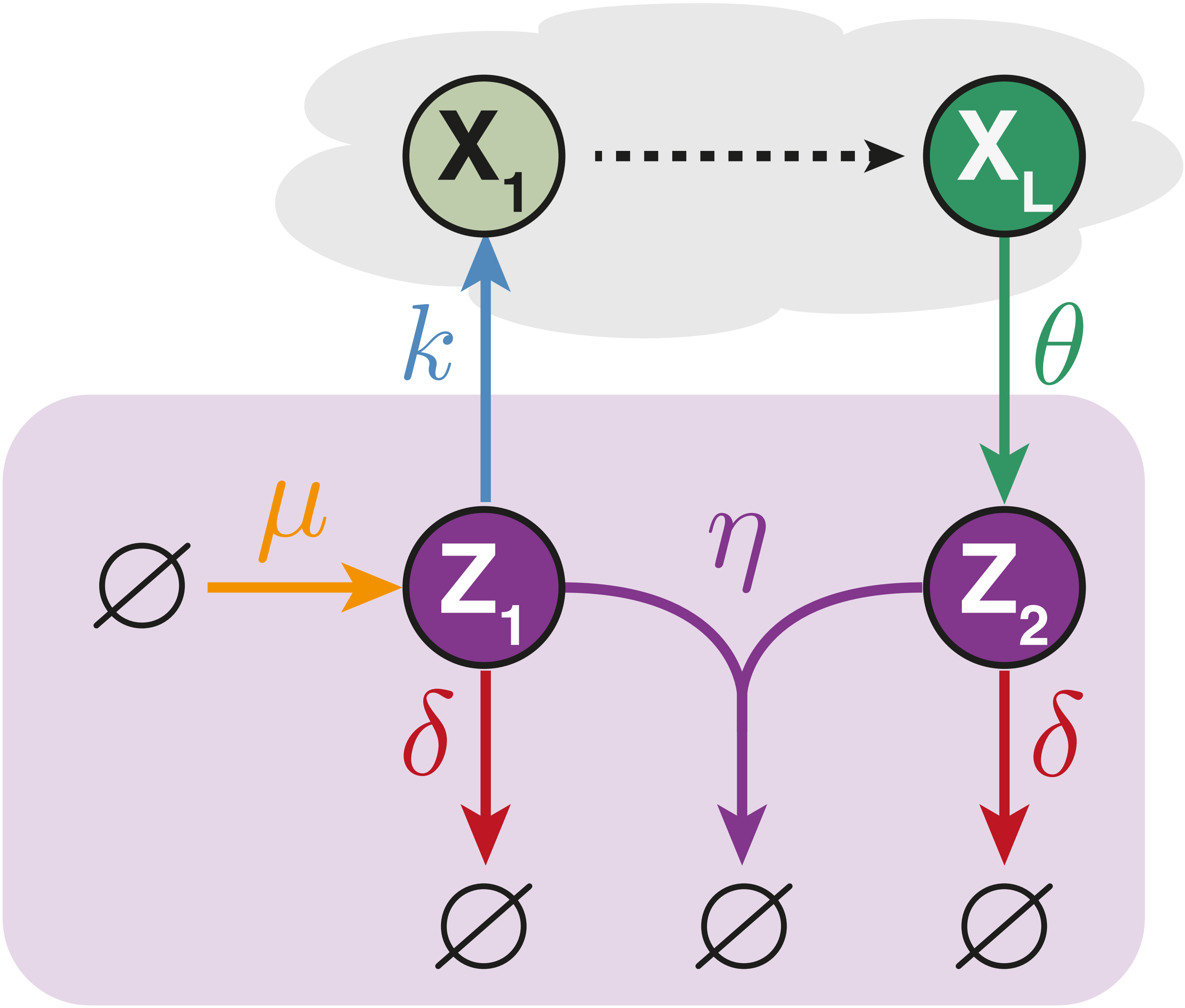

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Note: Removal reactions play the role of the unwanted internal resistance/friction force

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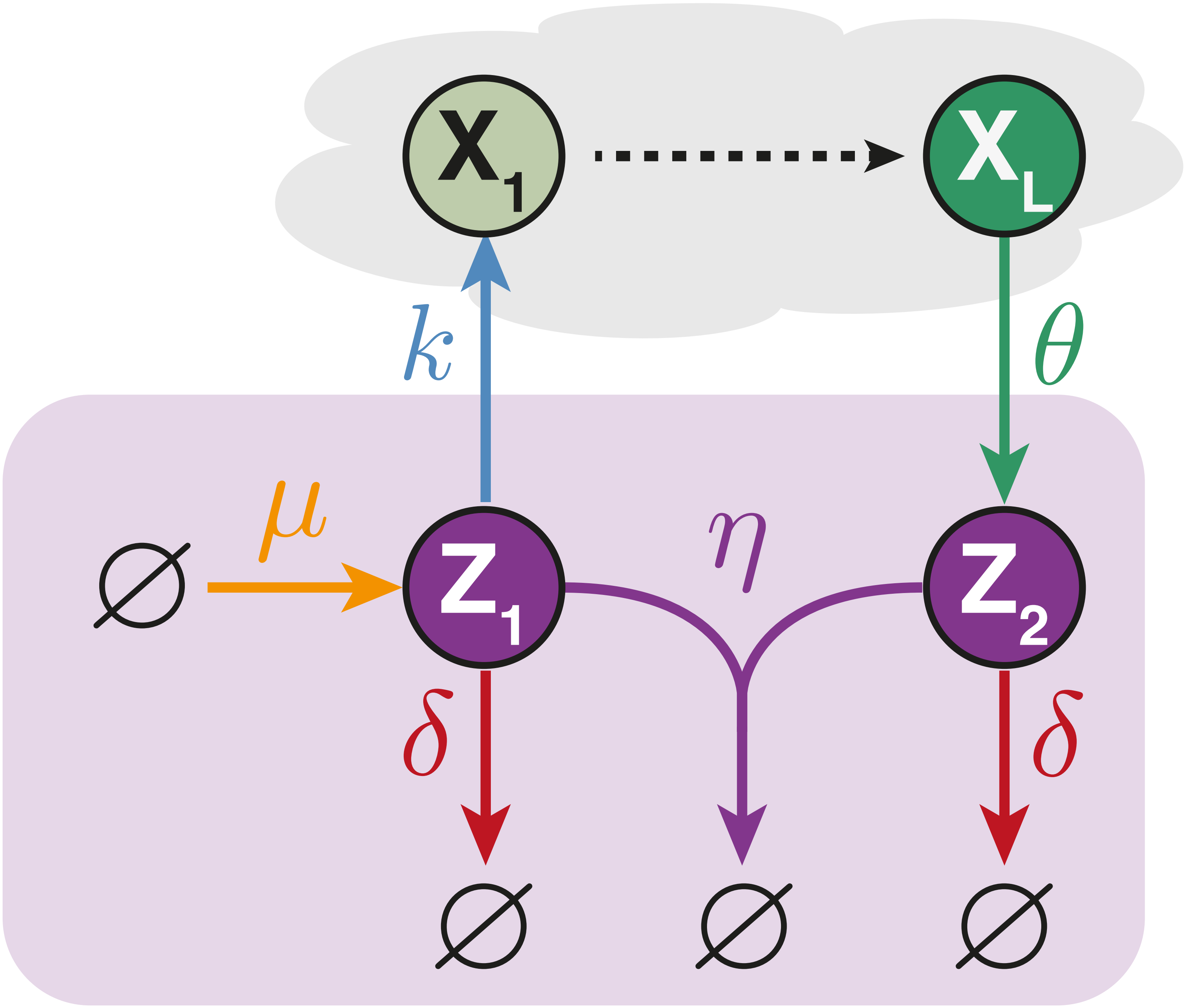

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Stochastic:

$$\dot{\mathbb{E}}[Z_1] = \mu - \eta\mathbb{E}[Z_1 Z_2] - \delta\mathbb{E}[Z_1]$$

$$\dot{\mathbb{E}}[Z_2] = \theta\mathbb{E}[X_L] - \eta\mathbb{E}[Z_1 Z_2] - \delta\mathbb{E}[Z_2]$$

$$\underset{\delta \to 0}{\overset{\text{Ergodicity}}{\Longrightarrow}} \quad \mathbb{E}[X_L] \xrightarrow[t\to\infty]{} \frac{\mu}{\theta}$$

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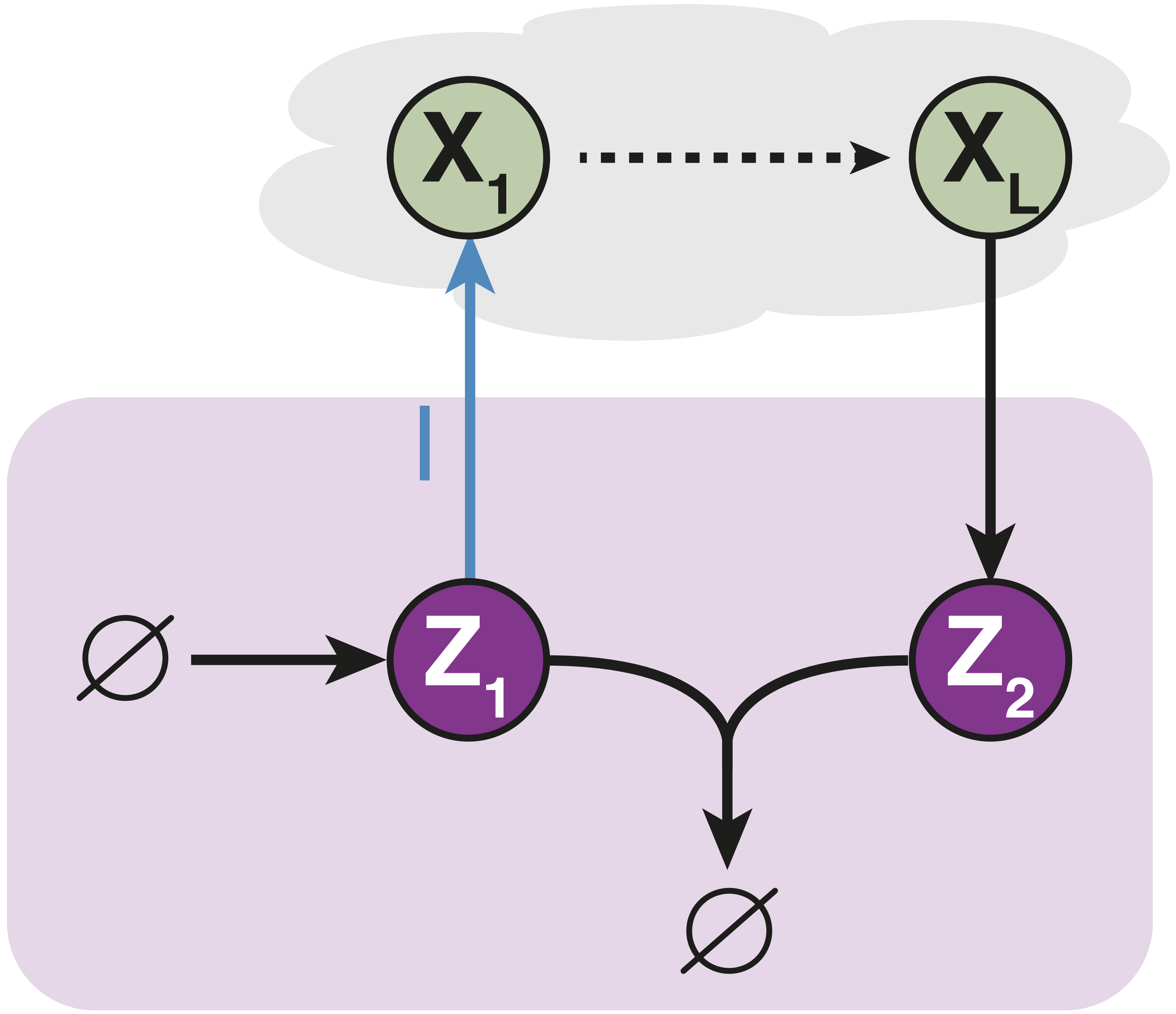
Molecular PID Controllers for Enhancing Dynamic Performance



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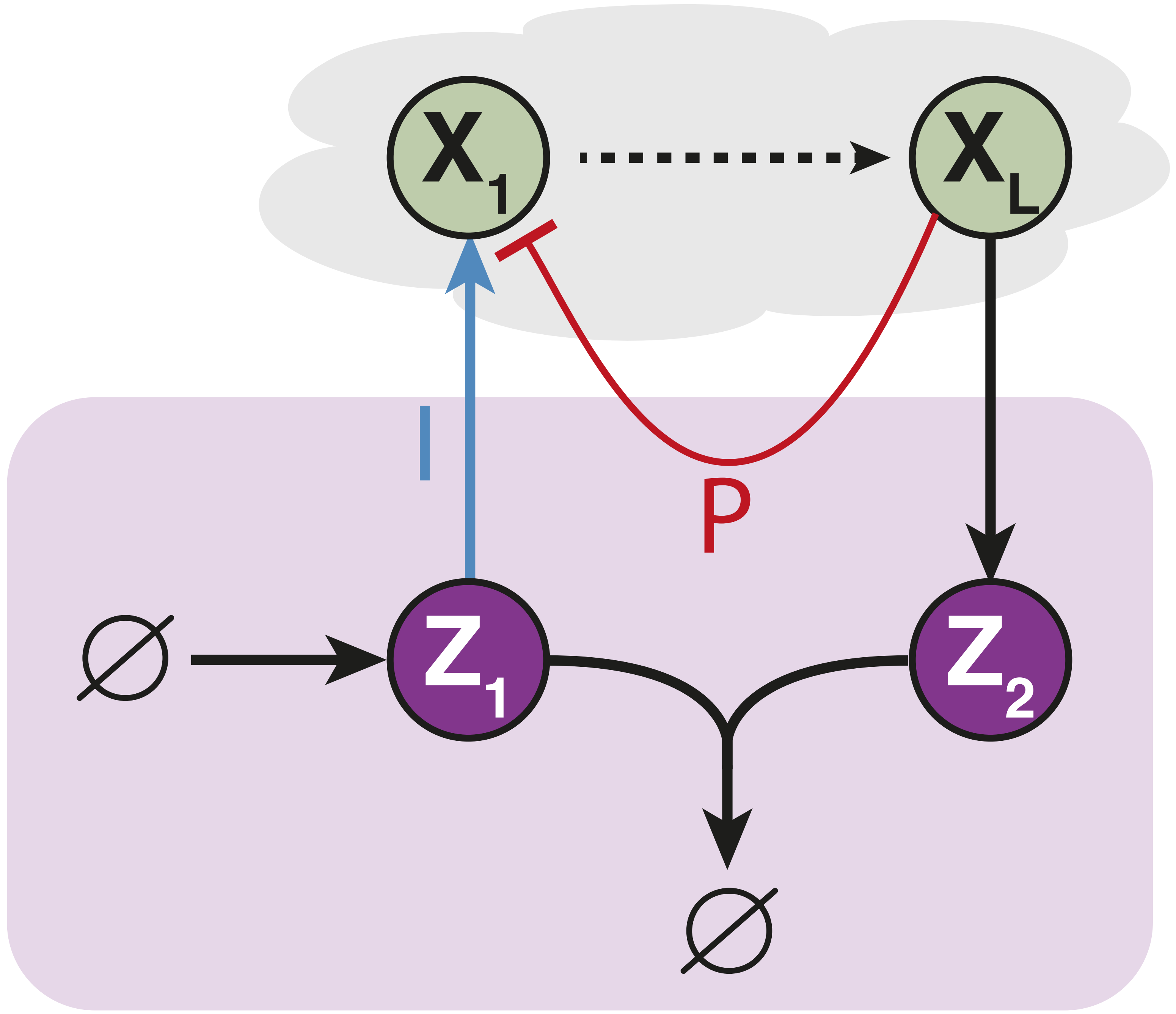

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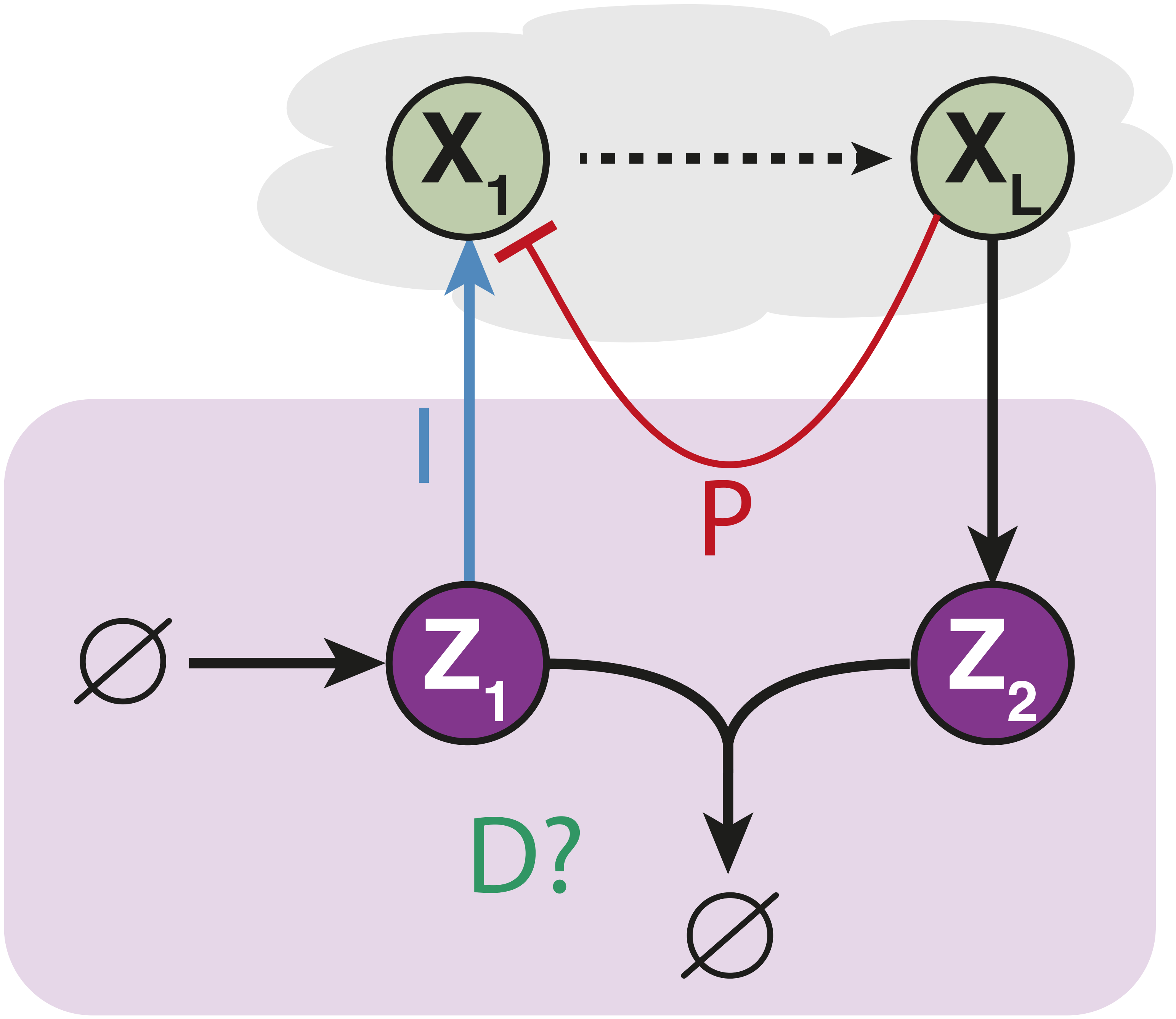

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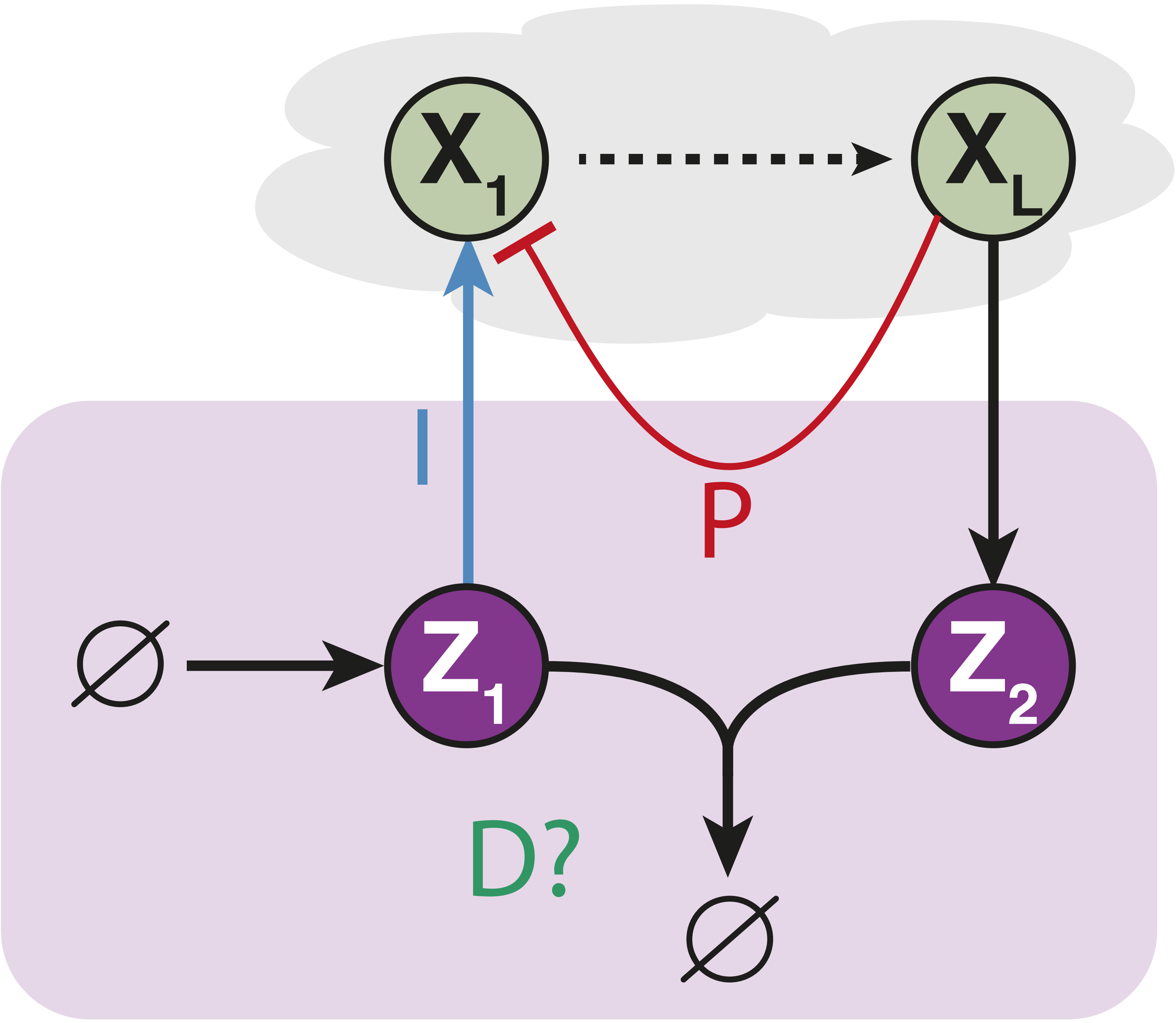
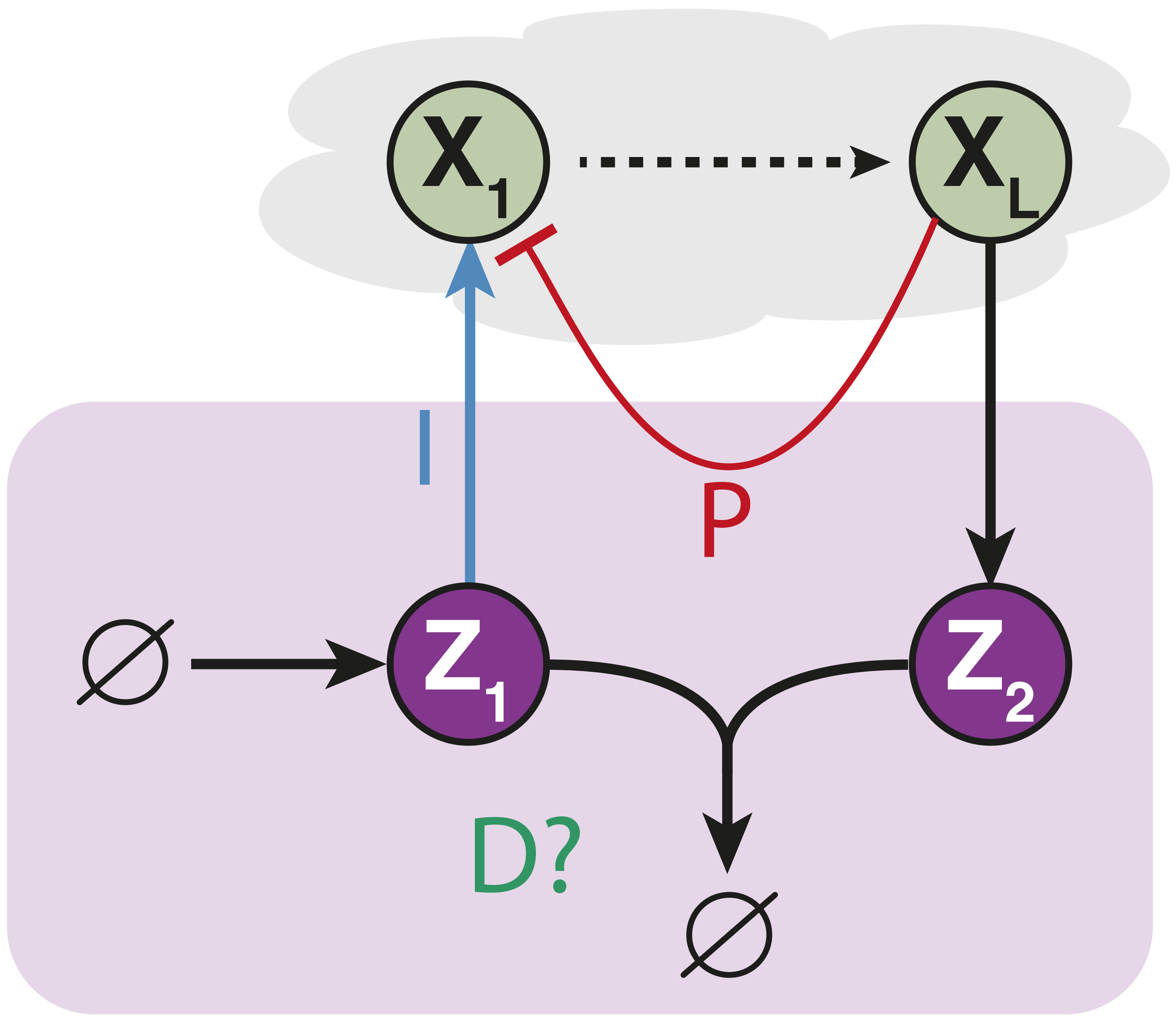


Two approaches to realize the D:

Molecular PID Controllers for Enhancing Dynamic Performance



Two approaches to realize the D:

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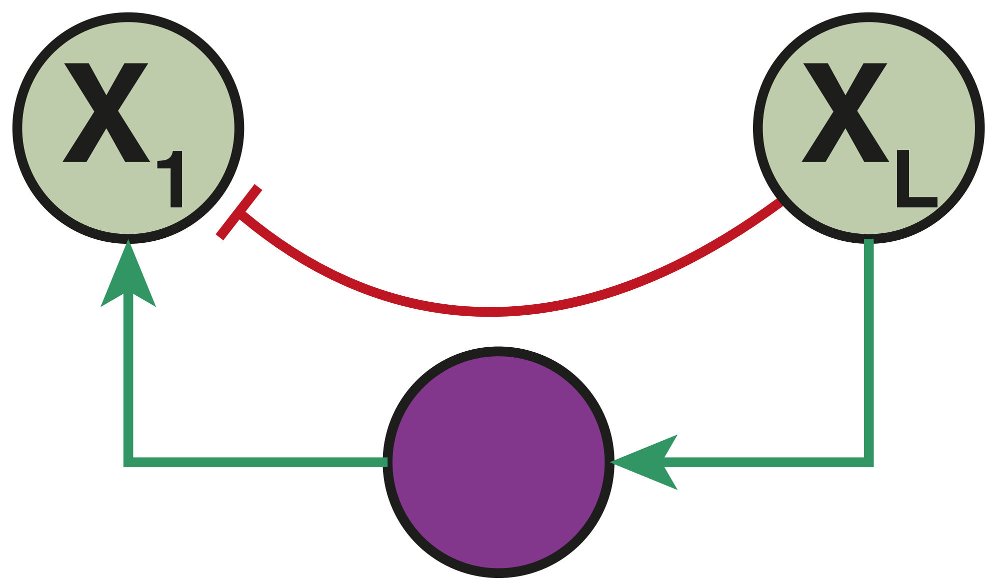

Filtered PD

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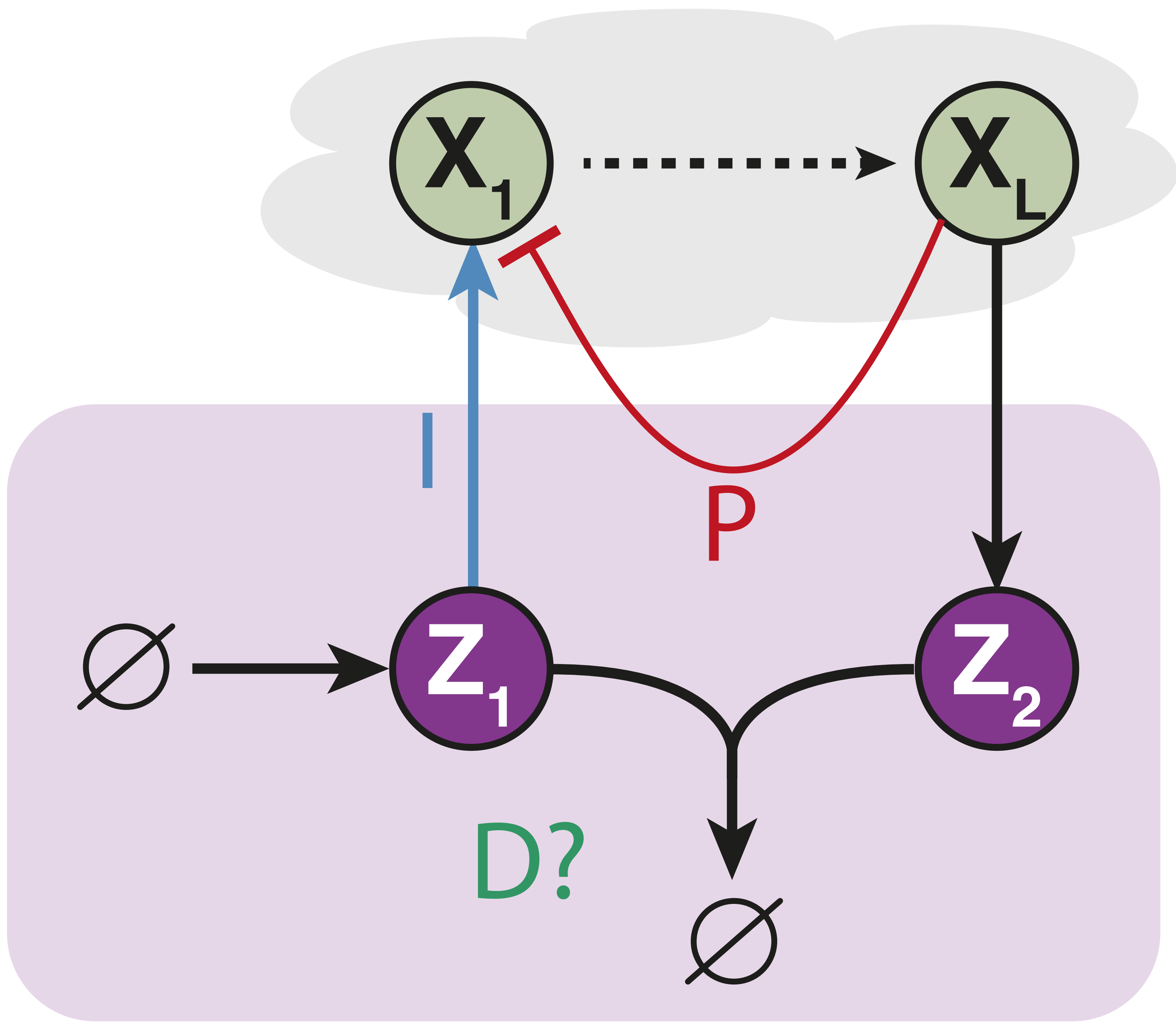

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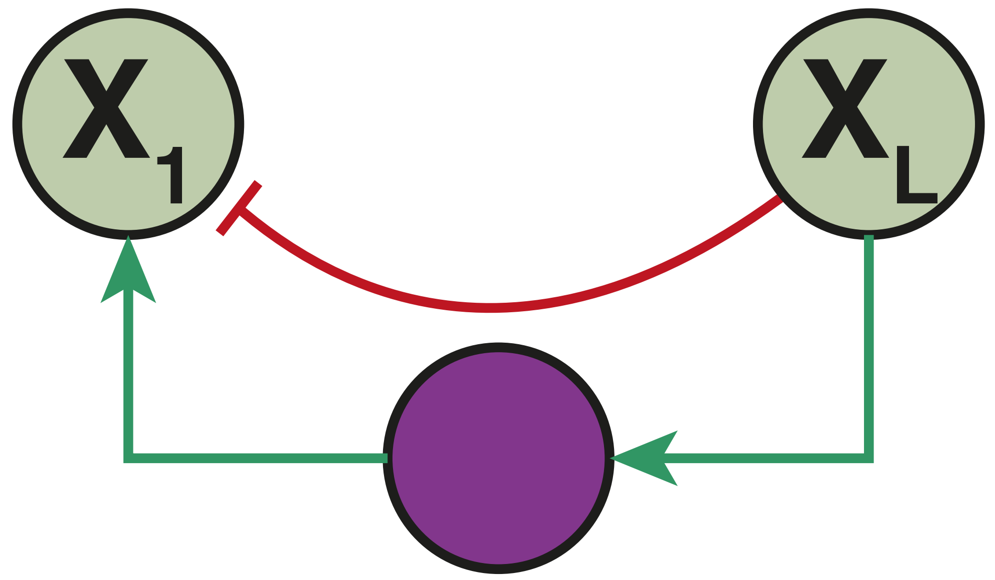
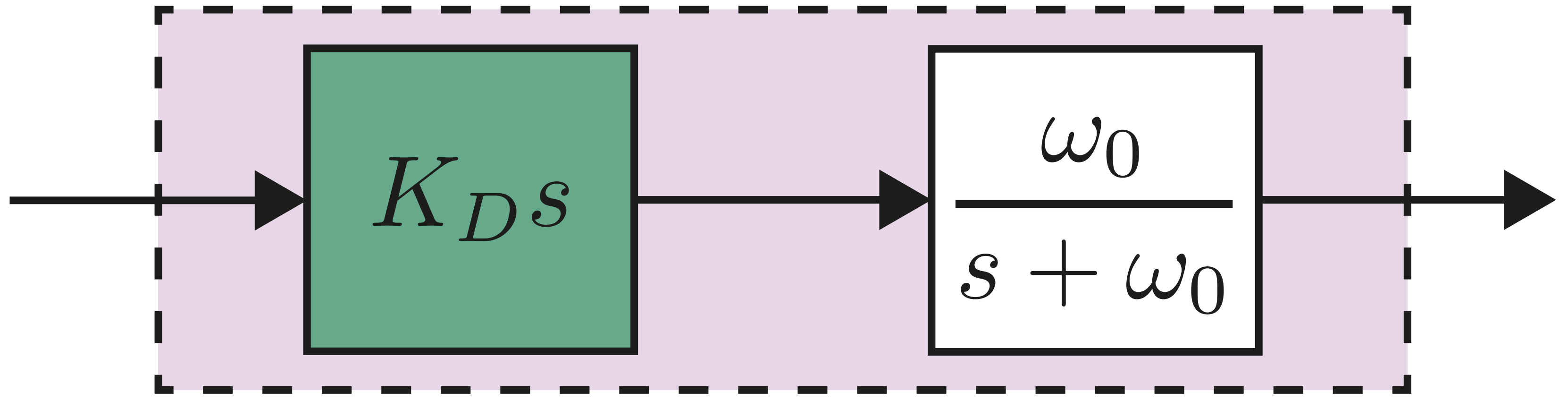


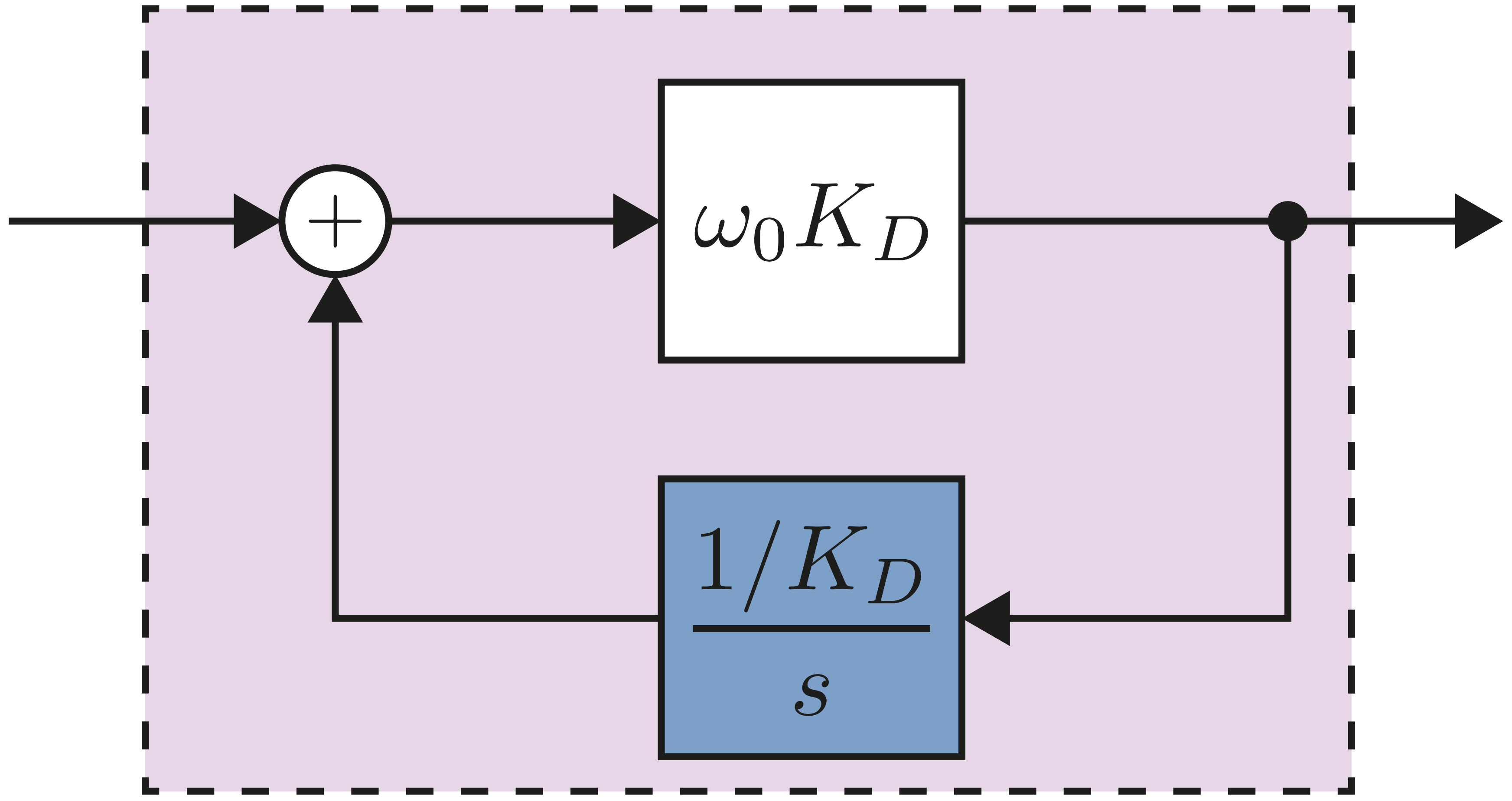
Filtered PD

(2) Integrator in Feedback

Filtered D



$$K_D s \frac{\omega_0}{s+\omega_0}$$



A Hierarchy of Molecular PID Controllers

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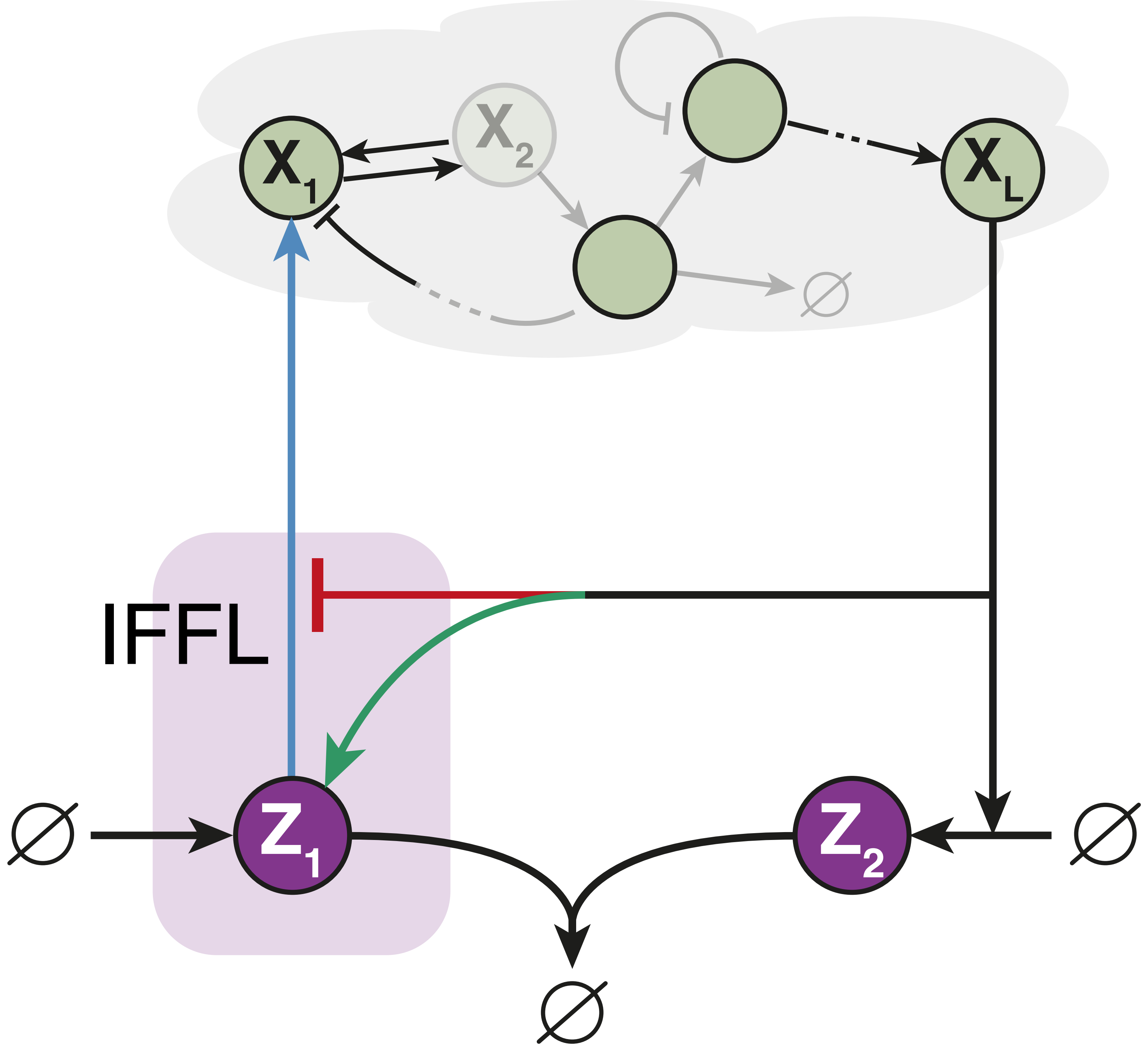
2nd- order
IFFL-based PID



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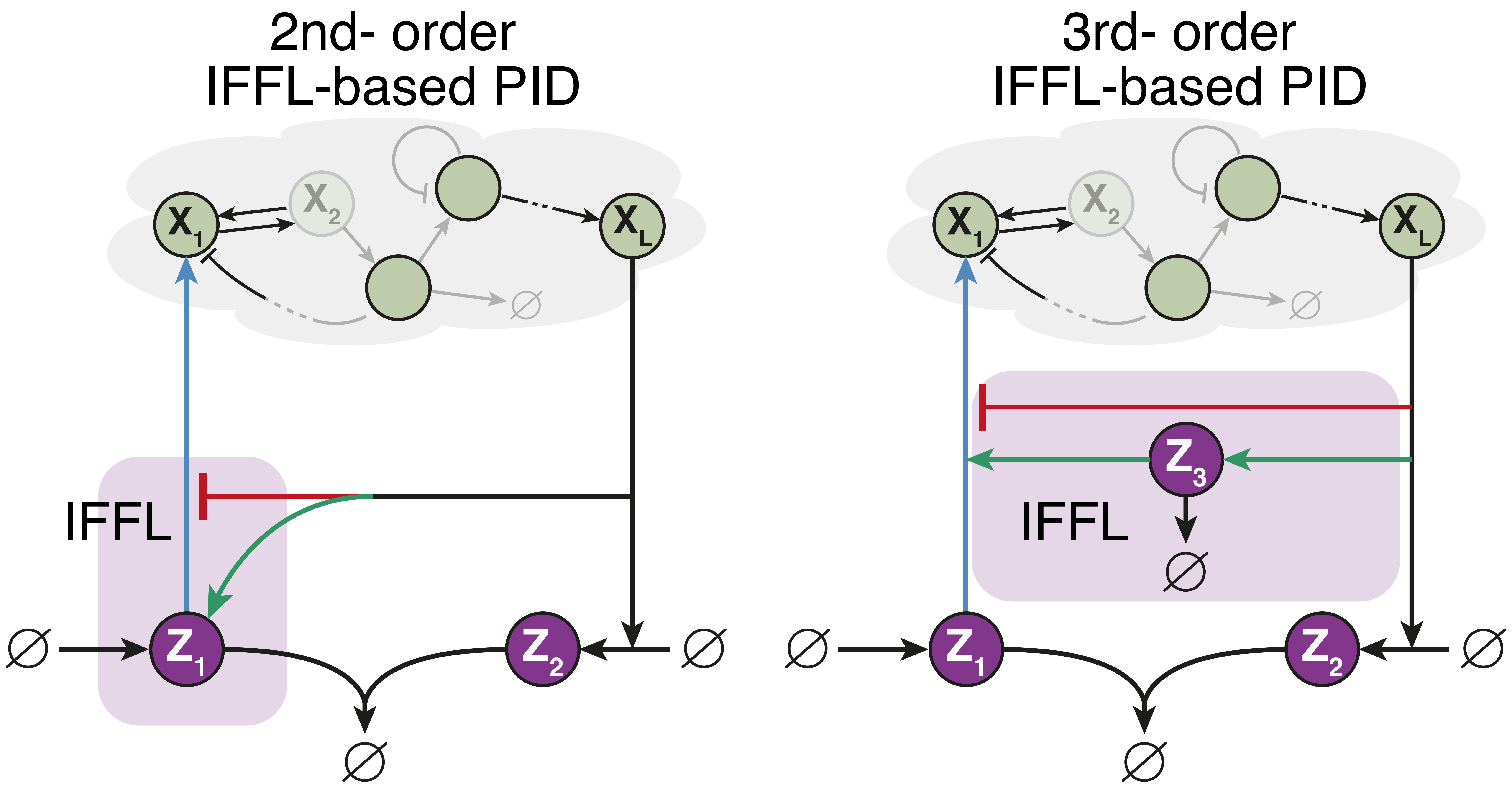

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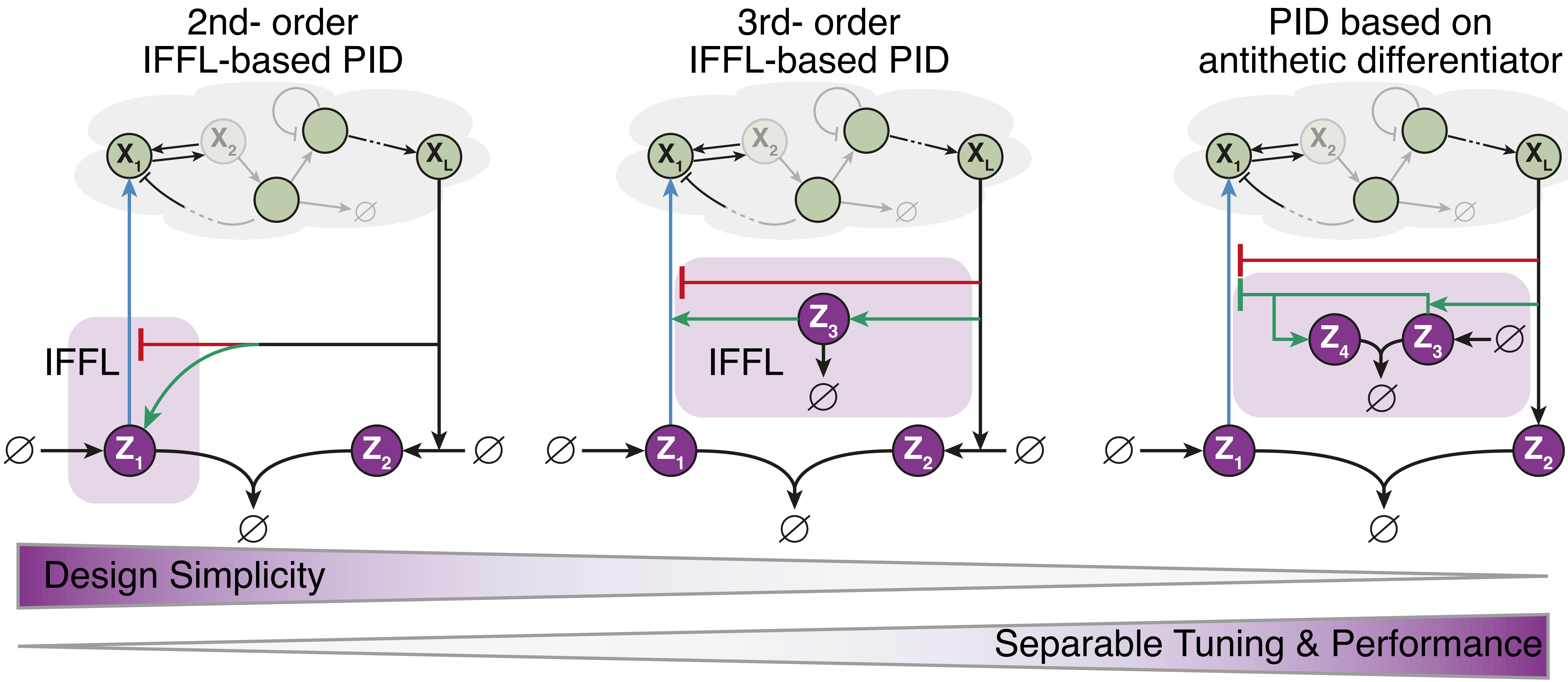

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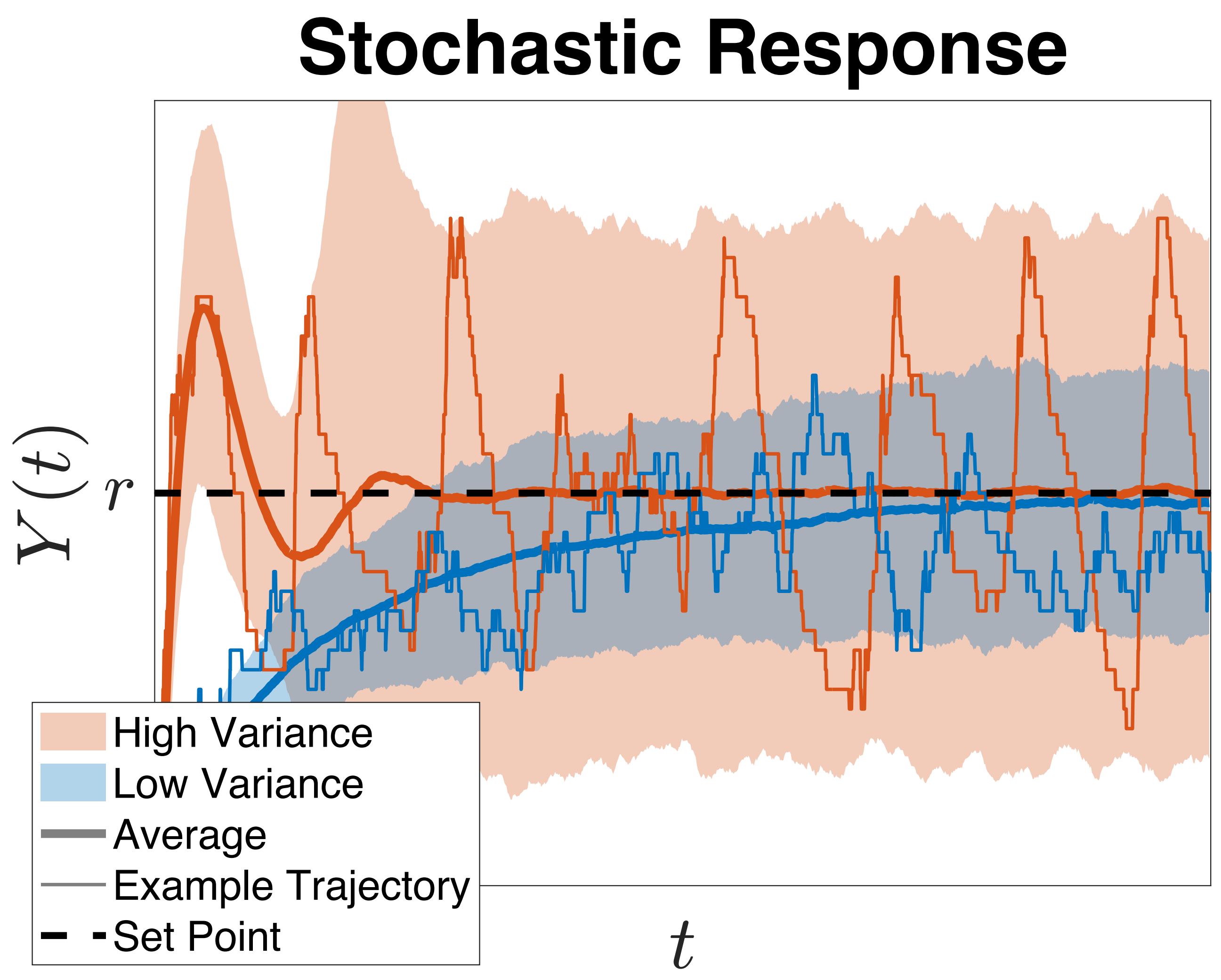

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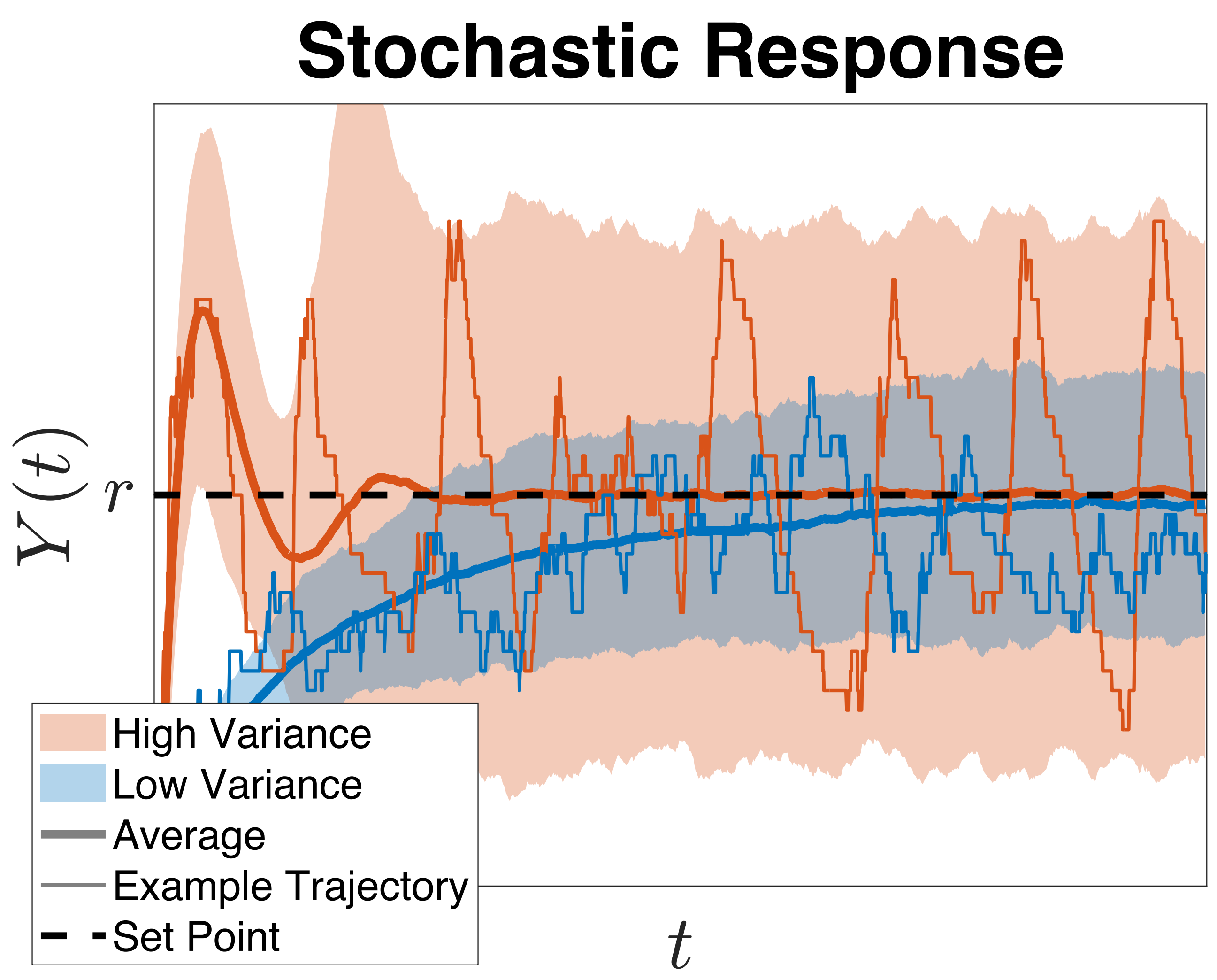

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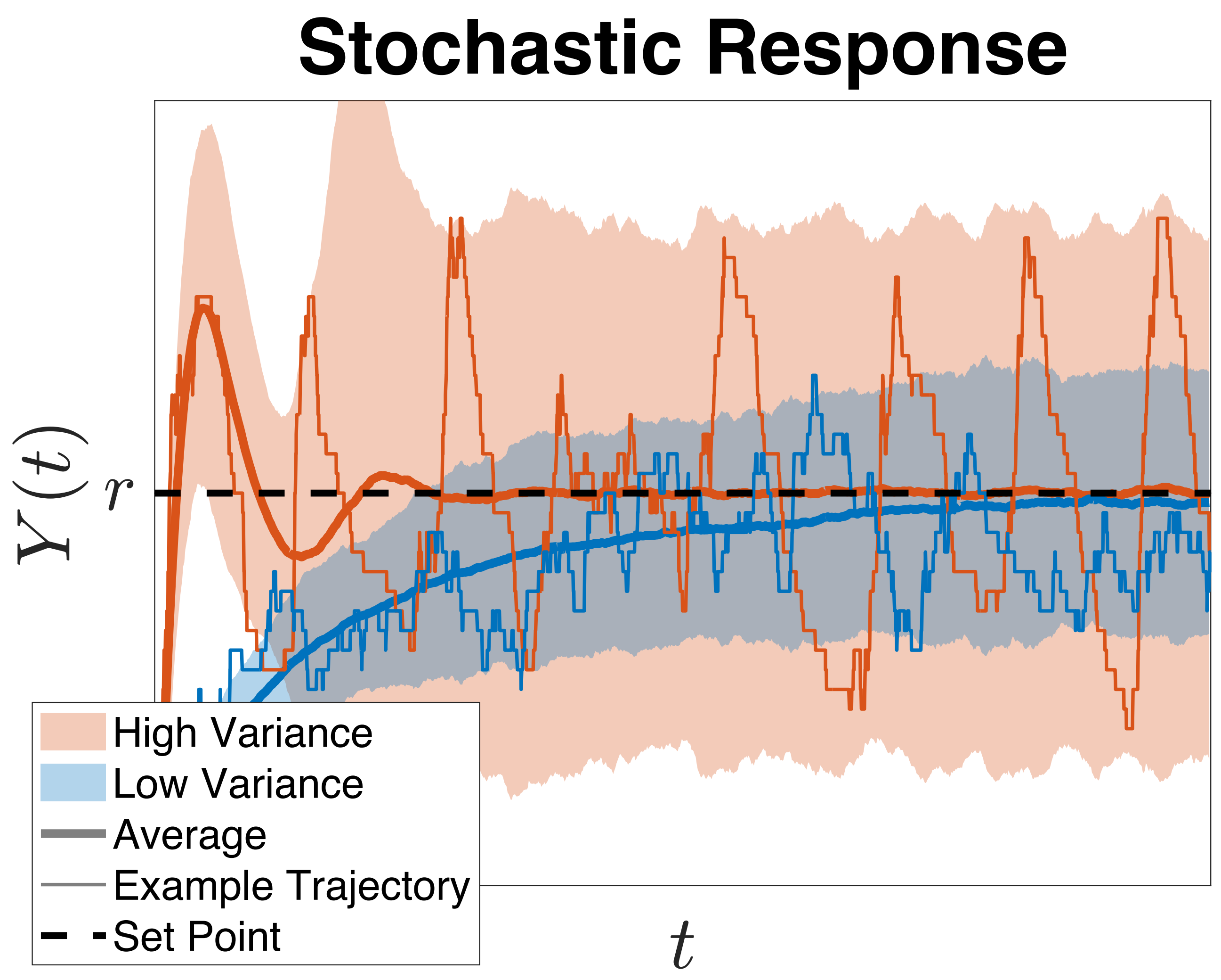

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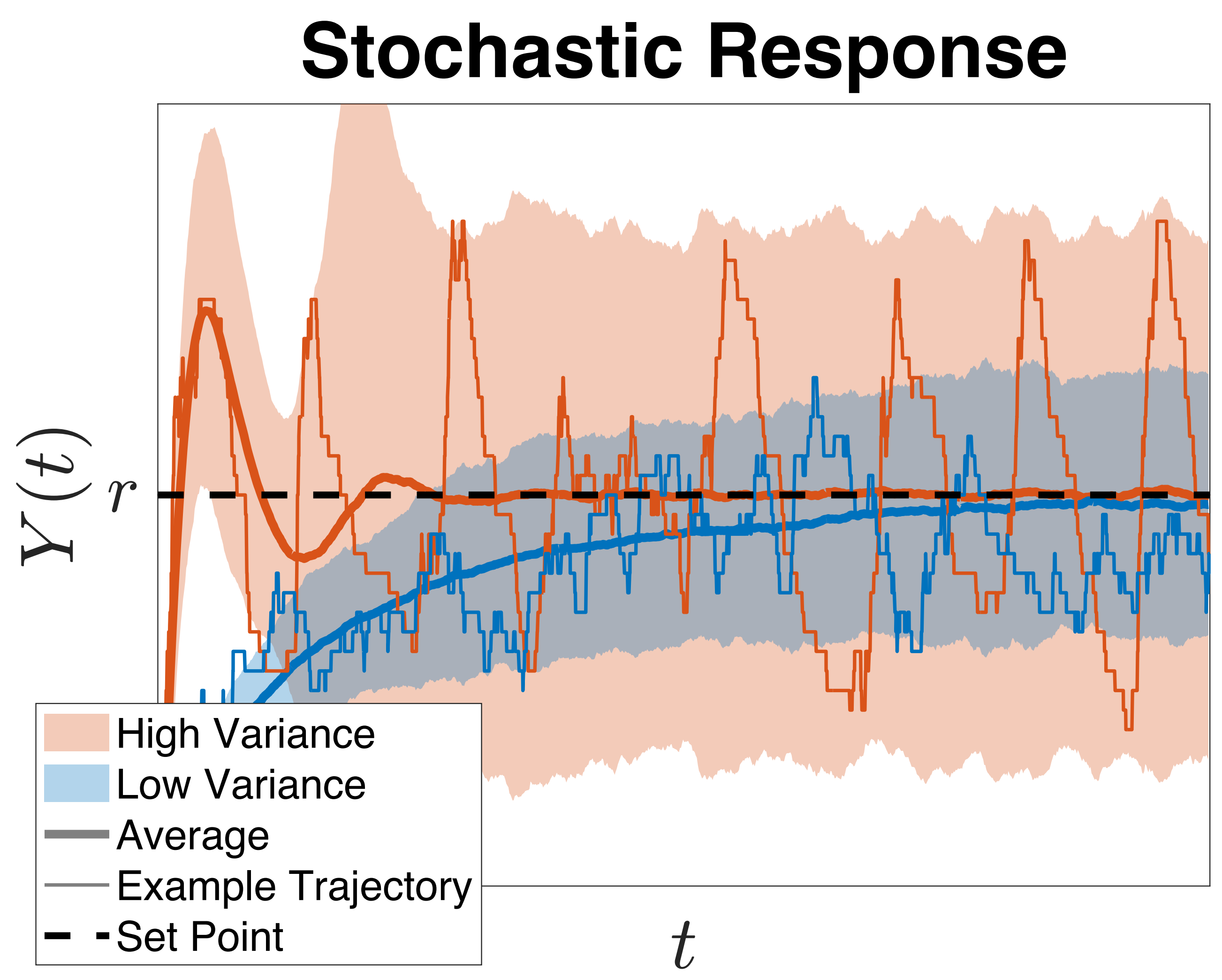

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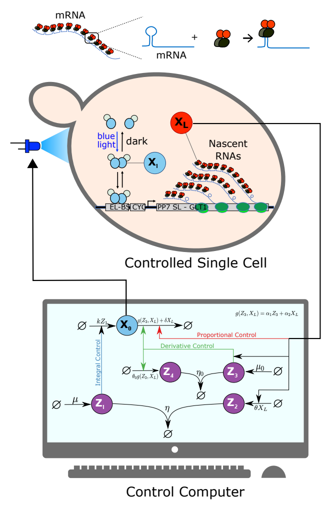
Experimental Results: **Cyberloop**



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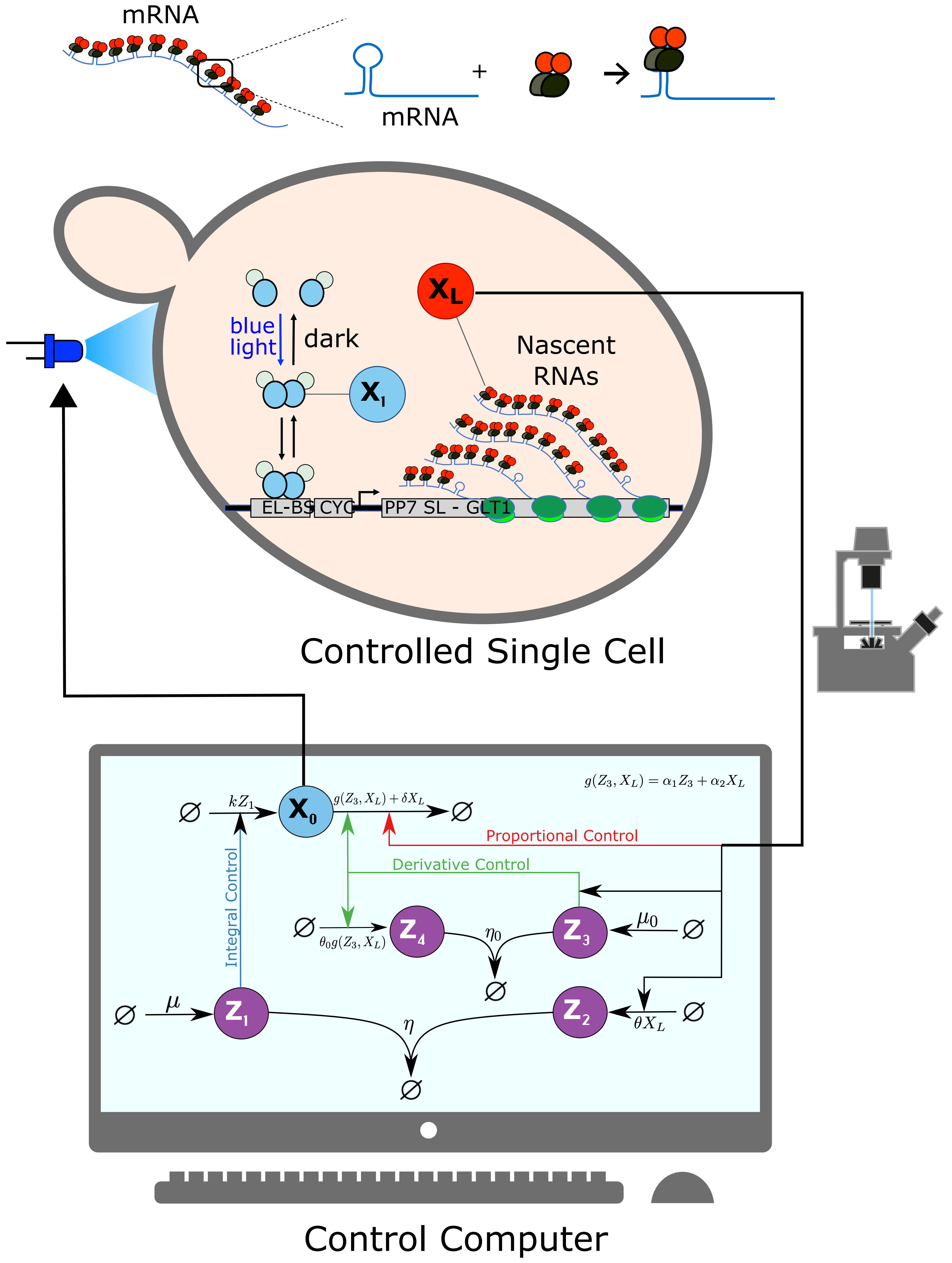
Cyberloop Experiments: A Hybrid Platform



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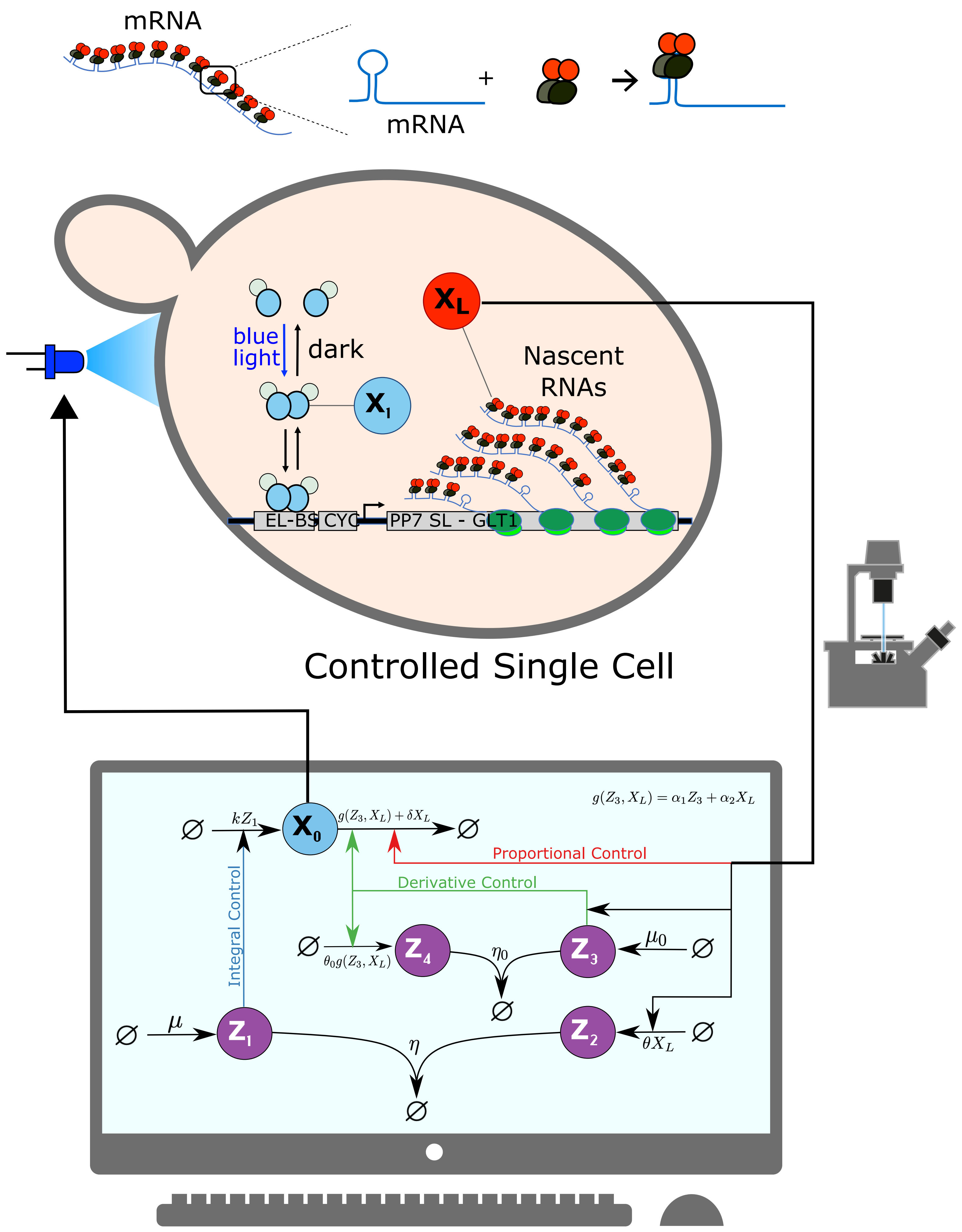

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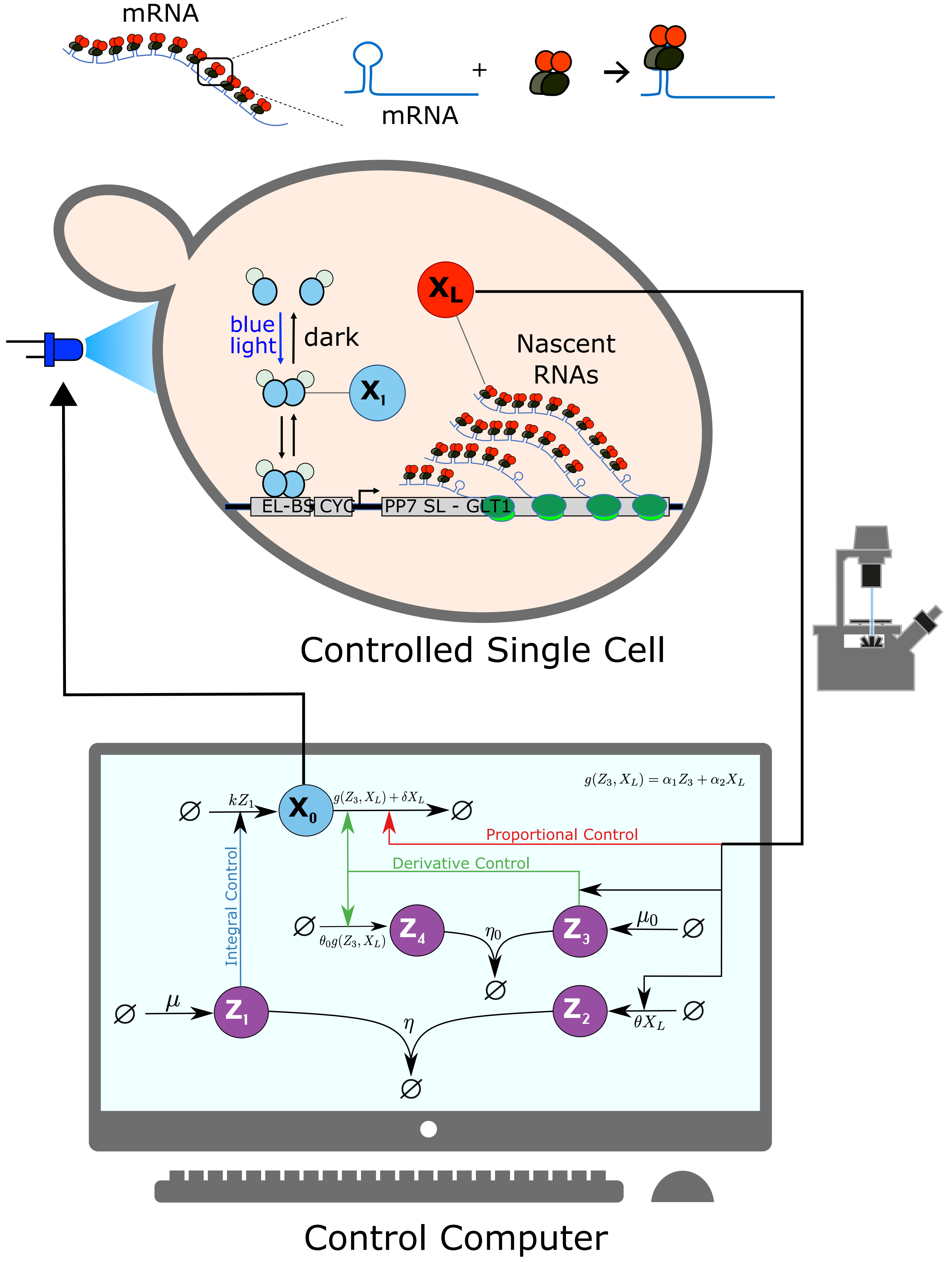

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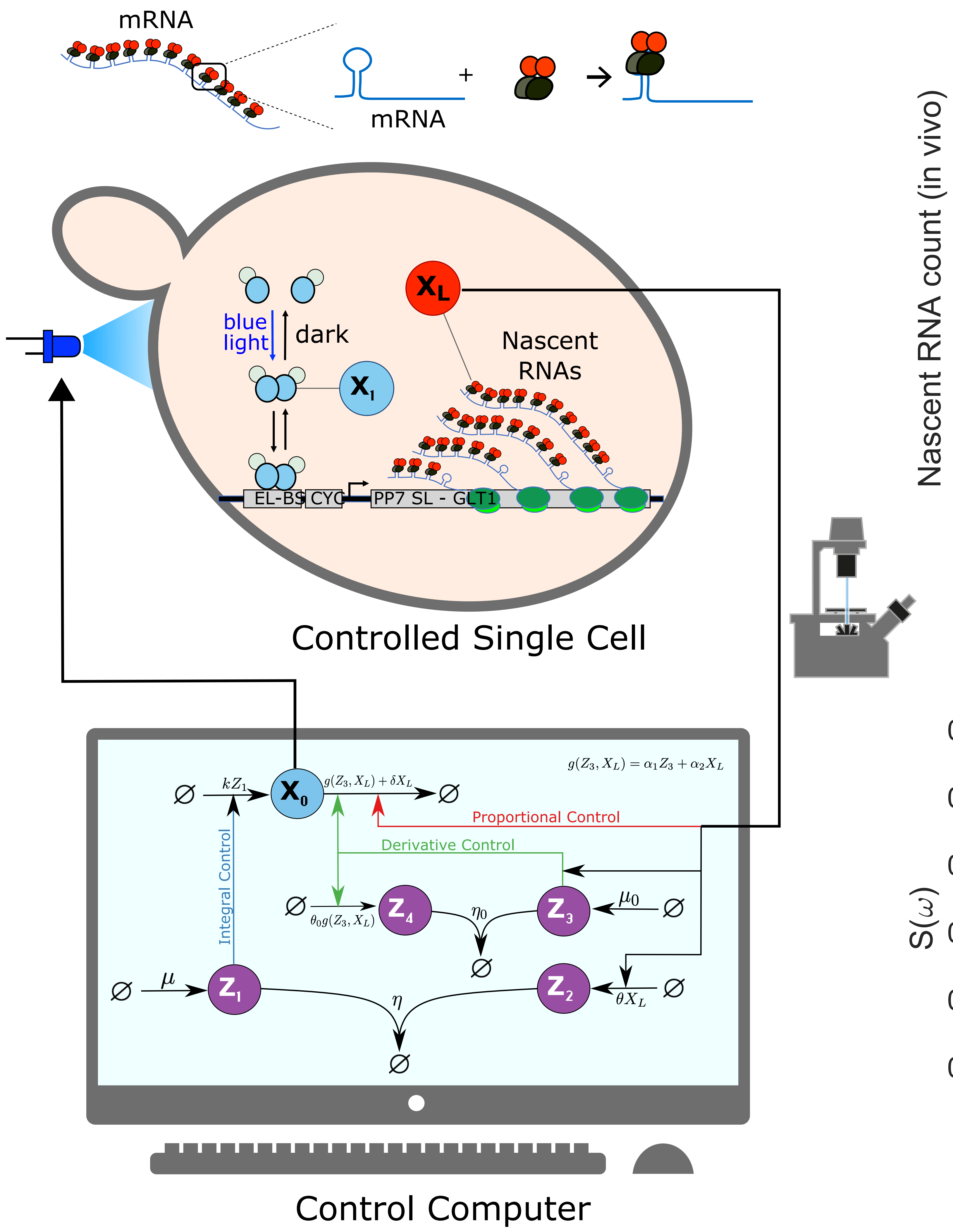

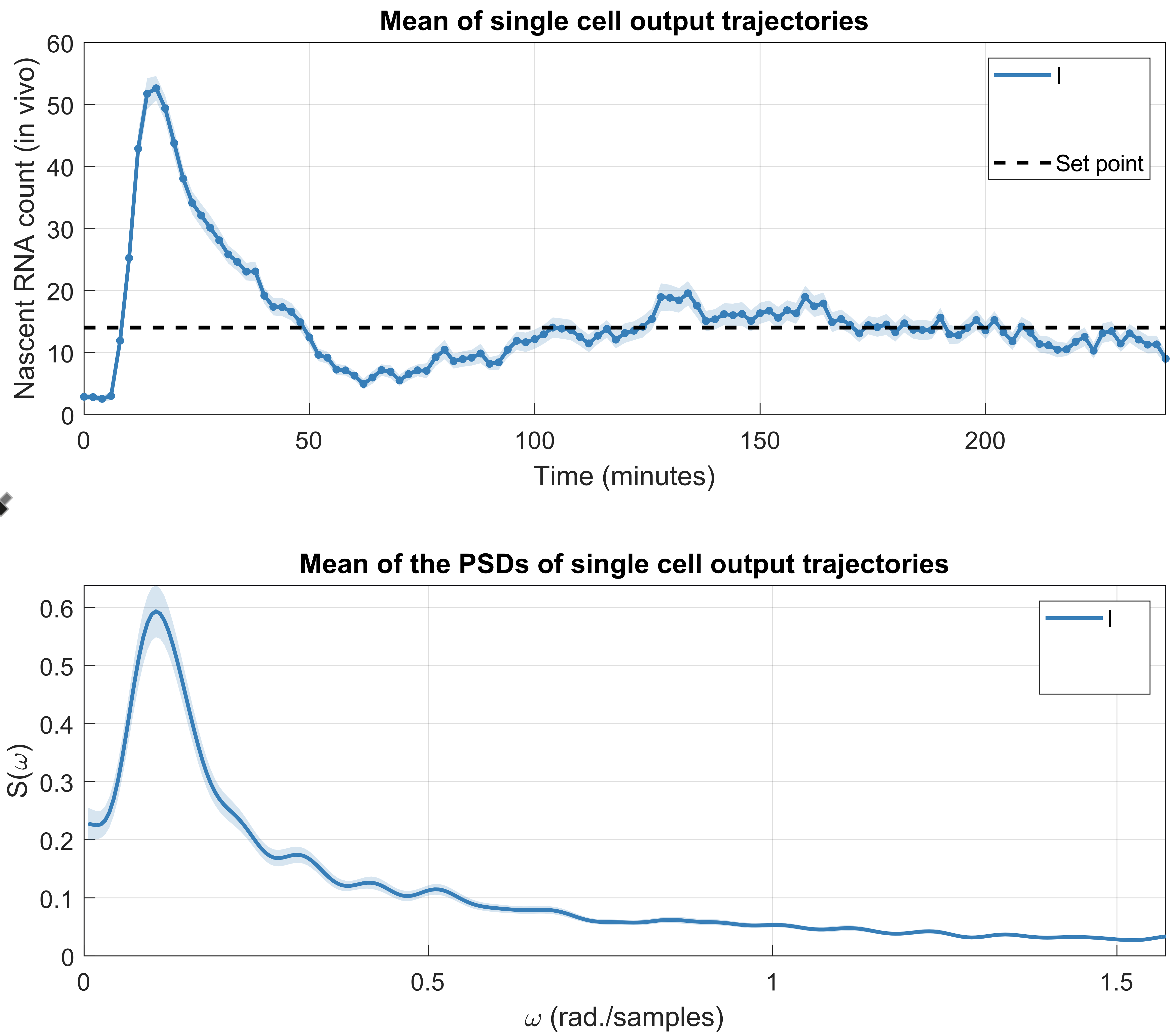

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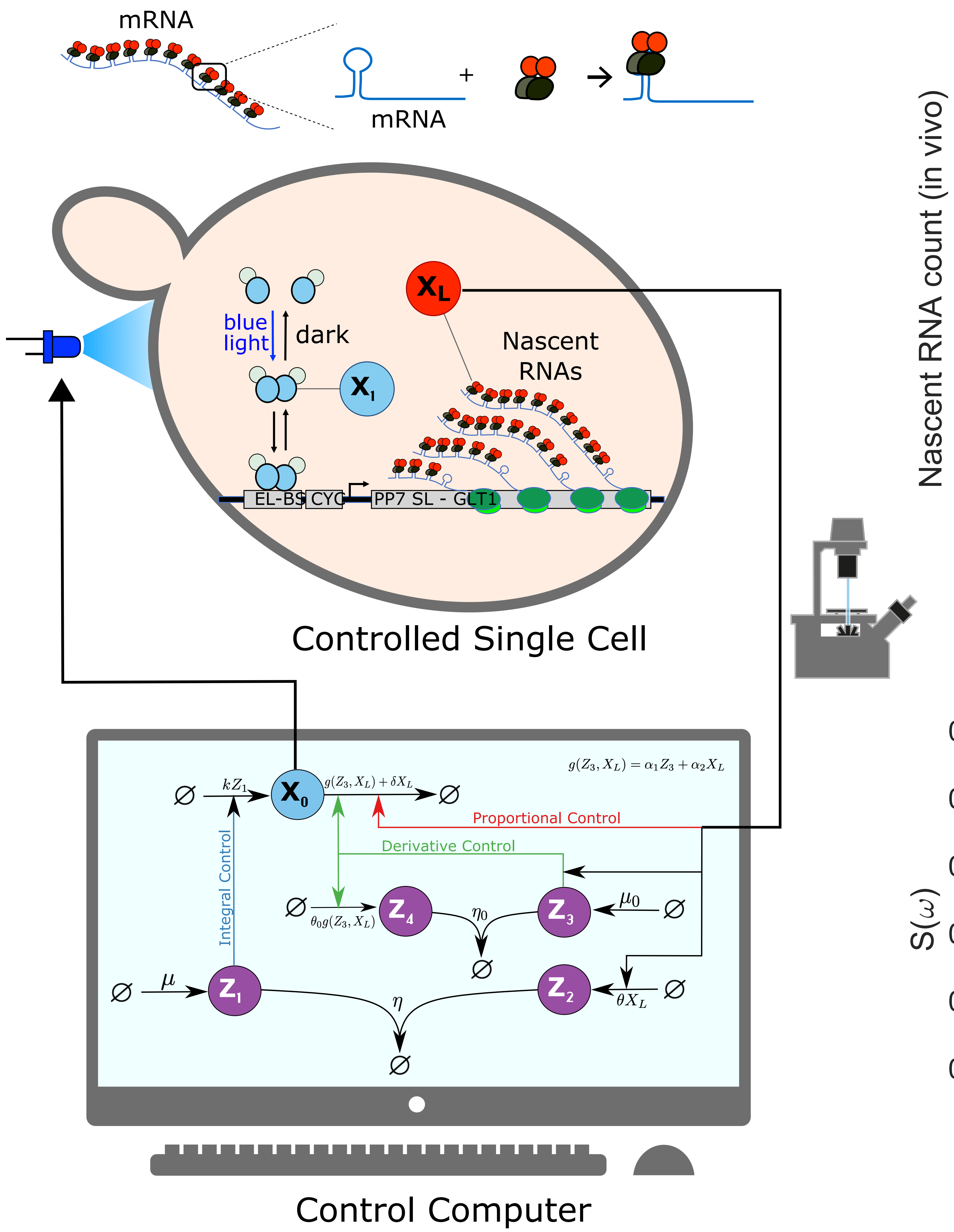
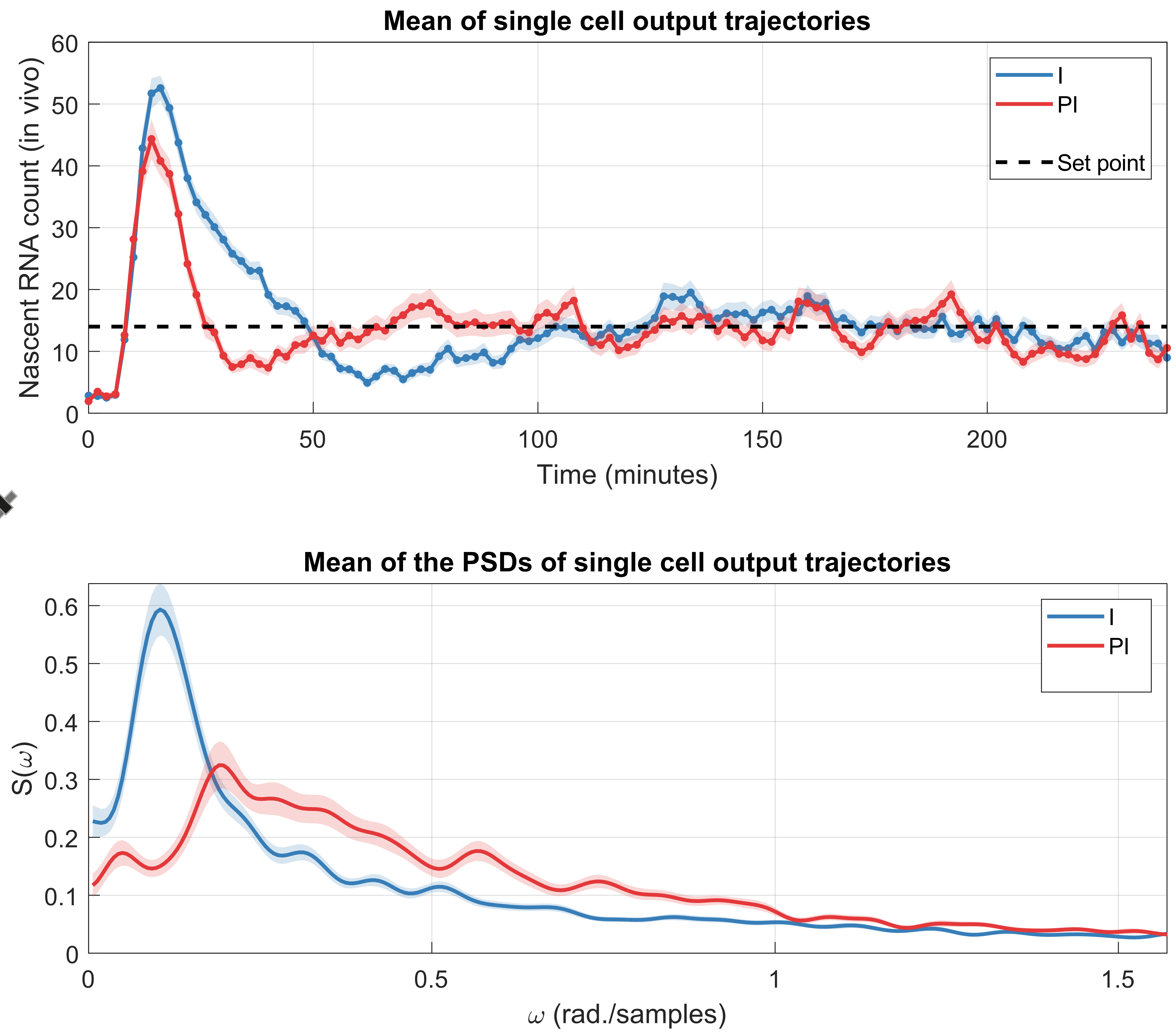
Cyberloop Experiments: A Hybrid Platform





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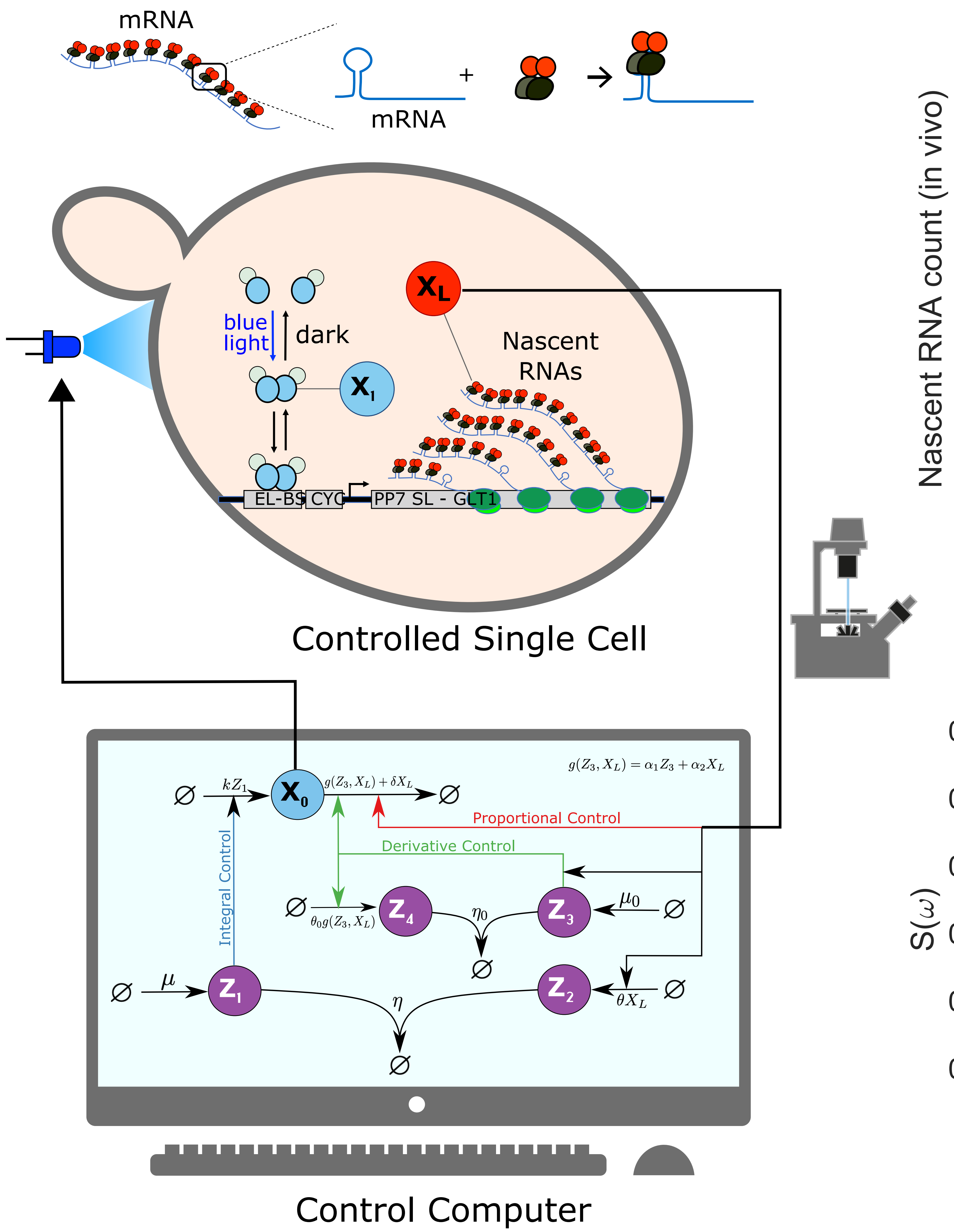

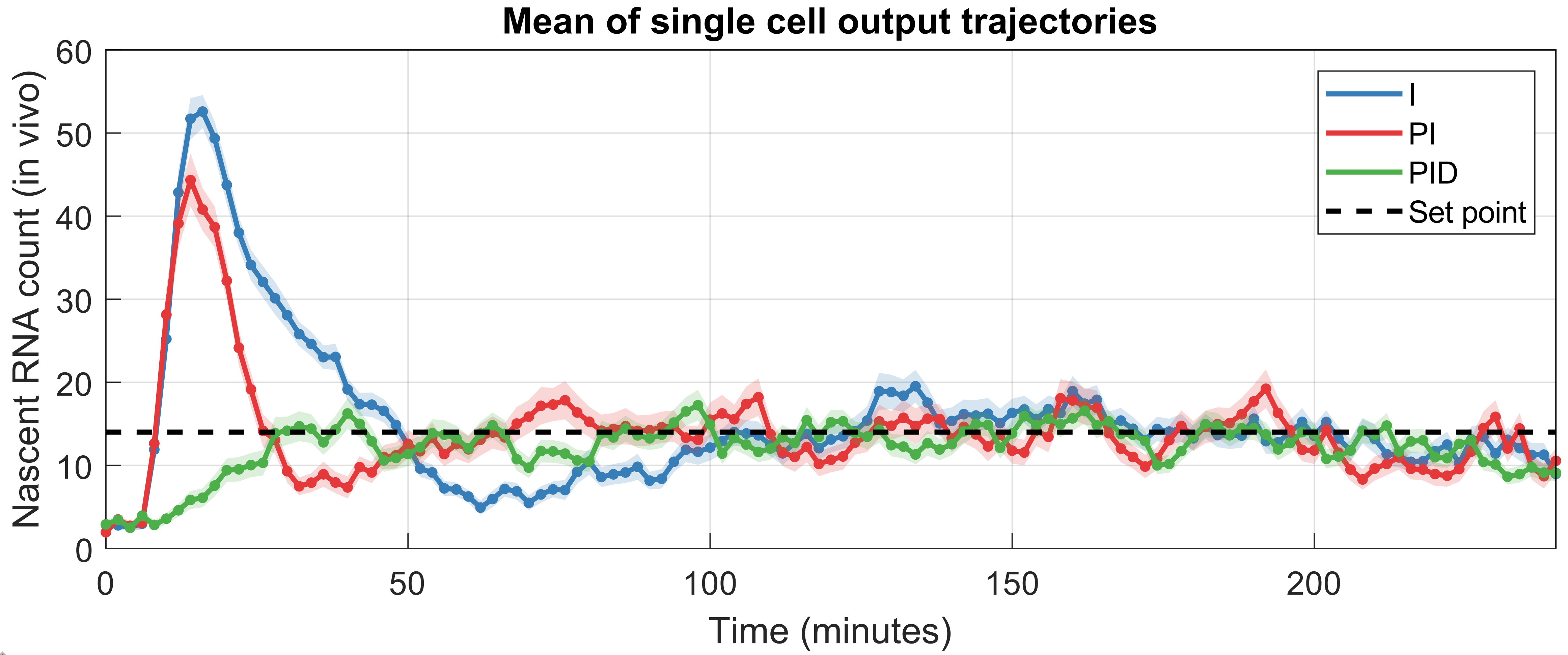

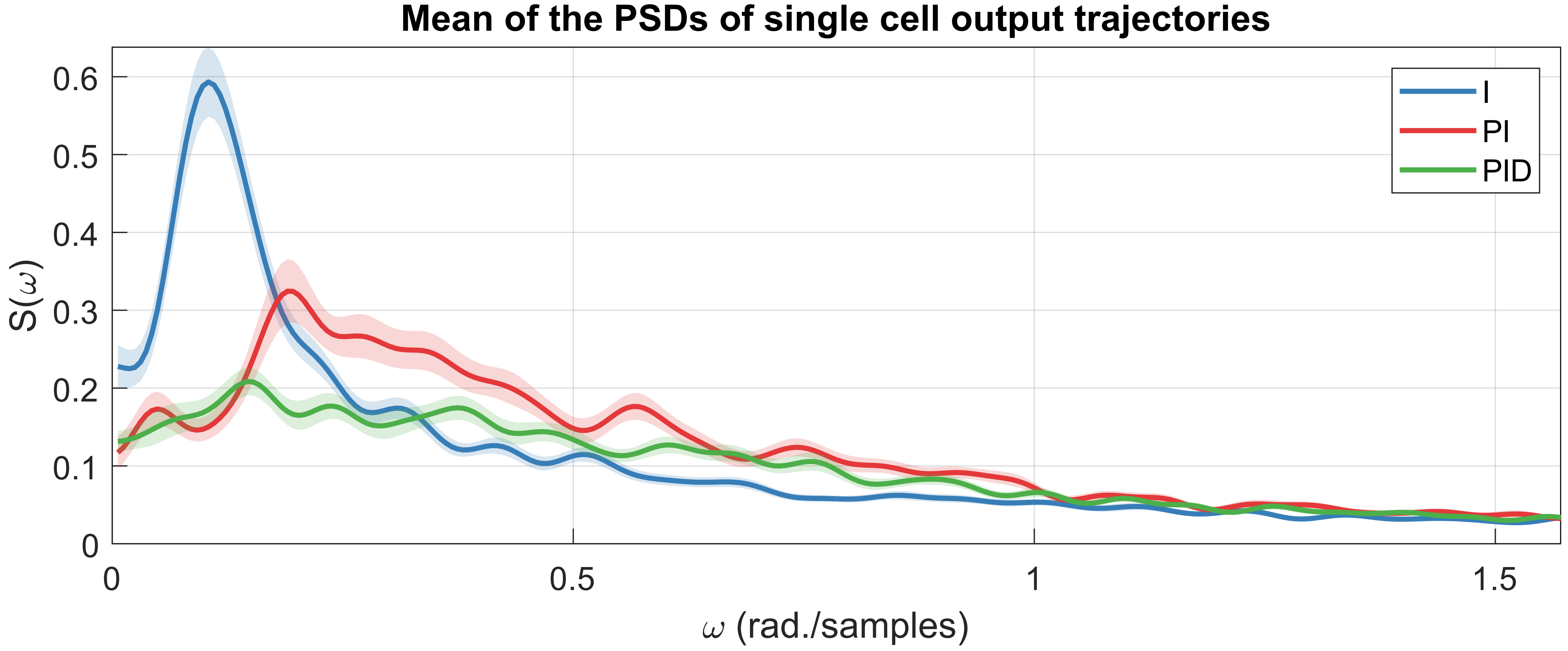

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Anti-windup Chemical Reaction Networks

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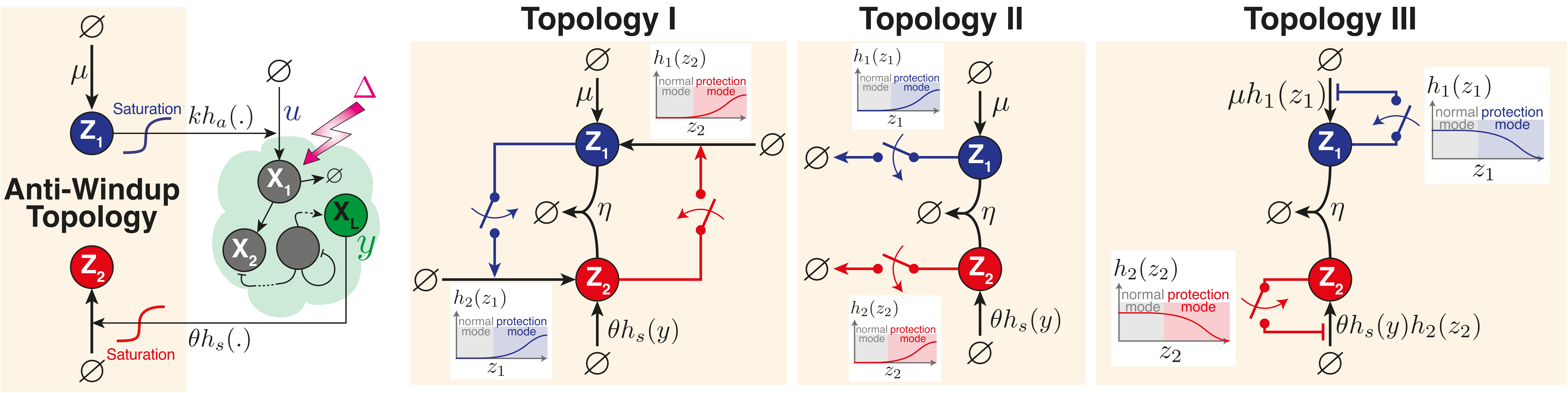

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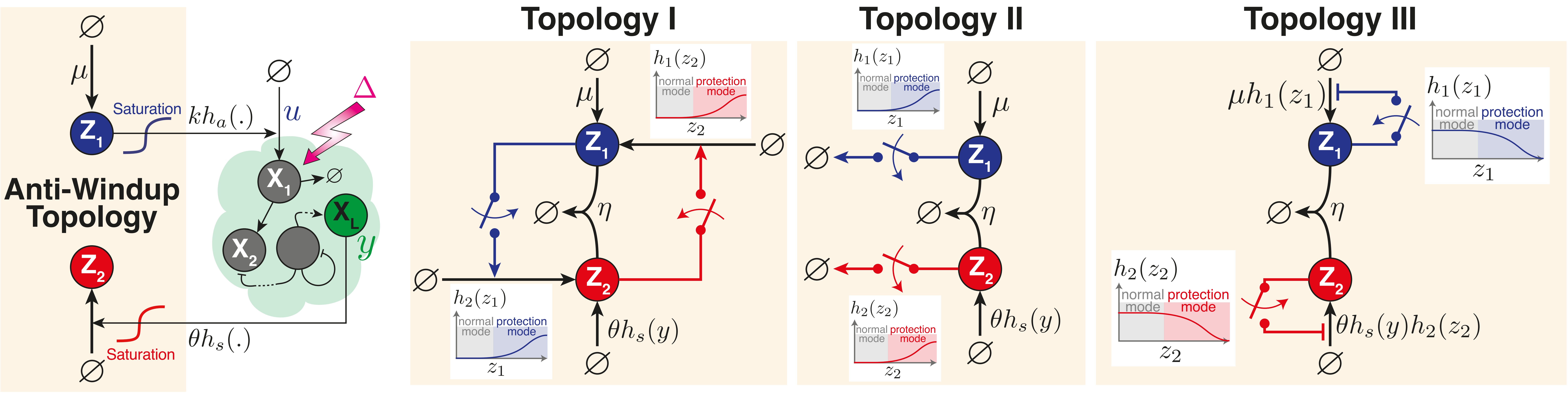

- Design guided by model reduction theory we developed for CRNs with fast sequestration kinetics

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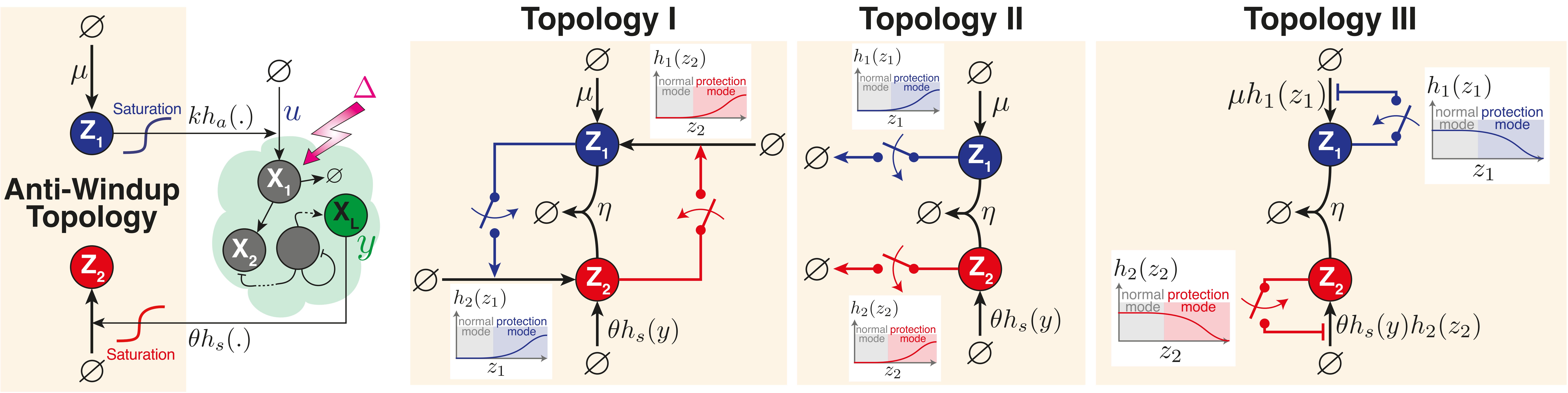

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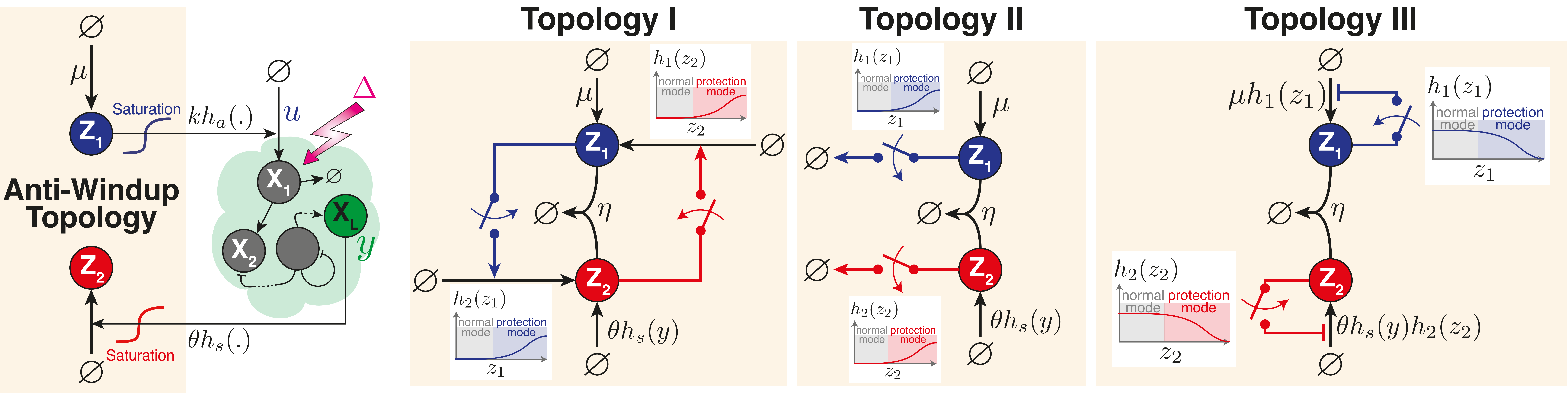
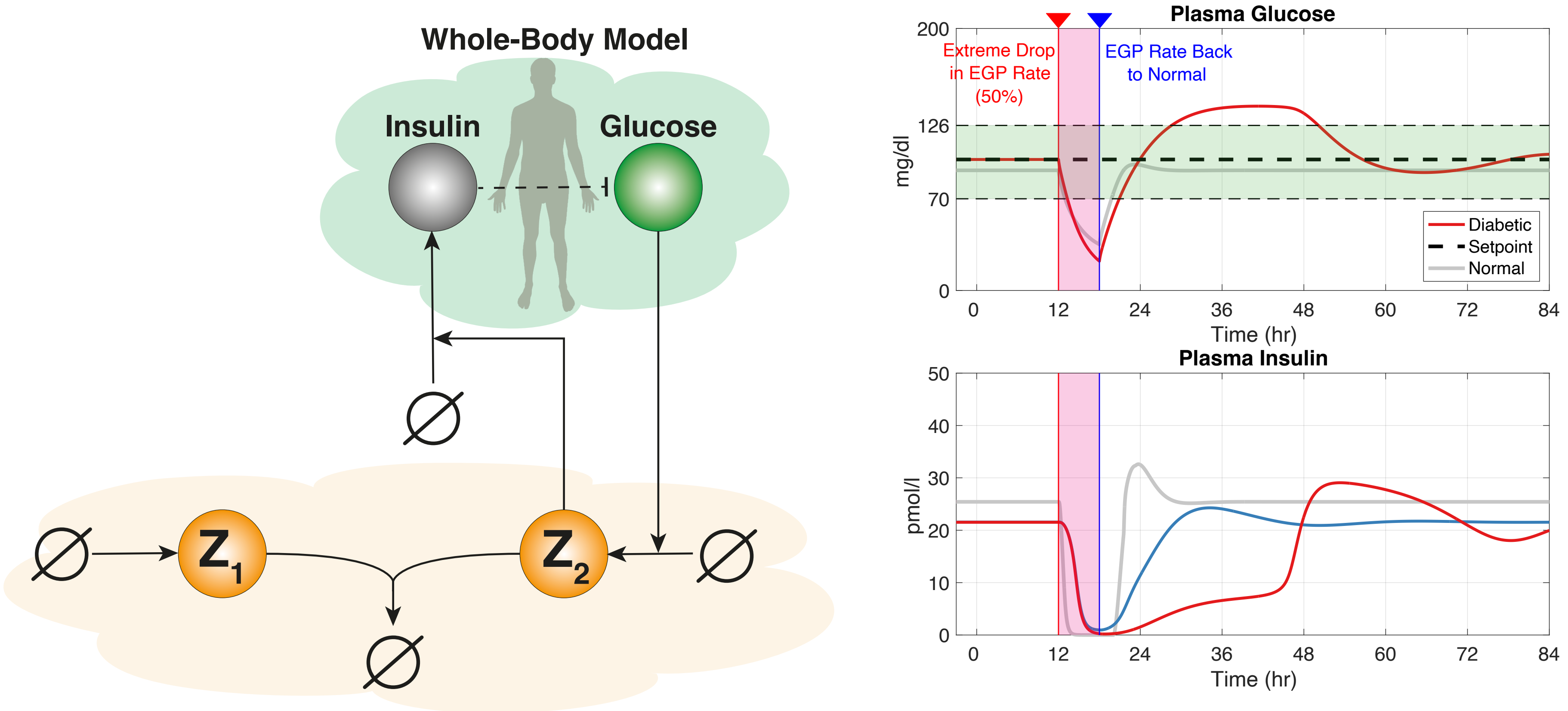


- Design guided by model reduction theory we developed for CRNs with fast sequestration kinetics
- Implements Conditional Integration and Reference Conditioning
- Successfully applied, in simulations, to regulate a high-dimensional FDA-approved model of diabetic patients



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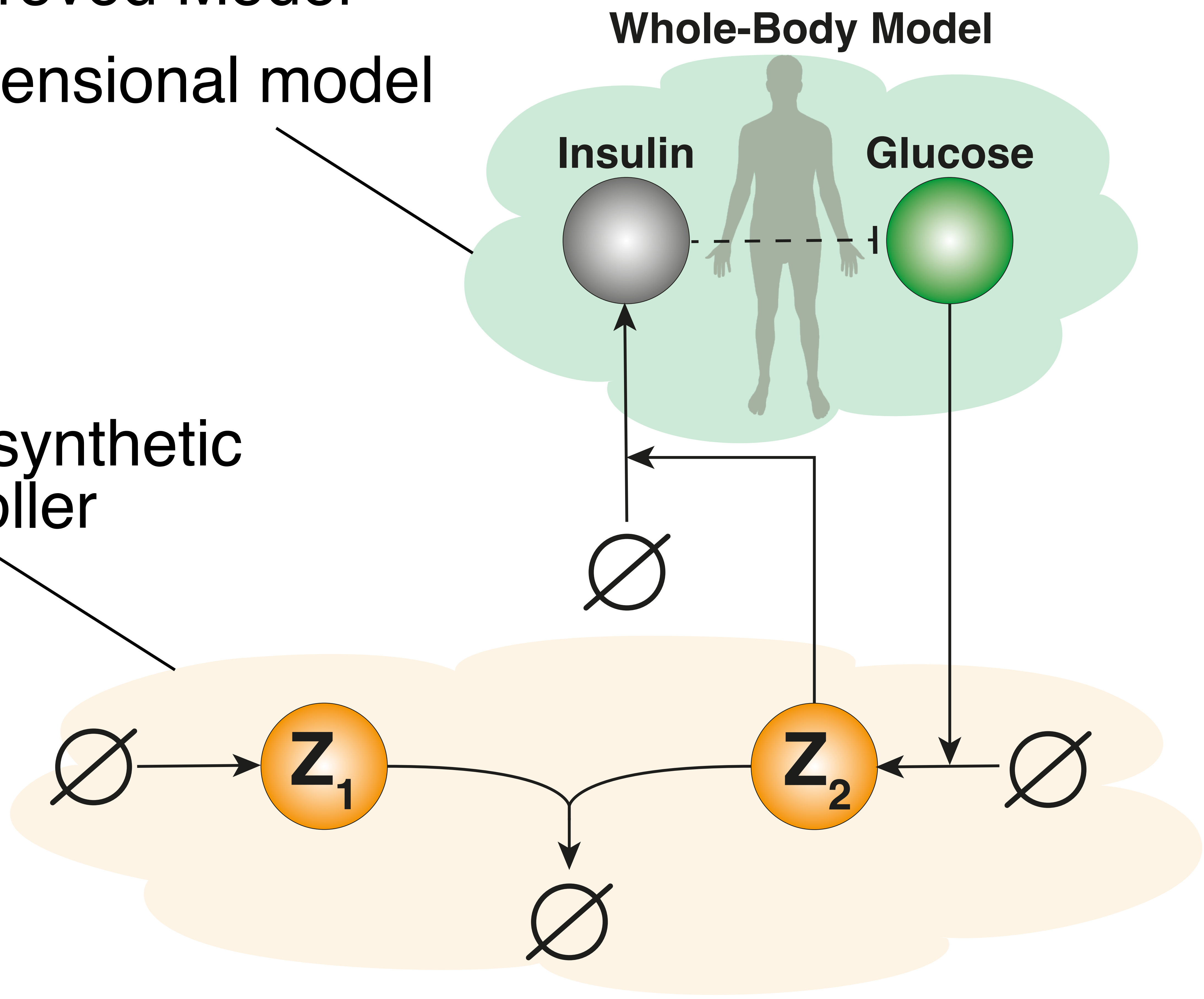
Windup in Integral Control of Blood Sugar

- FDA approved Model*
- High dimensional model



Implanted synthetic controller

*Meal Simulation Model of the Glucose-Insulin System. C. Dalla Man, R.A. Rizza, and C. Cobelli. *IEEE Transactions on Biomedical Engineering* (2007)

Windup in Integral Control of Blood Sugar

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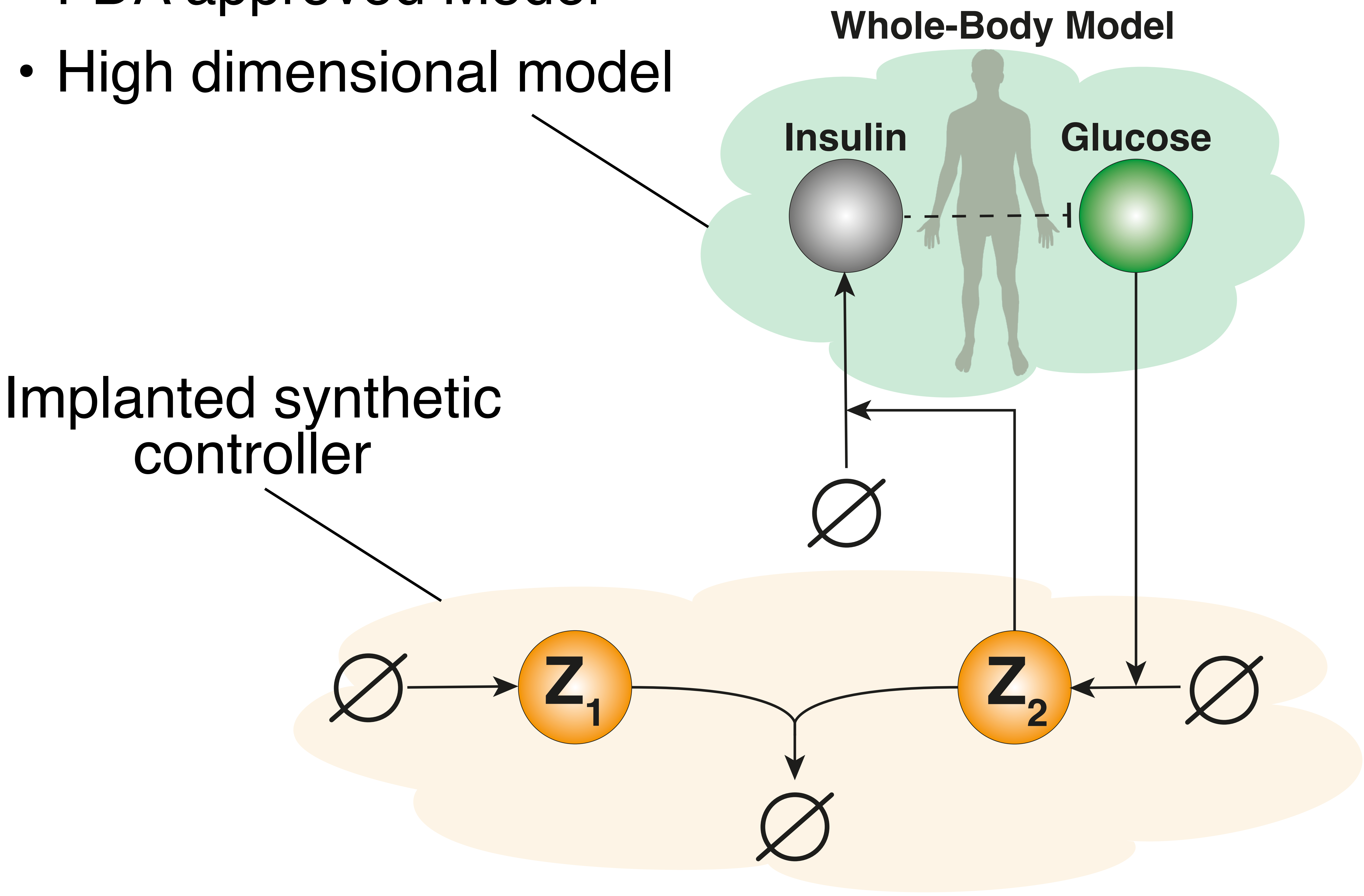

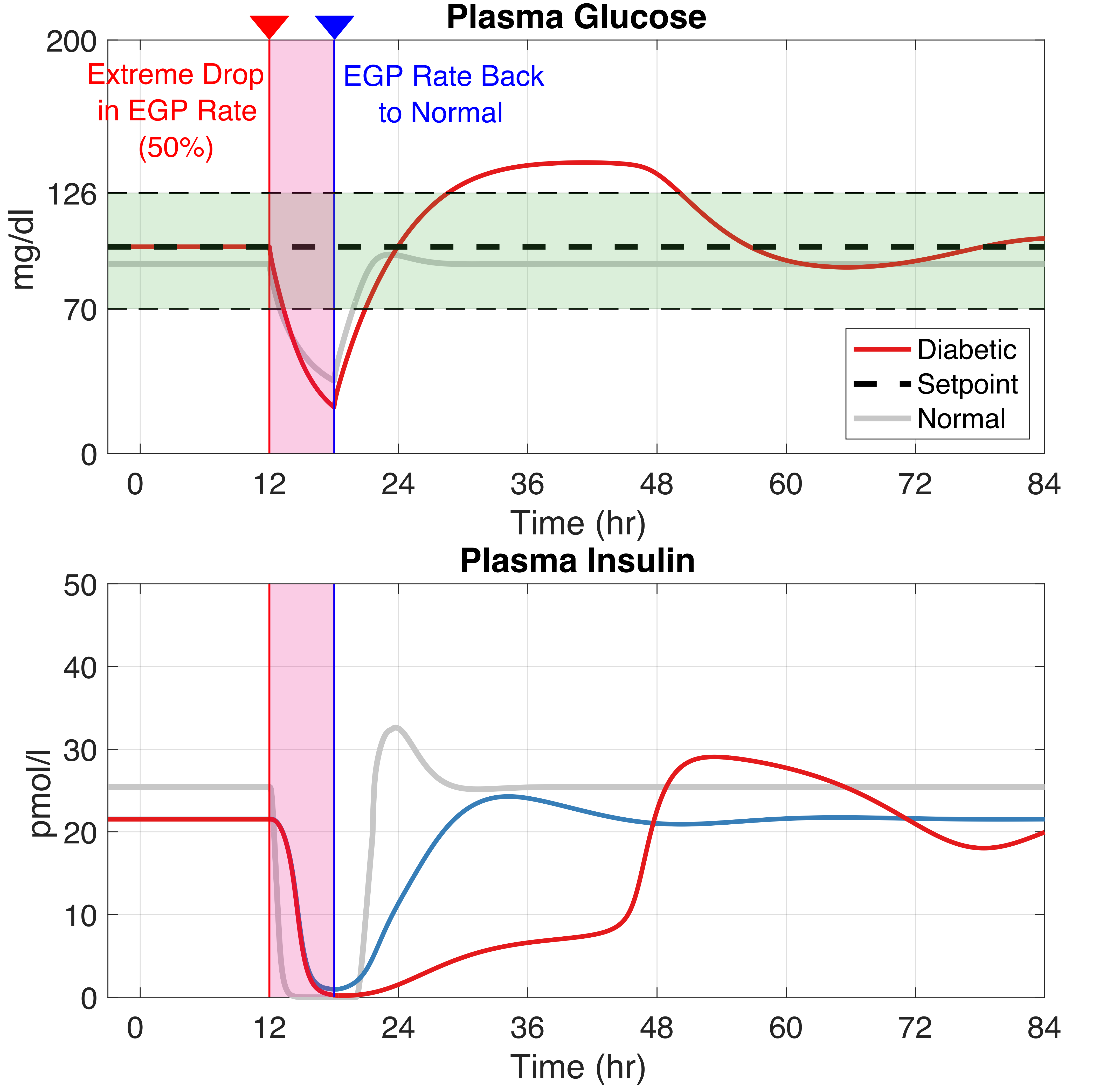

A Different Way to Implement Integrators

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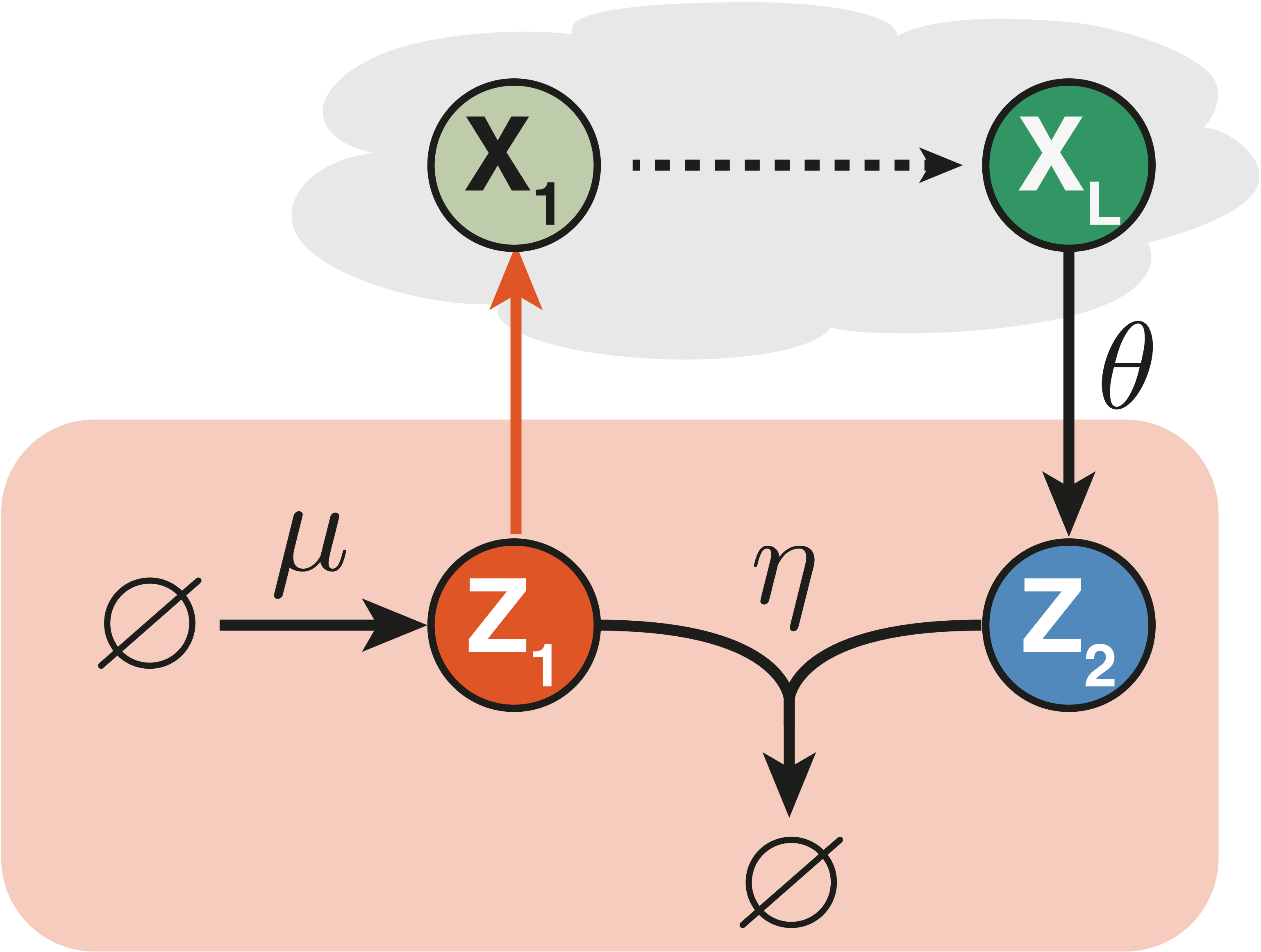

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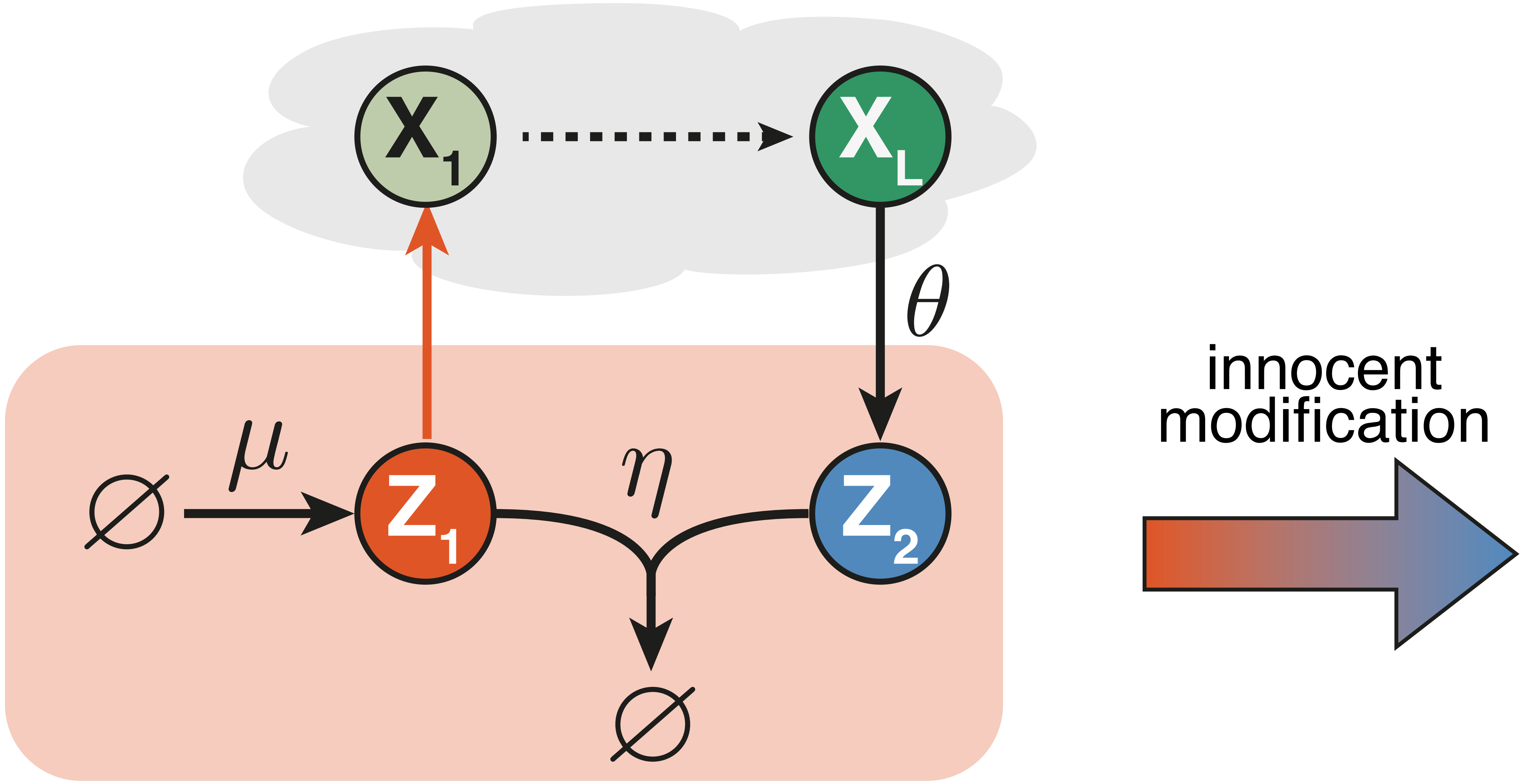

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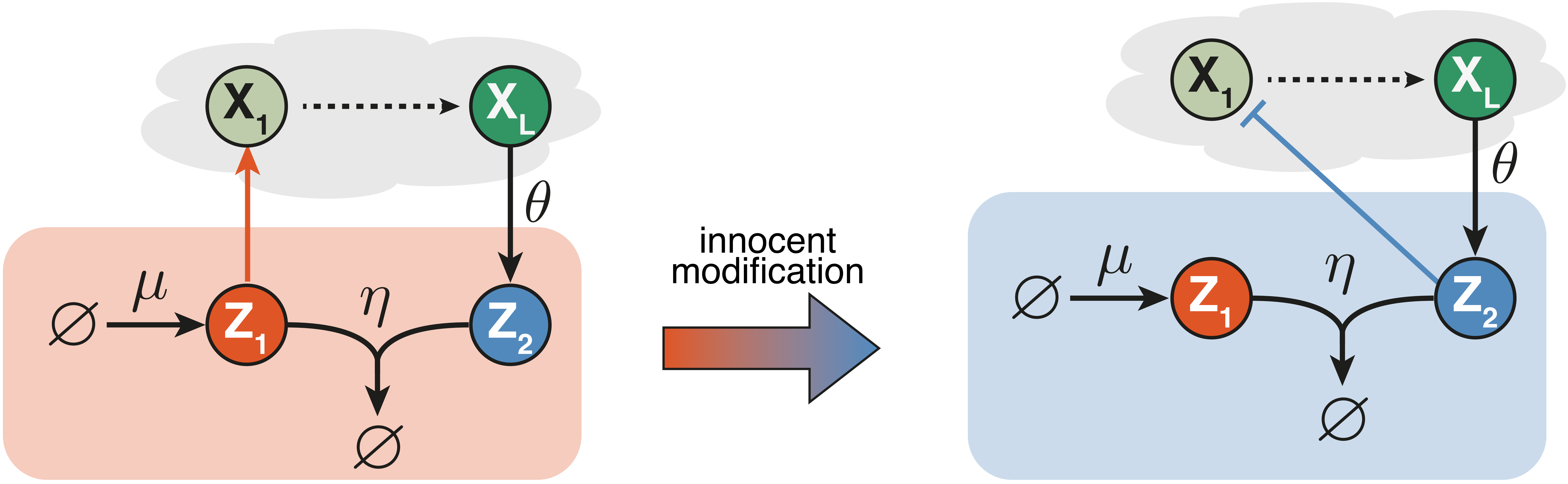

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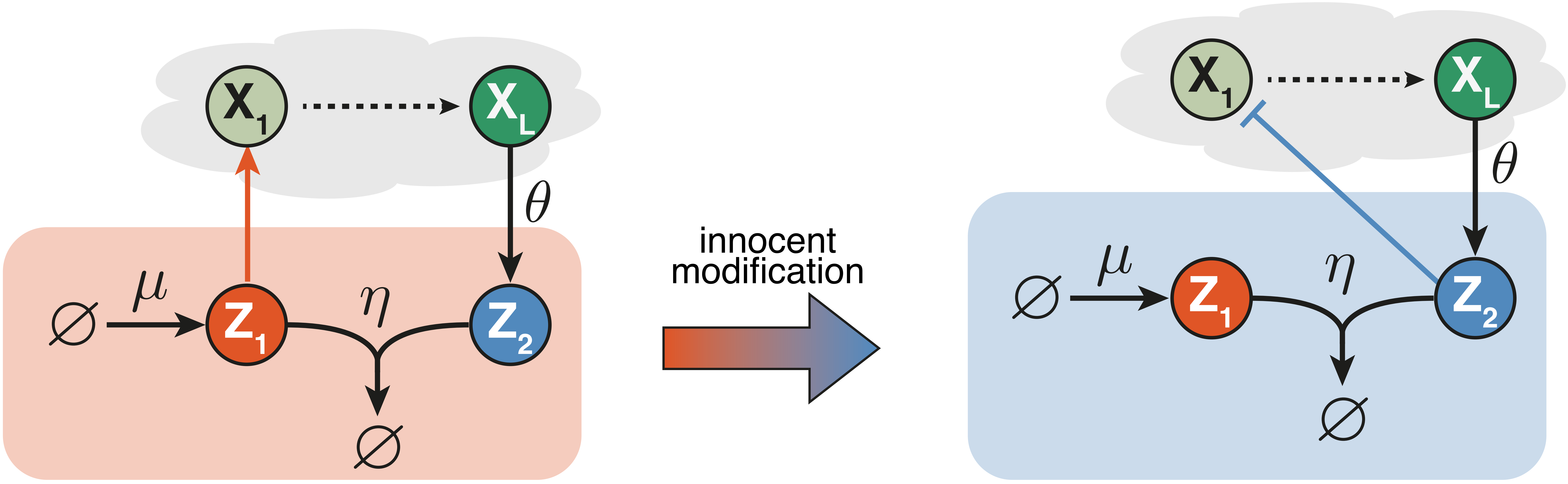

Why is this okay?

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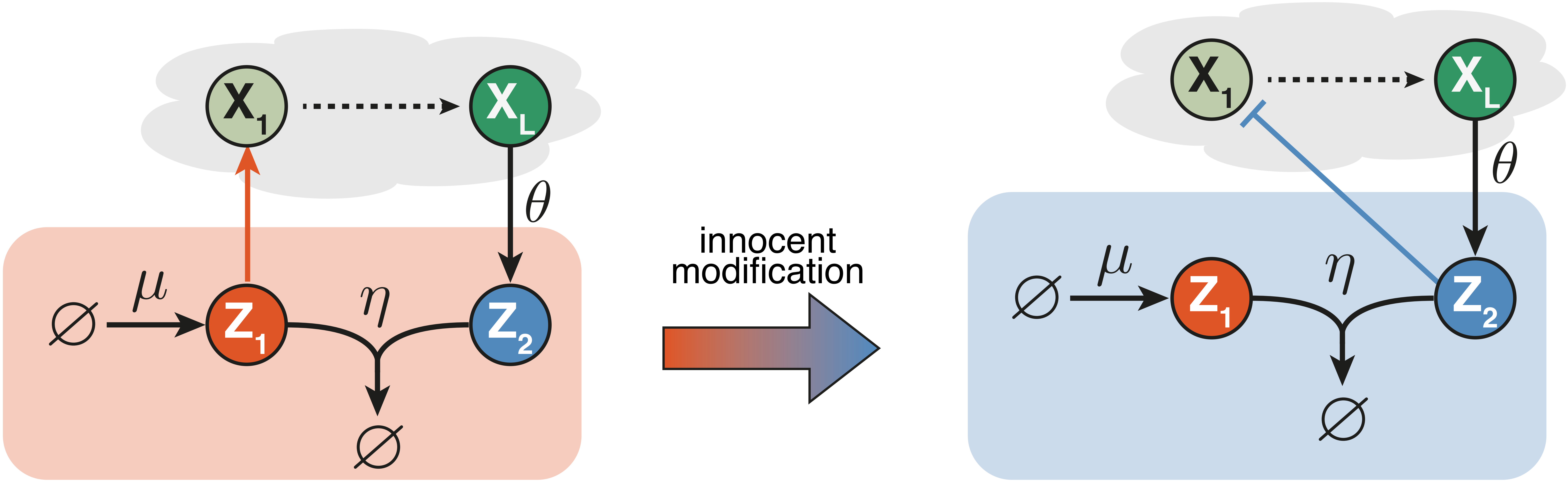

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Still overall negative feedback!

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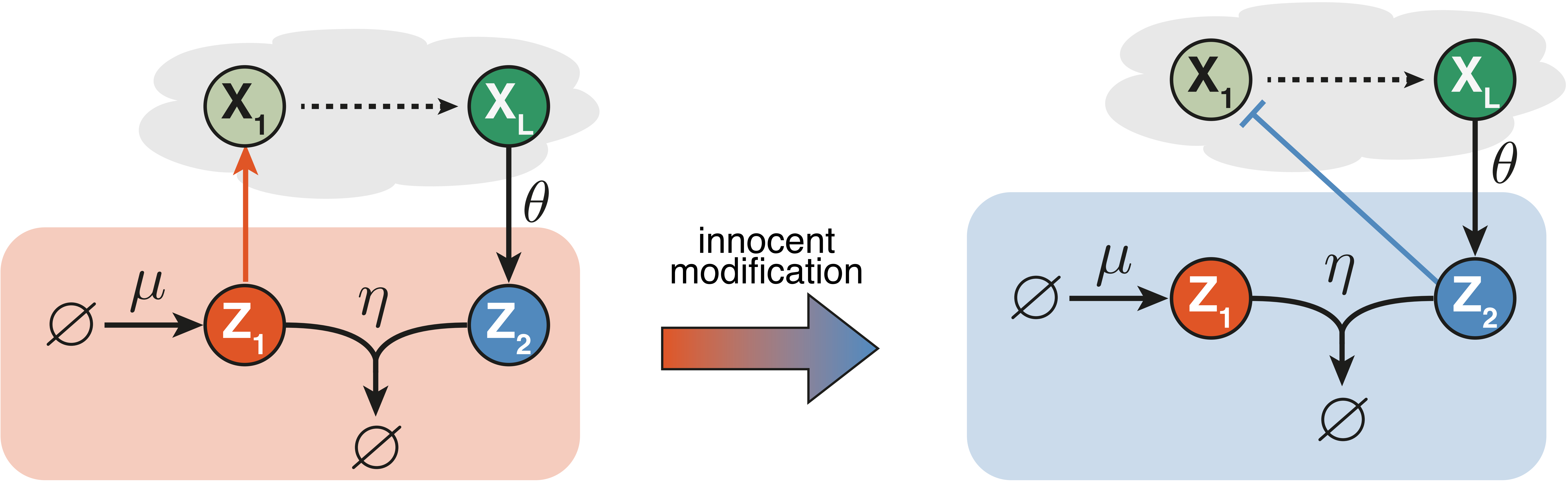

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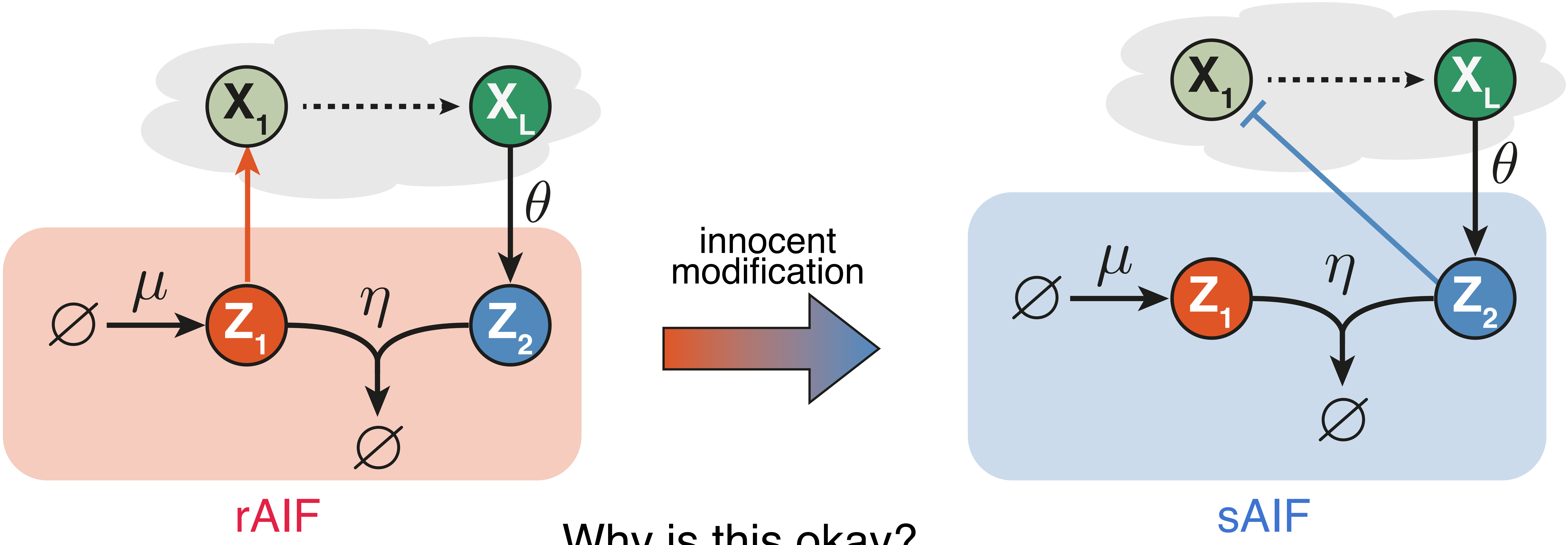

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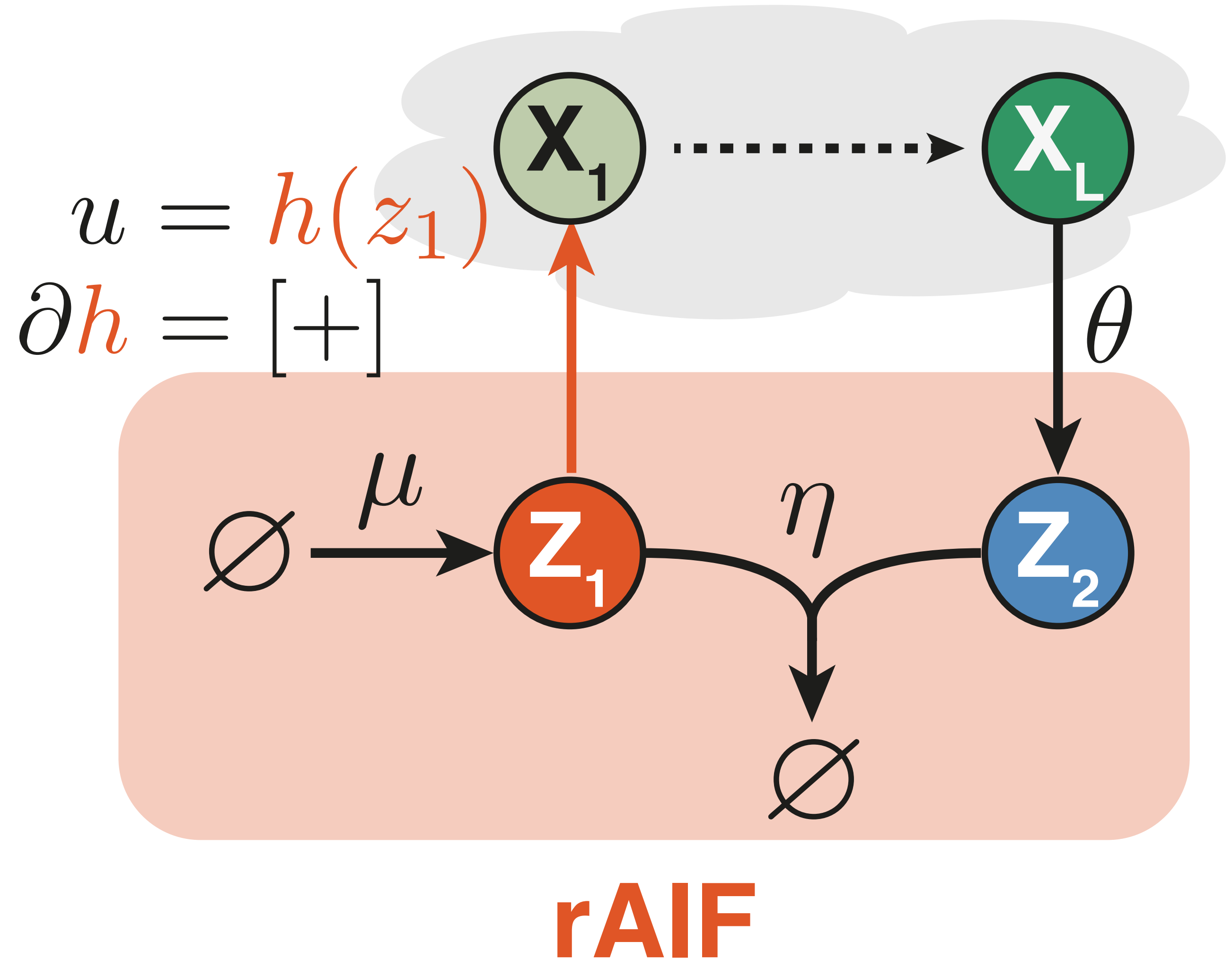
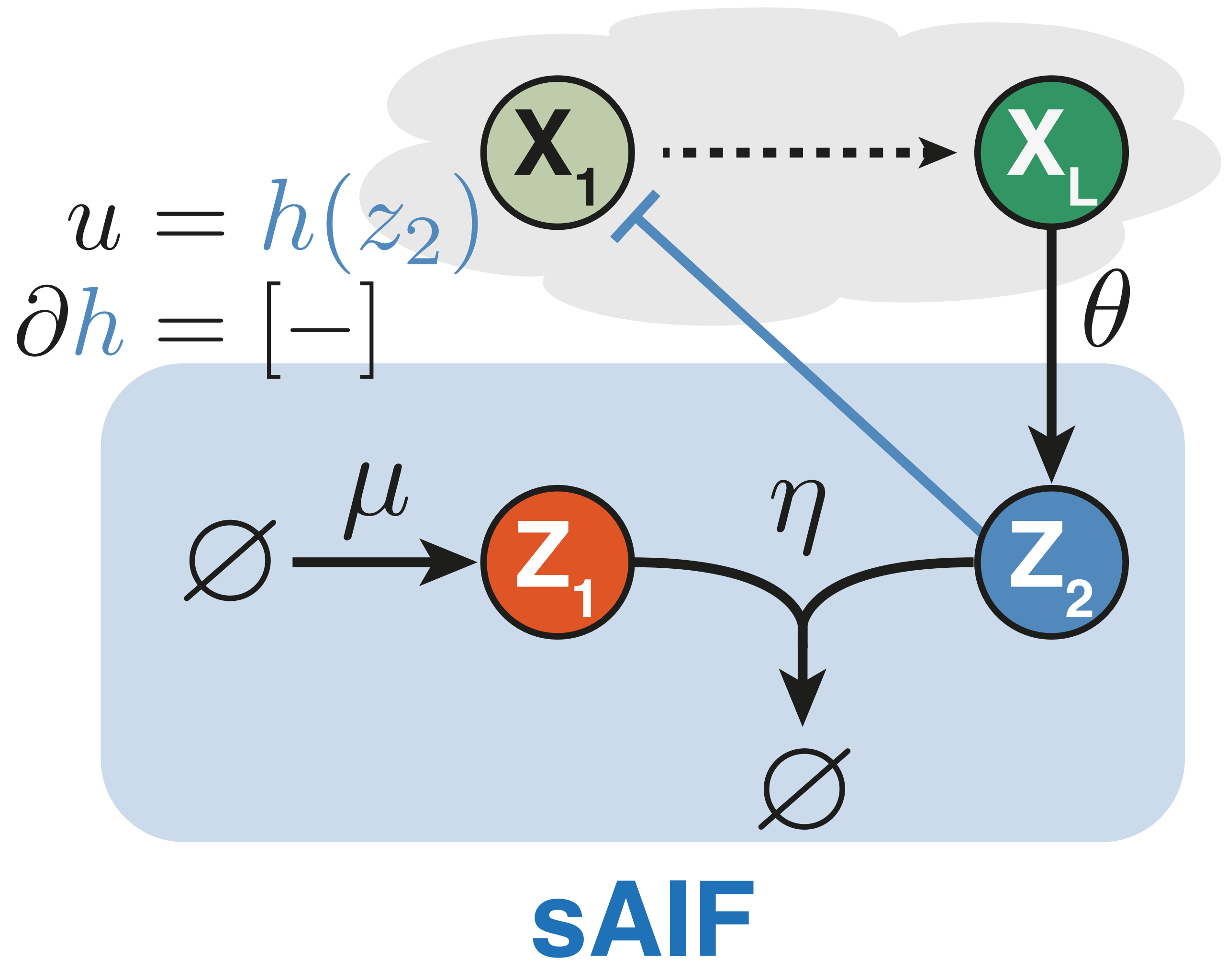
Control Architecture Underlying the sAIF Motif



$$\begin{cases} \dot{x} = f(x, u) \\ \dot{z}_1 = \mu - \eta z_1 z_2 \\ \dot{z}_2 = \theta x_L - \eta z_1 z_2 \\ u = h(z_1, z_2) \end{cases}$$



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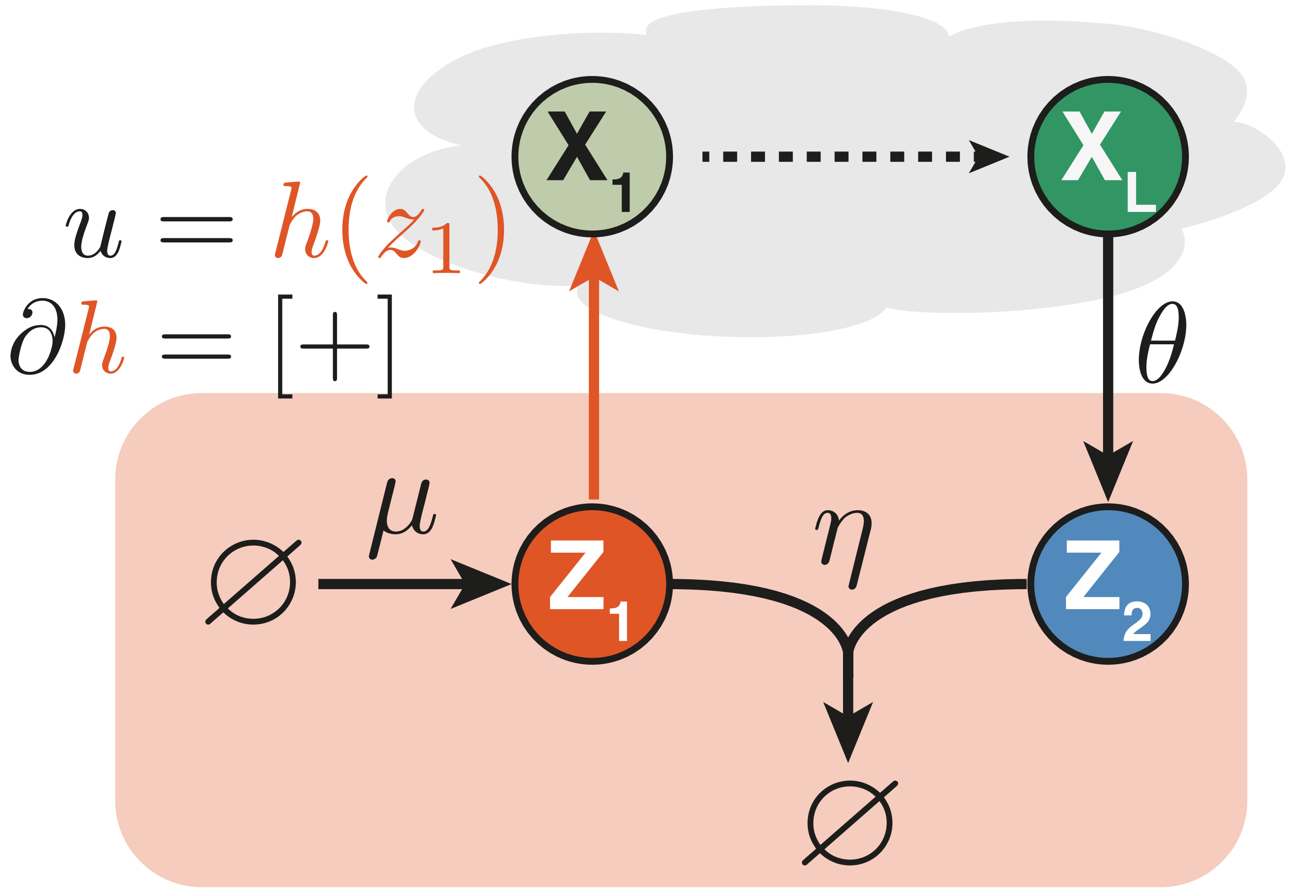

rAIF

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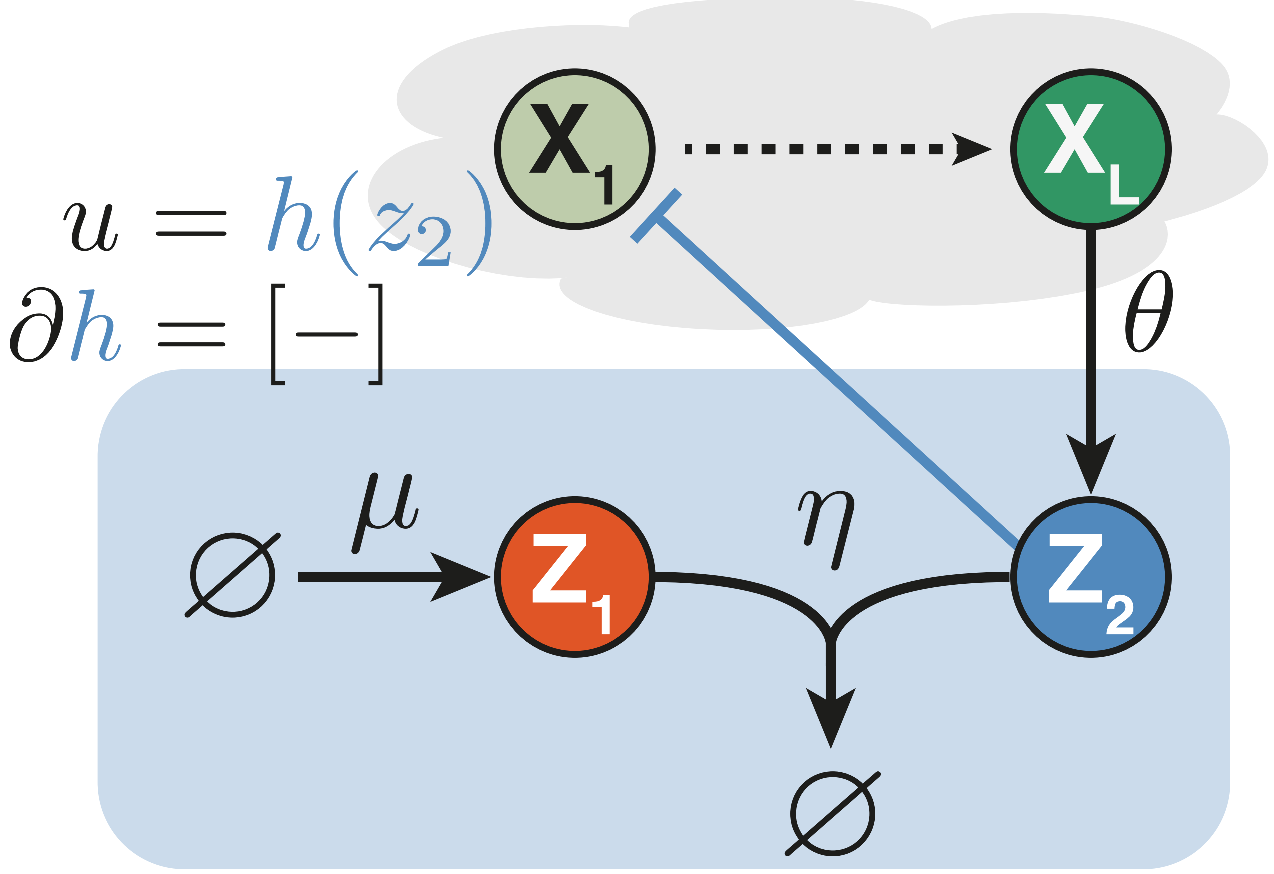

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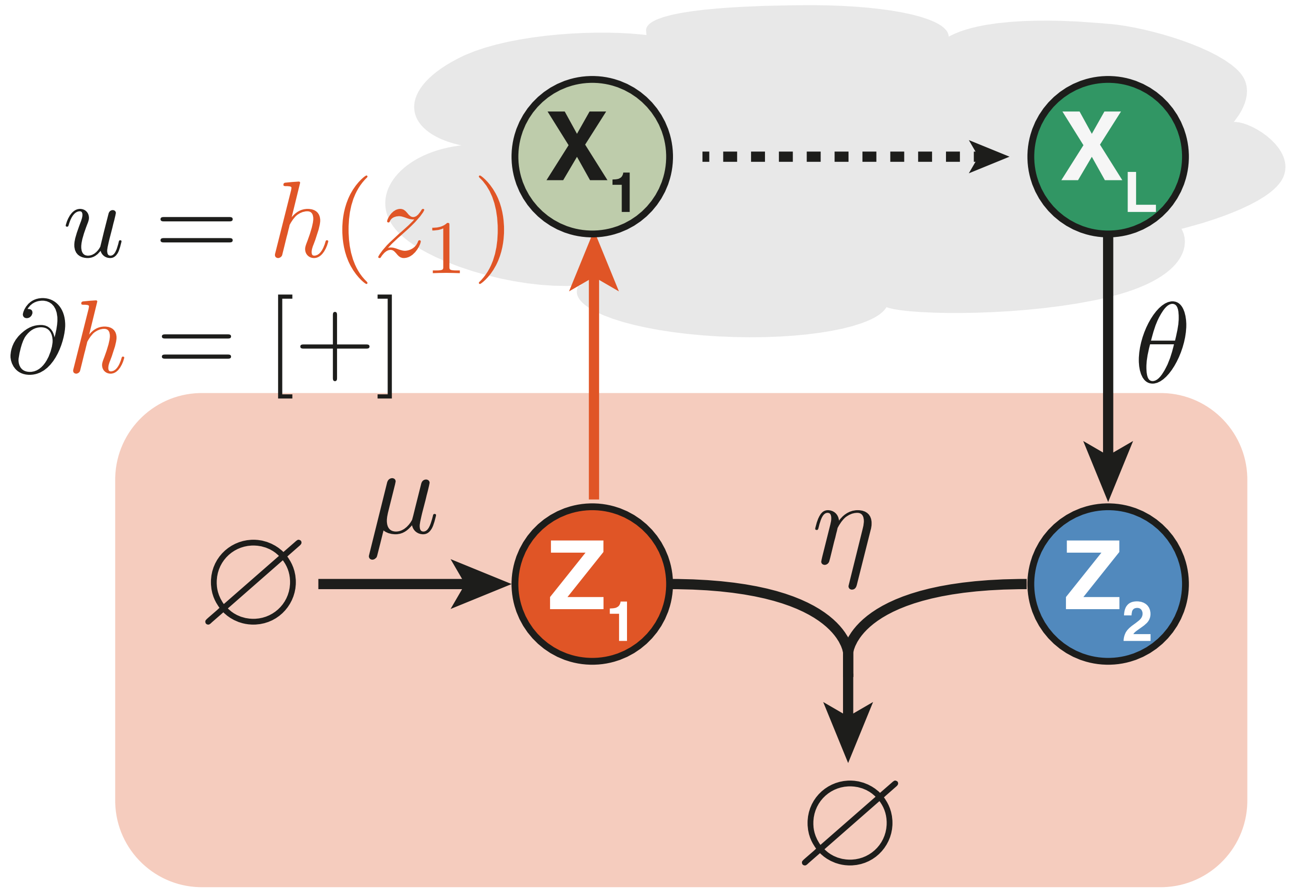

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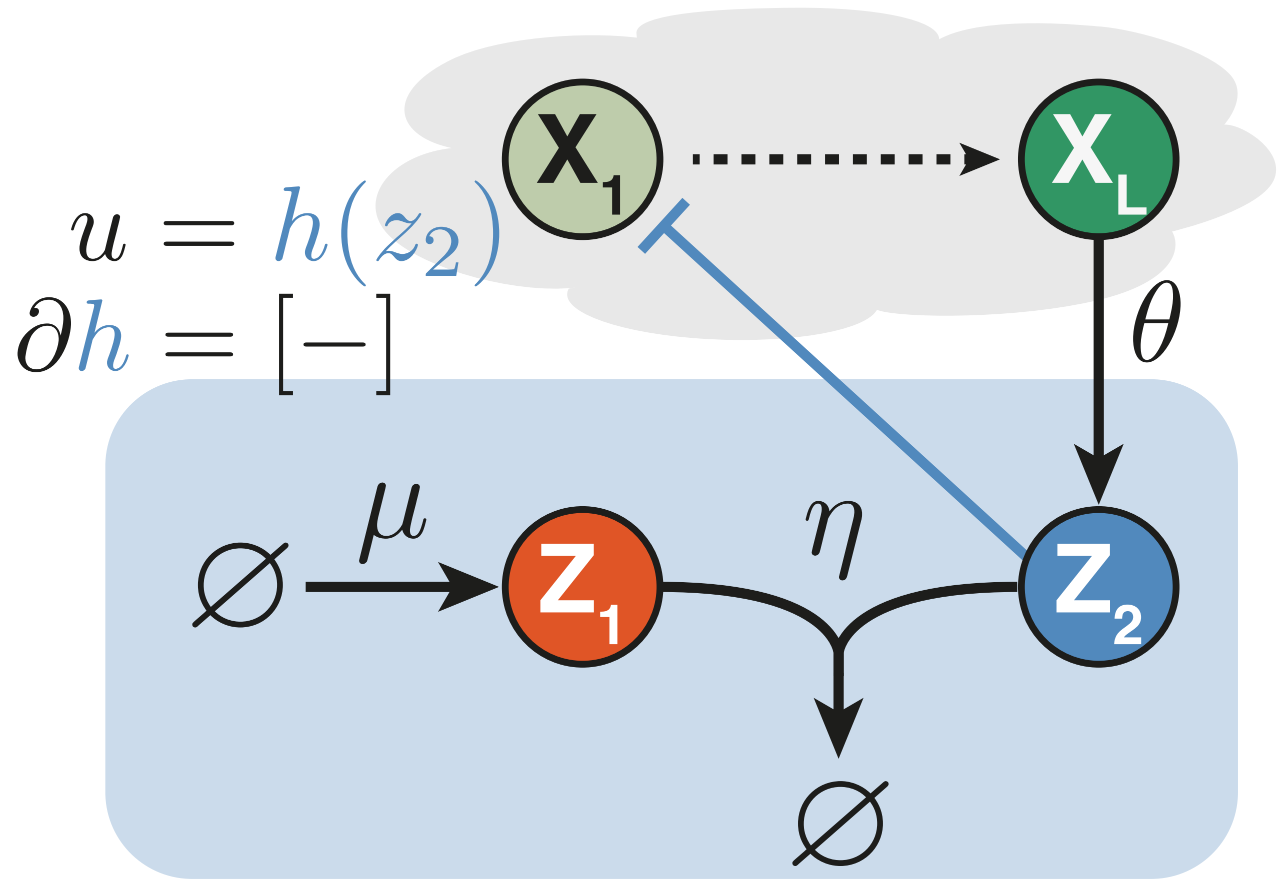

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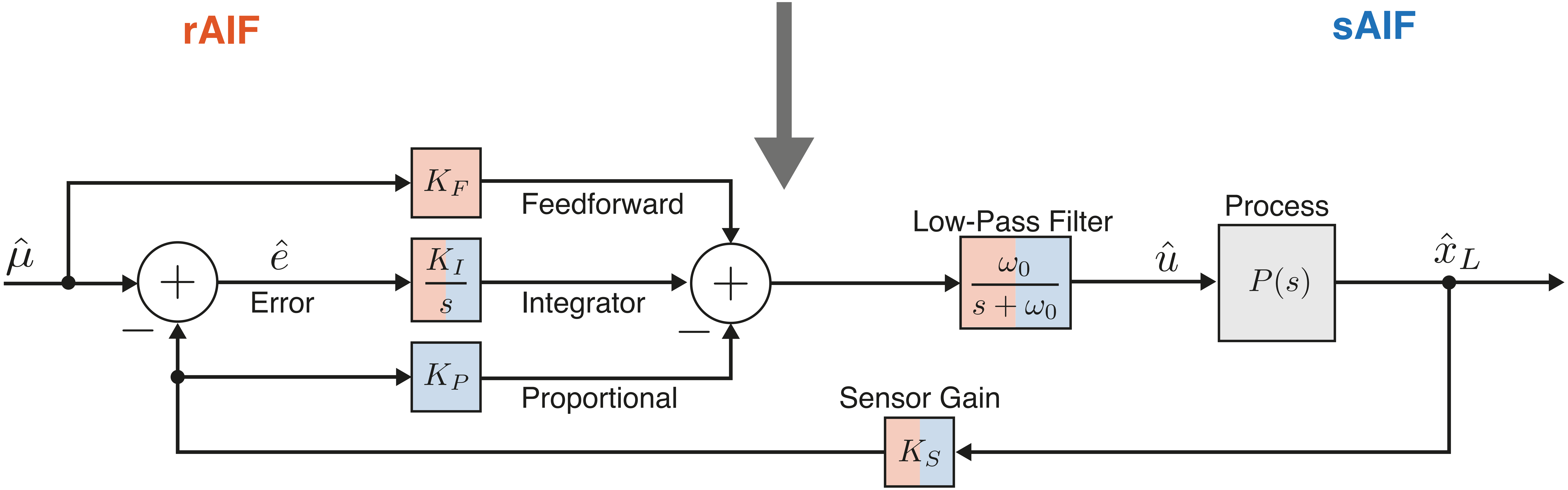

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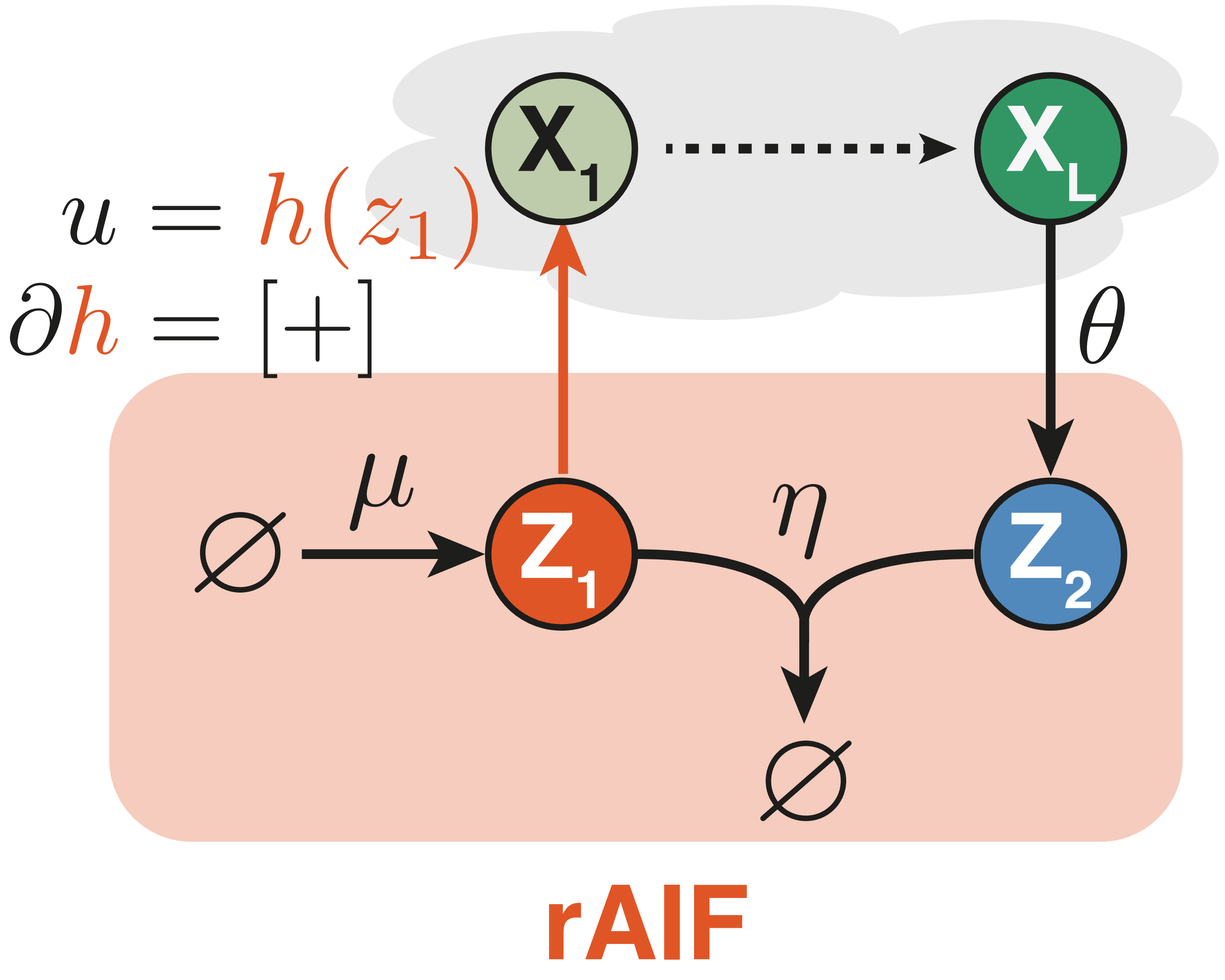

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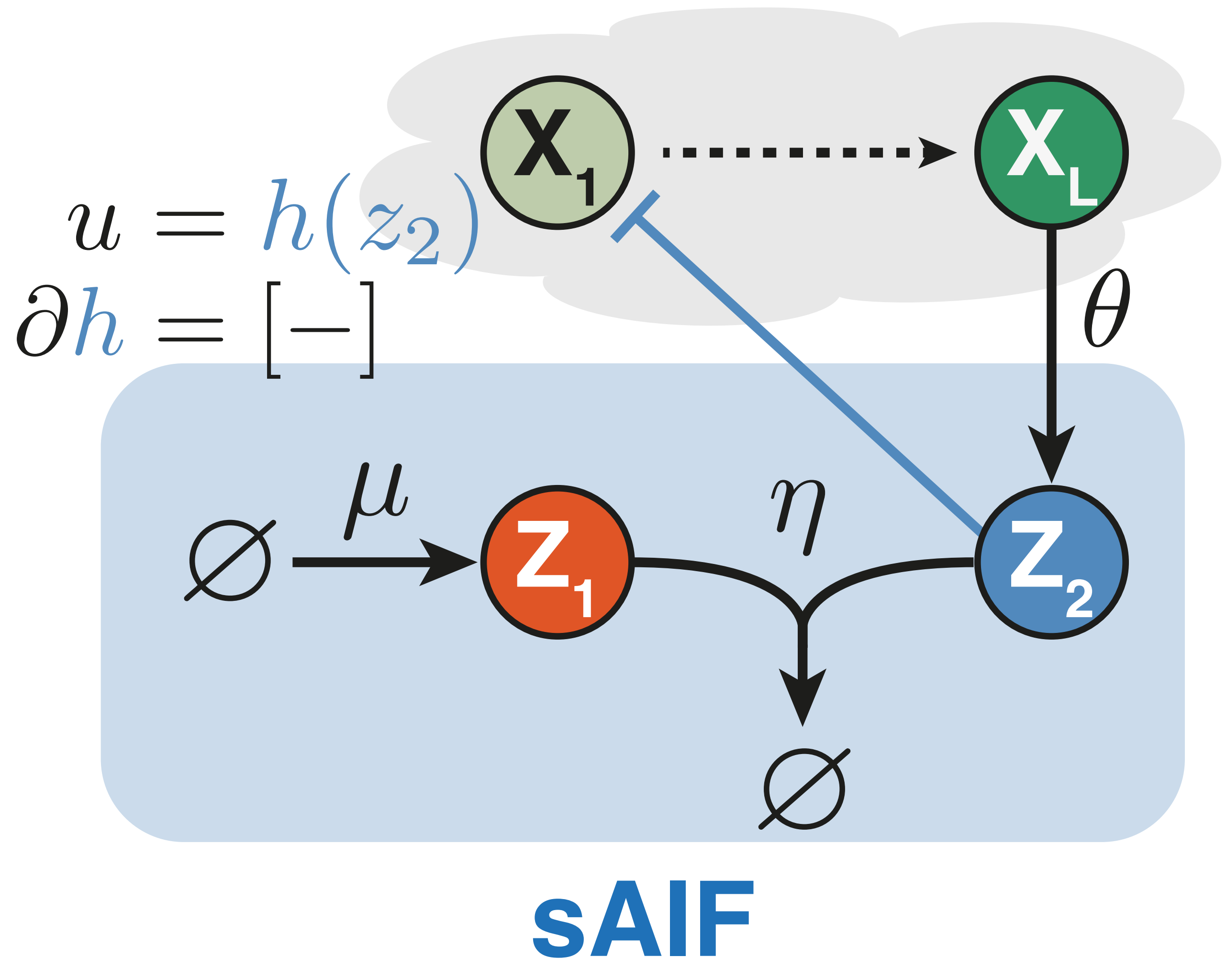
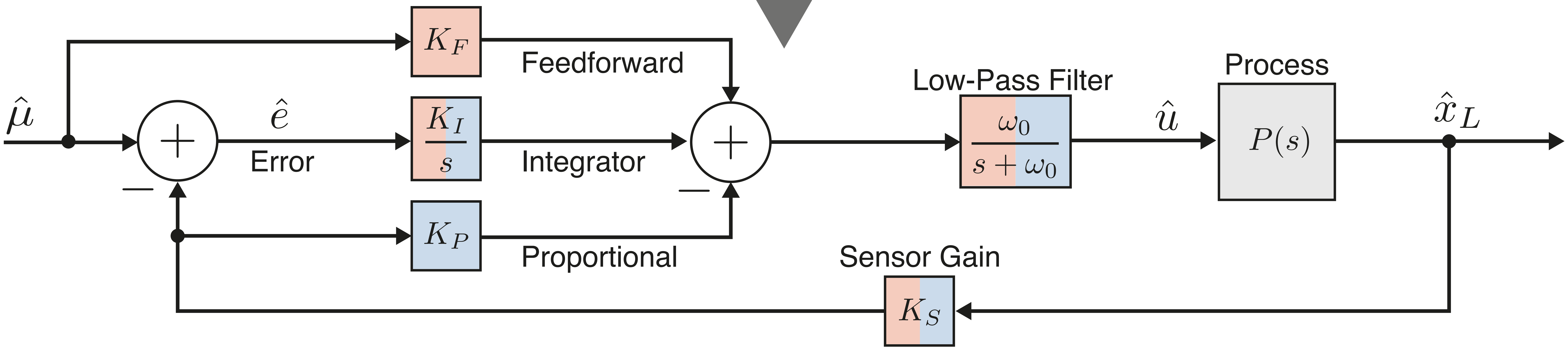


Mapping: $\mu, \eta, \theta, h, \bar{z}_1, \bar{z}_2 \rightarrow K_I, K_P, K_F, \omega_0, K_S$



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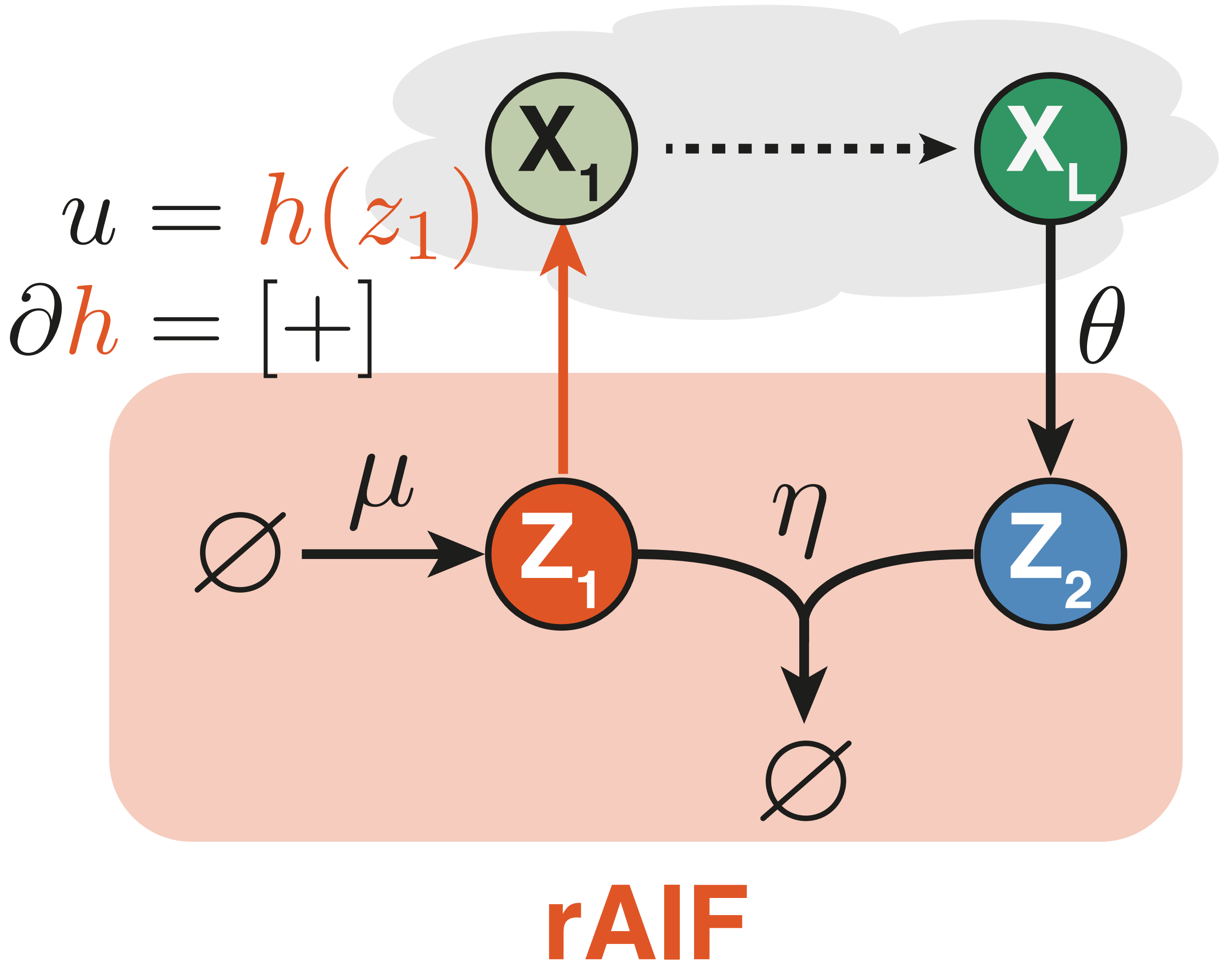

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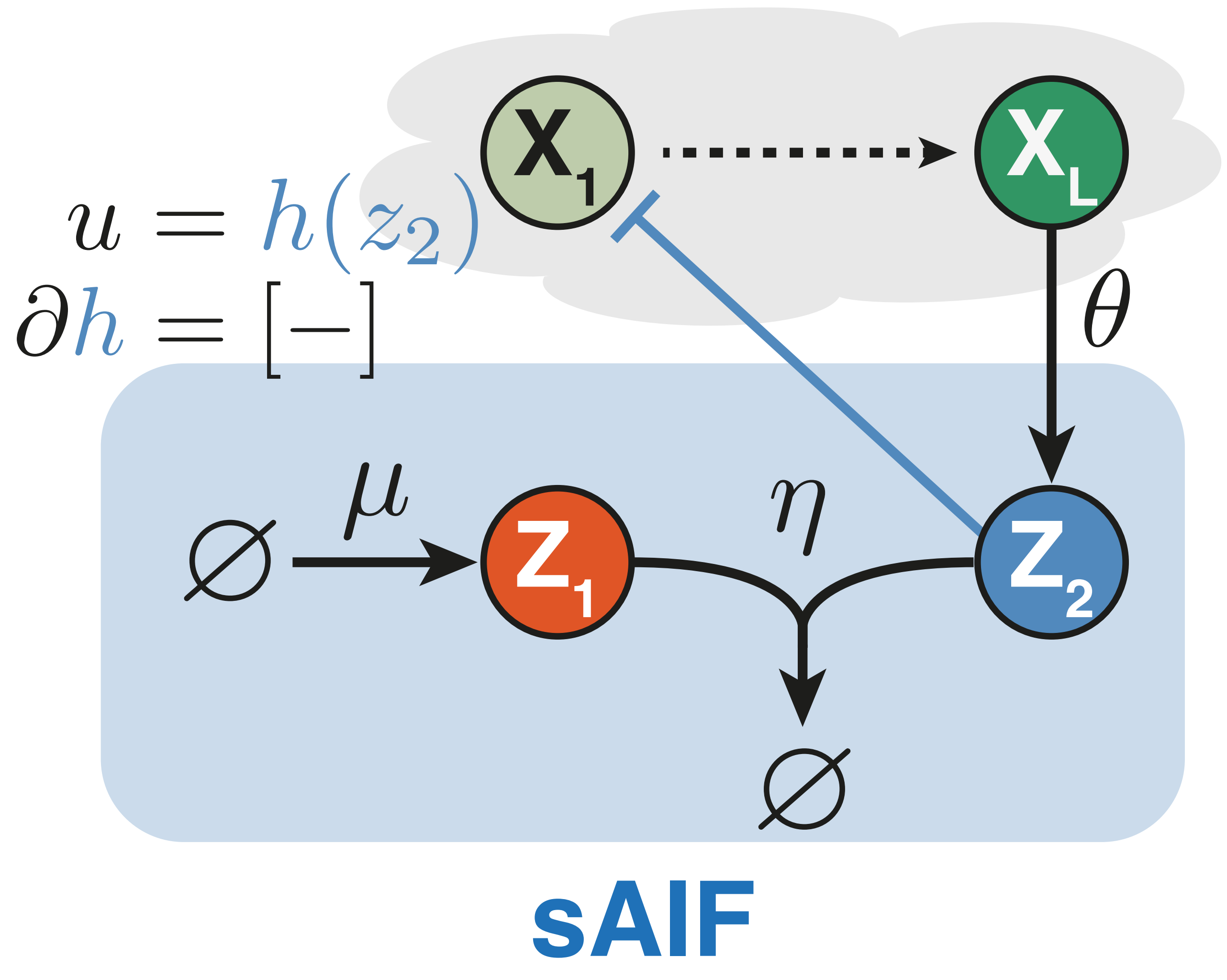

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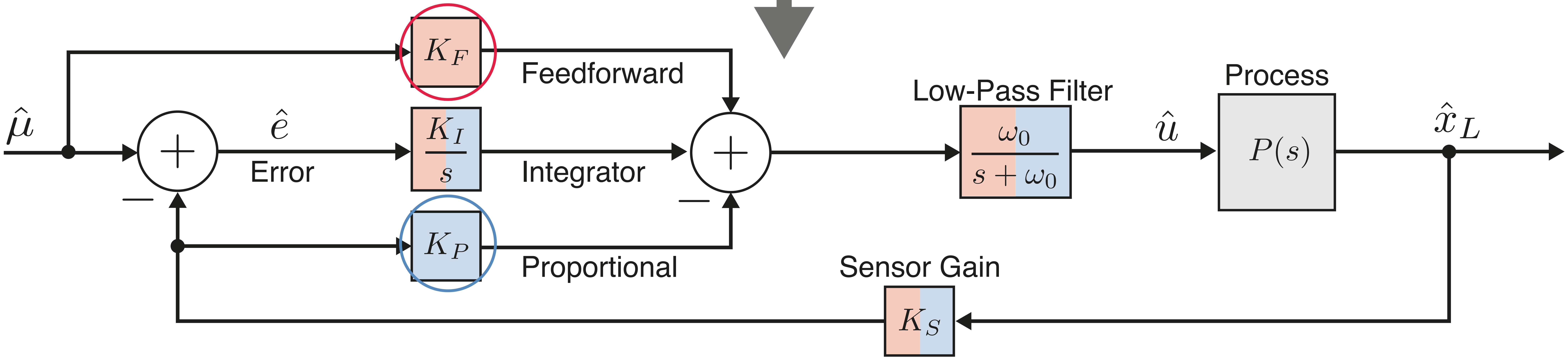

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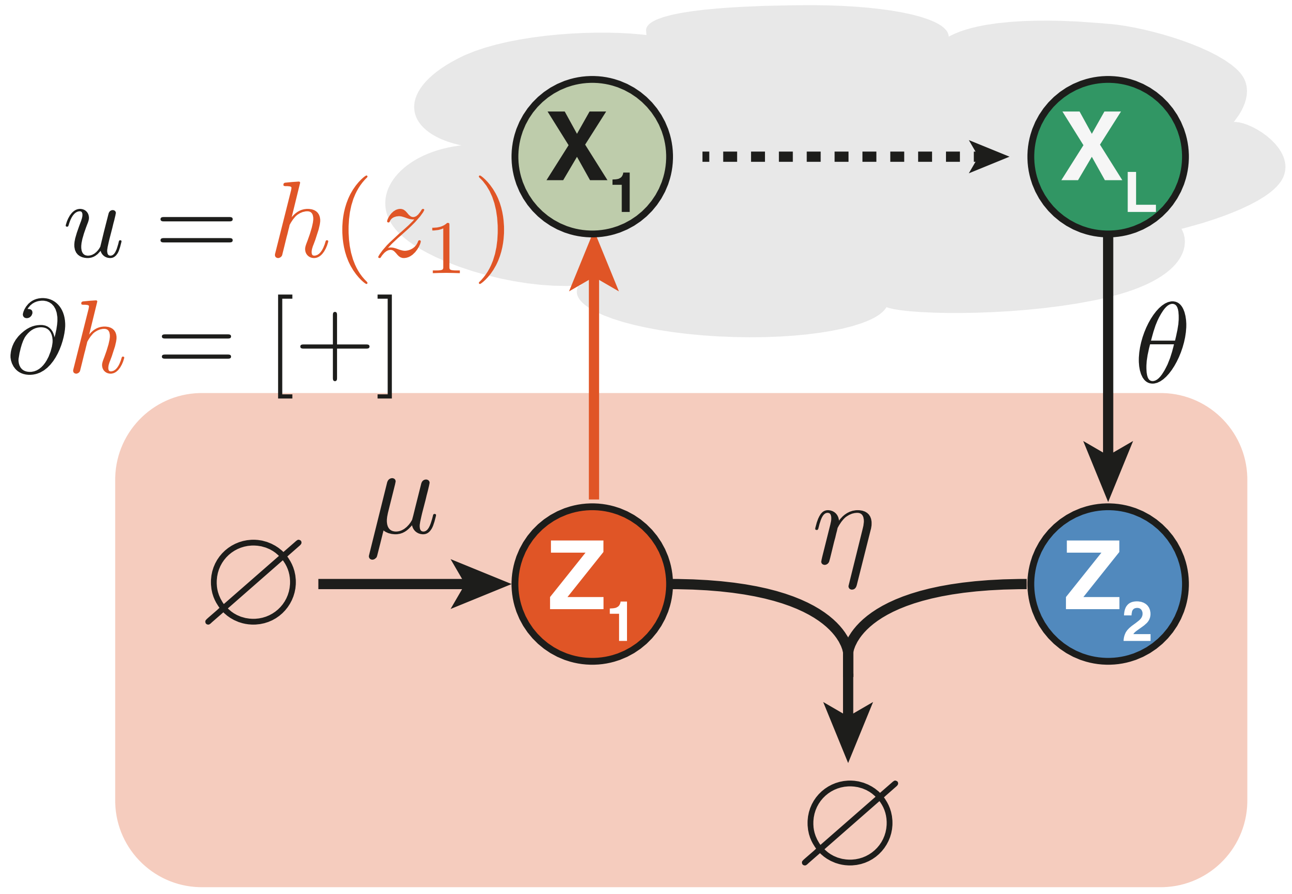

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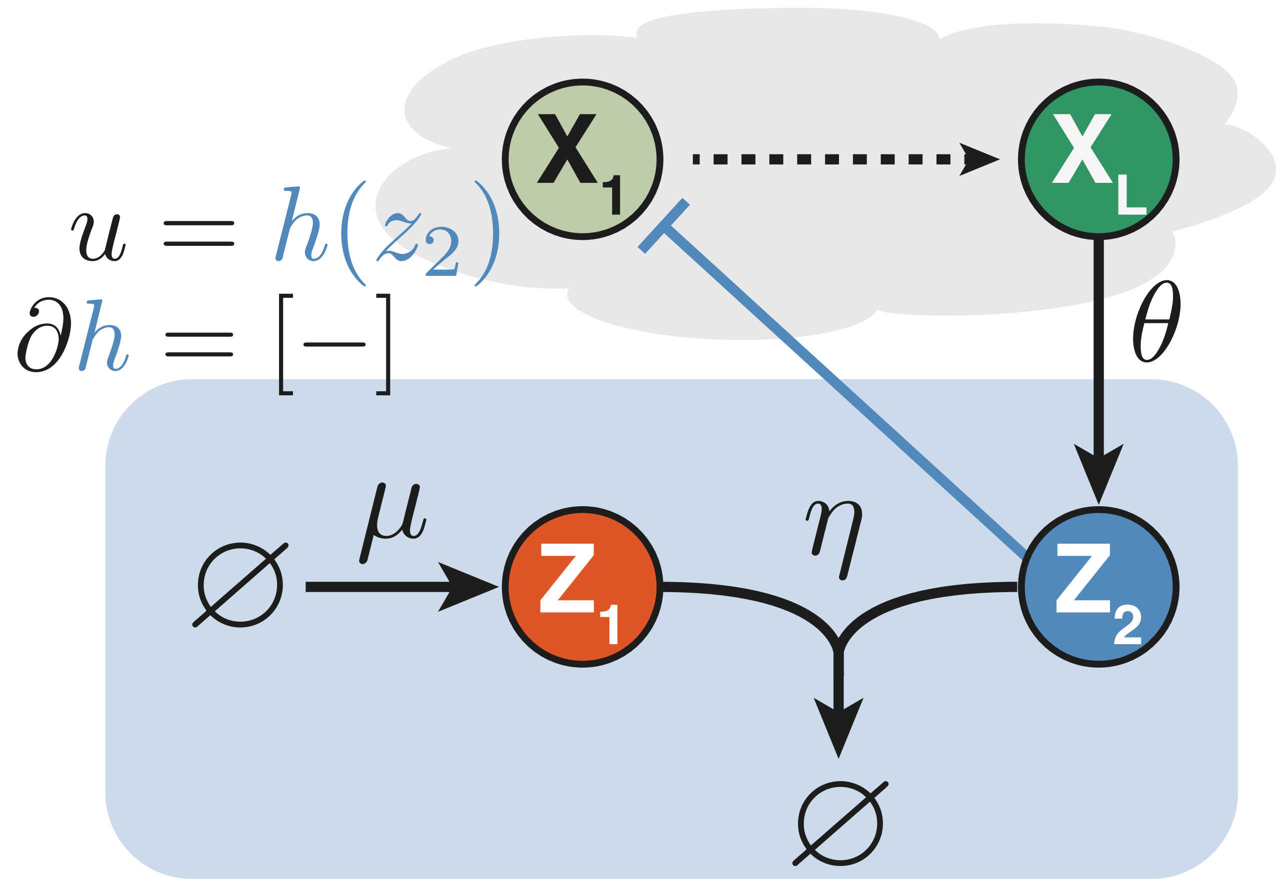
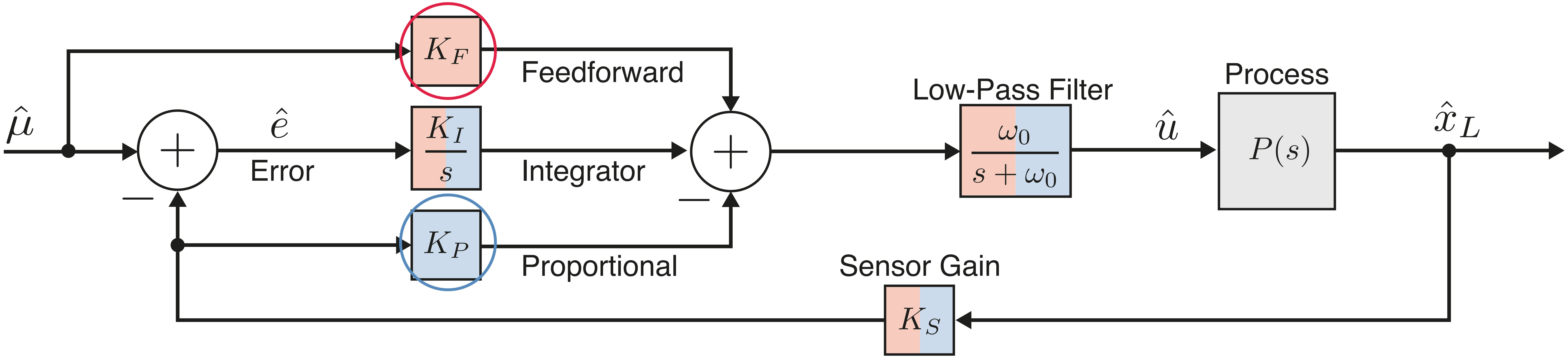


sAIF

$\Longrightarrow$ sAIF "hides" a proportional component under the integrator (minimal PI)



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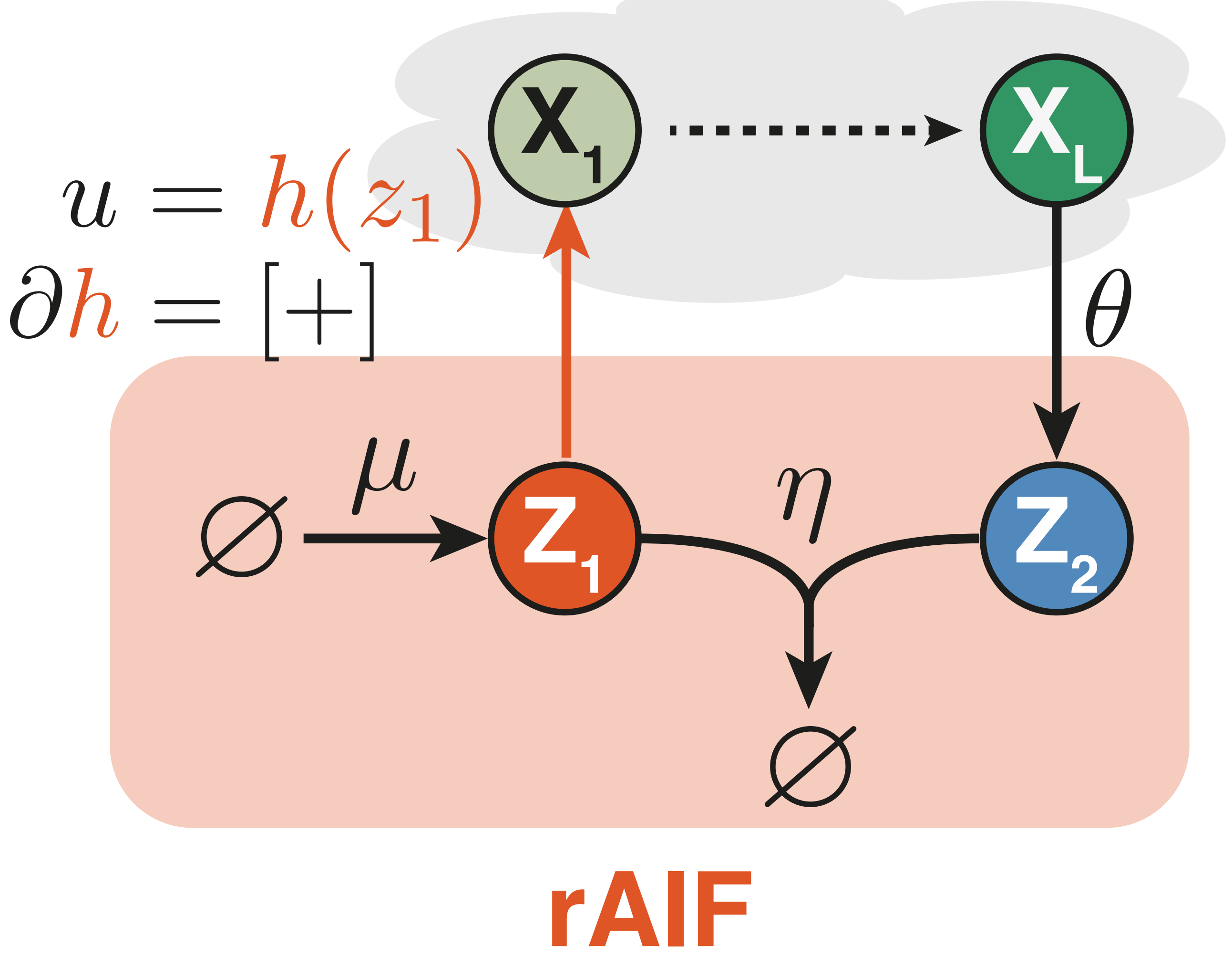

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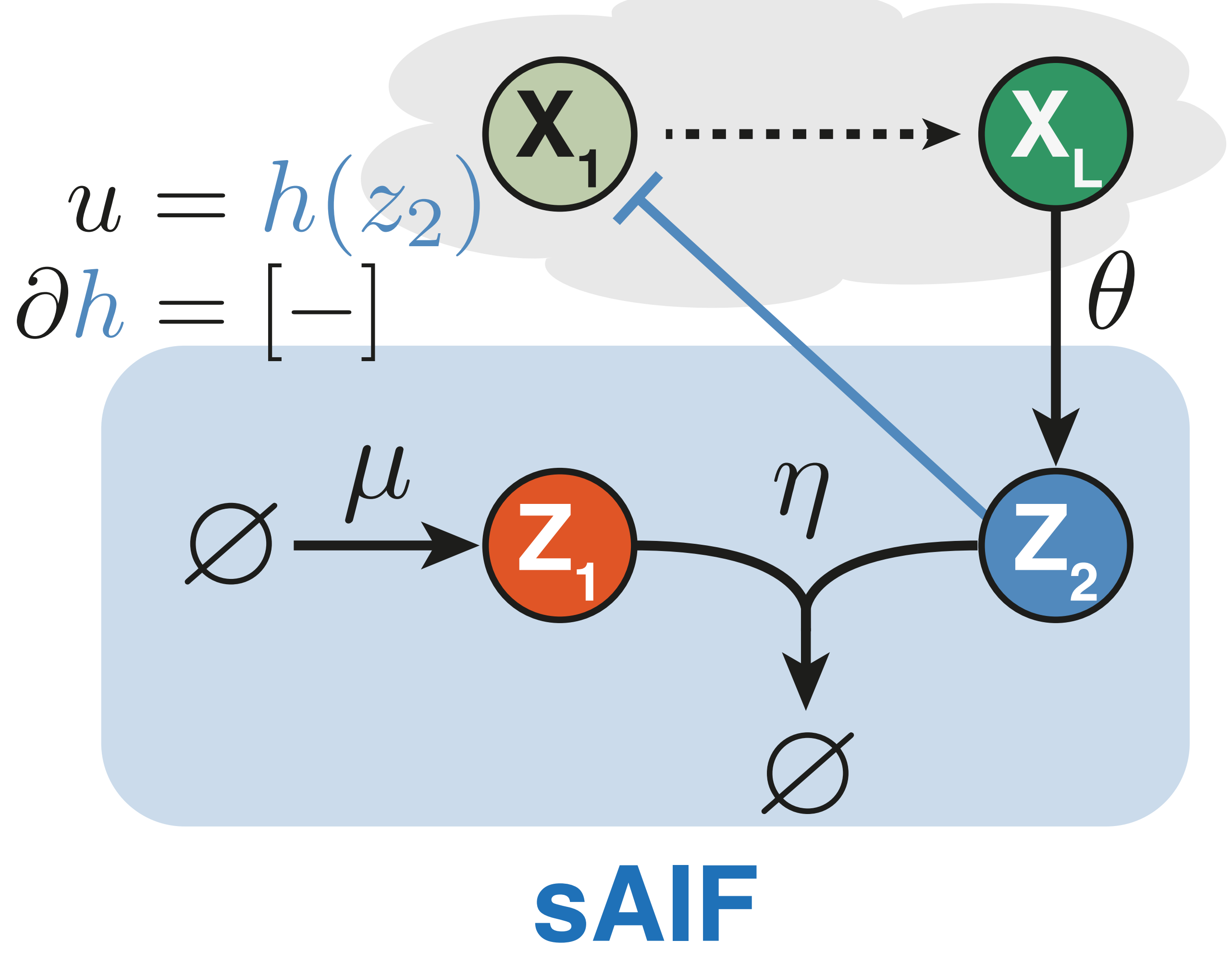
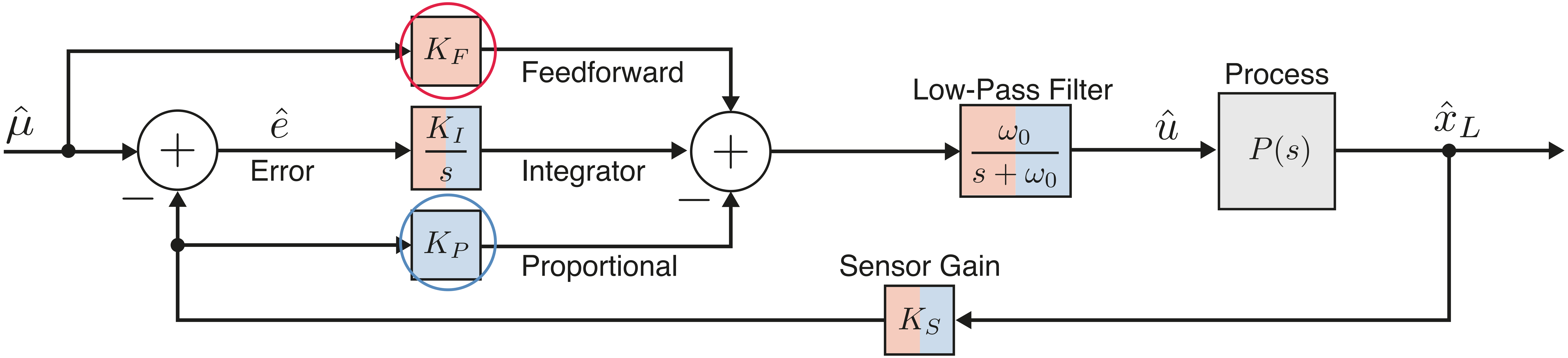


Does this simple modification bring with it the benefits of the P-component?



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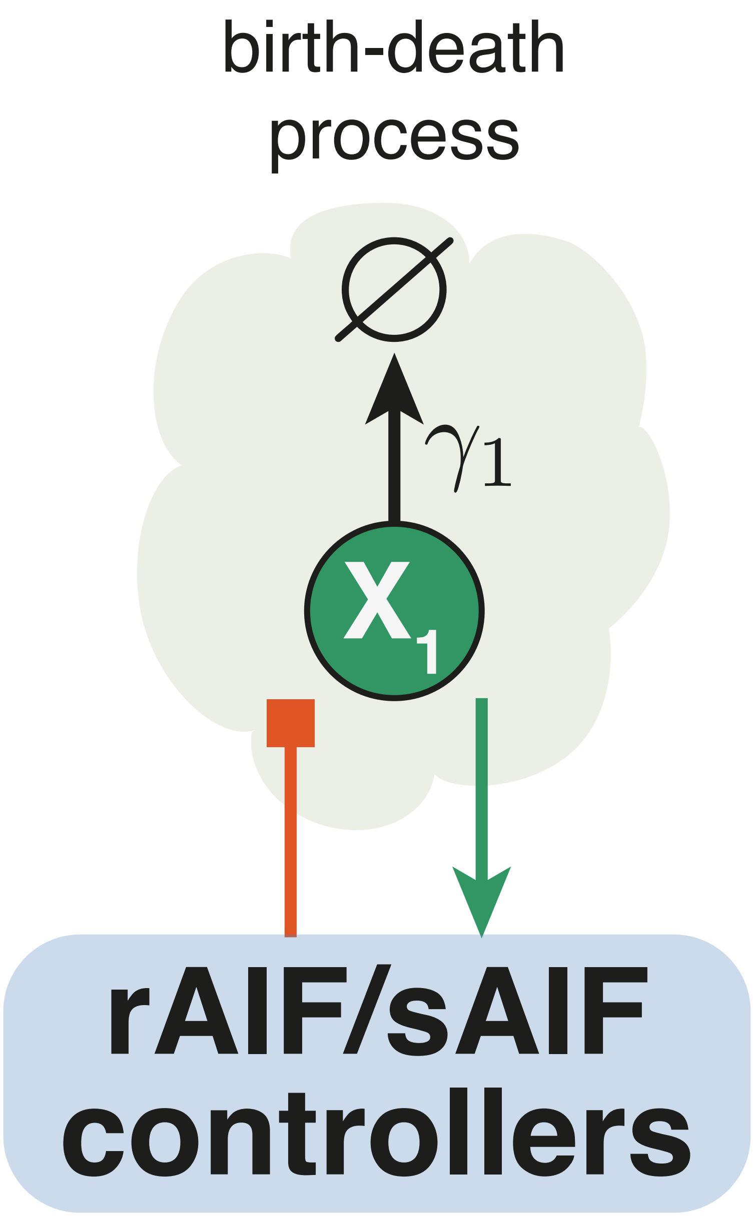
Dynamics of the rAIF Controller: Root Locus



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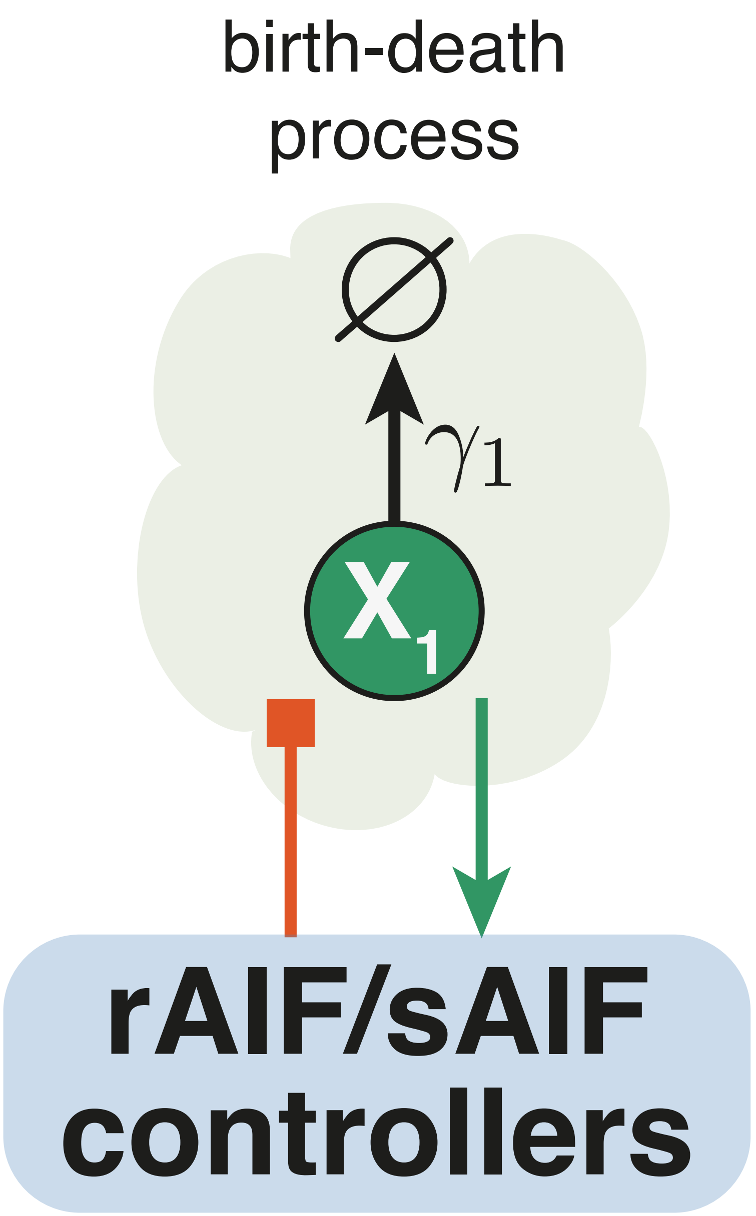

$$H(s) = \frac{\omega_0(K_F s + K_I)}{s^3 + (\omega_0 + \gamma_1)s^2 + \omega_0\left(\gamma_1 + K_P K_S\right)s + \omega_0 K_S K_I}$$

sAIF: $K_F = 0,\ K_P > 0$

rAIF: $K_F > 0,\ K_P = 0$

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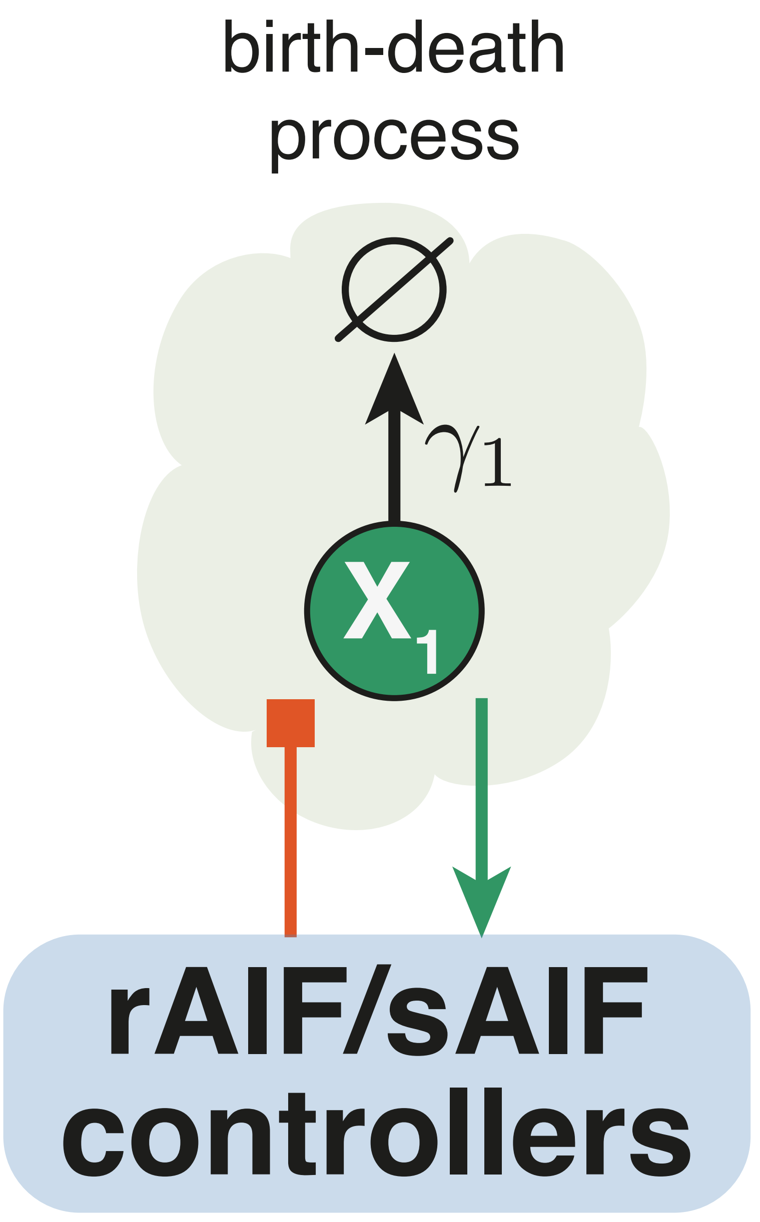

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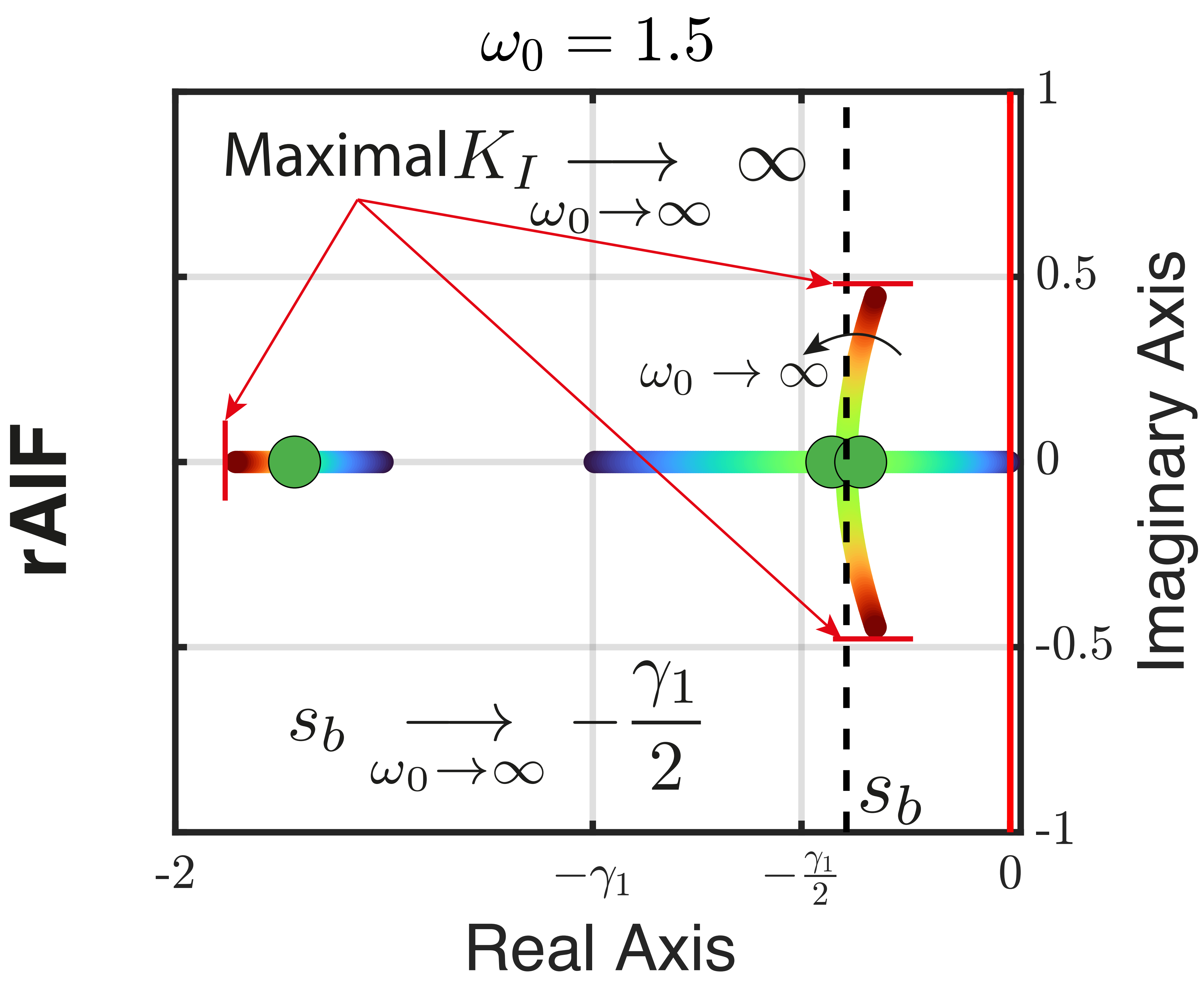

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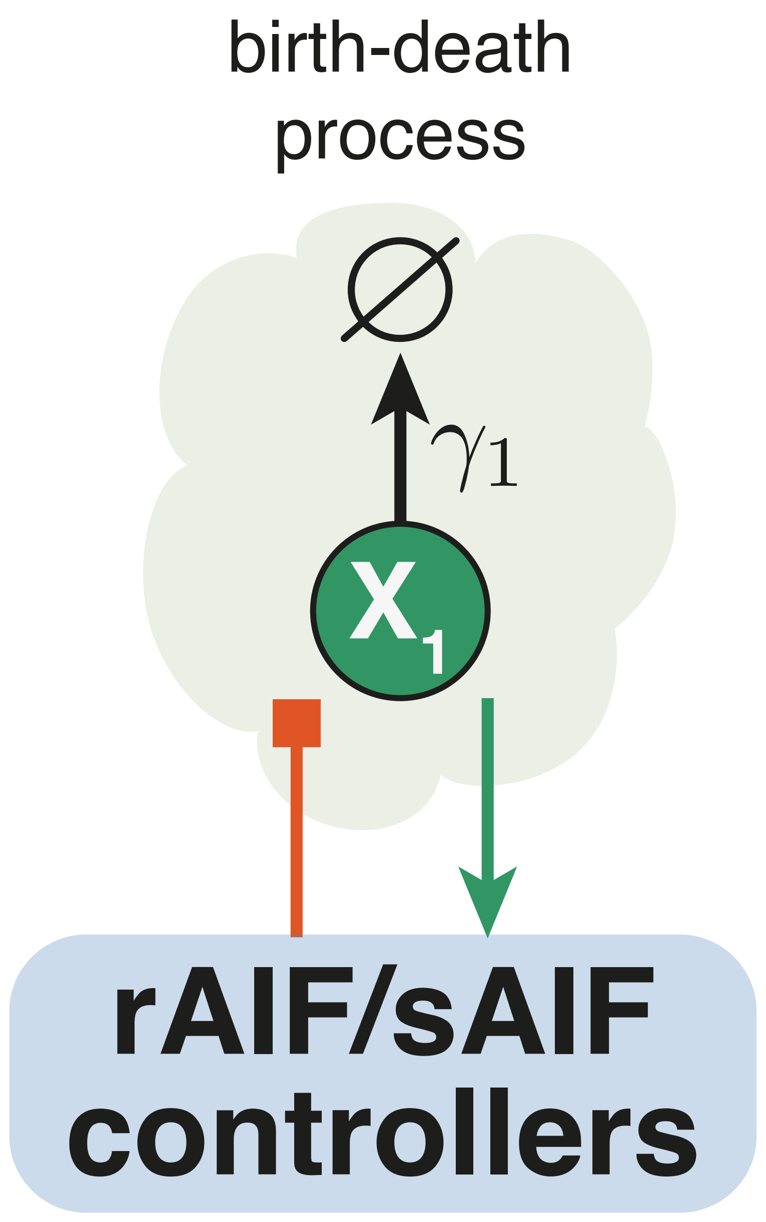

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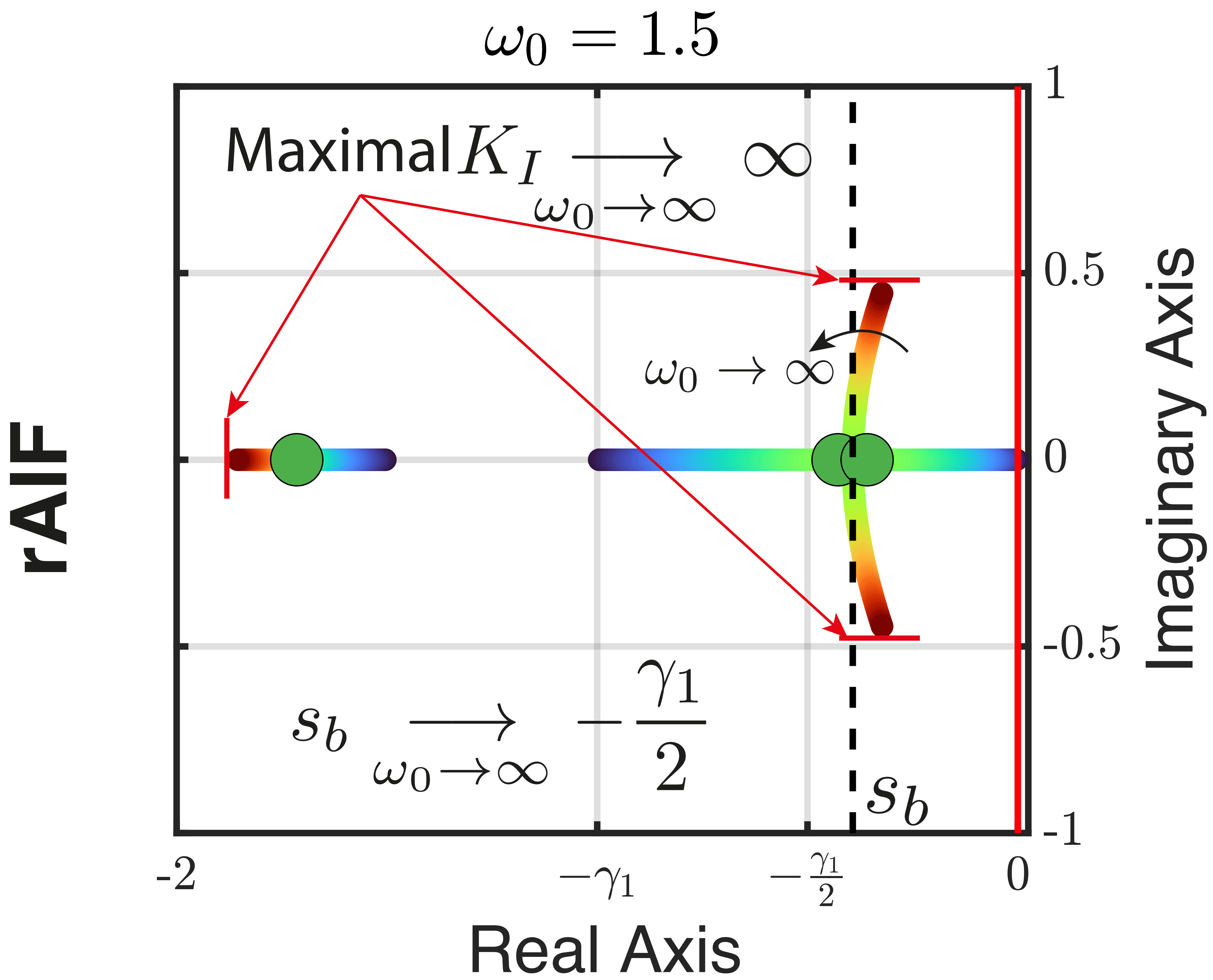
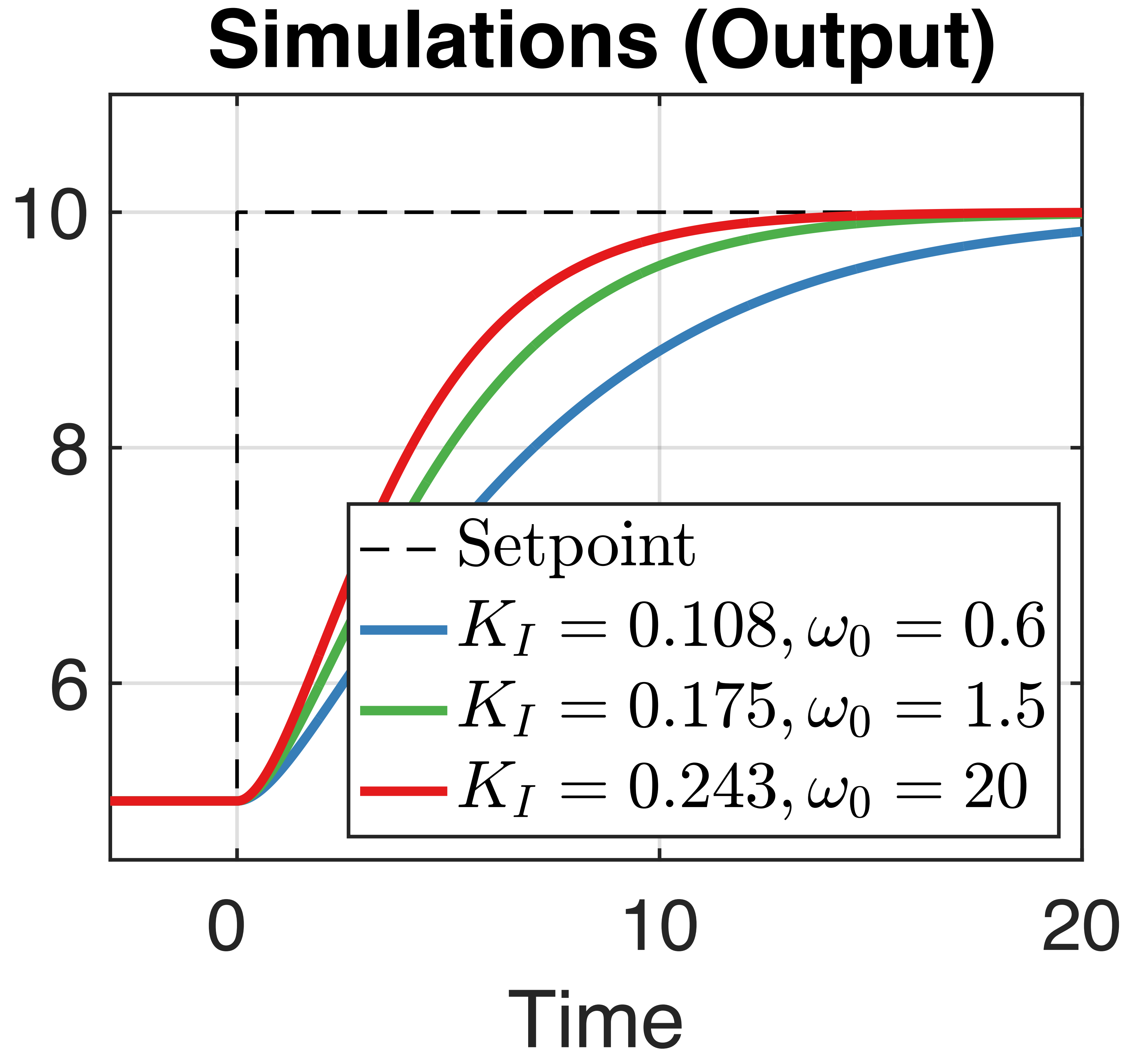
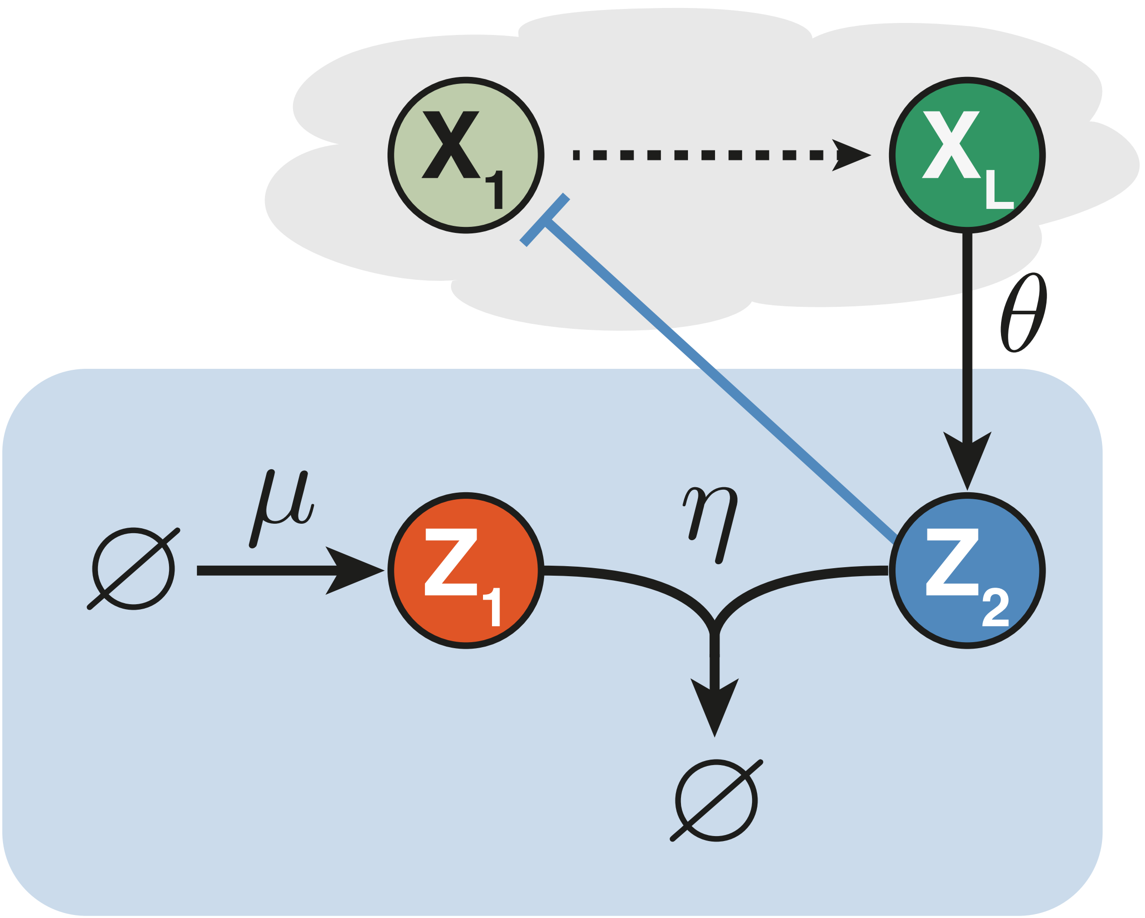




Dynamics of the sAIF Controller: PI Coverage



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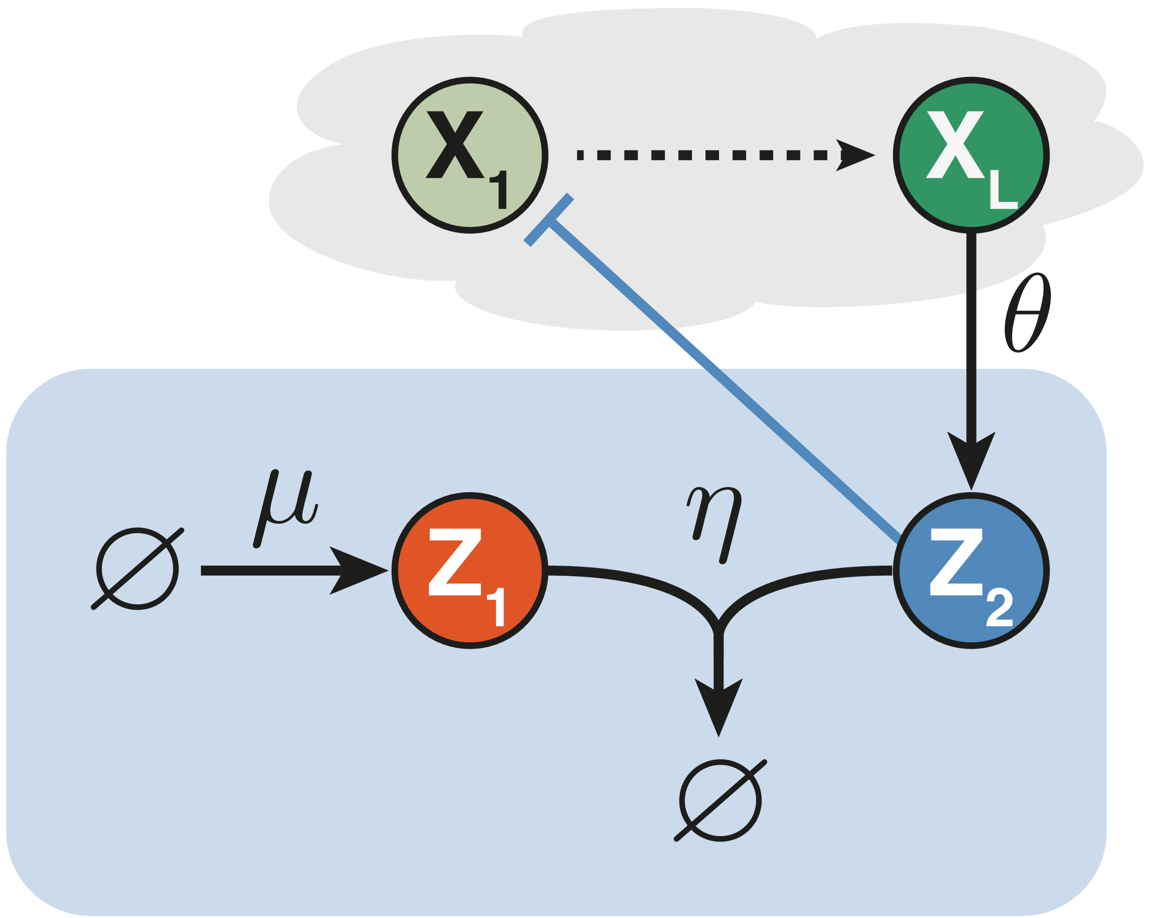

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$$h(z_2; x_1) = \begin{cases} \dfrac{\alpha}{1 + (z_2/\kappa)^n} & \textbf{(Repression)} \\ \alpha - \gamma z_2 x_1 & \textbf{(Degradation)} \end{cases}$$

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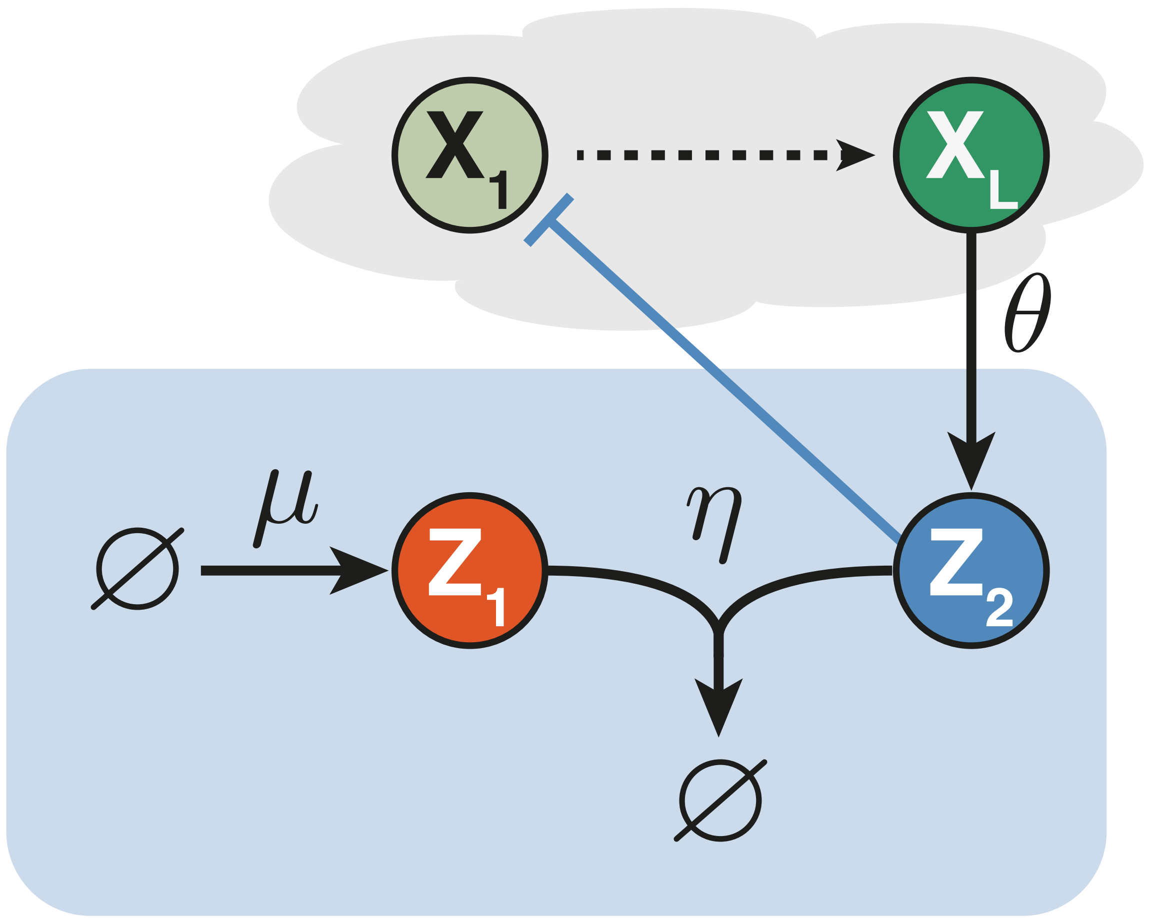

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$$\mathcal{S}_d = \left\{ (K_P, K_I, \omega_0) \in \mathbb{R}_+^3 : K_I < \omega_0 K_P \right\},$$

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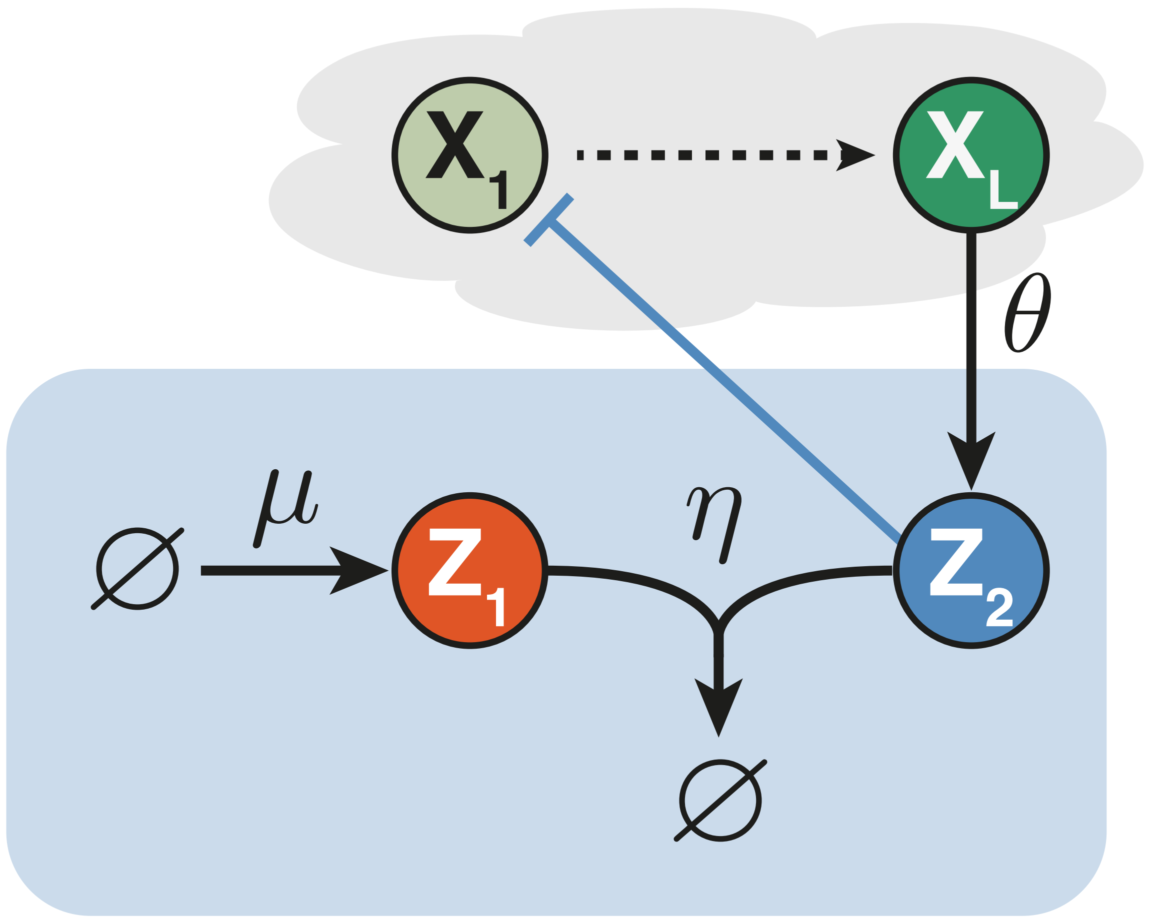

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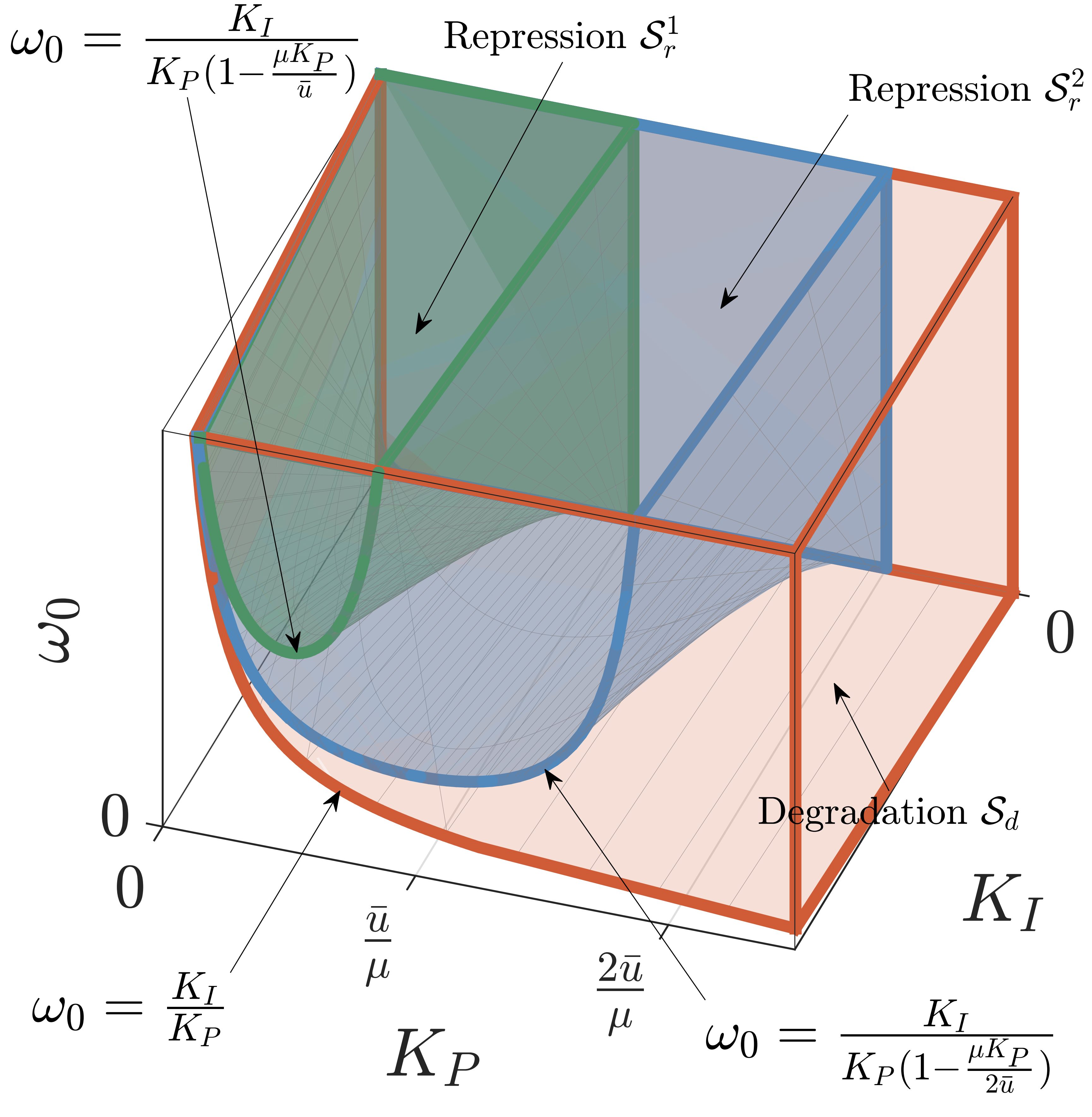
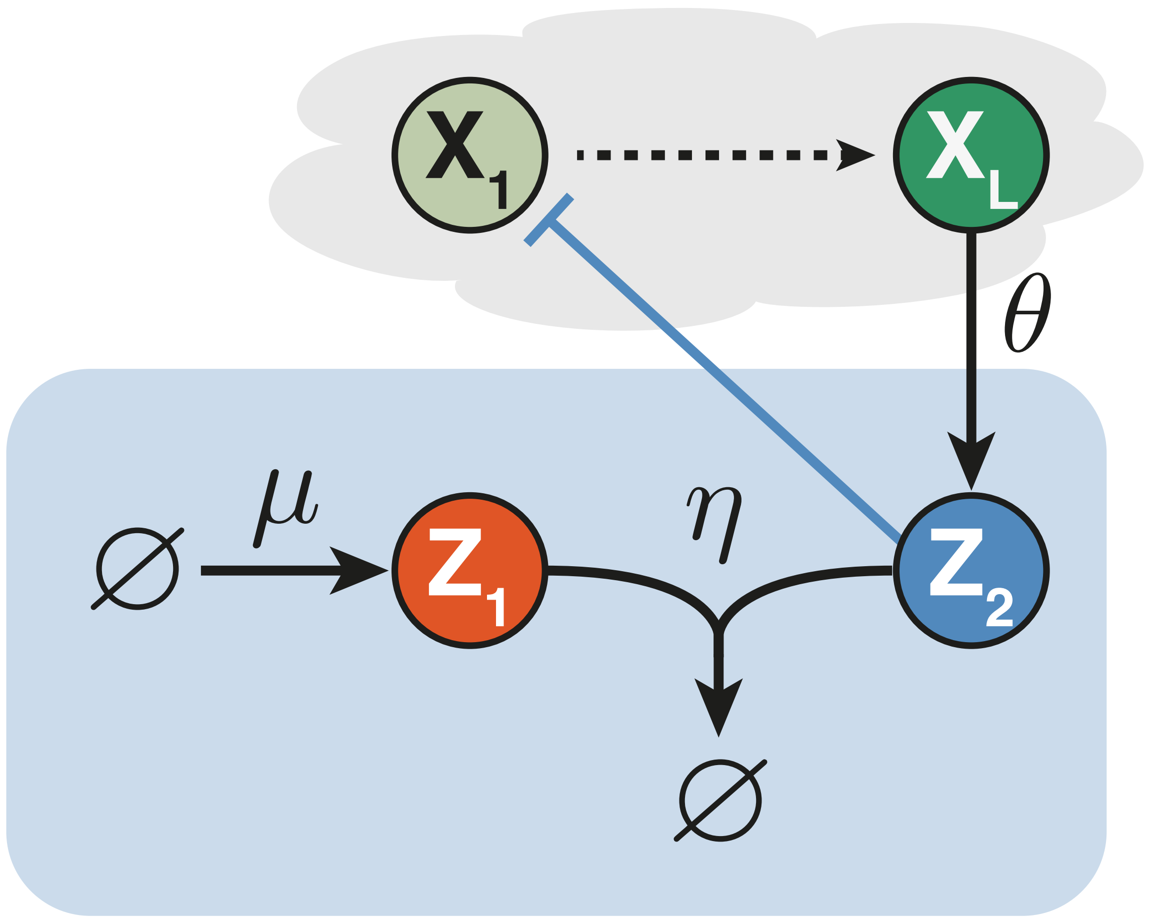


Dynamics of the sAIF Controller: PI Coverage



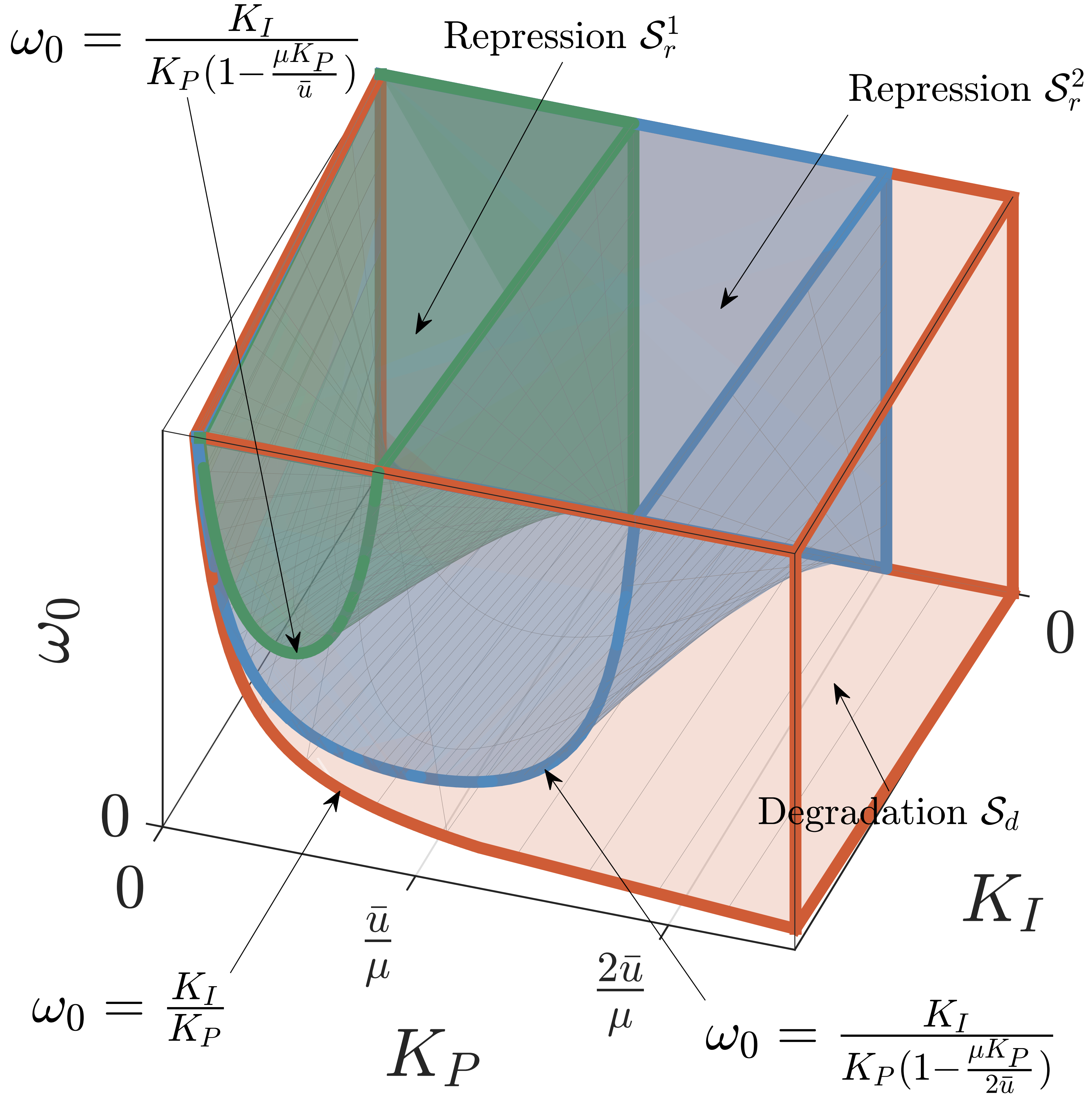
$$\begin{cases} \dot{z}_1 = \mu - \eta z_1 z_2 \\ \dot{z}_2 = \theta x_L - \eta z_1 z_2 \\ u = h(z_2; x_1) \end{cases}$$

$$h(z_2; x_1) = \begin{cases} \dfrac{\alpha}{1 + (z_2/\kappa)^n} & \textbf{(Repression)} \\ \alpha - \gamma z_2 x_1 & \textbf{(Degradation)} \end{cases}$$

$$\mathcal{S}_r^n = \left\{ (K_P, K_I, \omega_0) \in \mathbb{R}_+^3 : K_P < n\frac{\bar{u}}{\mu},\ K_I < \omega_0 K_P \left(1 - \frac{\mu K_P}{n\bar{u}}\right) \right\}$$

$$\mathcal{S}_d = \left\{ (K_P, K_I, \omega_0) \in \mathbb{R}_+^3 : K_I < \omega_0 K_P \right\},$$

$$\mathcal{S}_r^n \subset \mathcal{S}_r^{n+1} \subset \mathcal{S}_d$$



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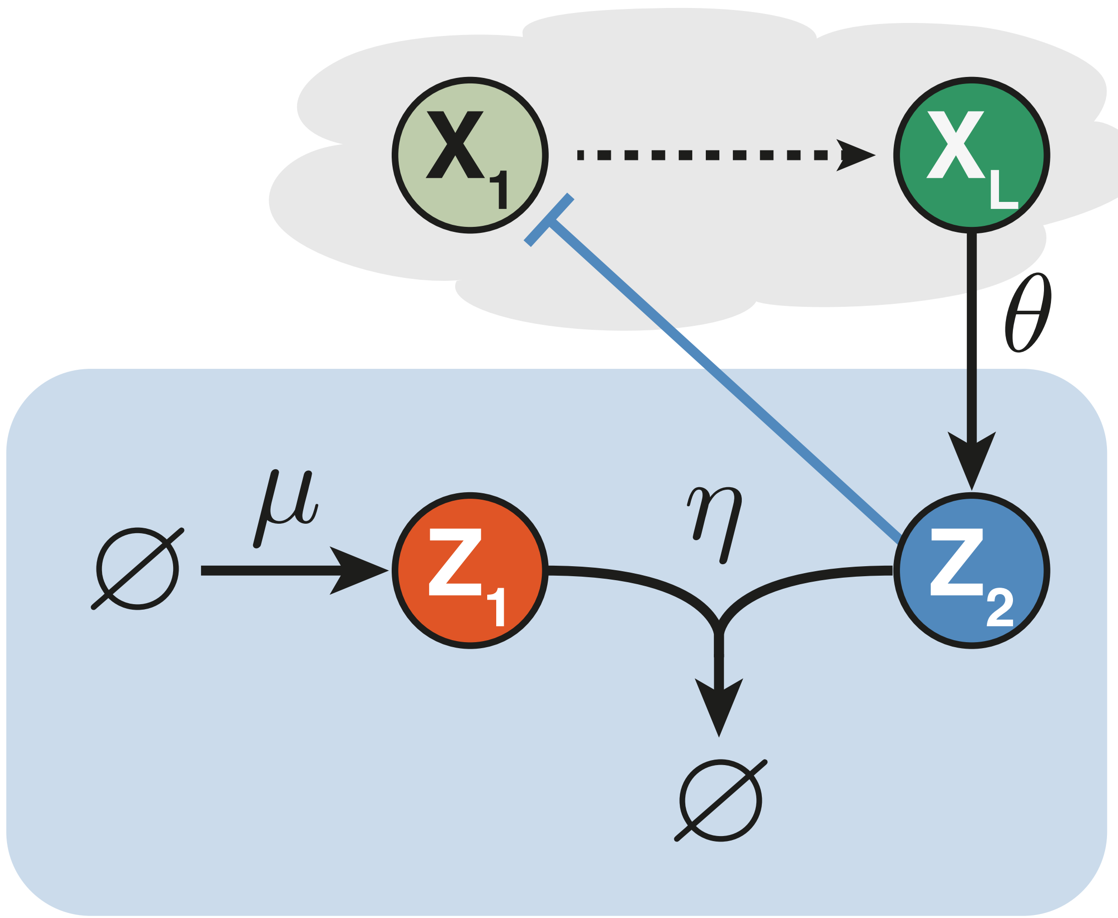

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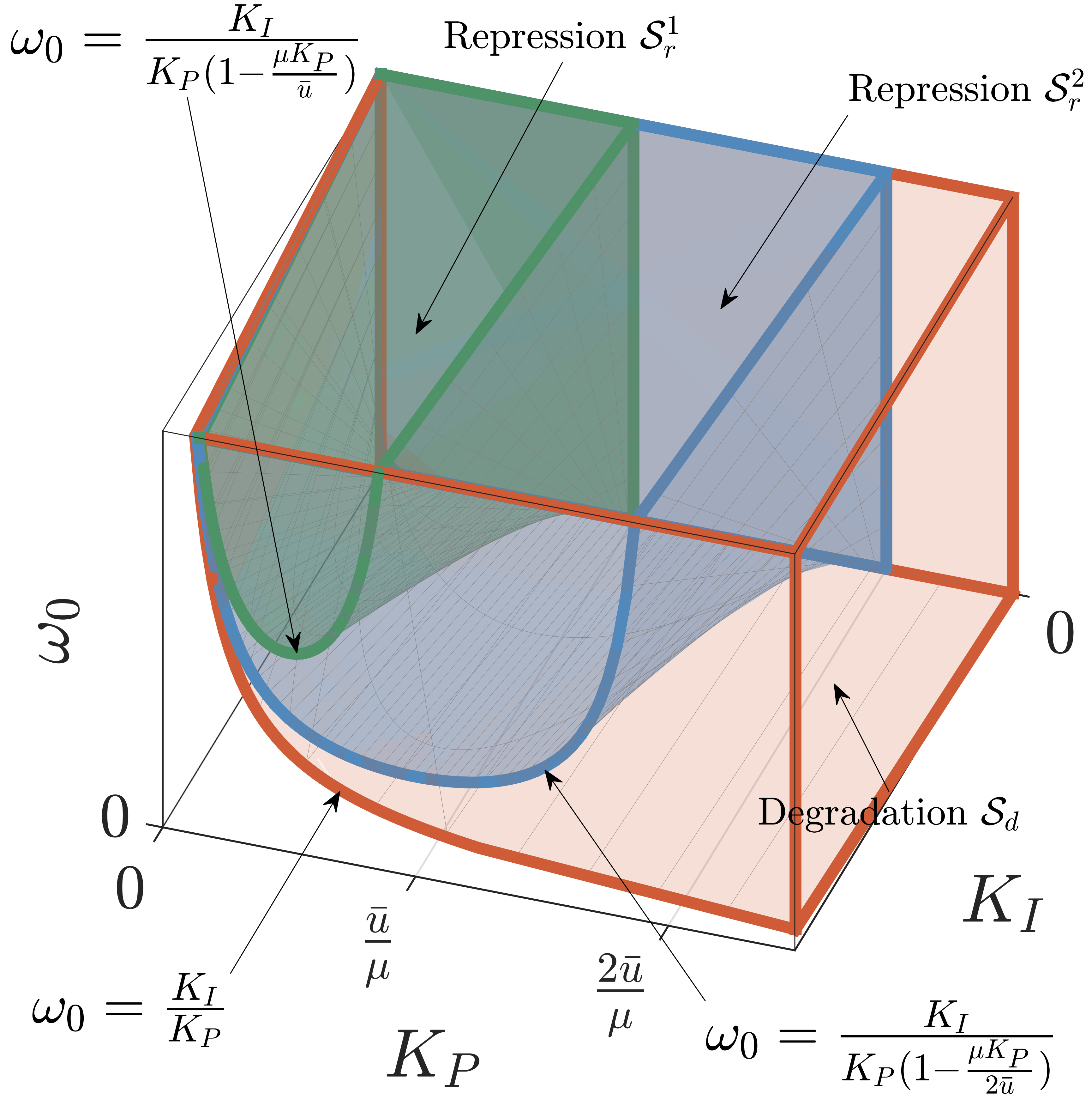
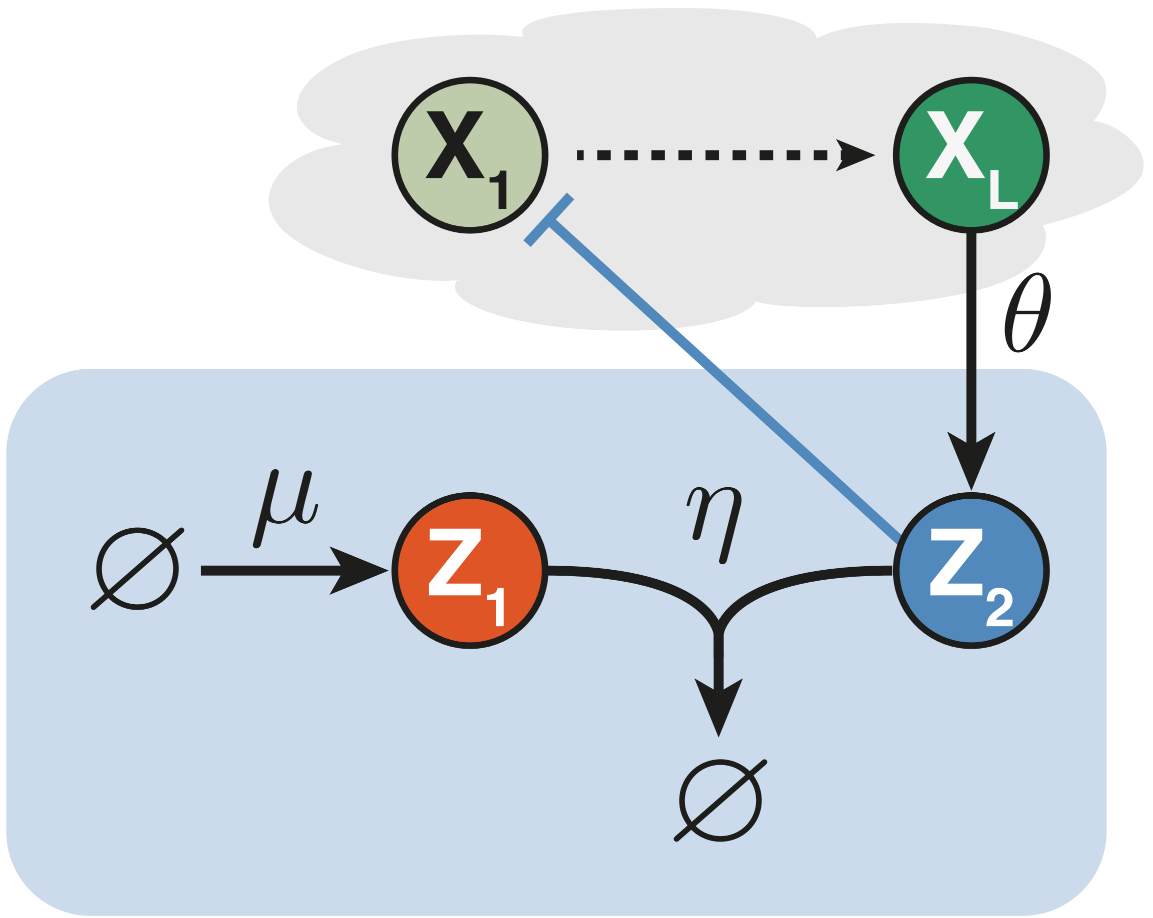


Dynamics of the sAIF Controller: Pole Placement



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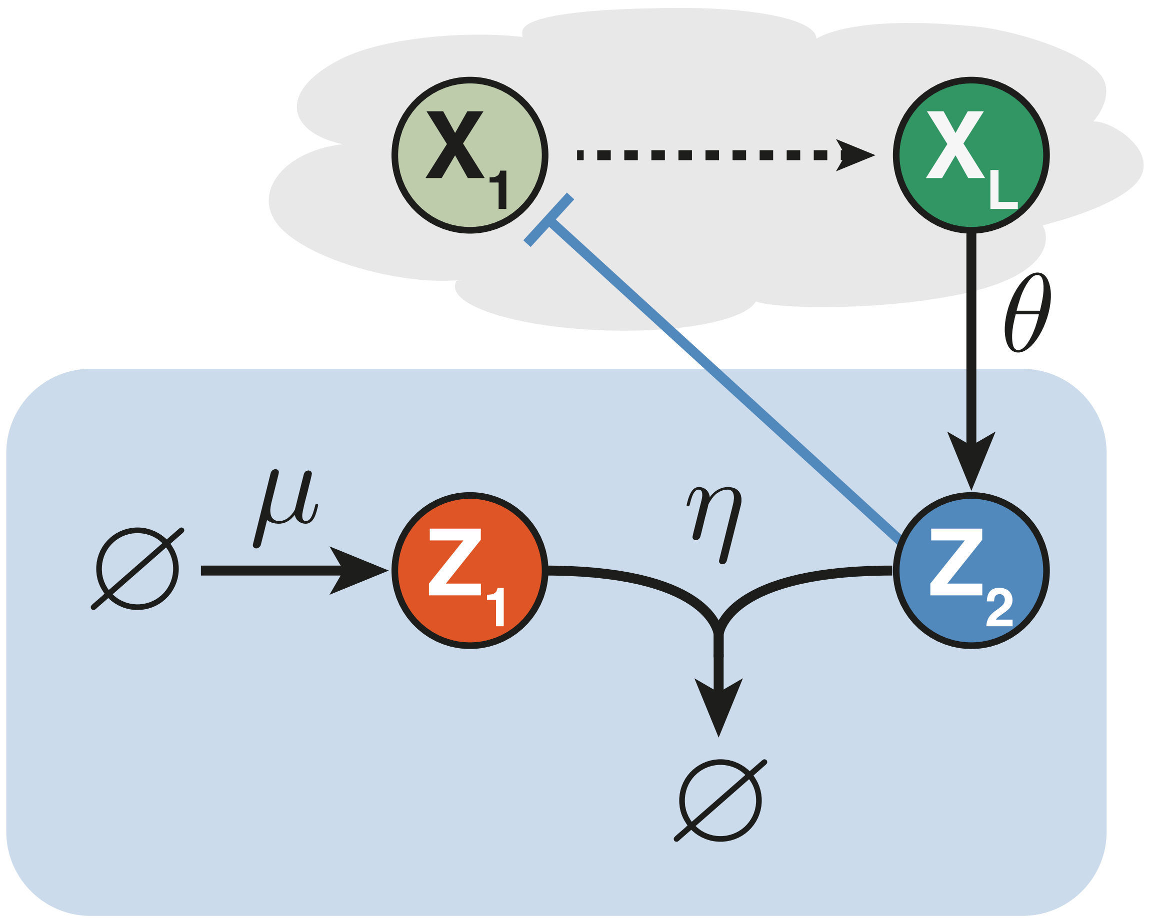

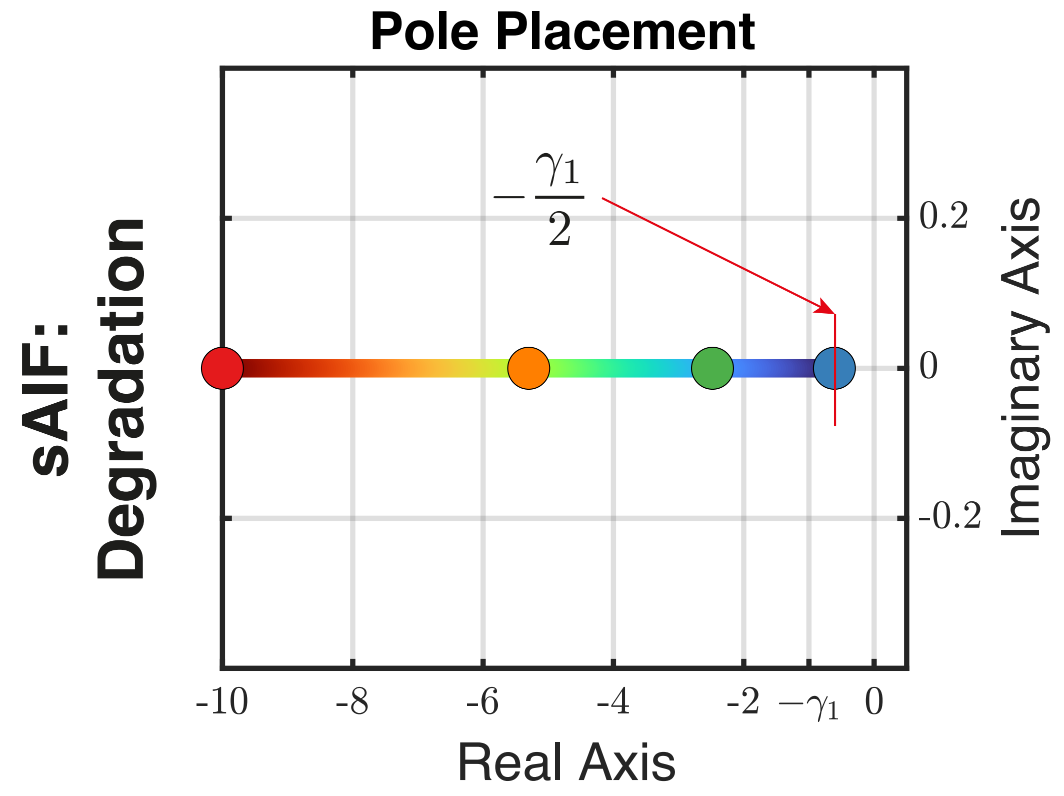

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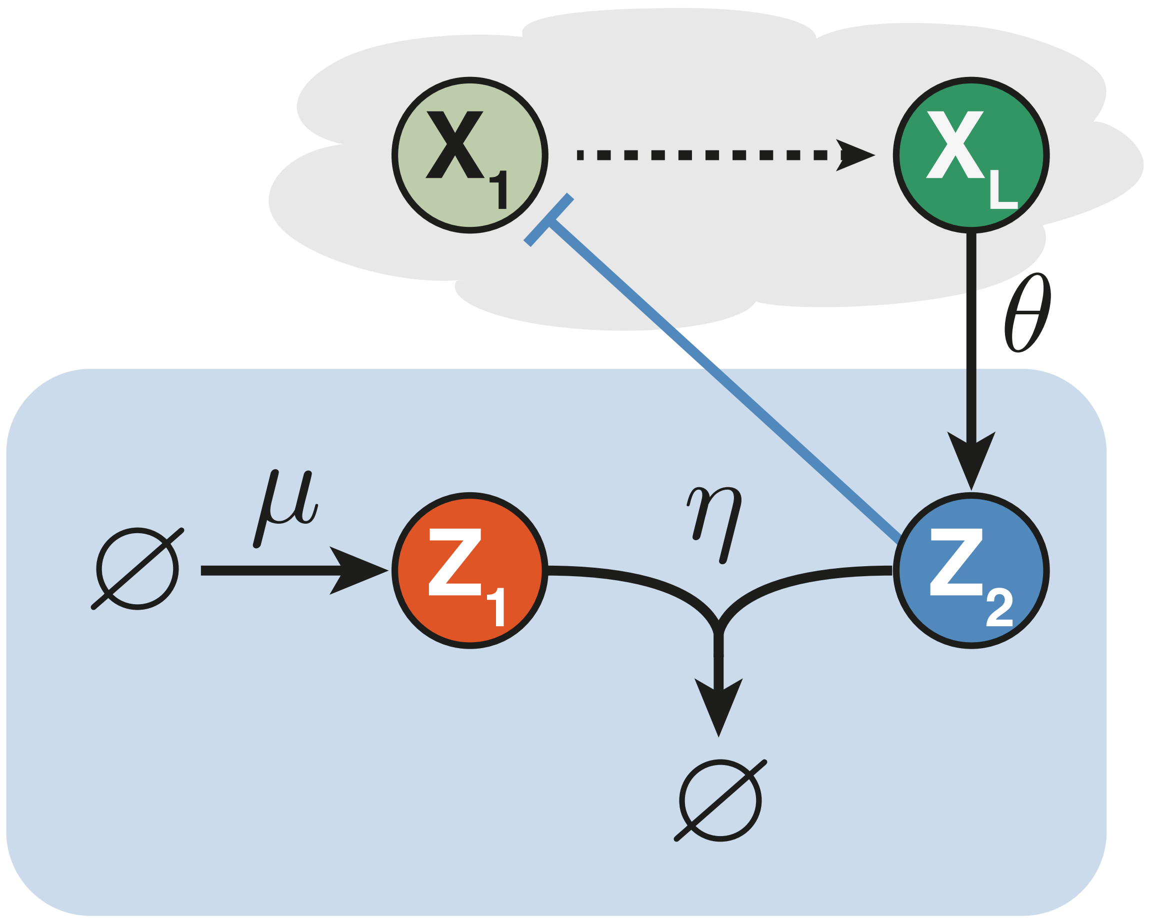

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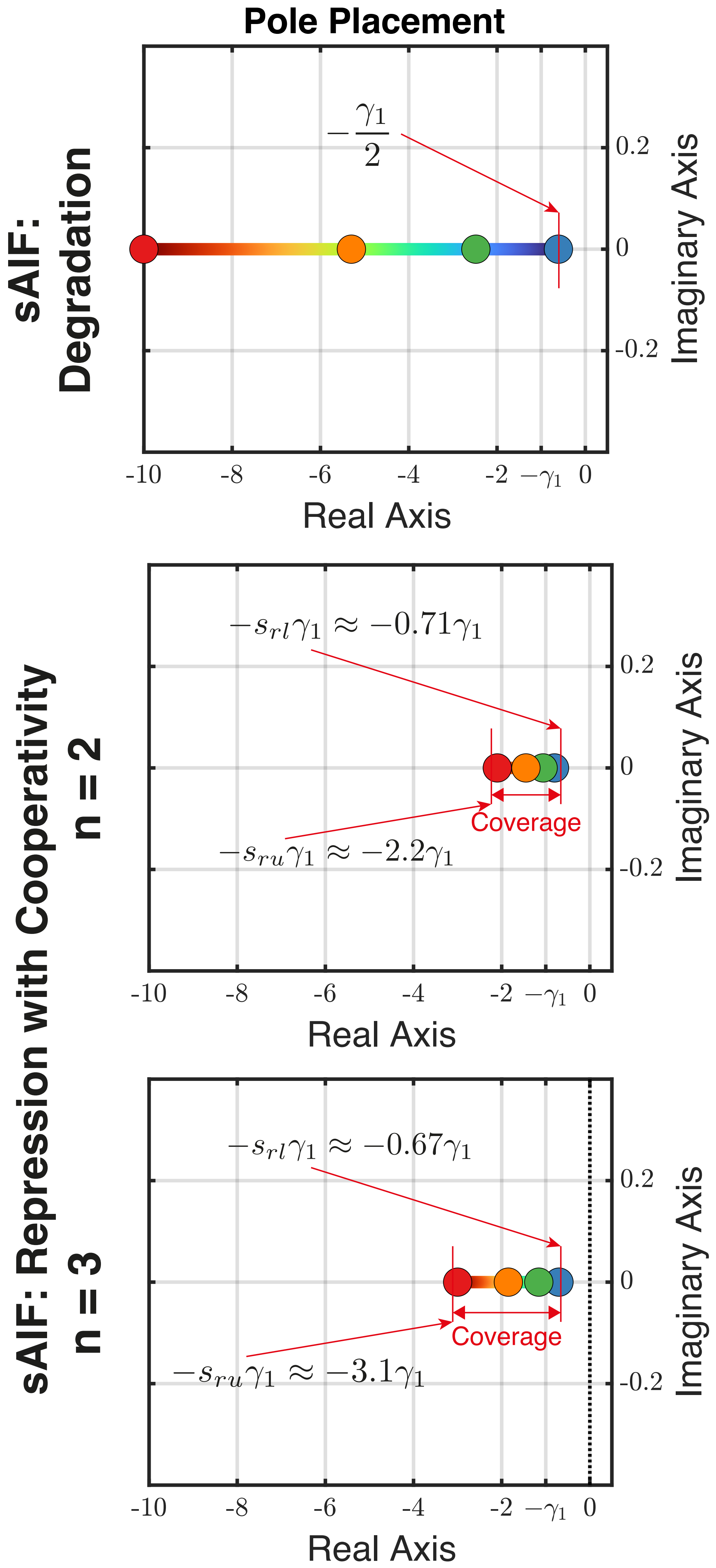

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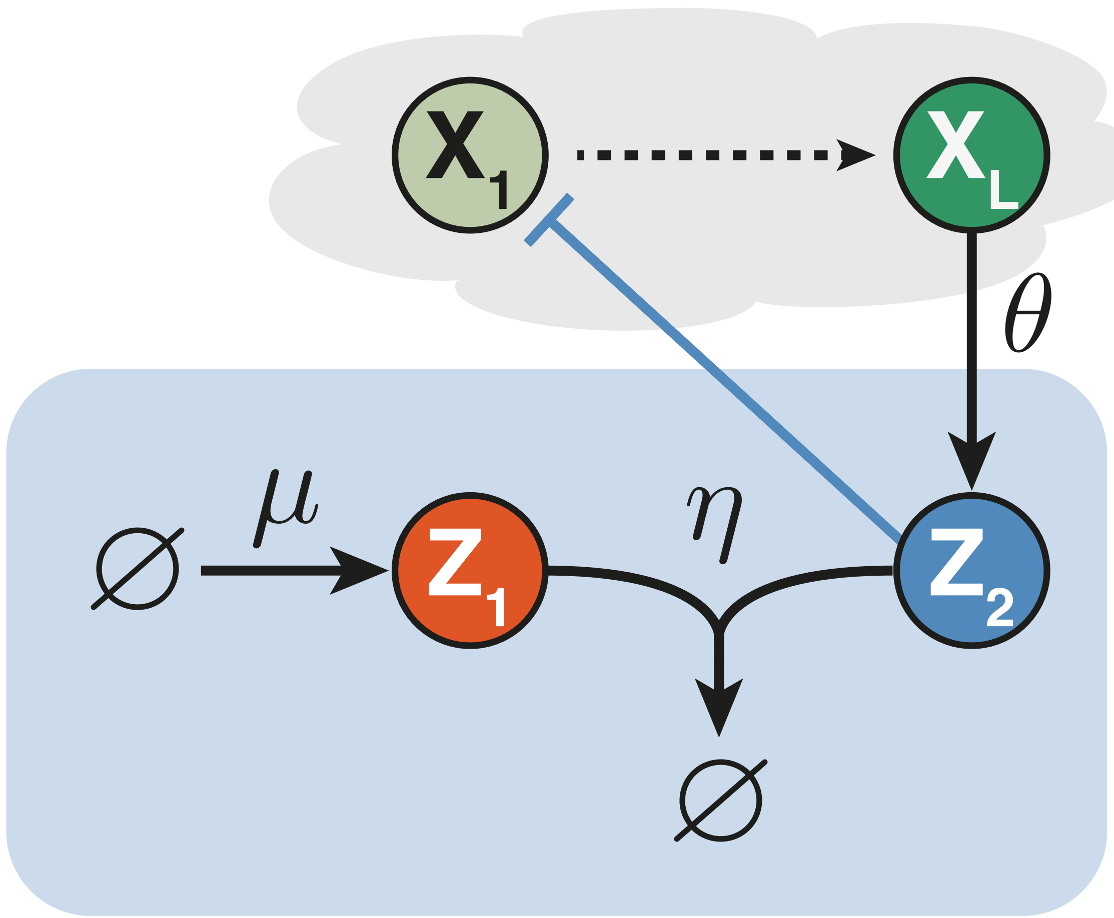

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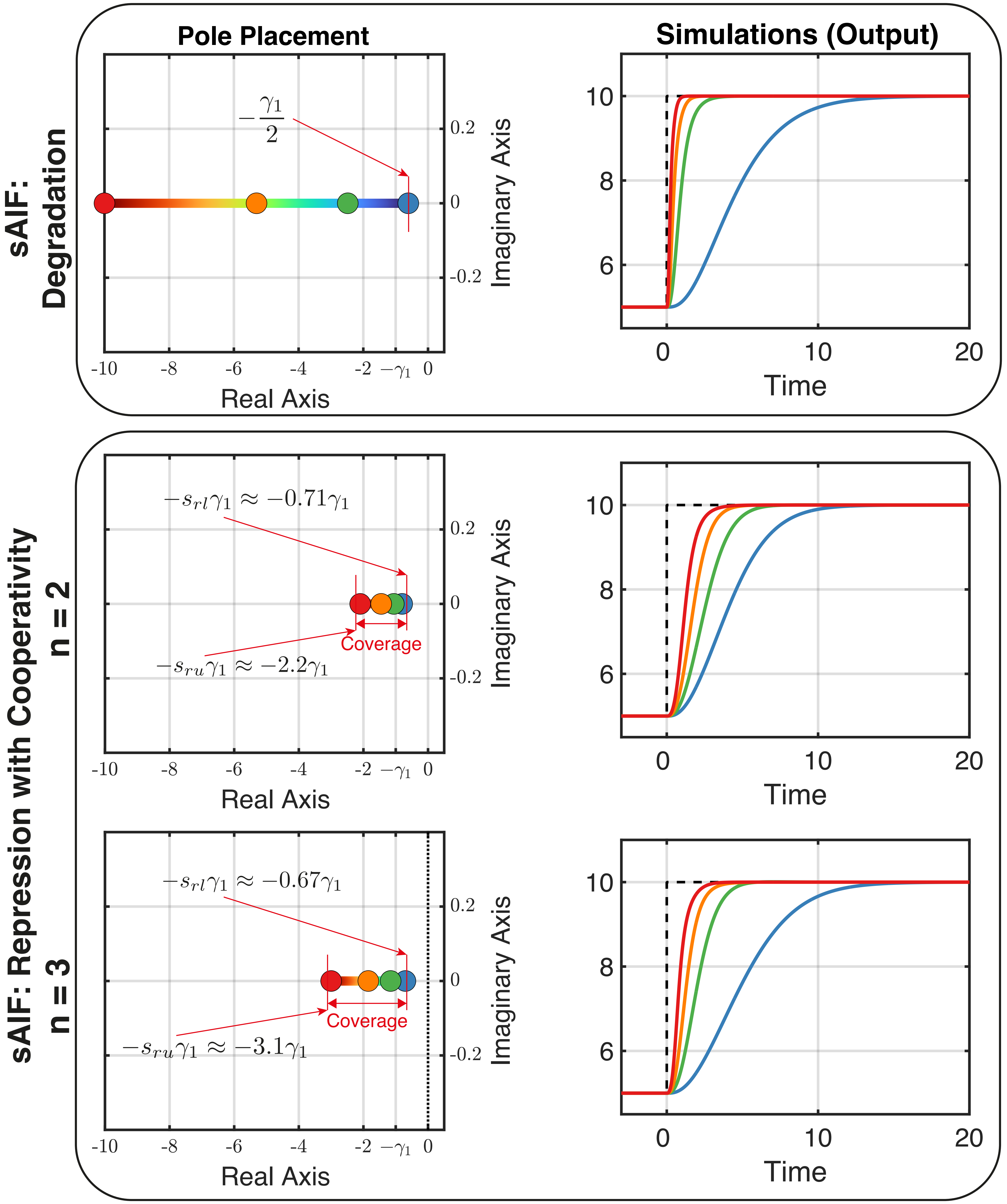
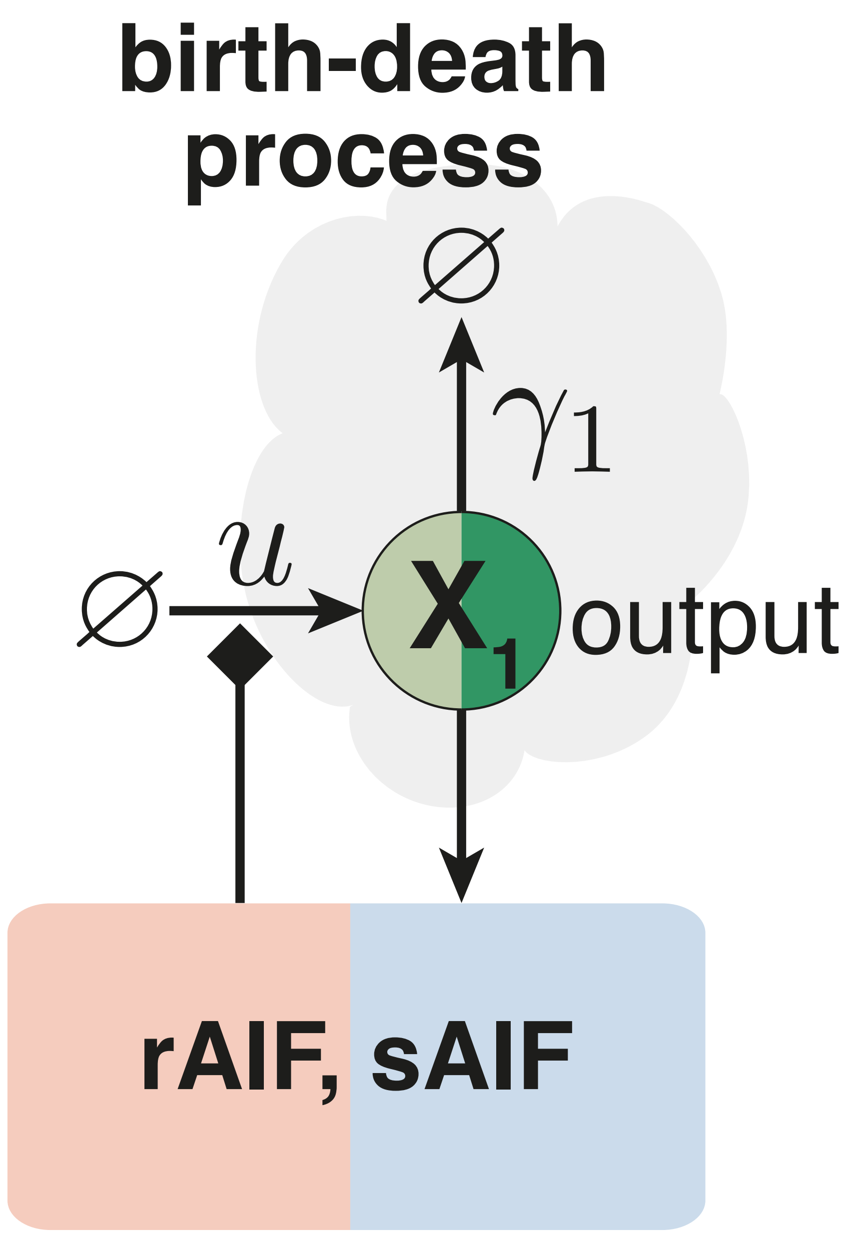
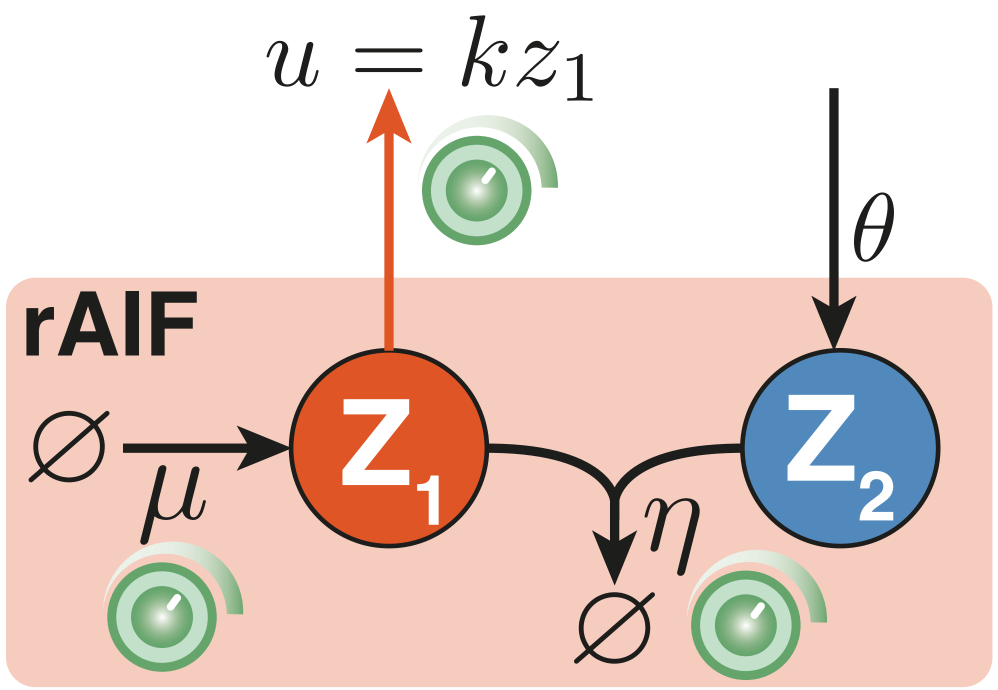
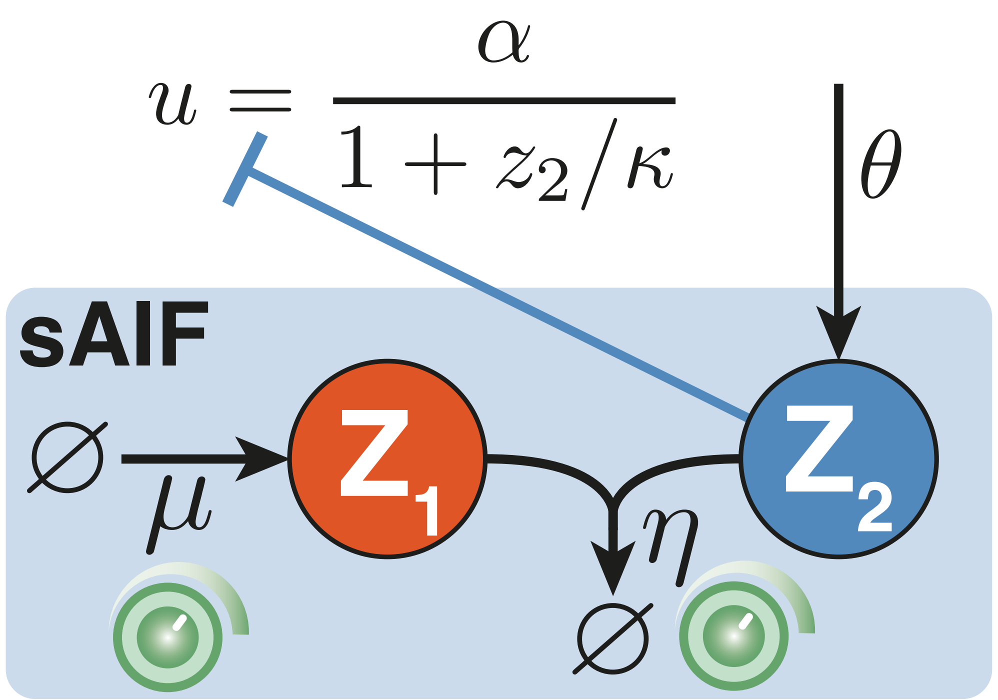


Stationary Intrinsic Noise Reduction: sAIF vs rAIF







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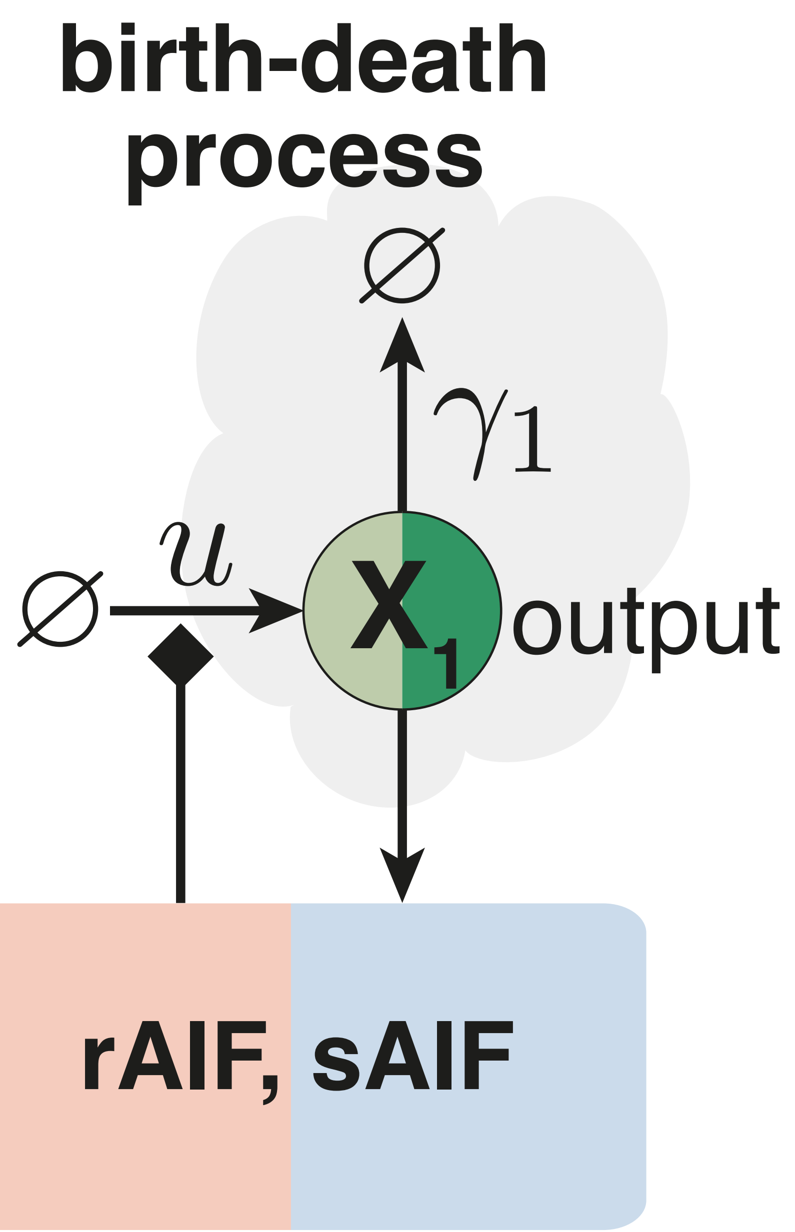

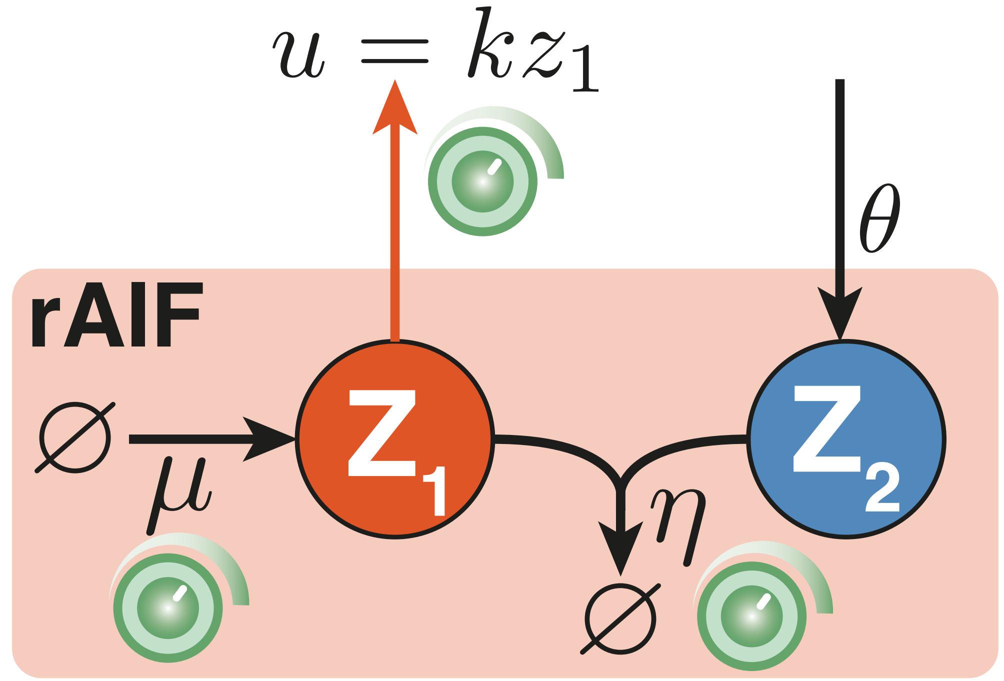

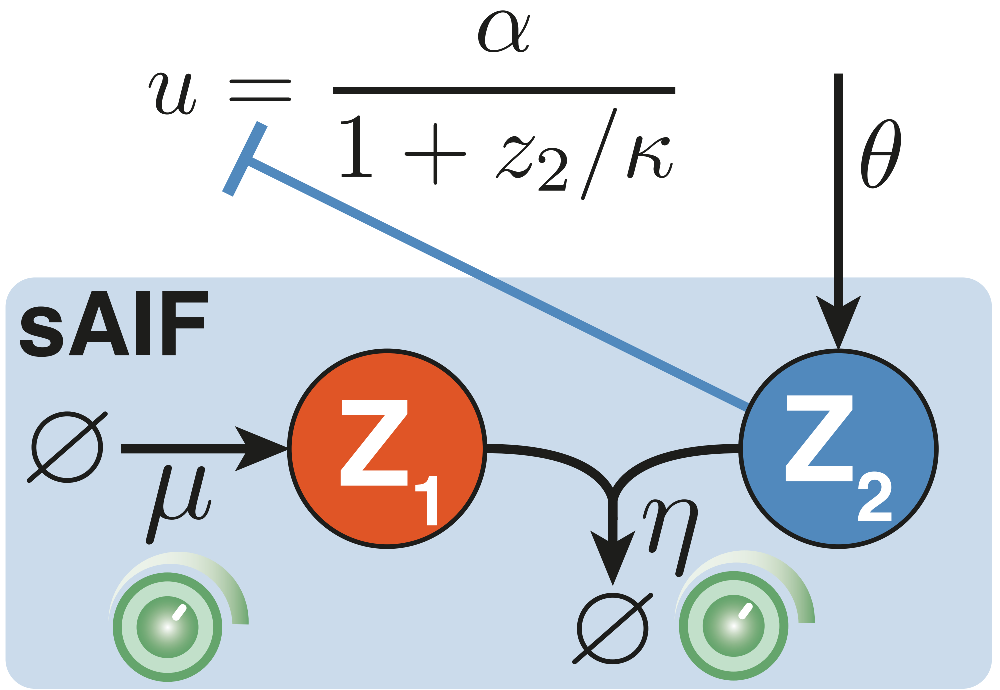

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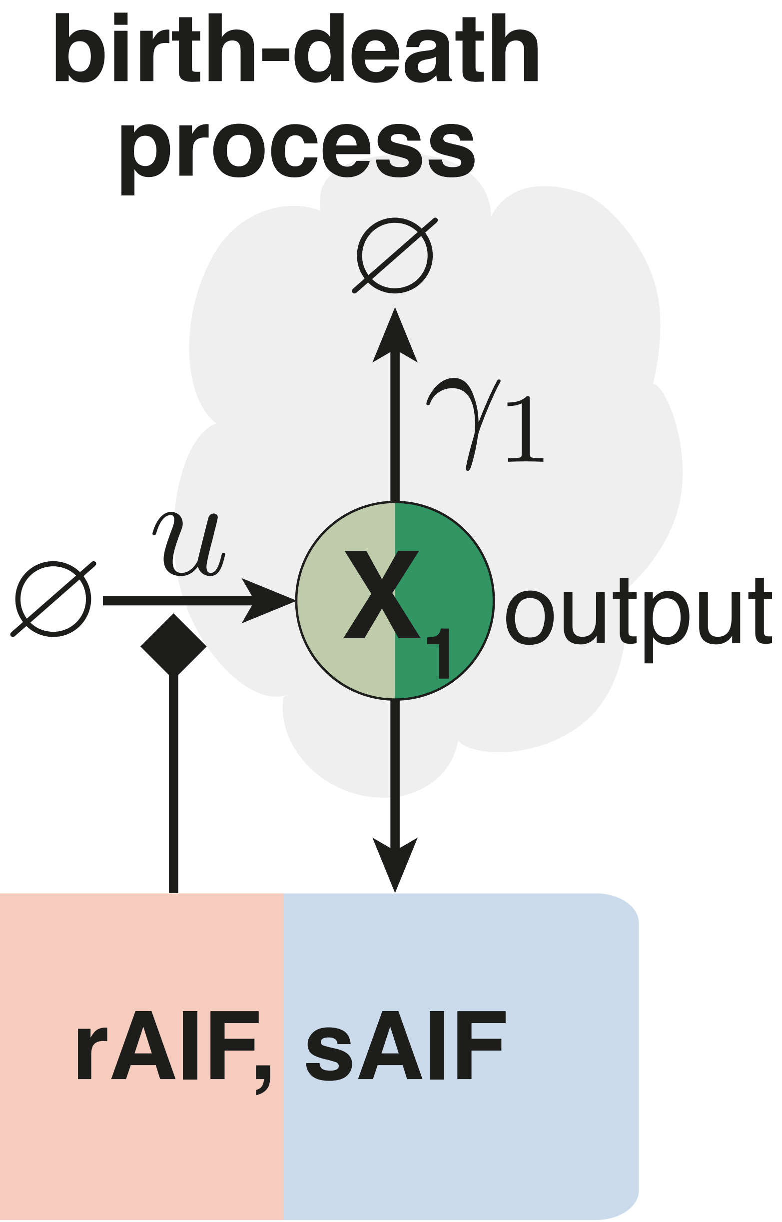

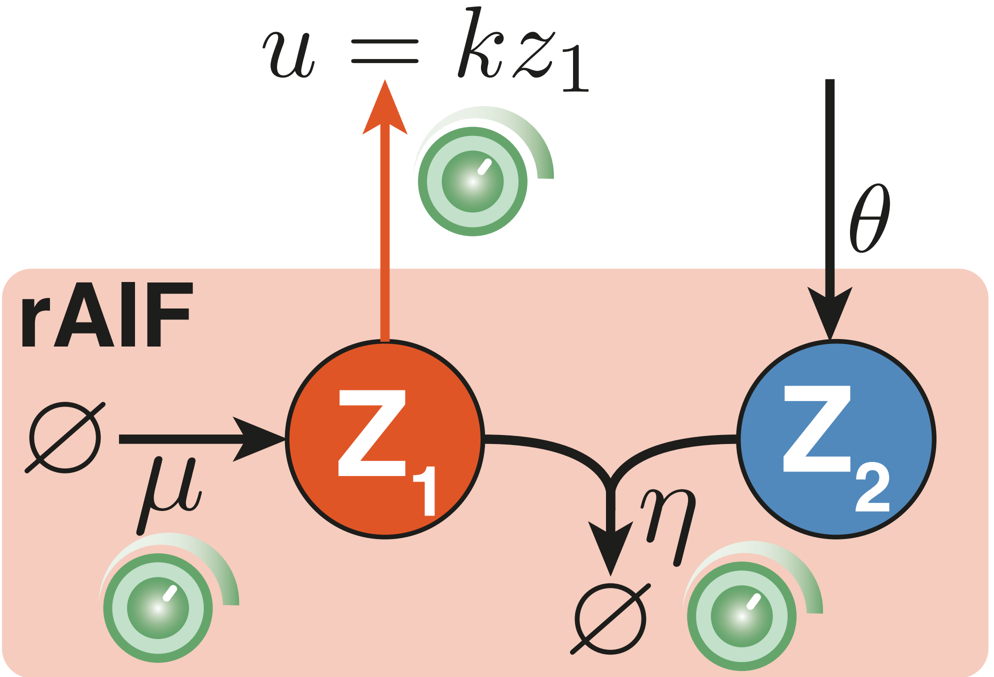

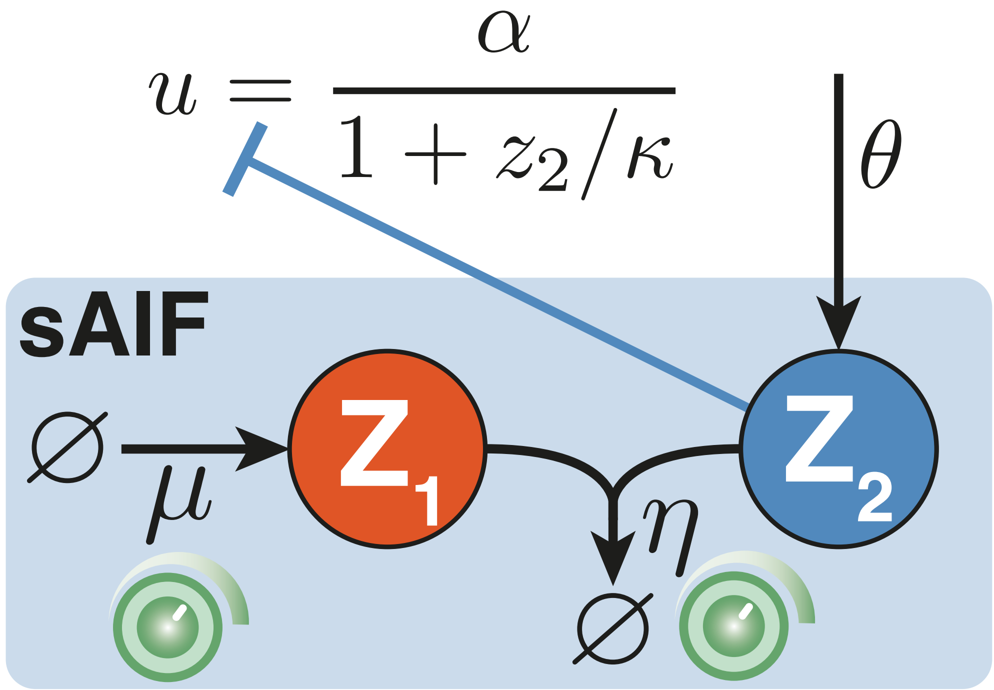

$$\mathrm{CV}_{\mathrm{rAIF}}[\bar{X}_1] \geq \mathrm{CV}_{\mathrm{ol}}[\bar{X}_1]$$

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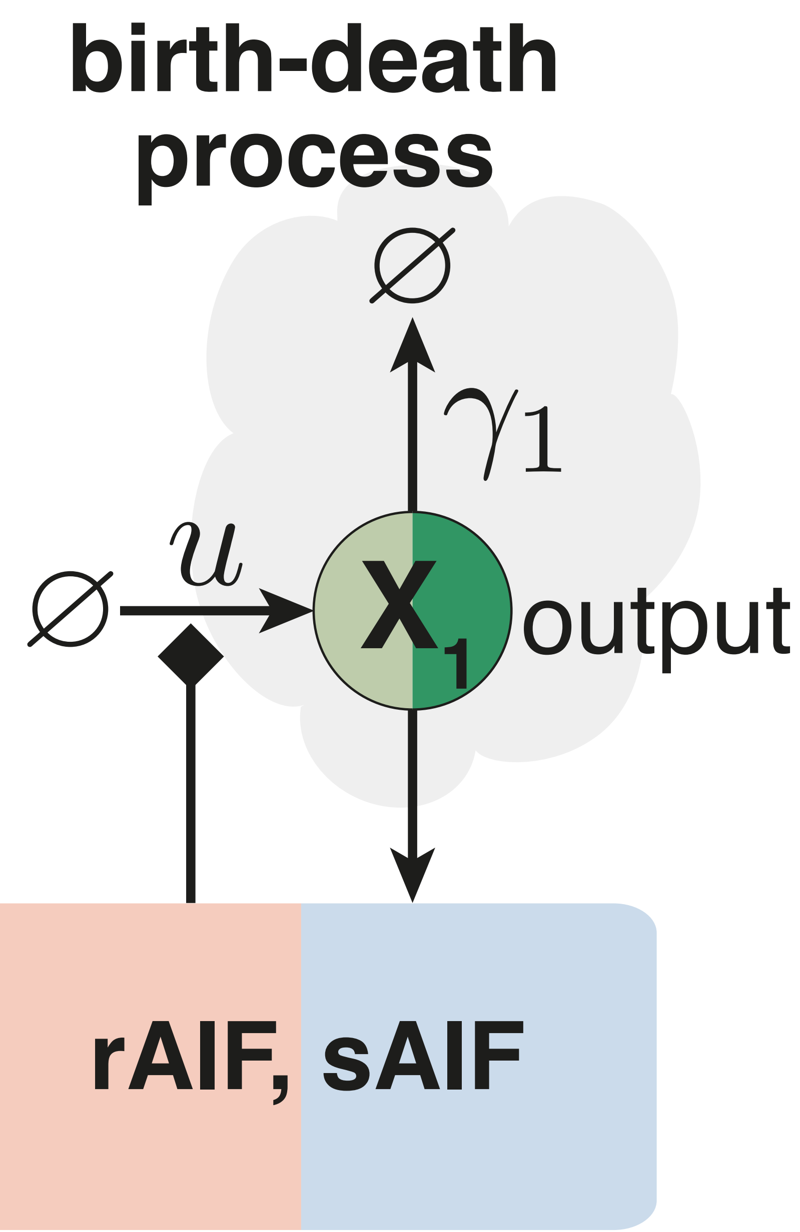

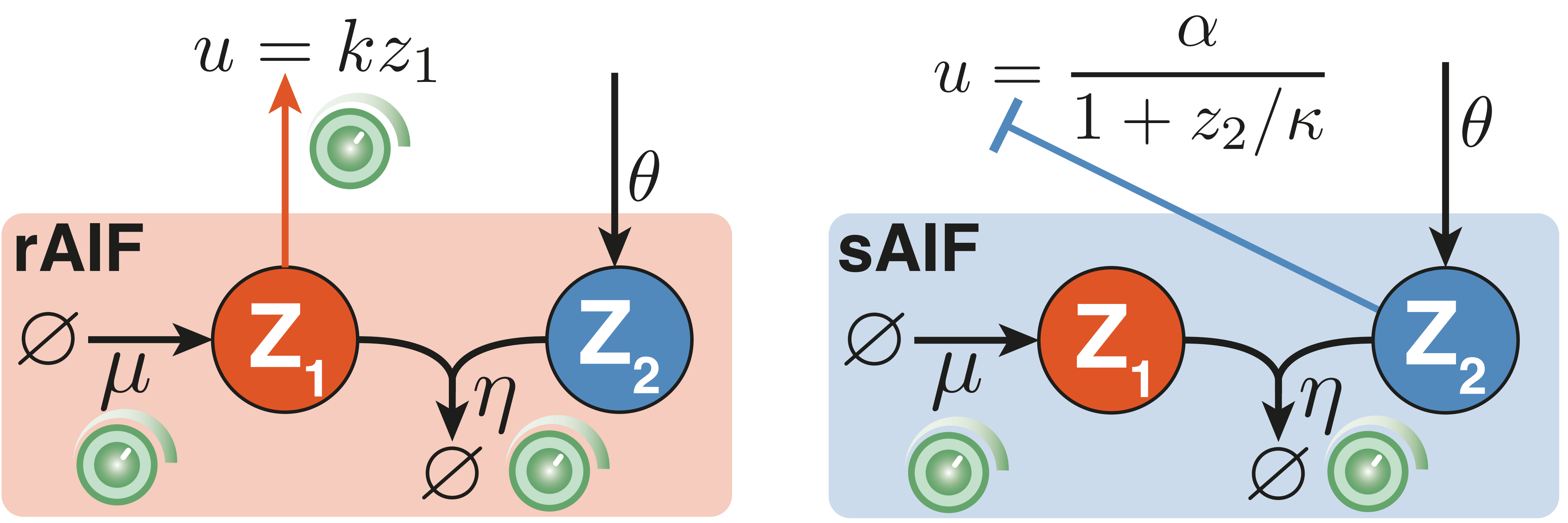

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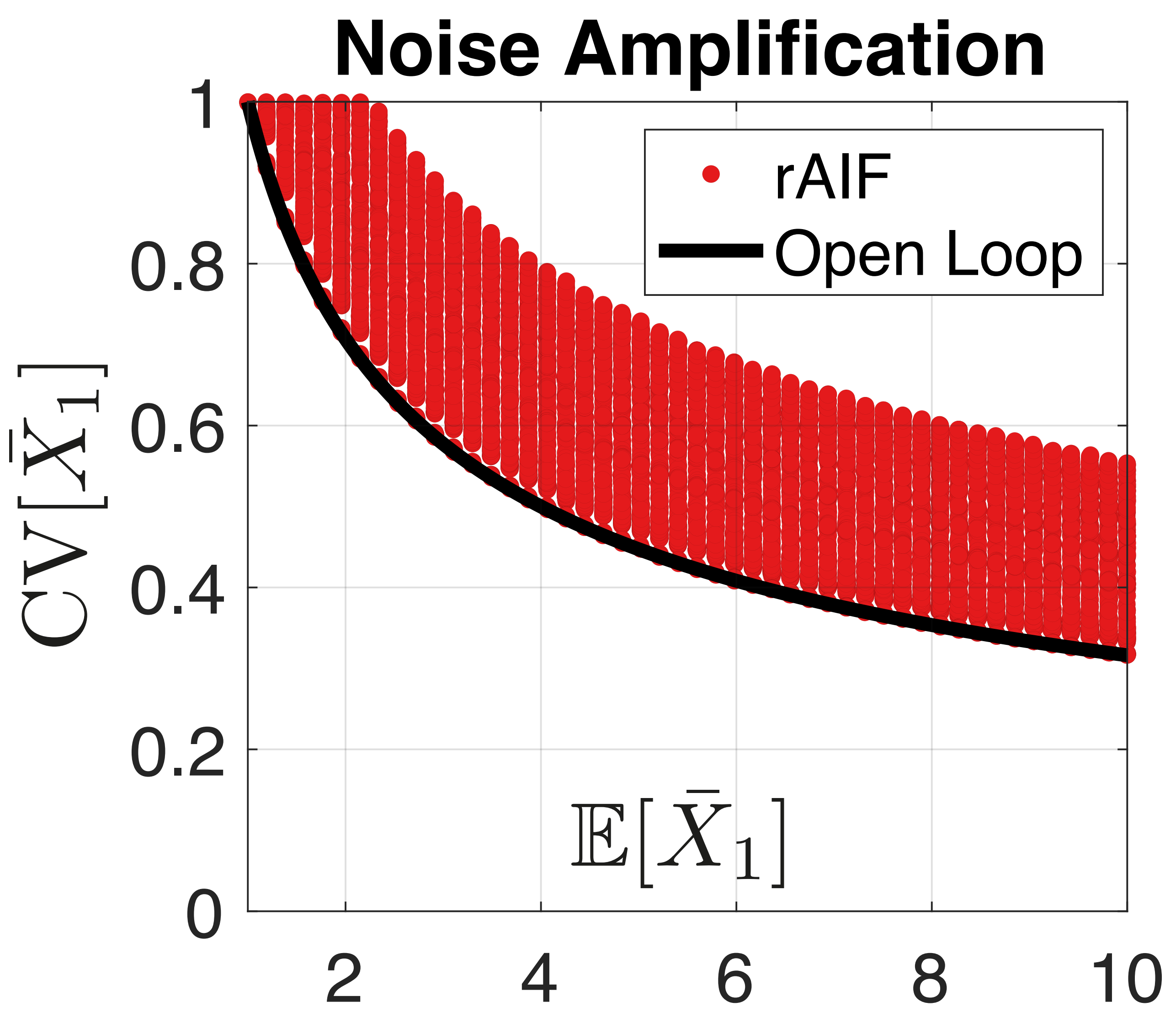

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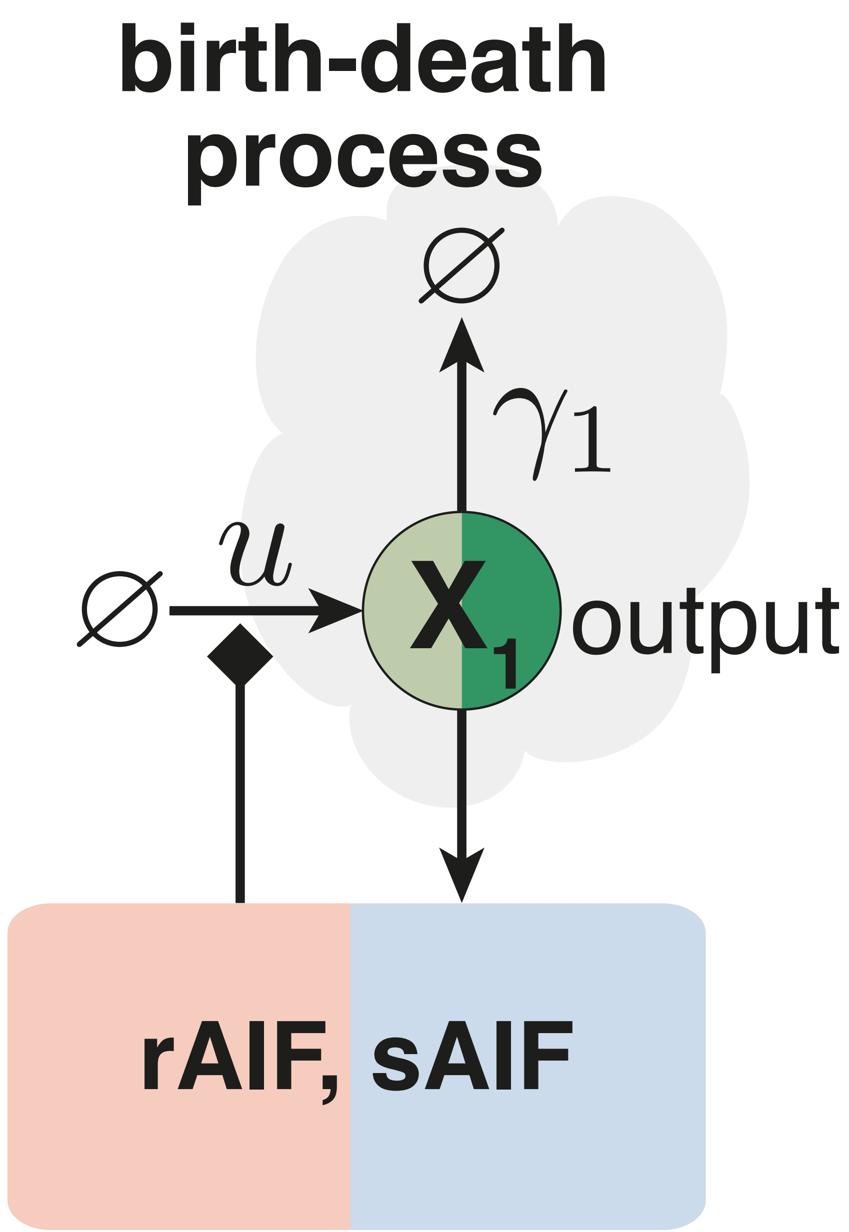

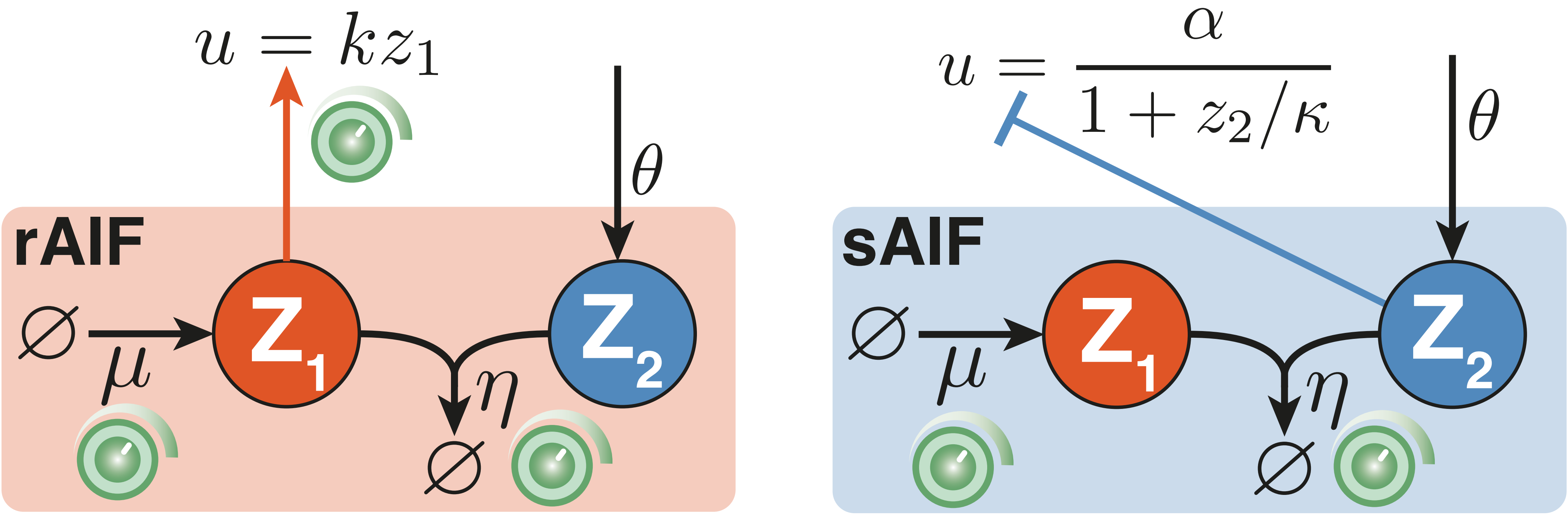

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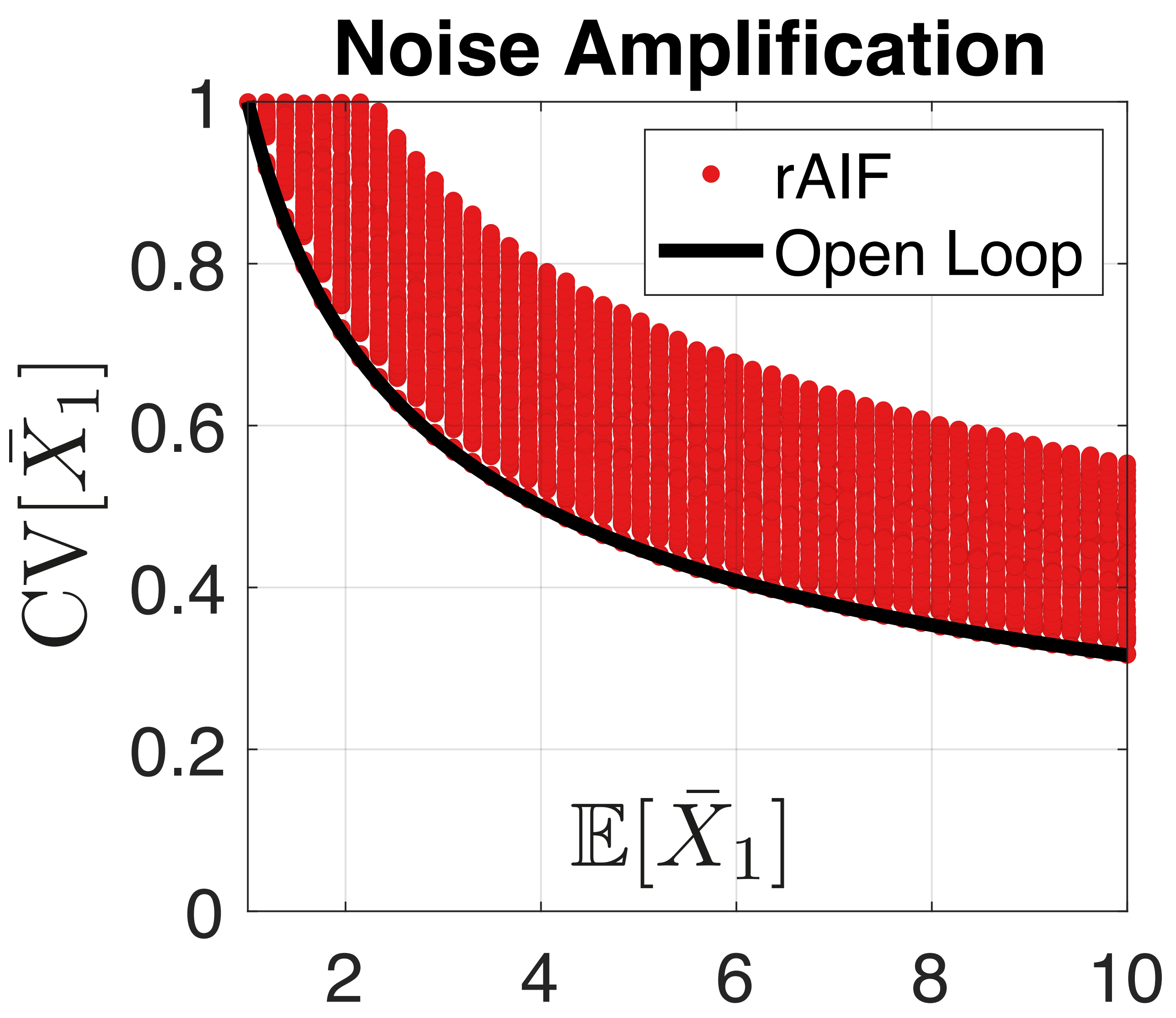

$$\mathrm{CV}_{\mathrm{sAIF}}[\bar{X}_1]^2 \approx \frac{1}{\mathbb{E}[\bar{X}_1]}\left[1 + \frac{K_P\omega_0\left(\frac{K_P}{\theta} - 1\right) + K_I\left(\omega_0 + \gamma_1 + \theta\right)}{\left(\gamma_1 + \omega_0\right)\left(\gamma_1 + K_P\right) - \theta K_I}\right]$$

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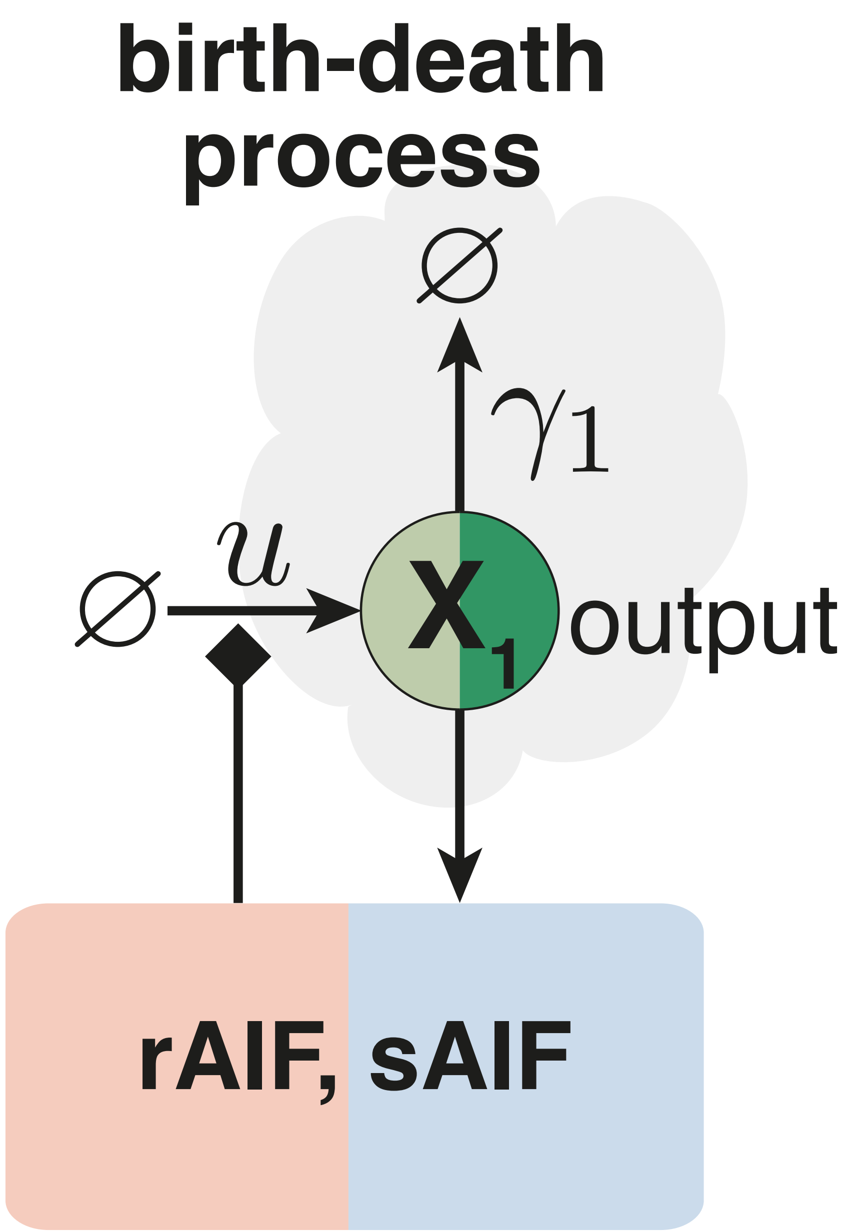

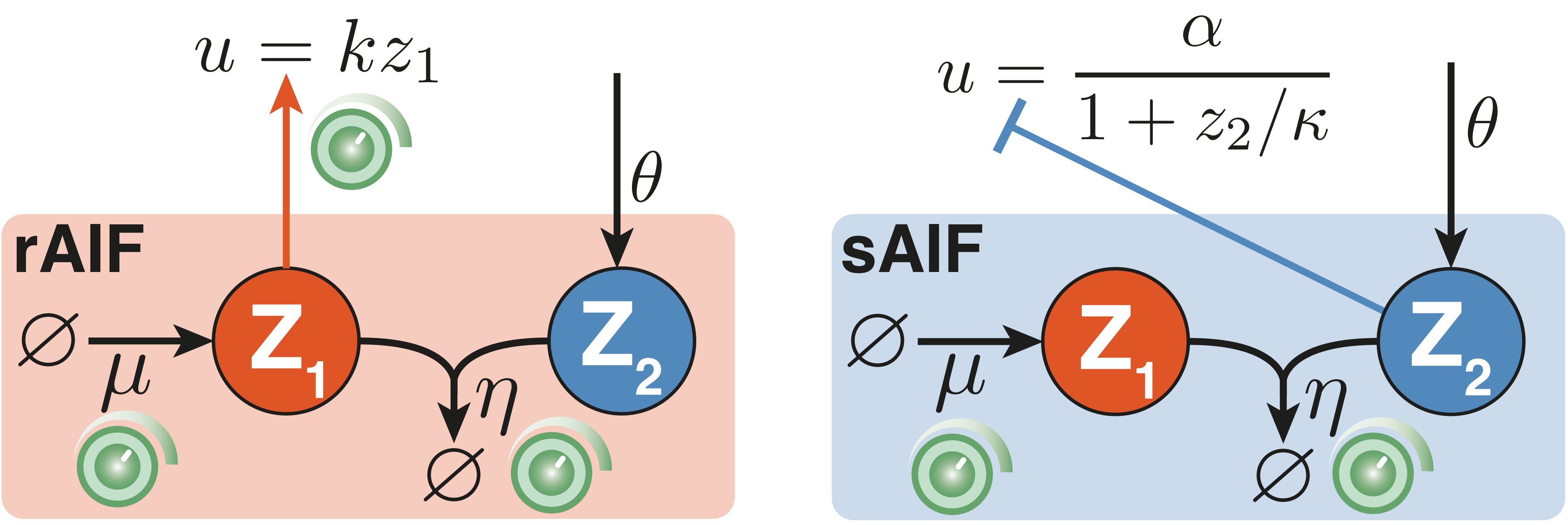

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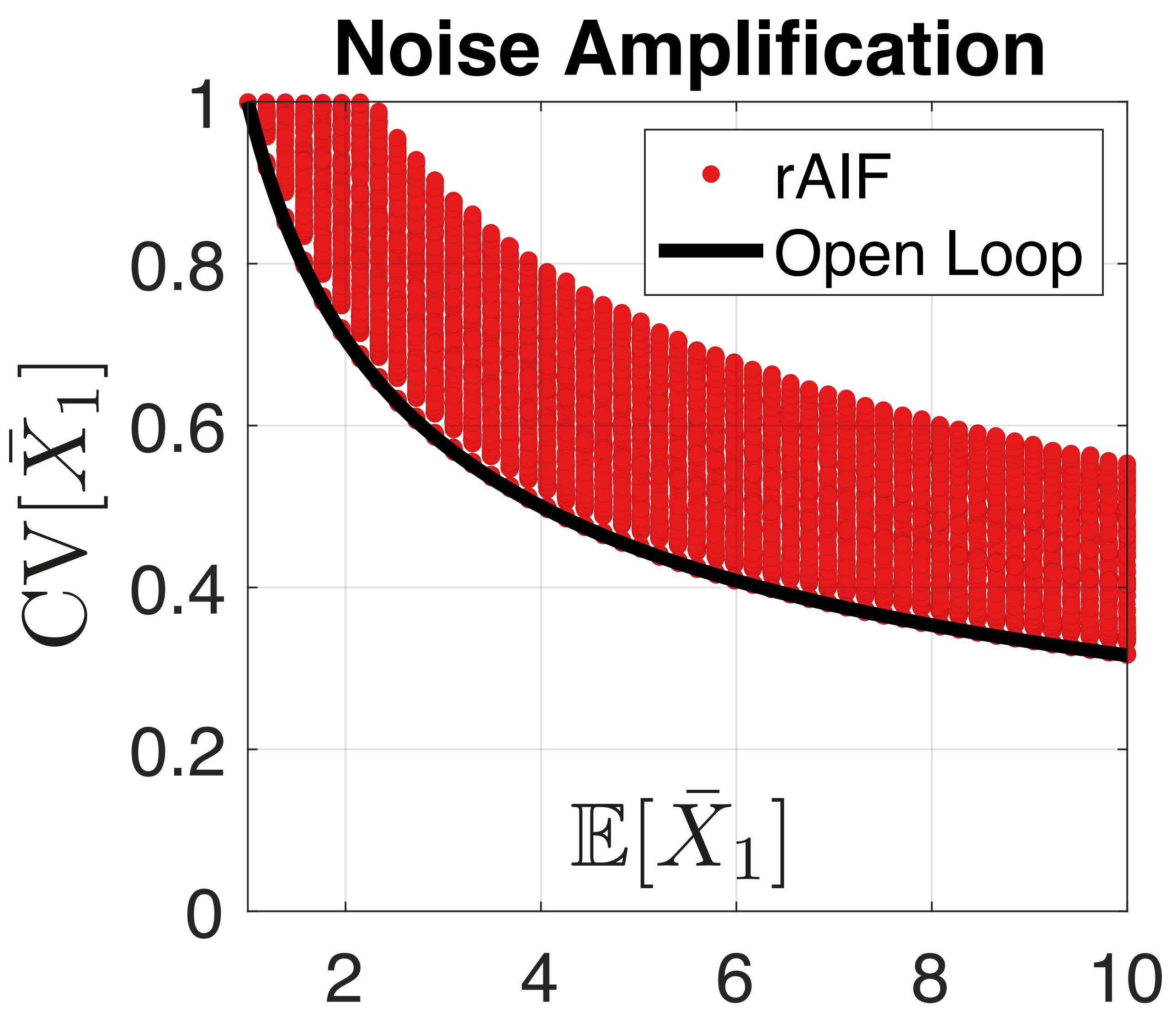

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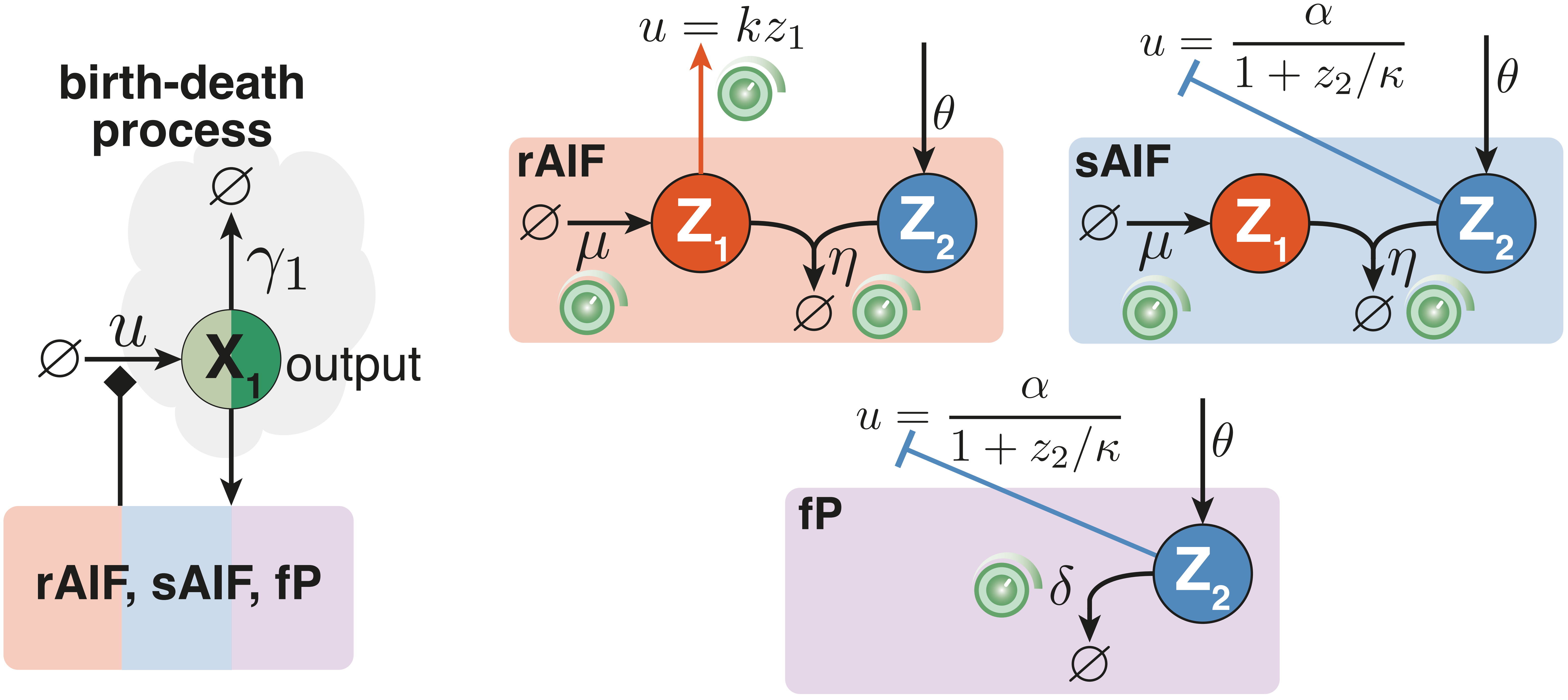

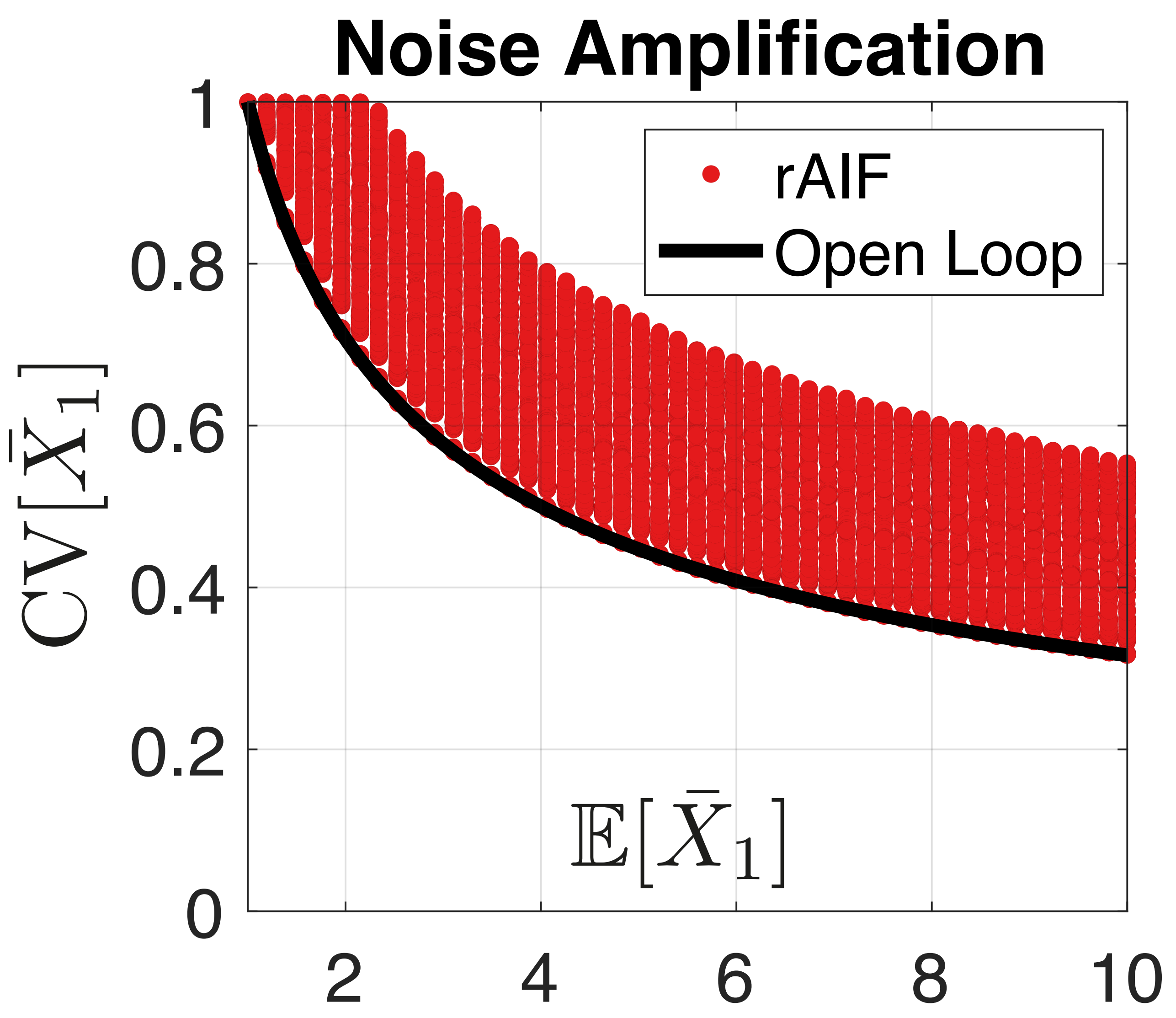

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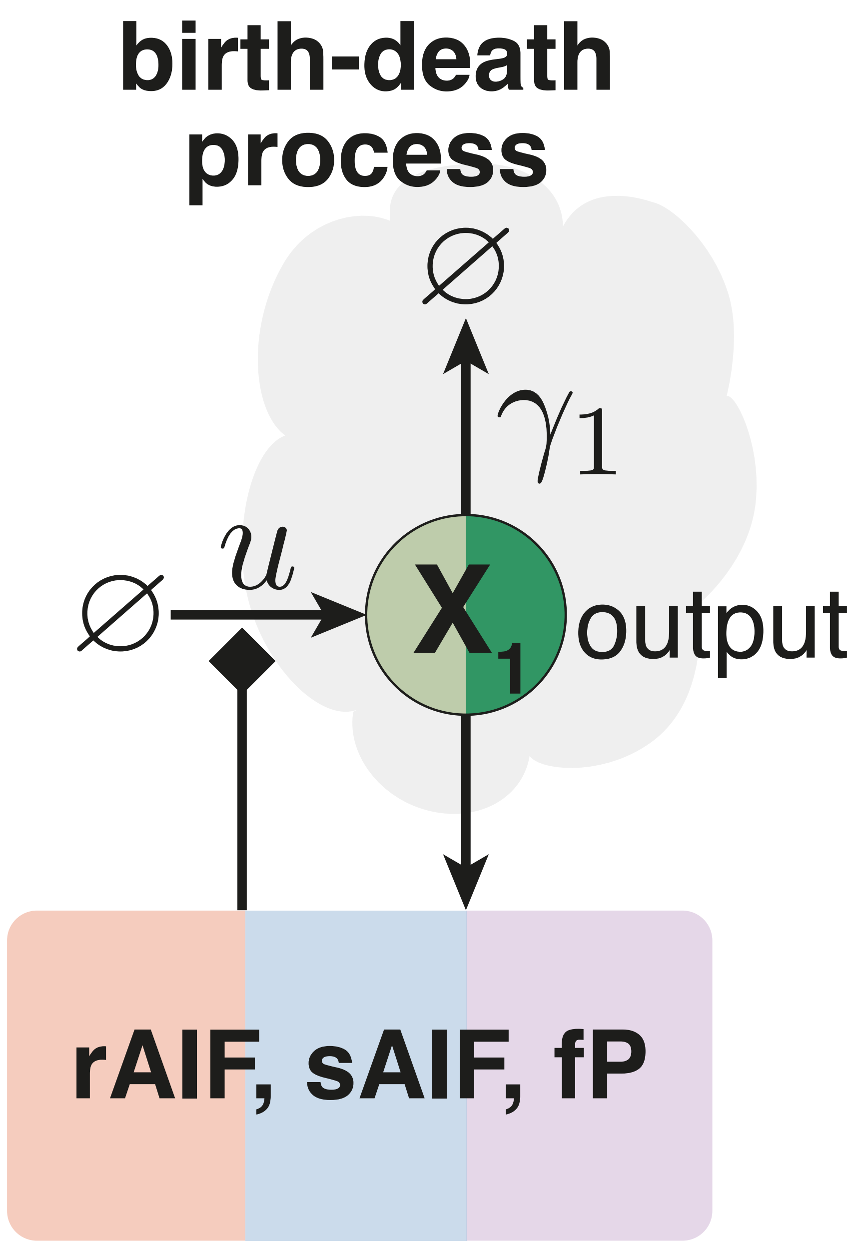

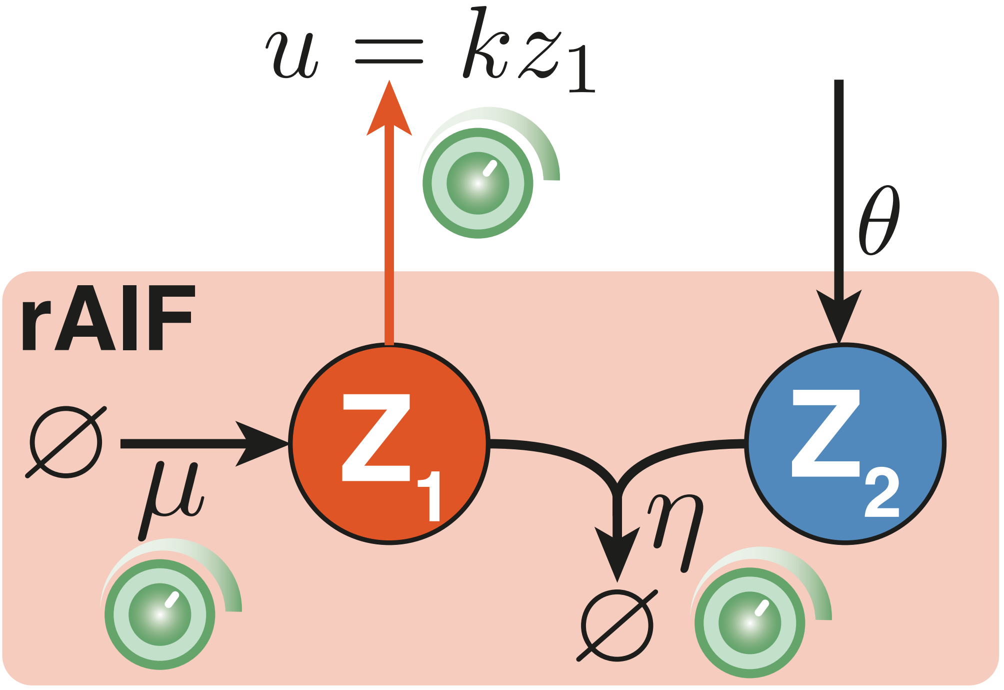

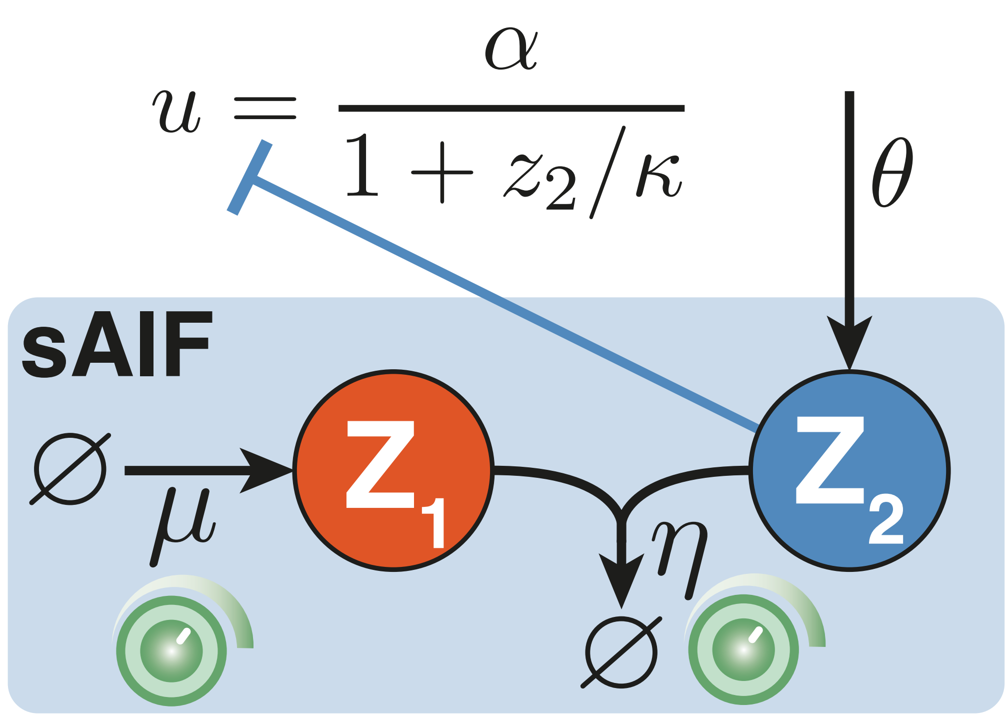

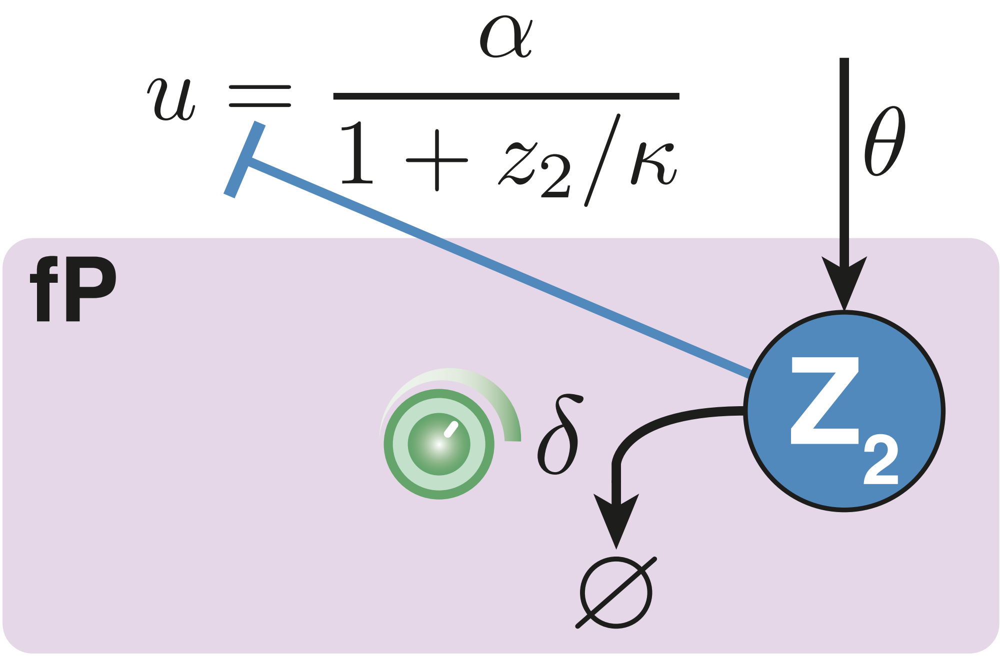

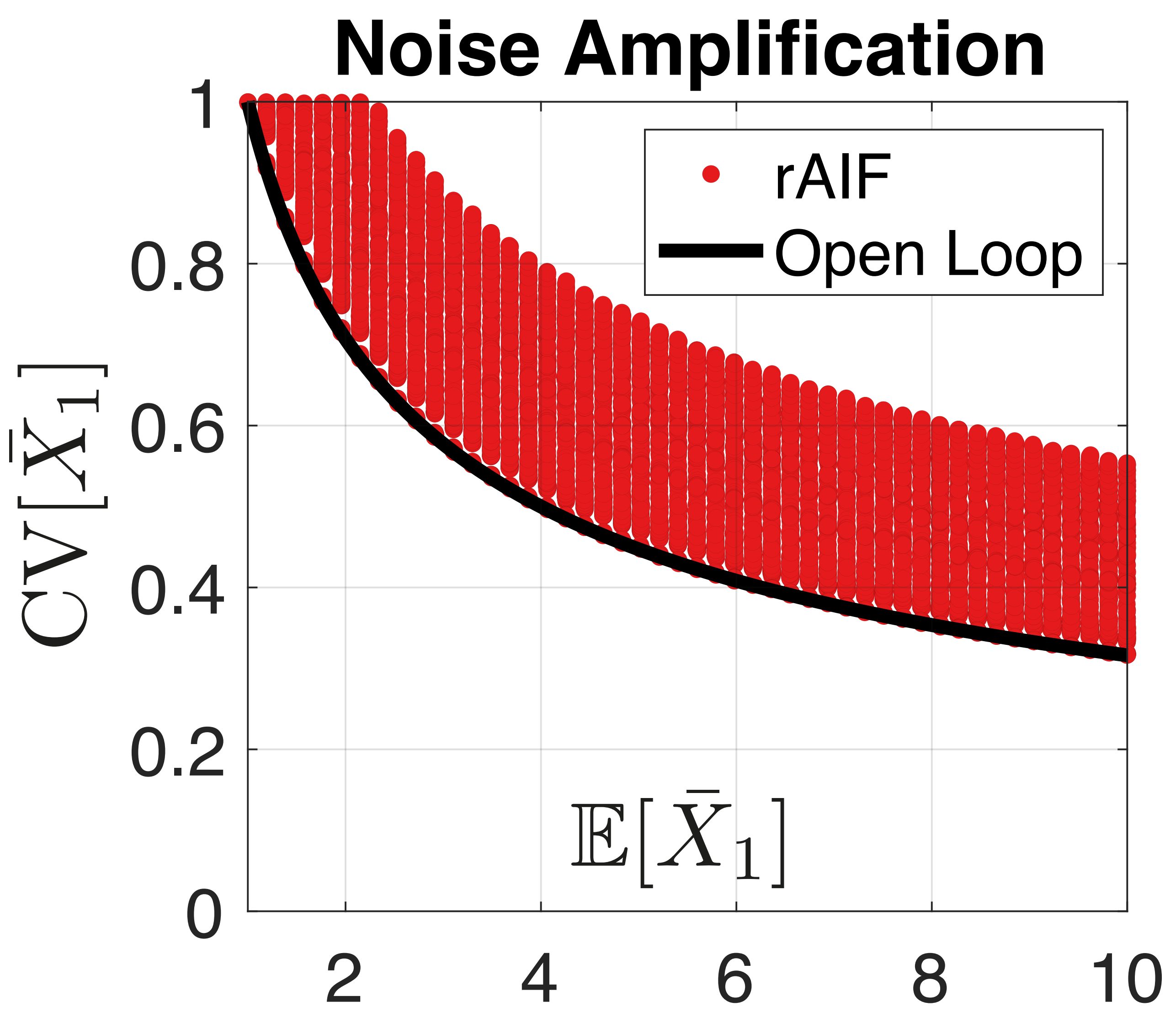

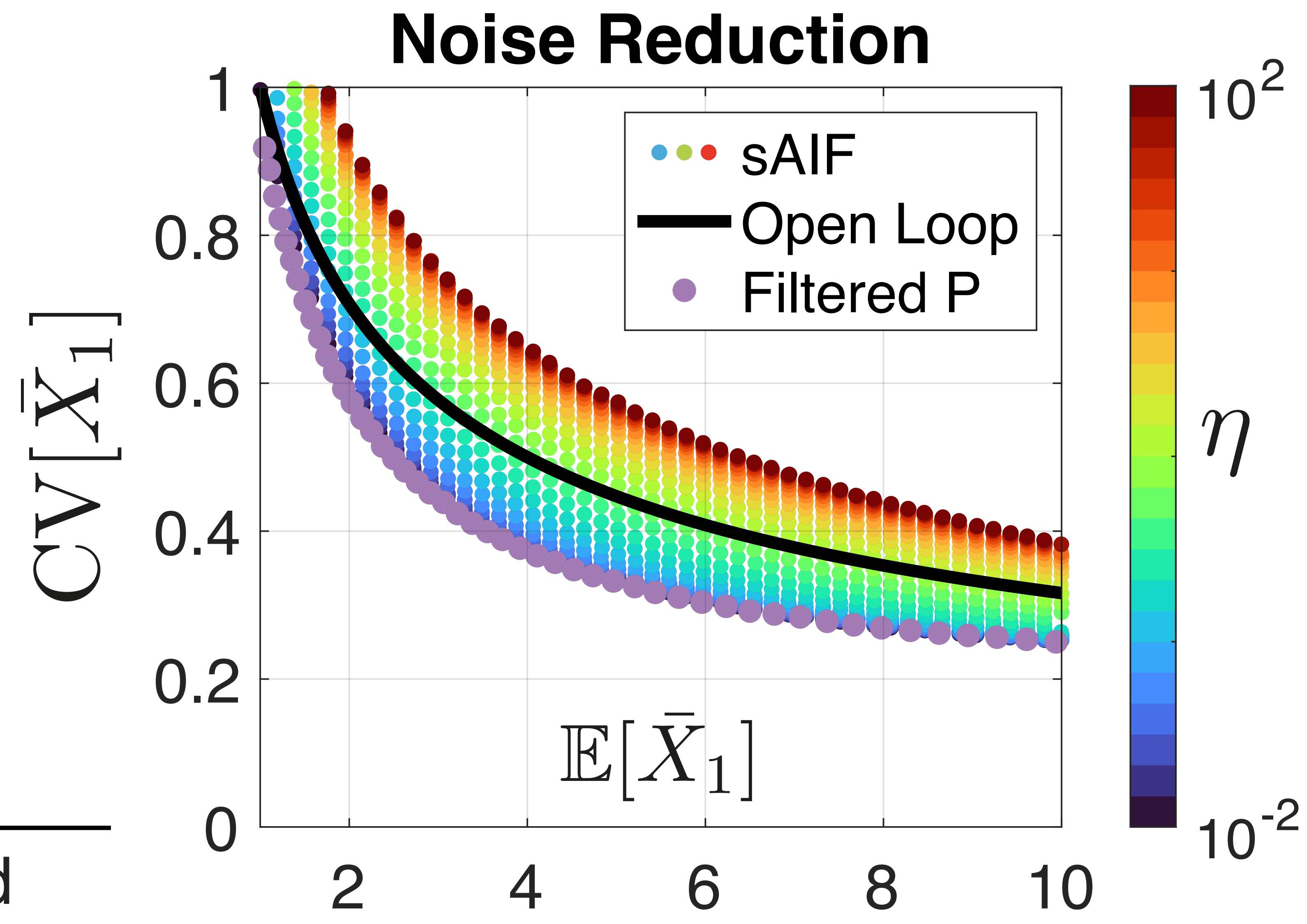

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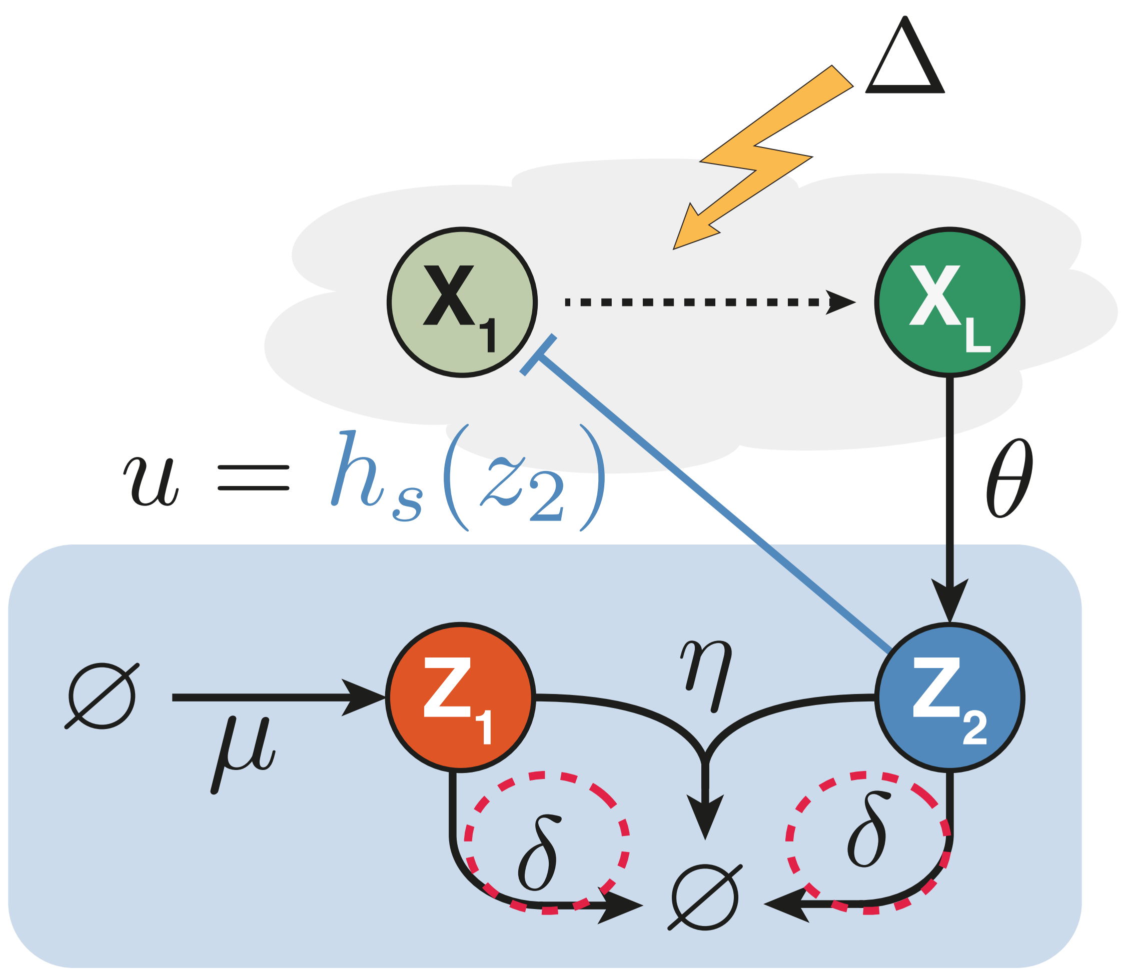
Steady-State Errors in Deterministic Non-ideal Settings



non-ideal sAIF

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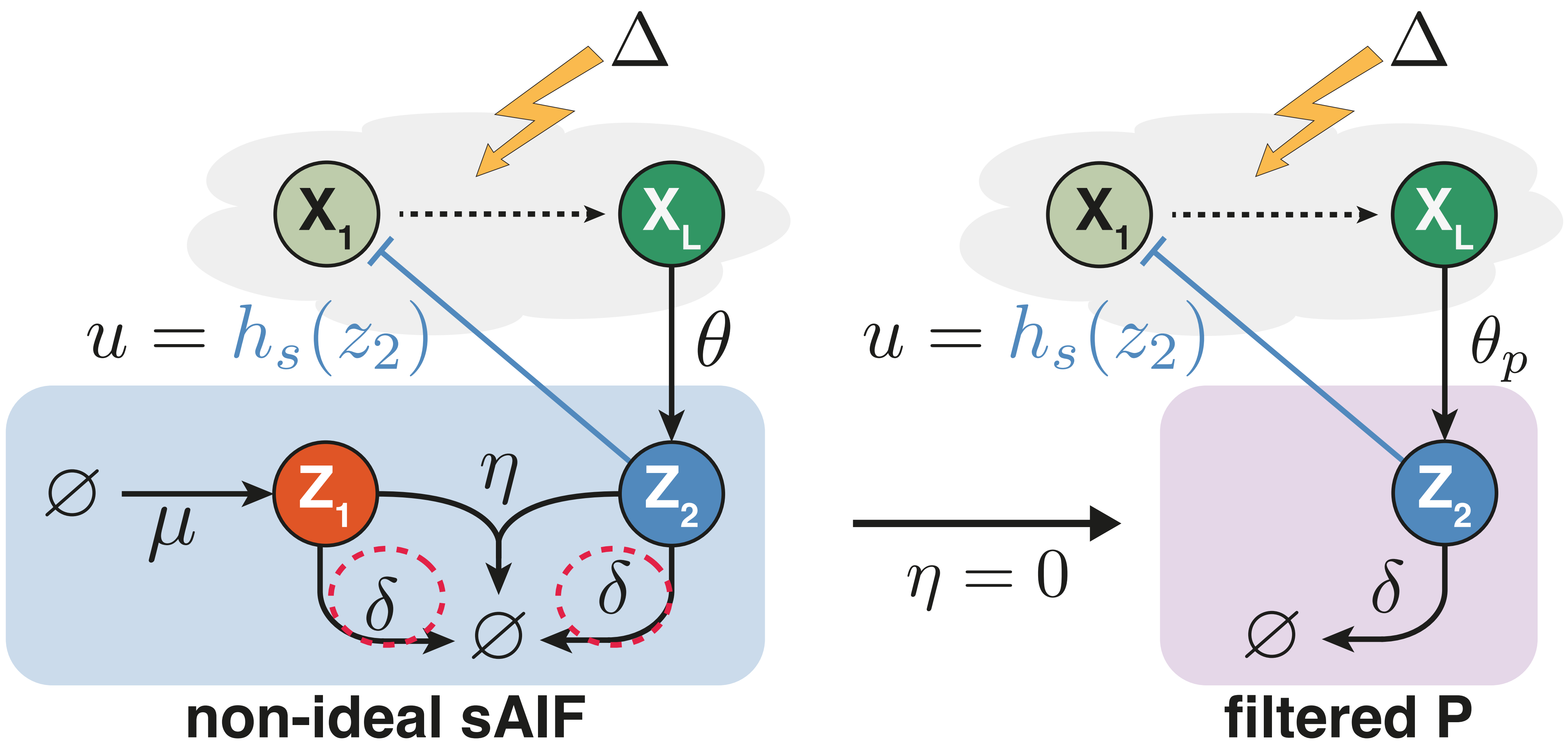
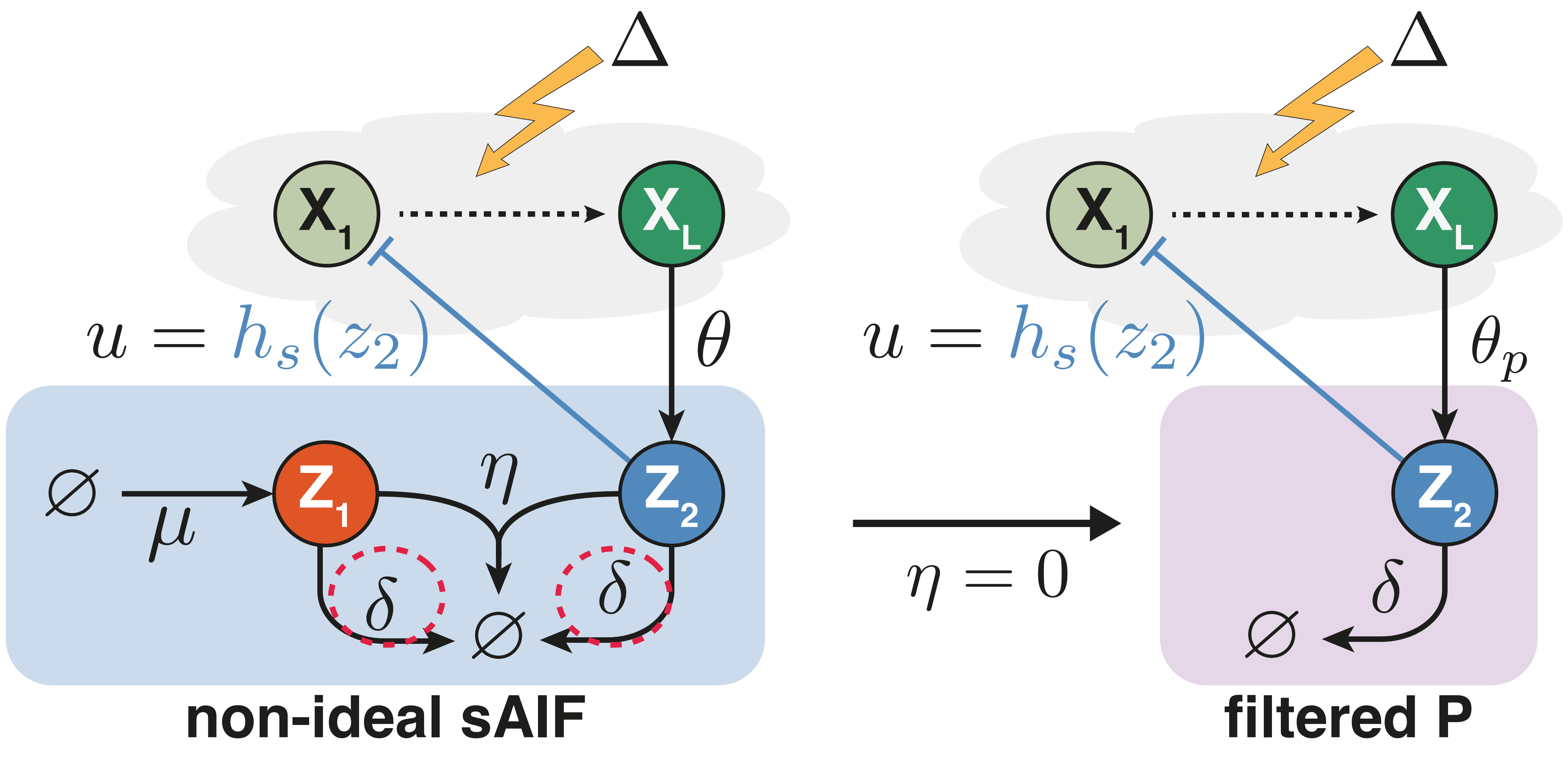


Question: Given that the non-ideal sAIF controller does not perfectly reject disturbances, why bother with additional circuitry?

Steady-State Errors in Deterministic Non-ideal Settings



Process: $x_L = \mathcal{P}_\Delta(u) \iff \dot{x} = f_\Delta(x, u)$

sAIF: $u = \mathcal{C}_s(x_L) \iff \begin{cases} \dot{z}_1 = \mu - \eta z_1 z_2 - \delta z_1 \\ \dot{z}_2 = \theta_s x_L - \eta z_1 z_2 - \delta z_2 \\ u = h_s(z_2) \end{cases}$

fP: $u = \mathcal{C}_p(x_L) \iff \begin{cases} \dot{z}_2 = \theta_p x_L - \delta z_2 \\ u = h_s(z_2) \end{cases}$

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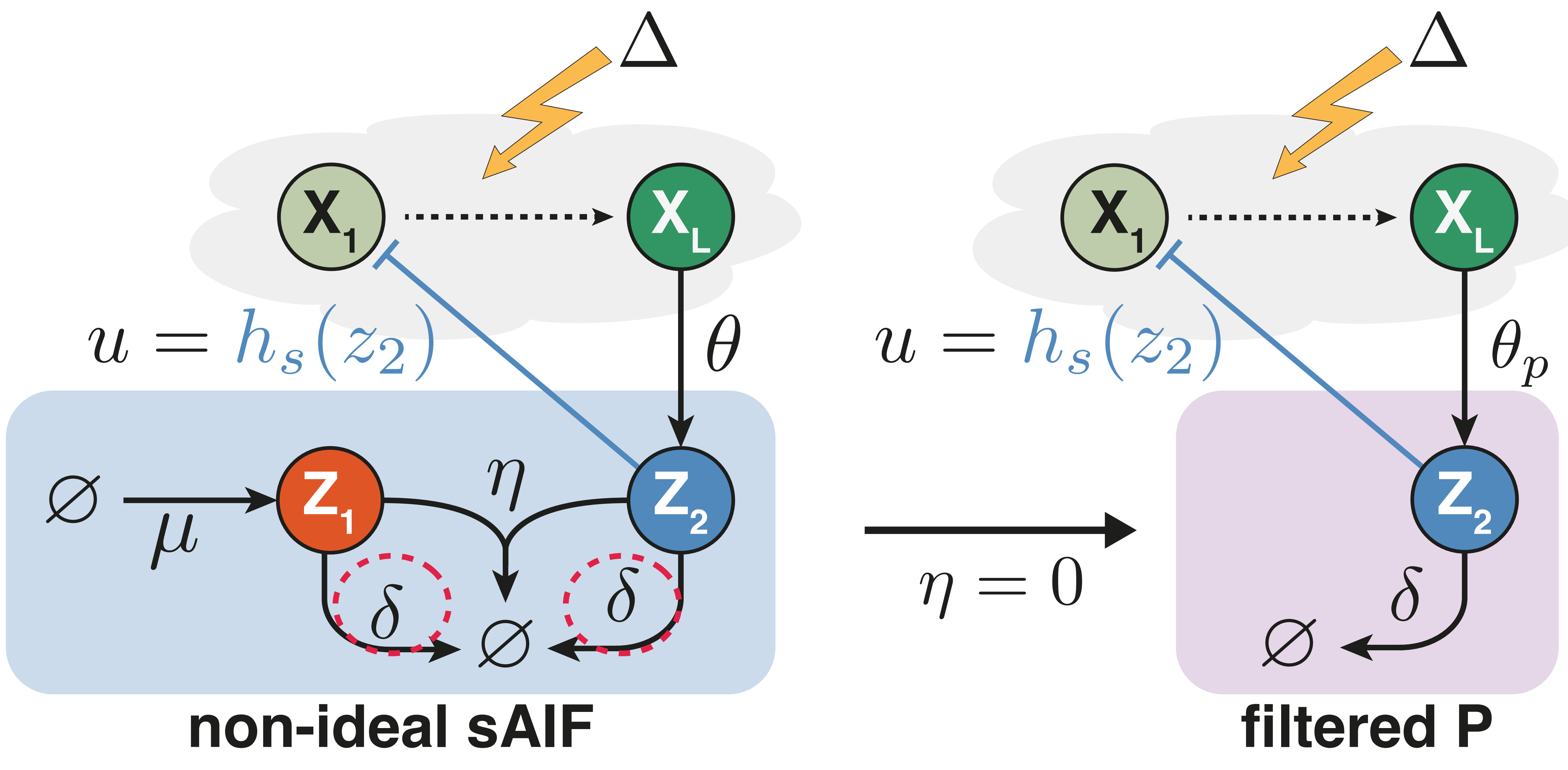

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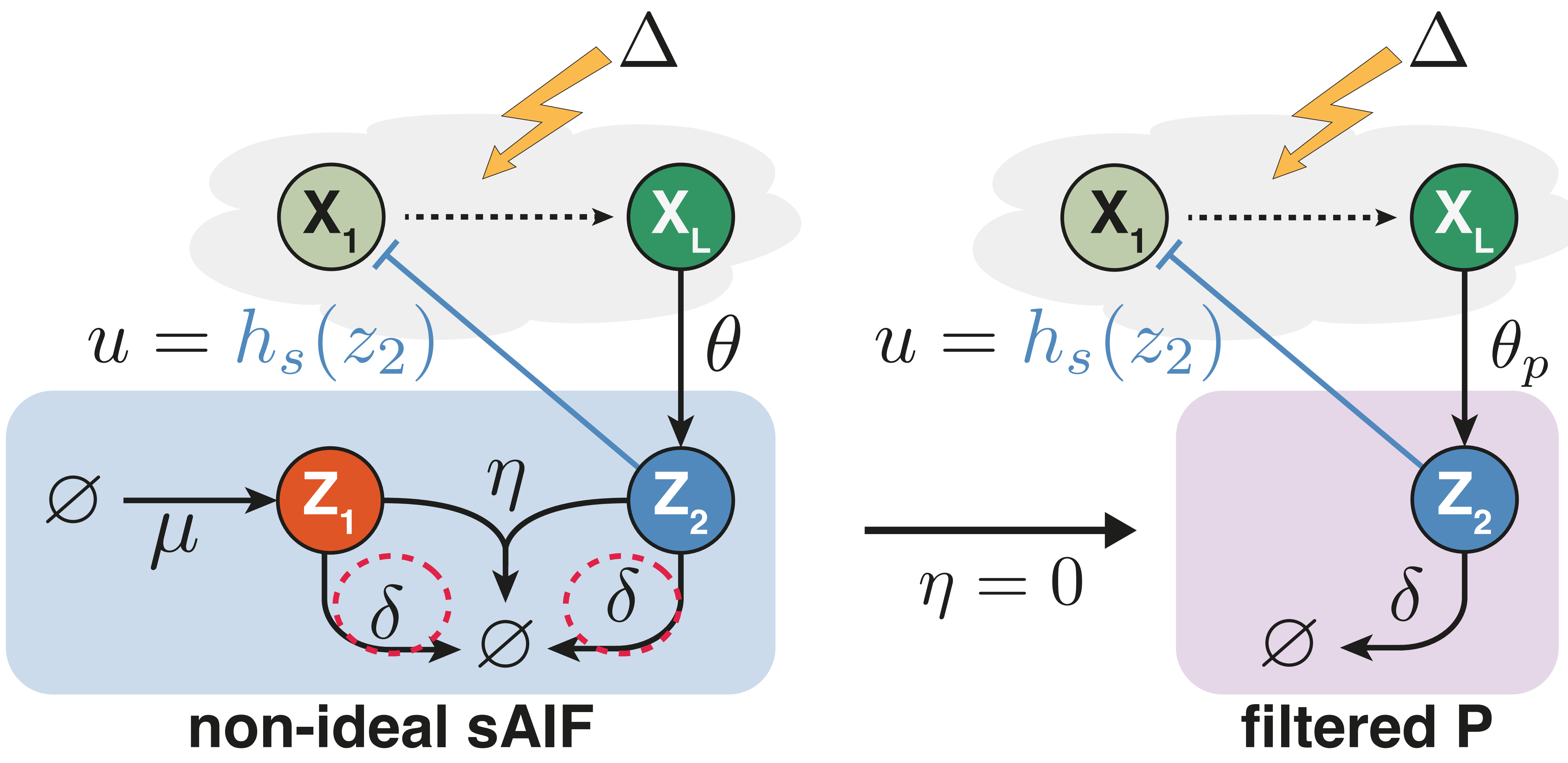
Theorem: Given a repressor Z_2, and a desired set point we have:

$$\left|\frac{\partial x_L}{\partial \Delta}\right|^{\text{sAIF}} < \left|\frac{\partial x_L}{\partial \Delta}\right|^{\text{fP}}$$ for any monotonic process.

Steady-State Errors in Deterministic Non-ideal Settings



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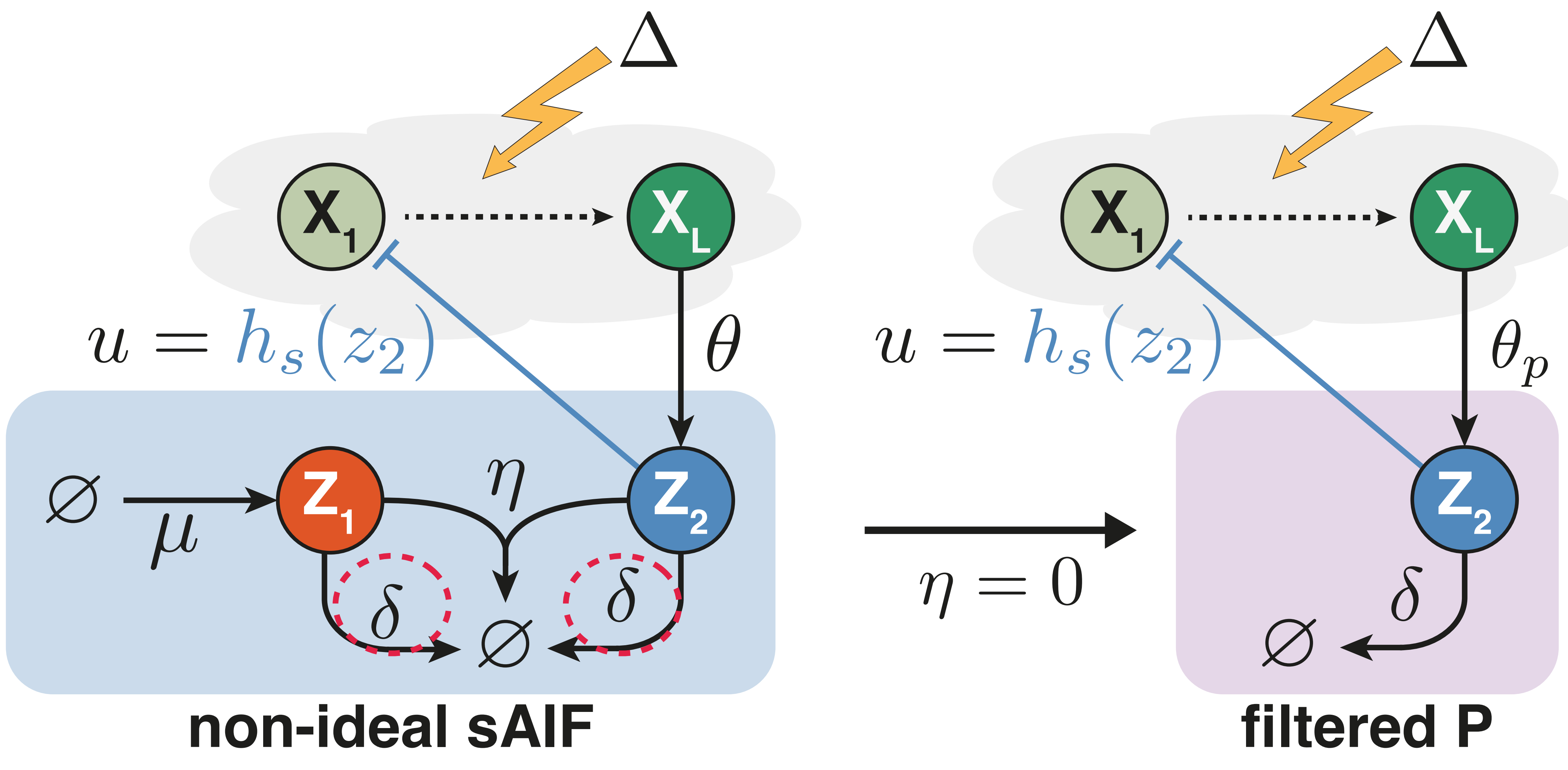
$$\frac{\partial}{\partial \eta}\left|\frac{\partial x_L}{\partial \Delta}\right|^{\text{sAIF}} < 0$$

Steady-State Errors in Deterministic Non-ideal Settings



Process: $x_L = \mathcal{P}_\Delta(u) \iff \dot{x} = f_\Delta(x,u)$

sAIF: $u = \mathcal{C}_s(x_L) \iff \begin{cases} \dot{z}_1 = \mu - \eta z_1 z_2 - \delta z_1 \\ \dot{z}_2 = \theta_s x_L - \eta z_1 z_2 - \delta z_2 \\ u = h_s(z_2) \end{cases}$

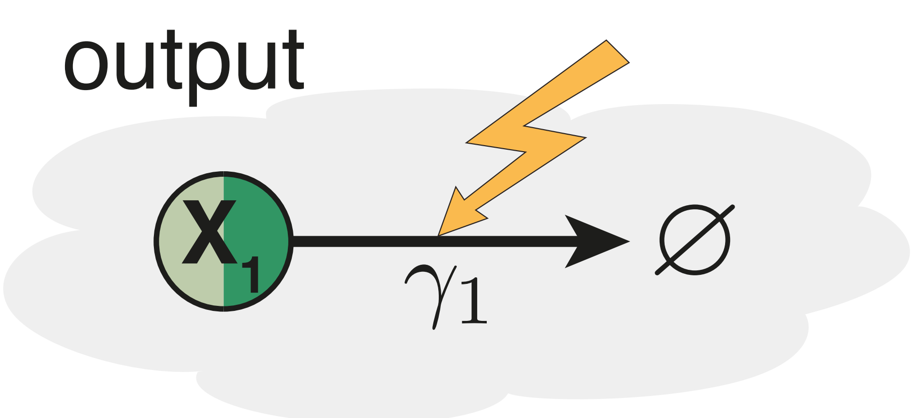
fP: $u = \mathcal{C}_p(x_L) \iff \begin{cases} \dot{z}_2 = \theta_p x_L - \delta z_2 \\ u = h_s(z_2) \end{cases}$

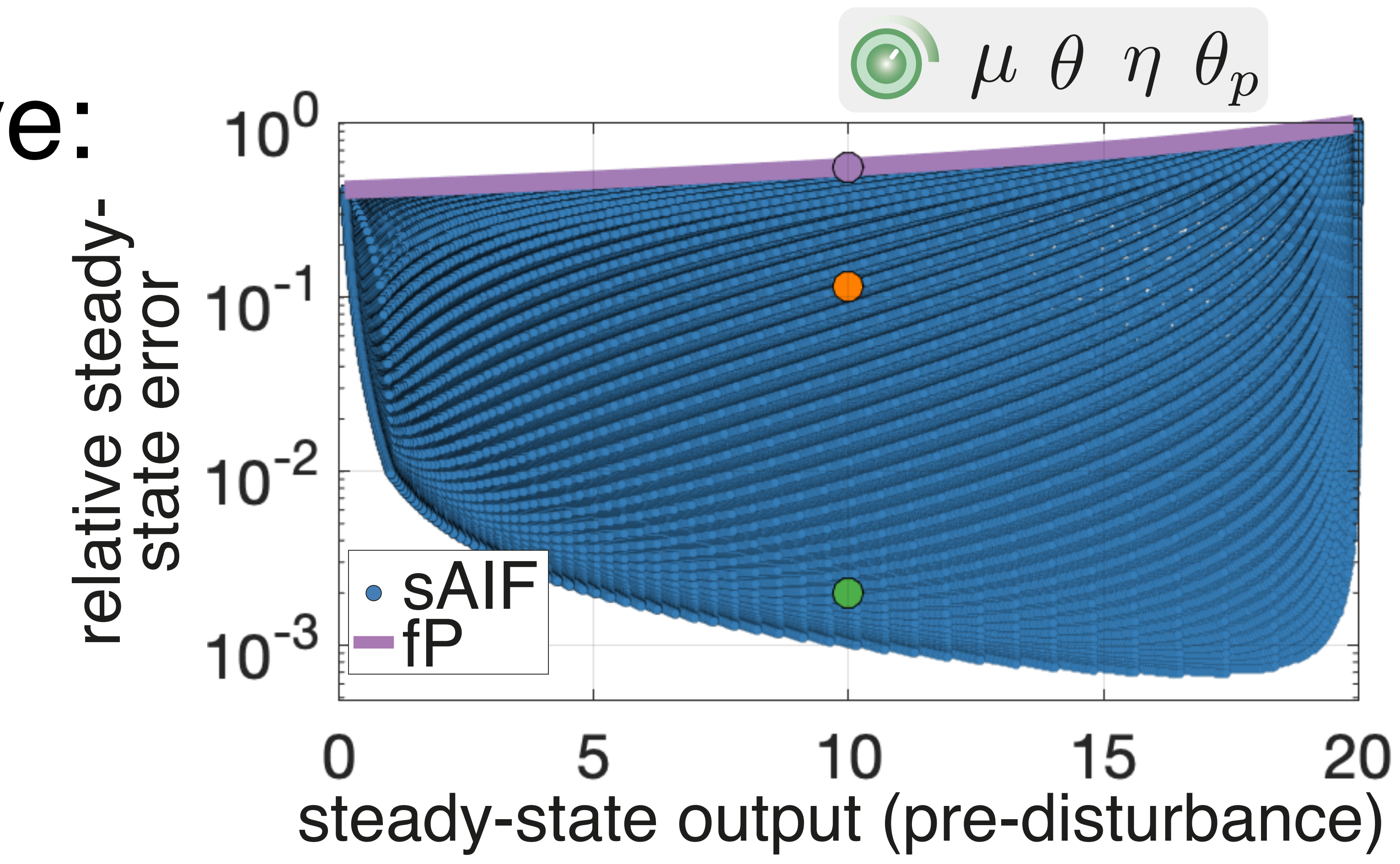
Question: Given that the non-ideal sAIF controller does not perfectly reject disturbances, why bother with additional circuitry?

Theorem: Given a repressor Z_2, and a desired set point we have:

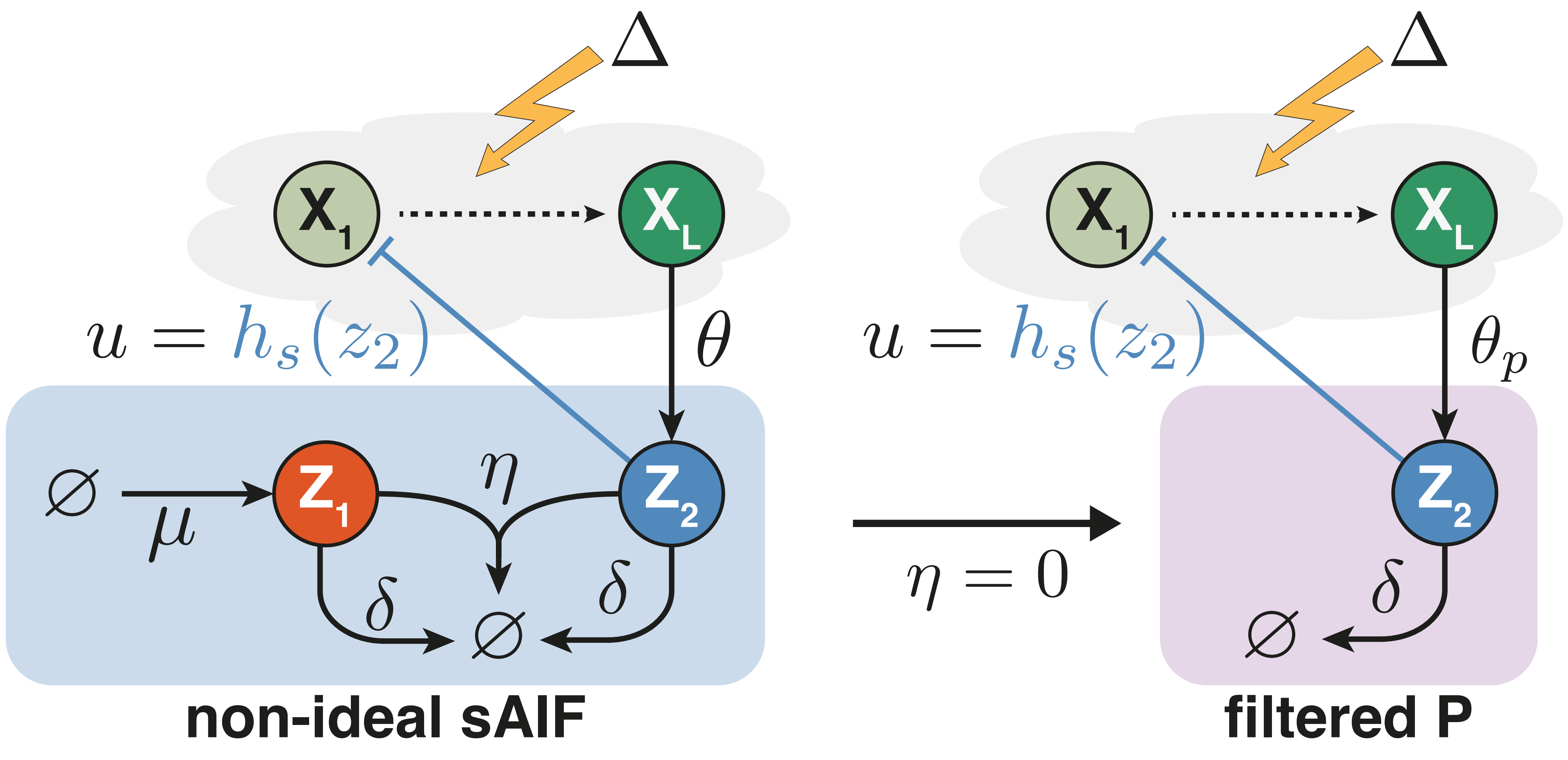
$$\left|\frac{\partial x_L}{\partial \Delta}\right|^{\text{sAIF}} < \left|\frac{\partial x_L}{\partial \Delta}\right|^{\text{fP}}$$ for any monotonic process.

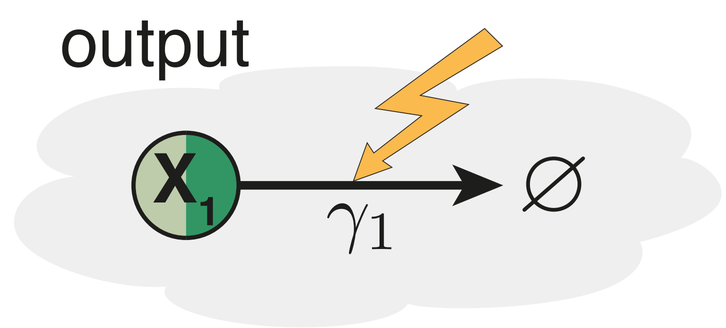
$$\frac{\partial}{\partial \eta}\left|\frac{\partial x_L}{\partial \Delta}\right|^{\text{sAIF}} < 0$$



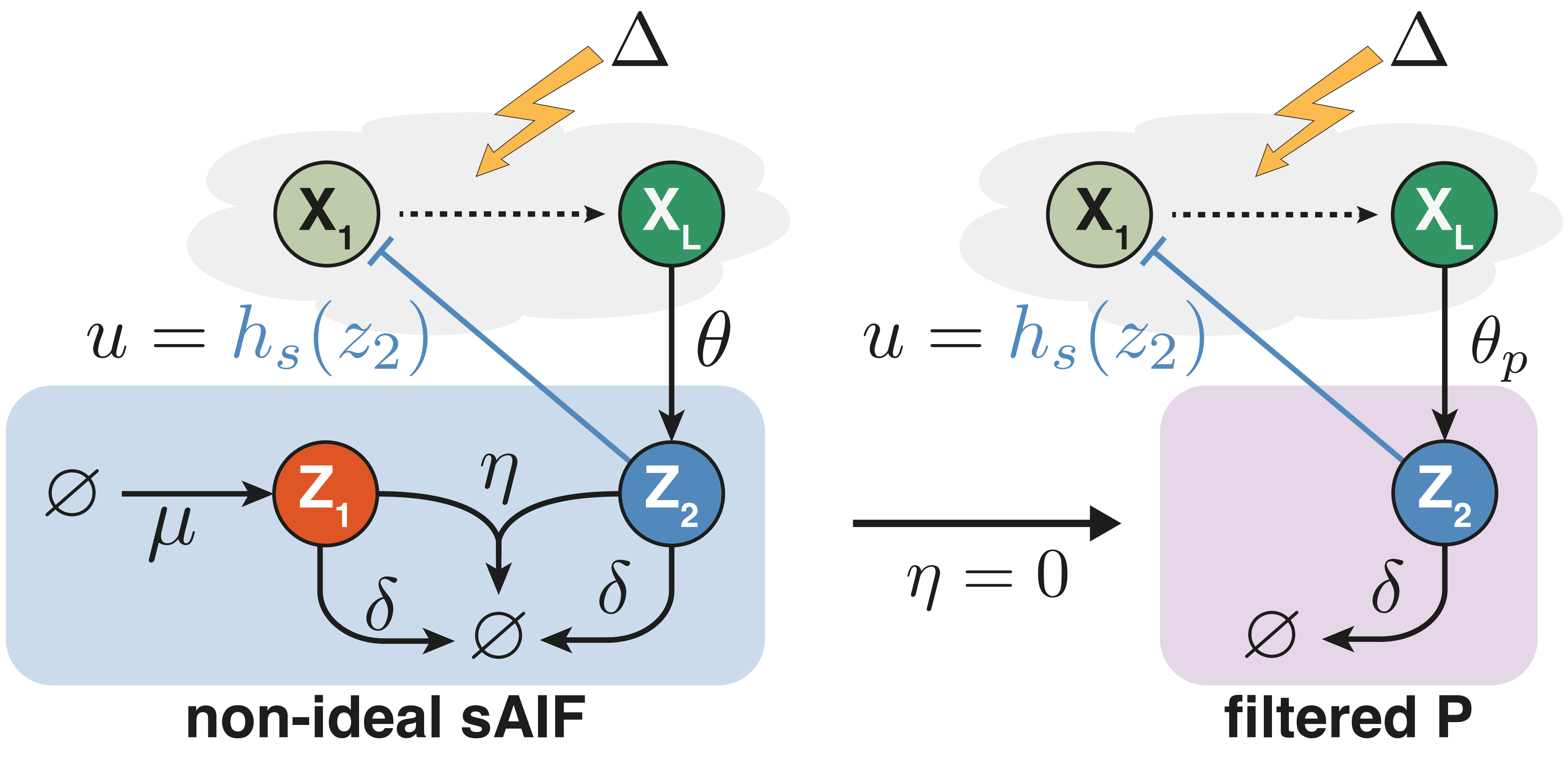


Stationary Noise in Stochastic Non-ideal Settings

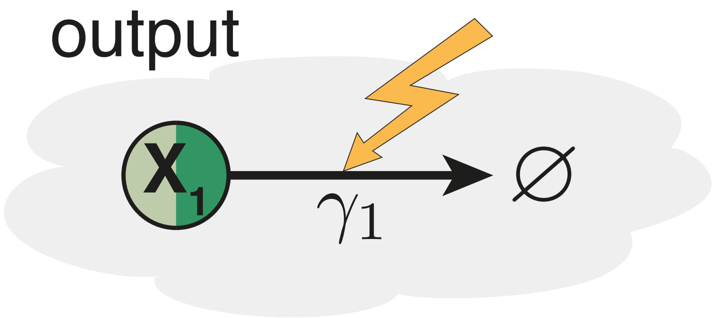




Stationary Noise in Stochastic Non-ideal Settings



For a given desired set point r, $\left.\frac{\partial \mathrm{Var}[\bar{X}_1]}{\partial \eta}\right|_{\mathbb{E}[\bar{X}_1]=r} = \ ?$



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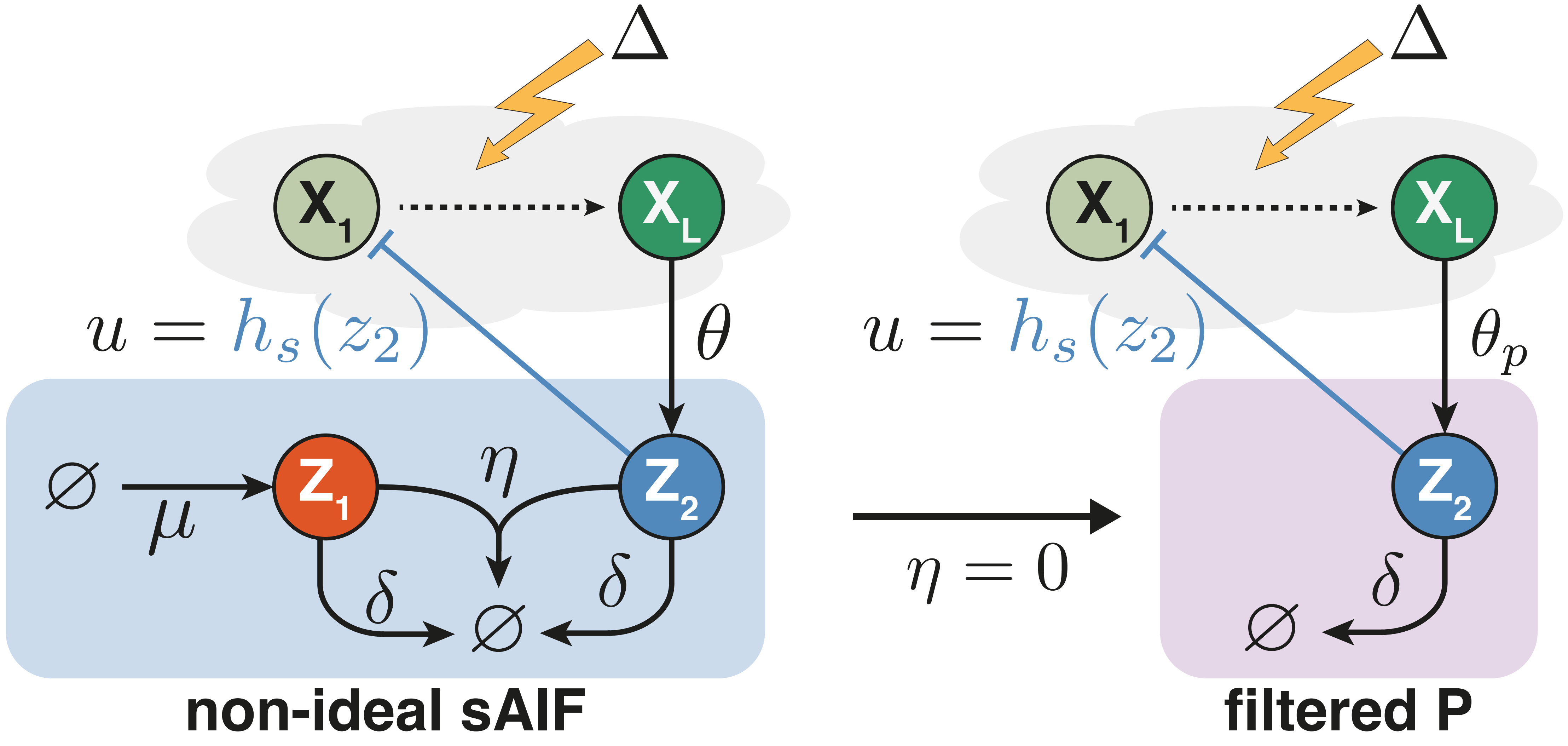

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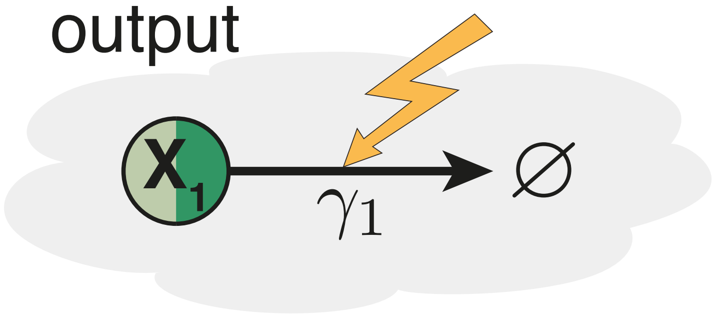
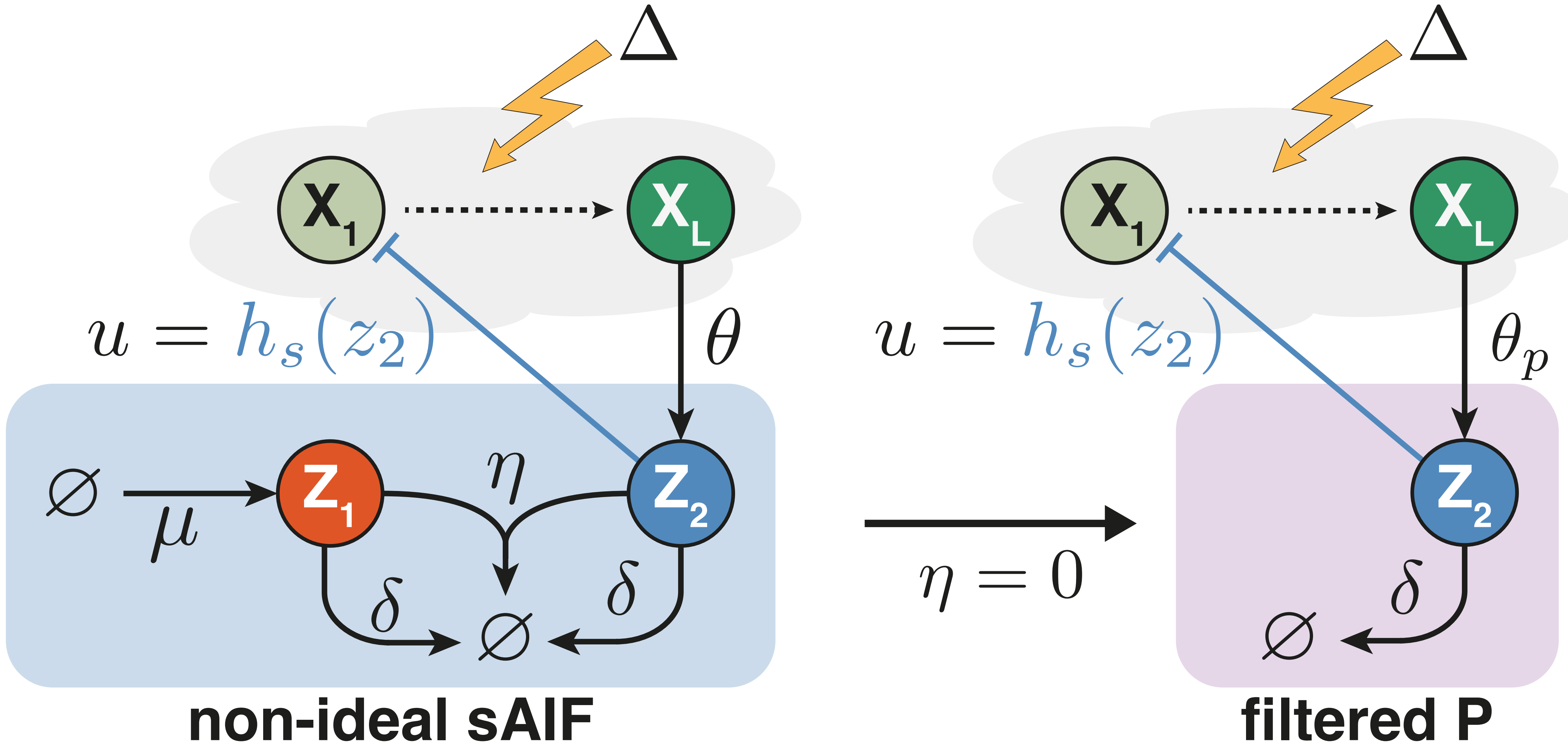
$$\left.\frac{\partial \mathrm{Var}[\bar{X}_1]}{\partial \eta}\right|_{\substack{\eta = 0 \\ \mathbb{E}[\bar{X}_1] = r}} \approx -\frac{\mathbb{E}[\bar{Z}_2]\,\mu\, r\,\left|h'(\mathbb{E}[\bar{Z}_2])\right|\,\left(\gamma_1 + \left|h'(\mathbb{E}[\bar{Z}_2])\right|\right)}{\delta\,(\delta+\gamma_1)^2\,\left(\mathbb{E}[\bar{Z}_2]\,\left|h'(\mathbb{E}[\bar{Z}_2])\right| + \gamma_1\, r\right)} \leq 0$$

output



Stationary Noise in Stochastic Non-ideal Settings



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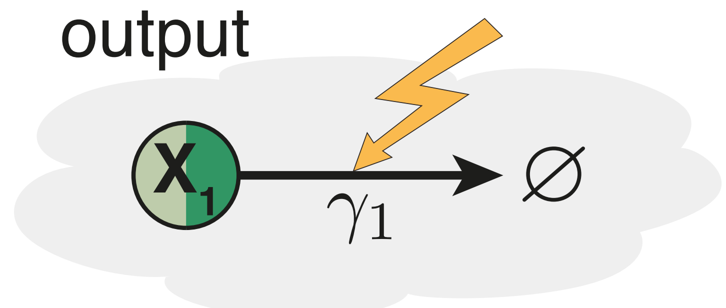
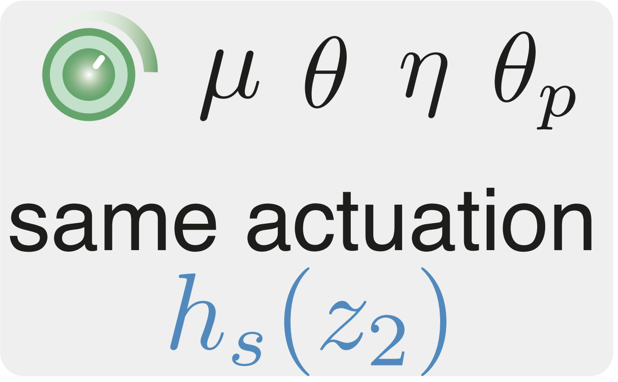
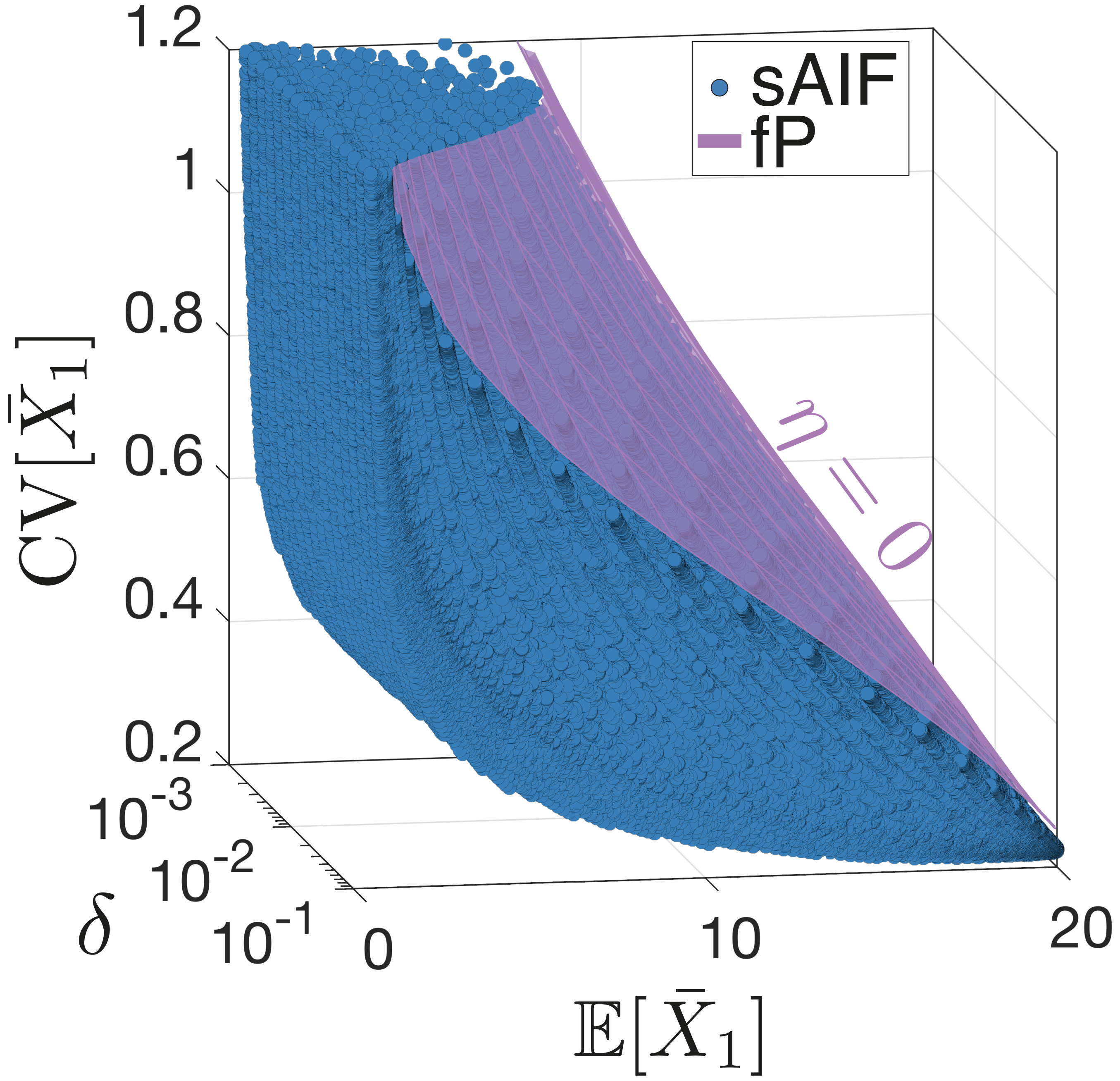
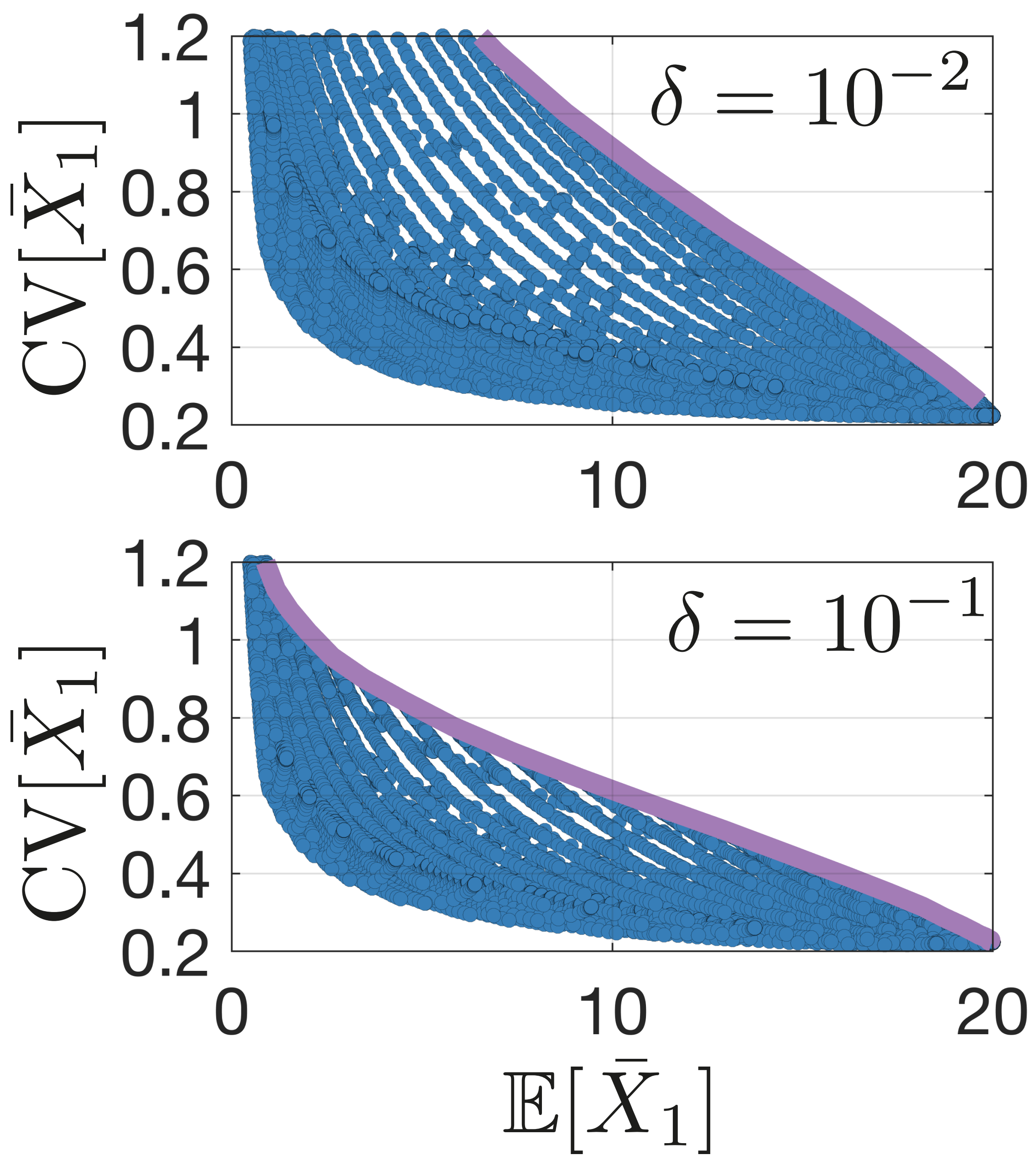
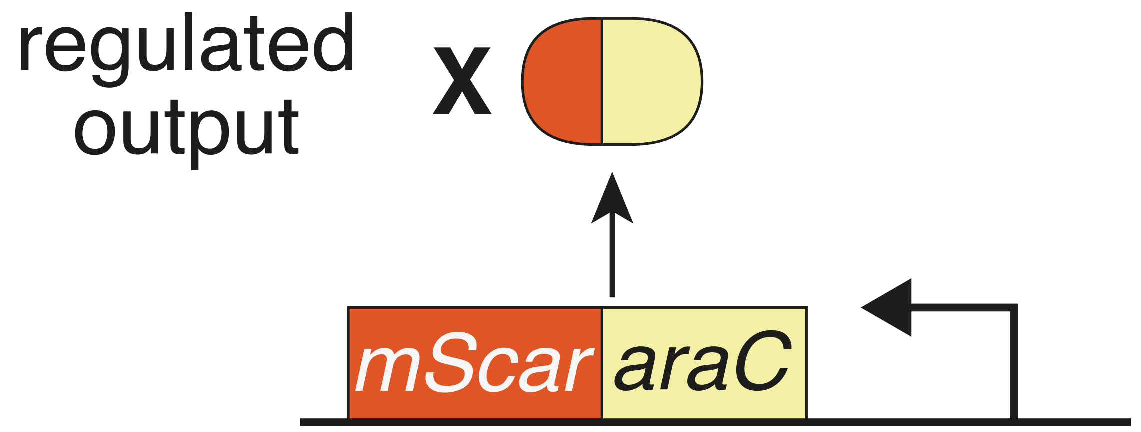








Genetic Implementations



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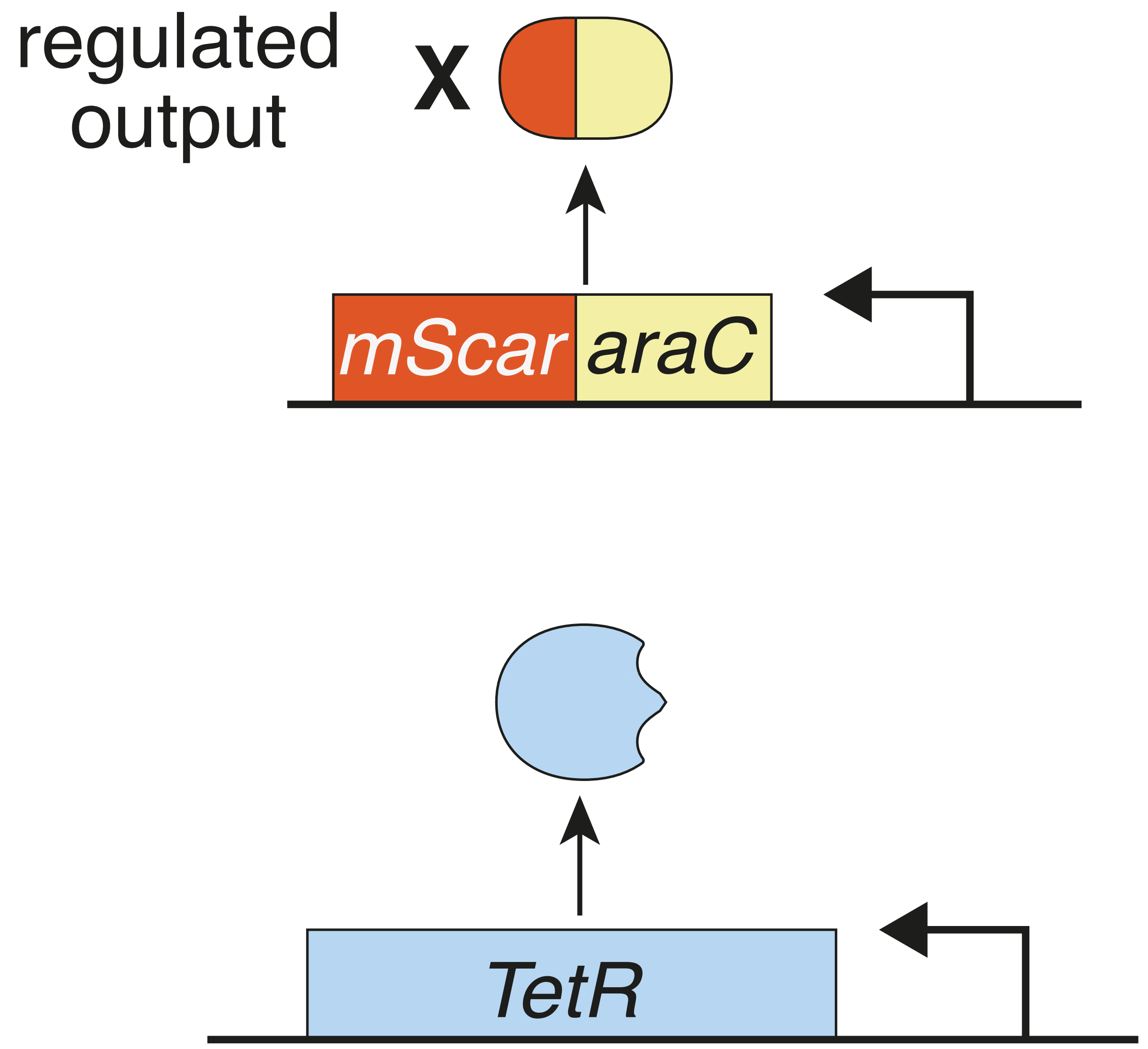
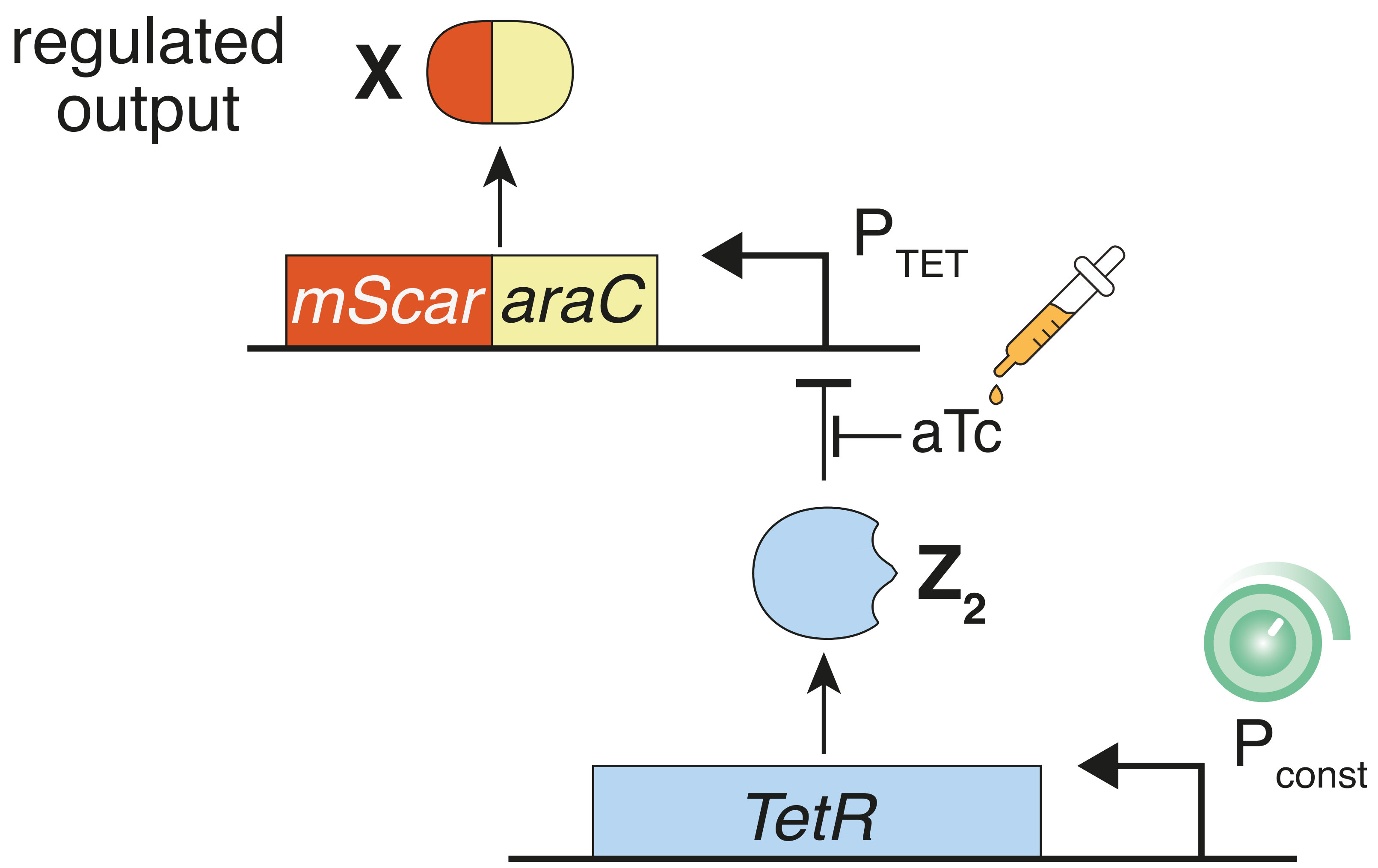
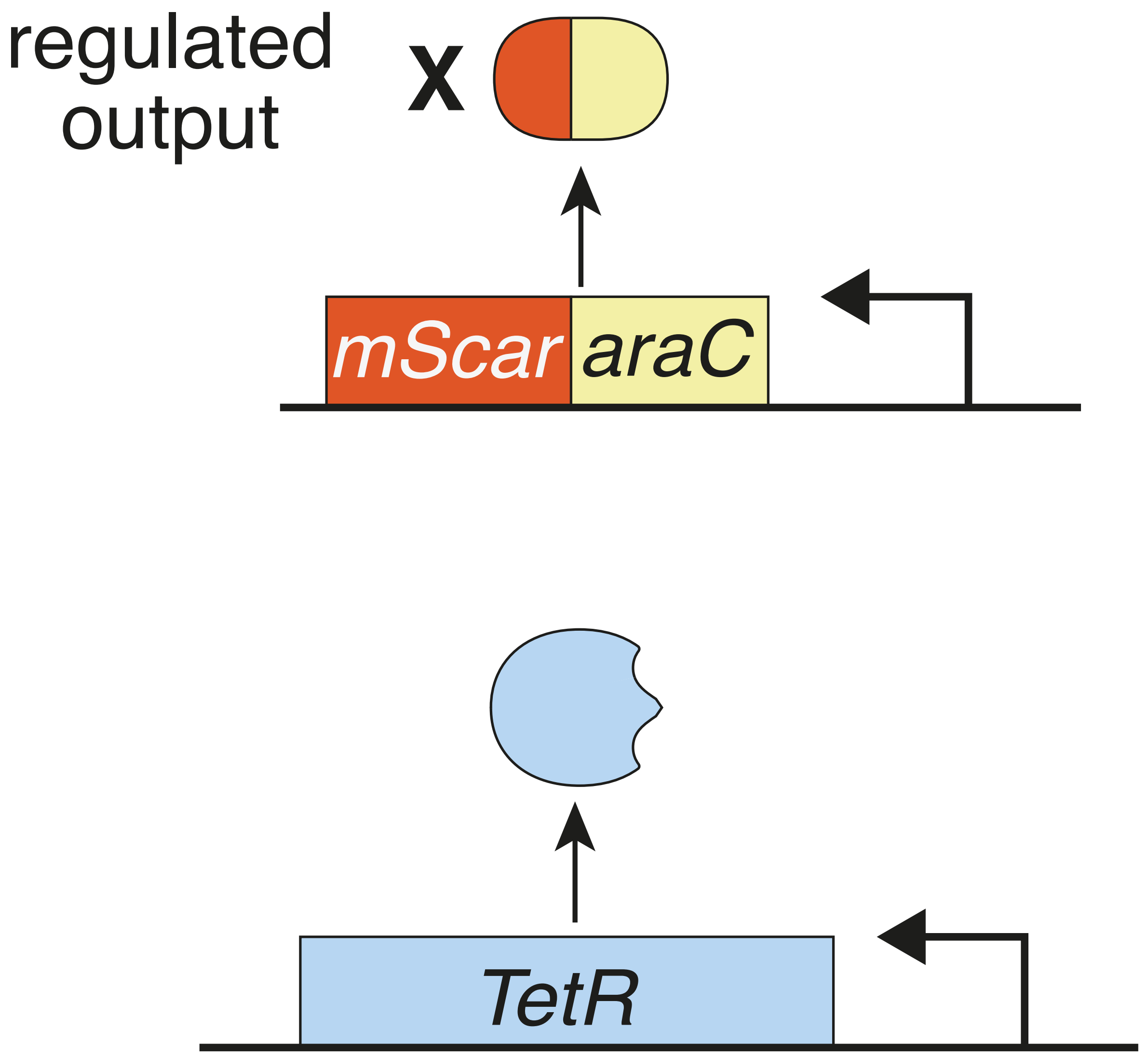


Genetic Implementations

open loop control





Genetic Implementations

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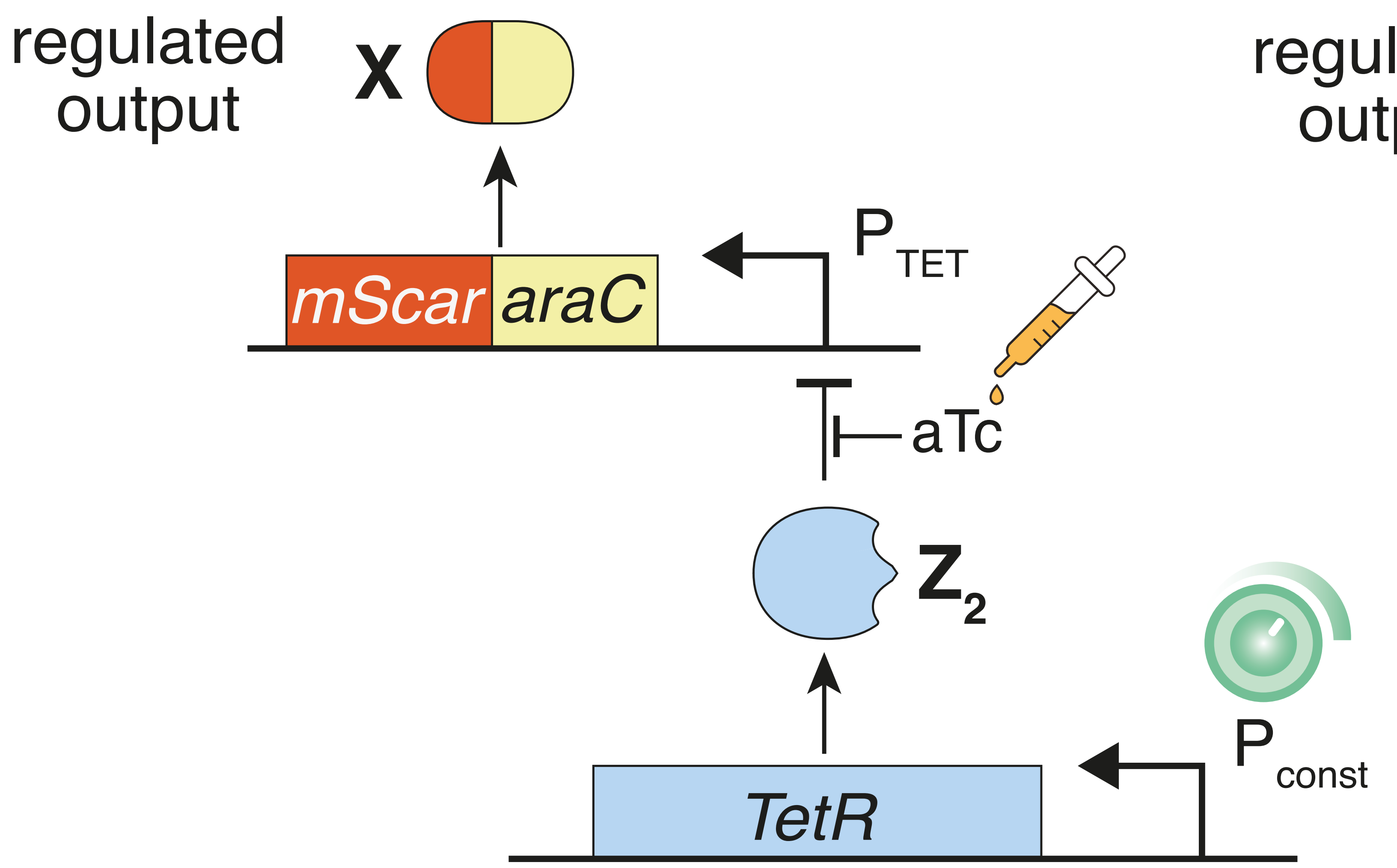
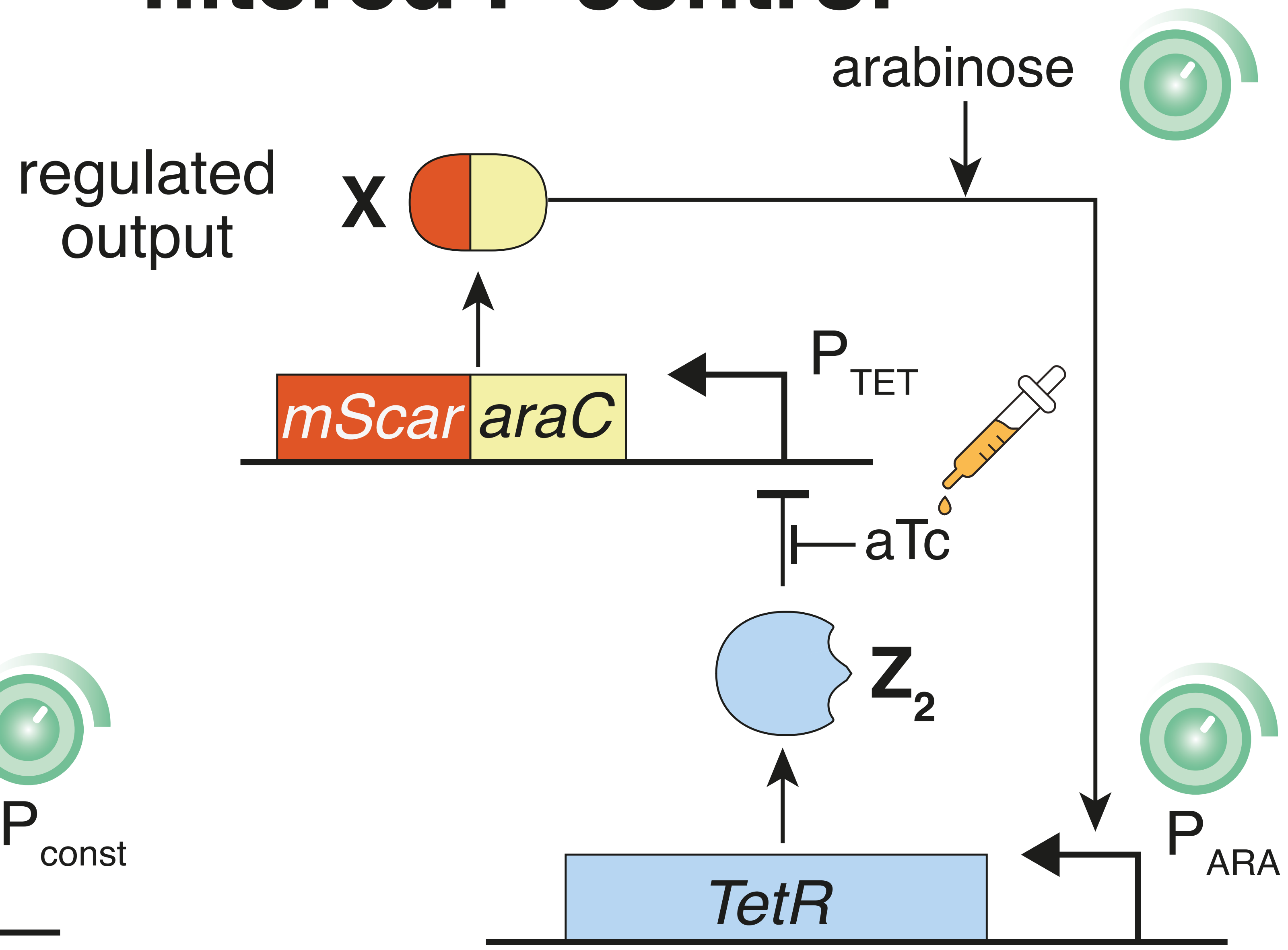
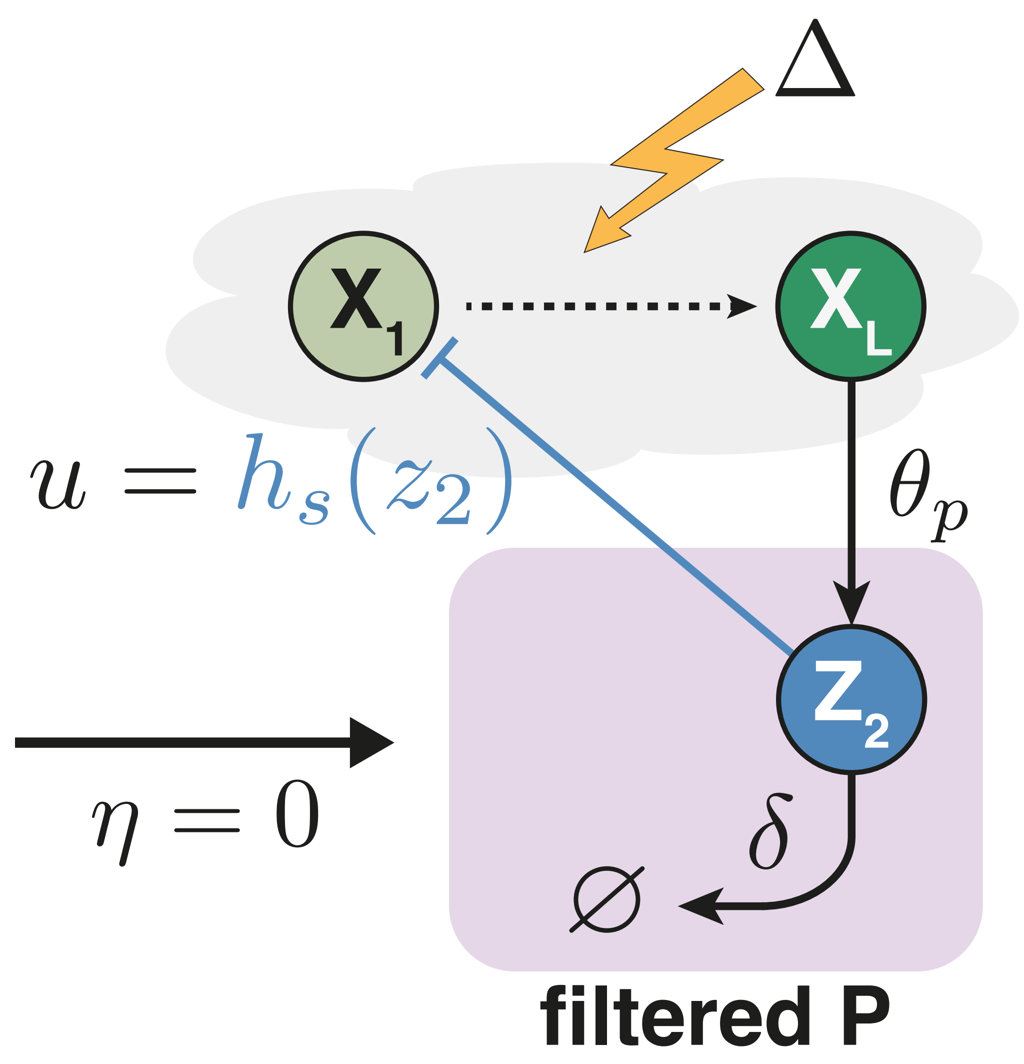


filtered P control





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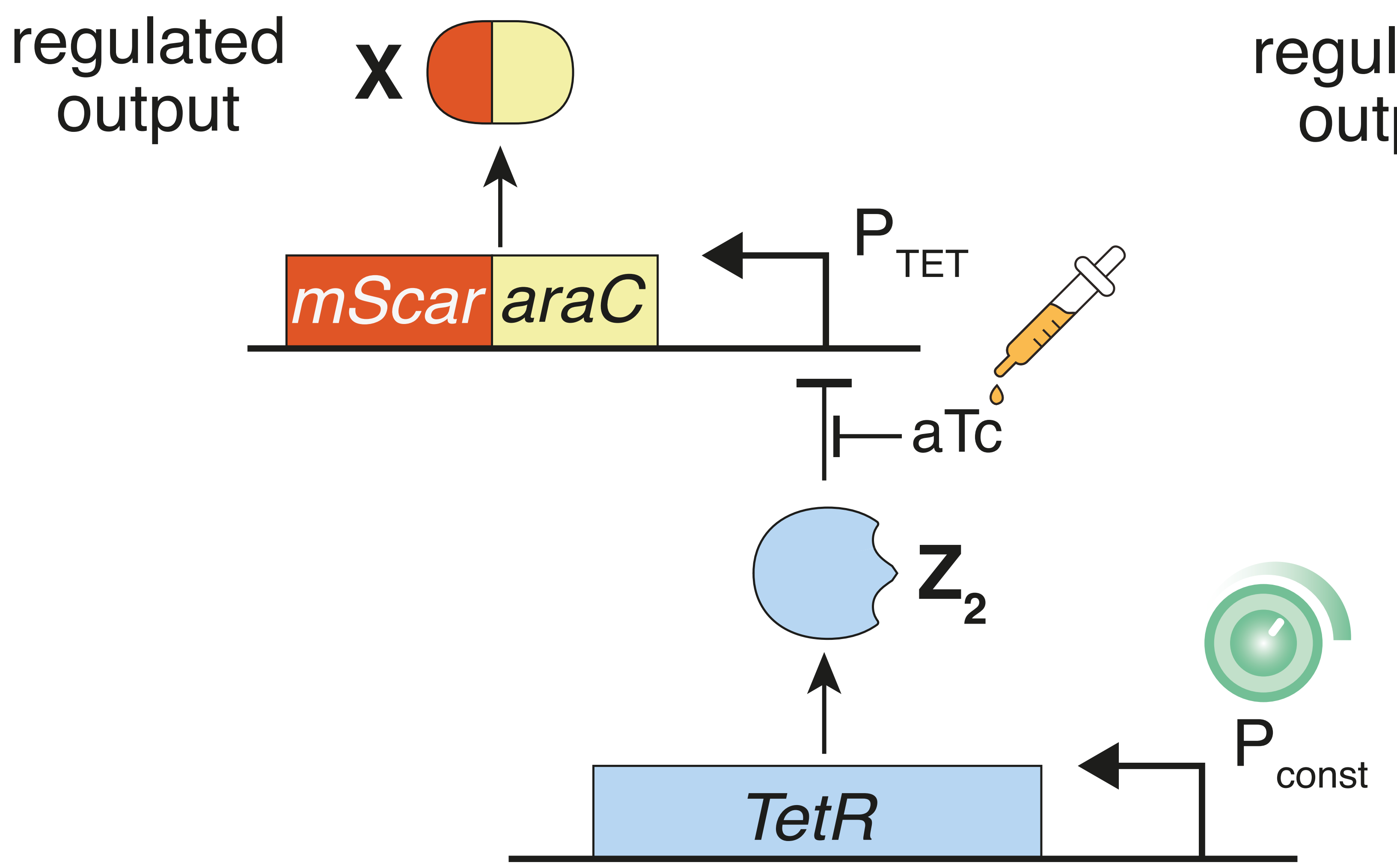

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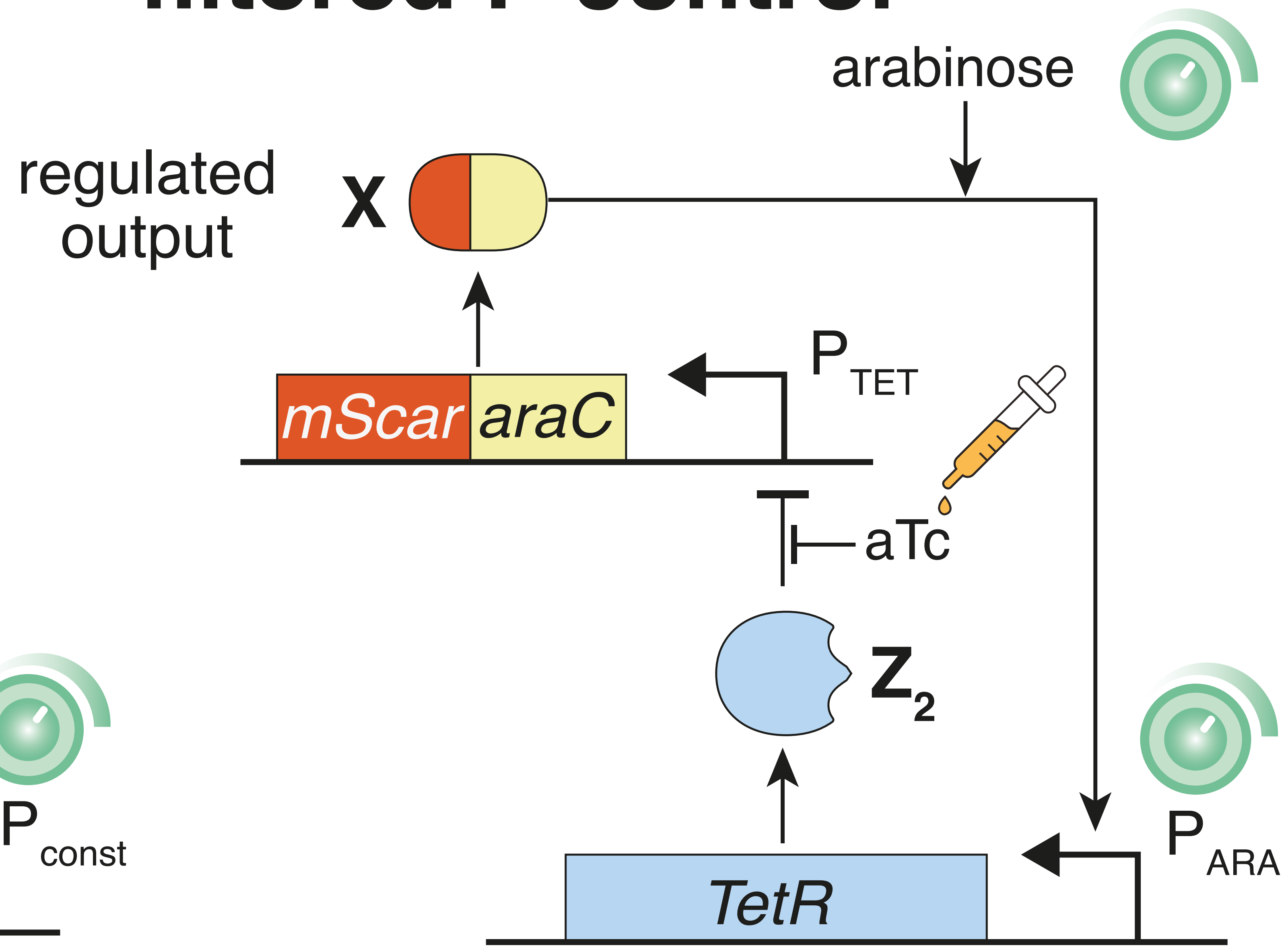
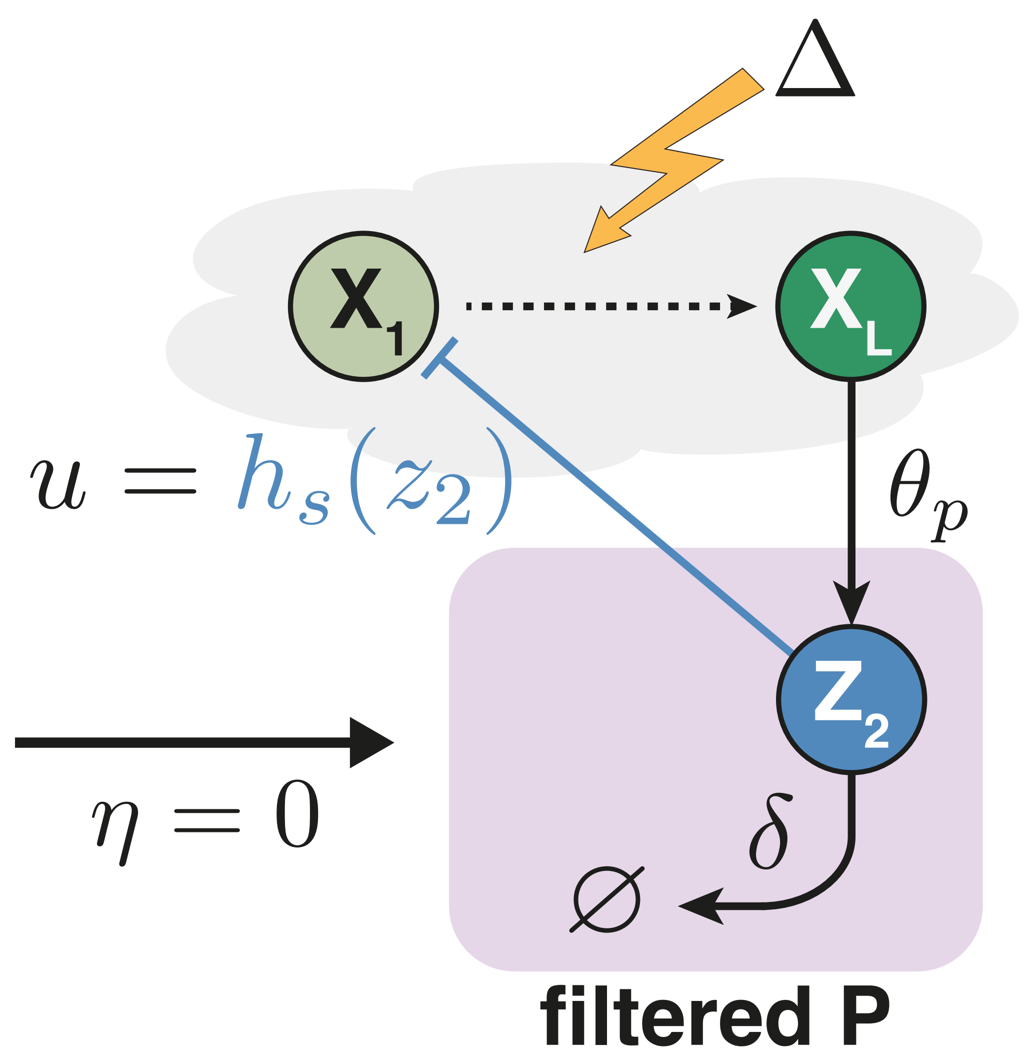
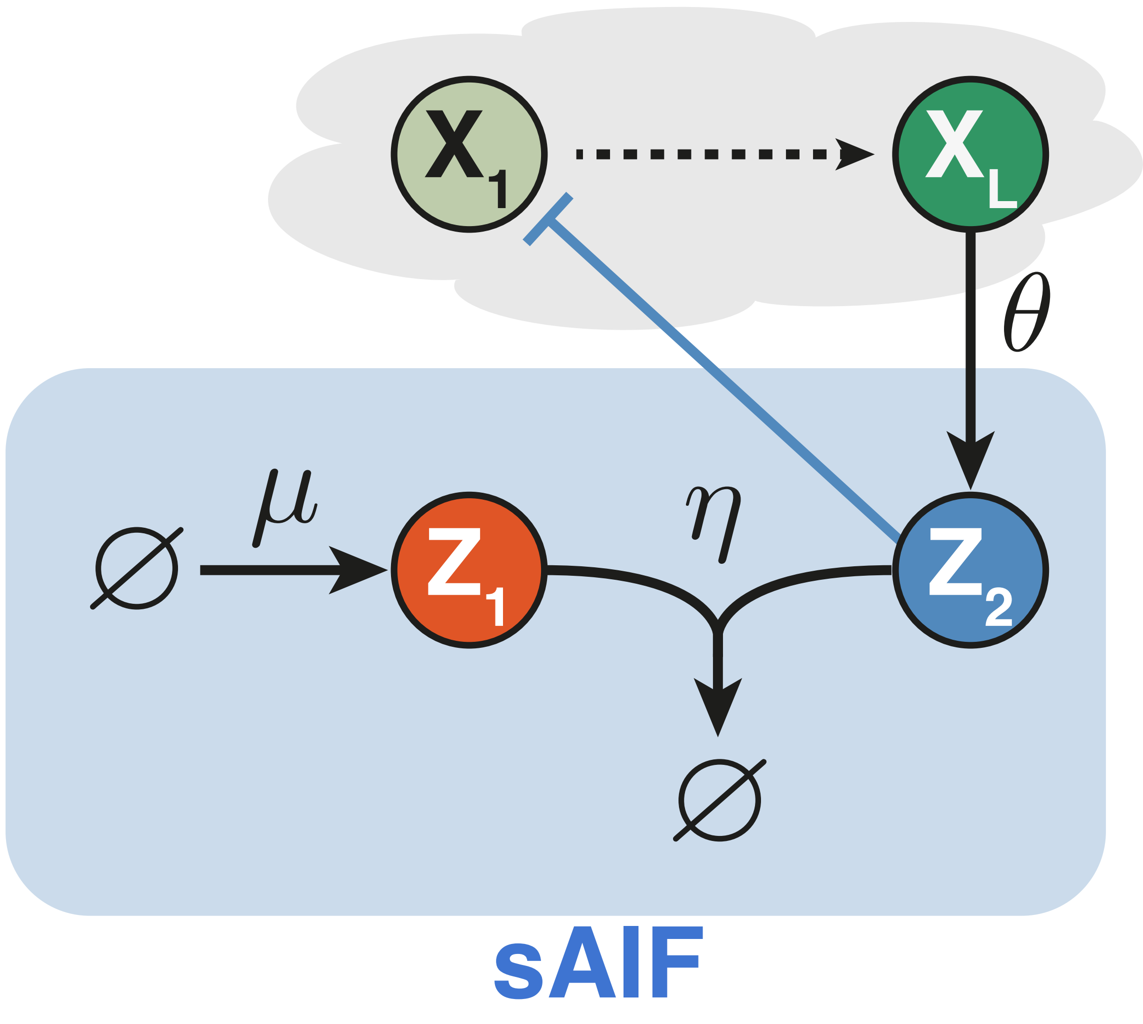
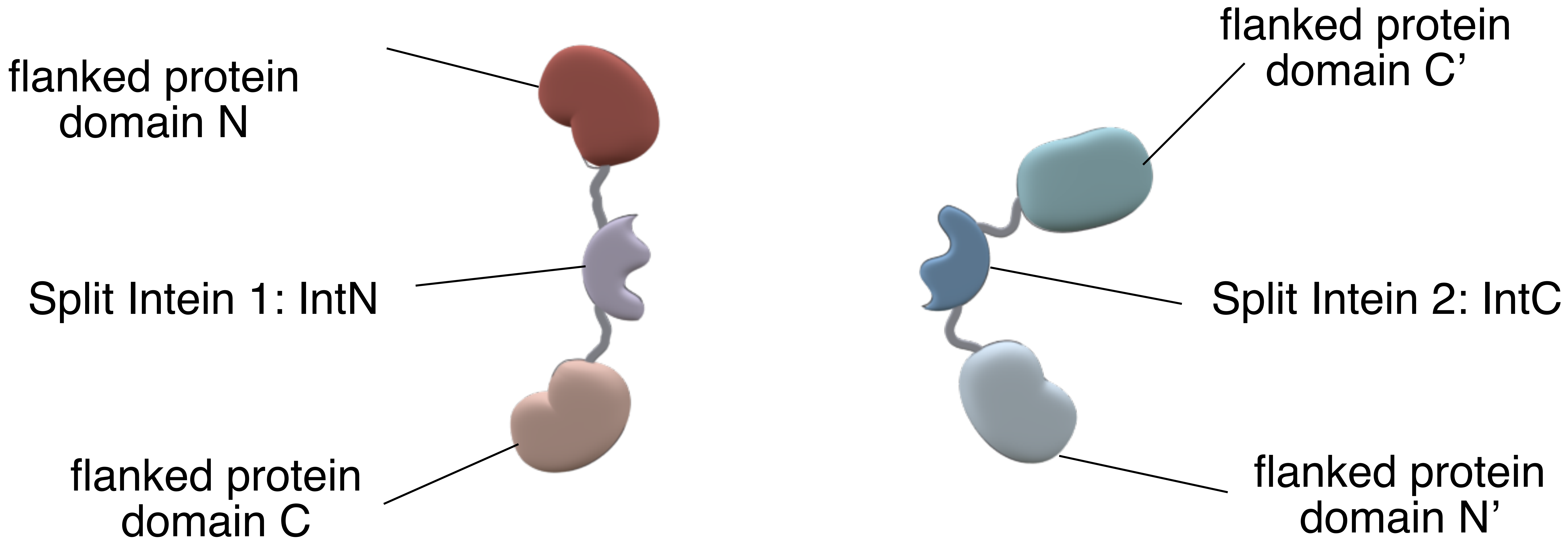


?





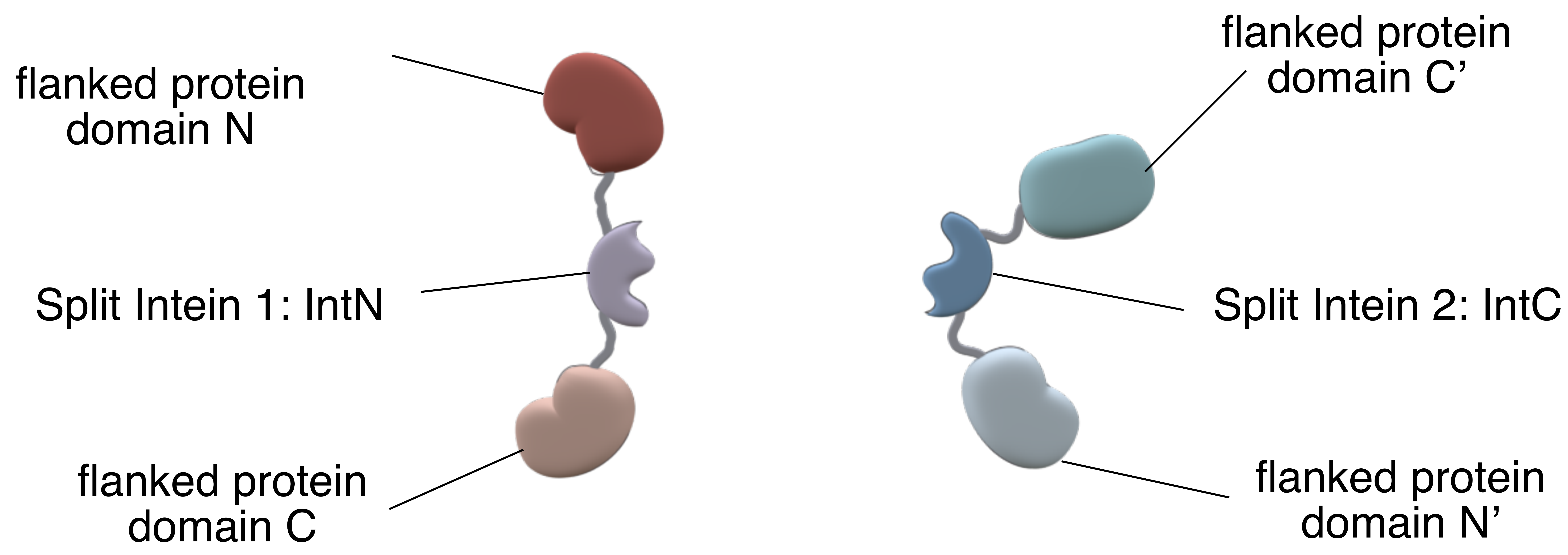
Inteins: A Swiss Army Knife for Synthetic Biology



Anastassov, S., **Filo, M.**, & Khammash, M. (2024). Inteins: A Swiss army knife for synthetic biology. *Biotechnology Advances*.

Inteins: A Swiss Army Knife for Synthetic Biology

Each protein domain has a specific function



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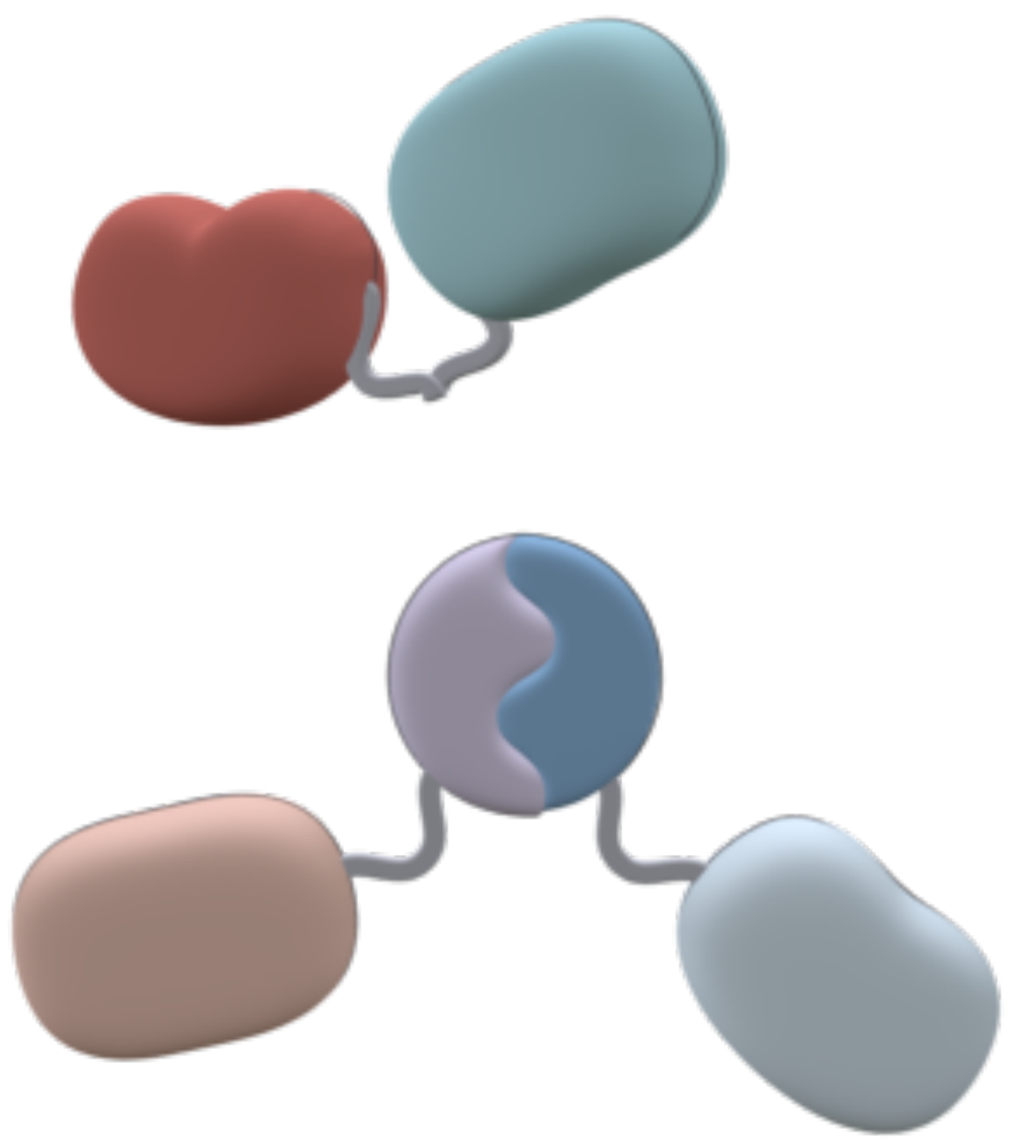
Inteins: A Swiss Army Knife for Synthetic Biology

Splicing reaction yields new protein products with new functions!



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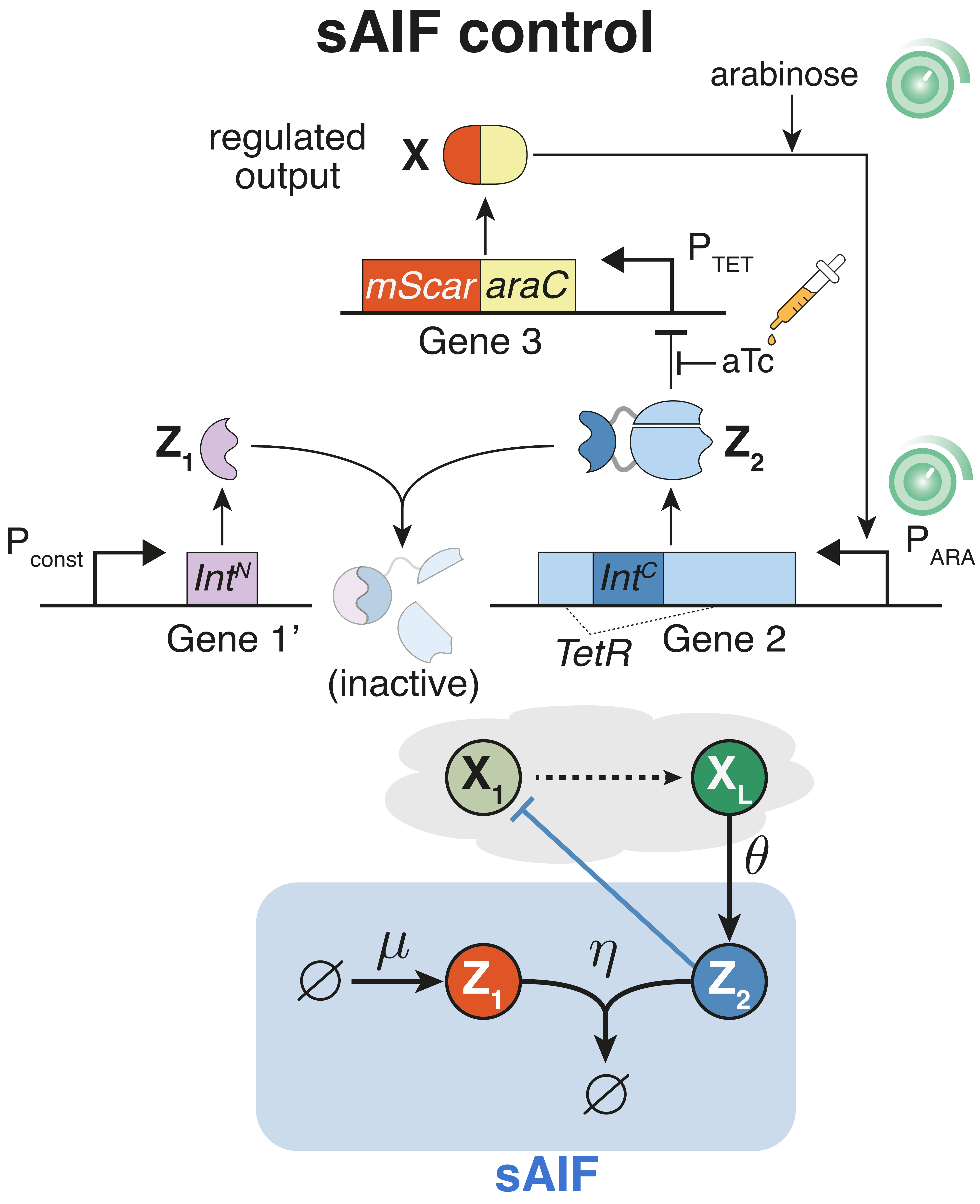
Minimal PI: Genetic Implementation with Inteins



Minimal PI: Genetic Implementation with Inteins

Minimal PI: Noise

rAIF was seen experimentally to achieve RPA but with a significant increase in noise.

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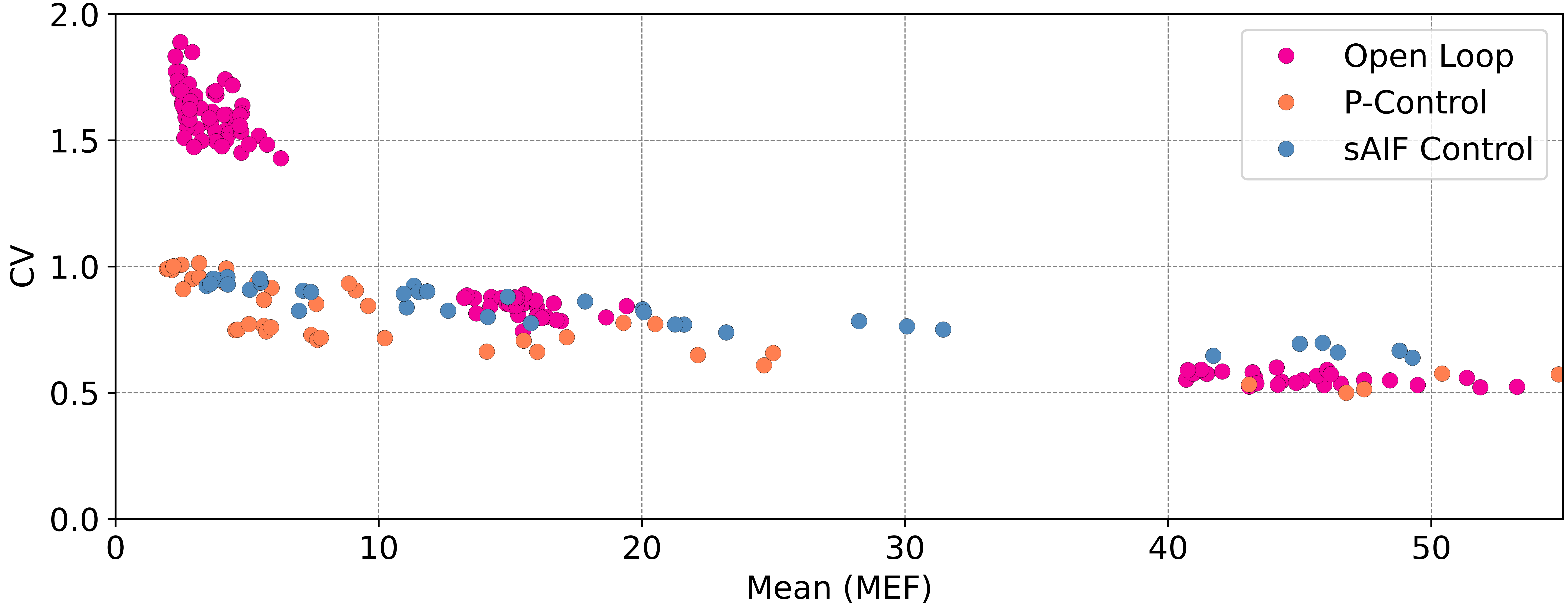

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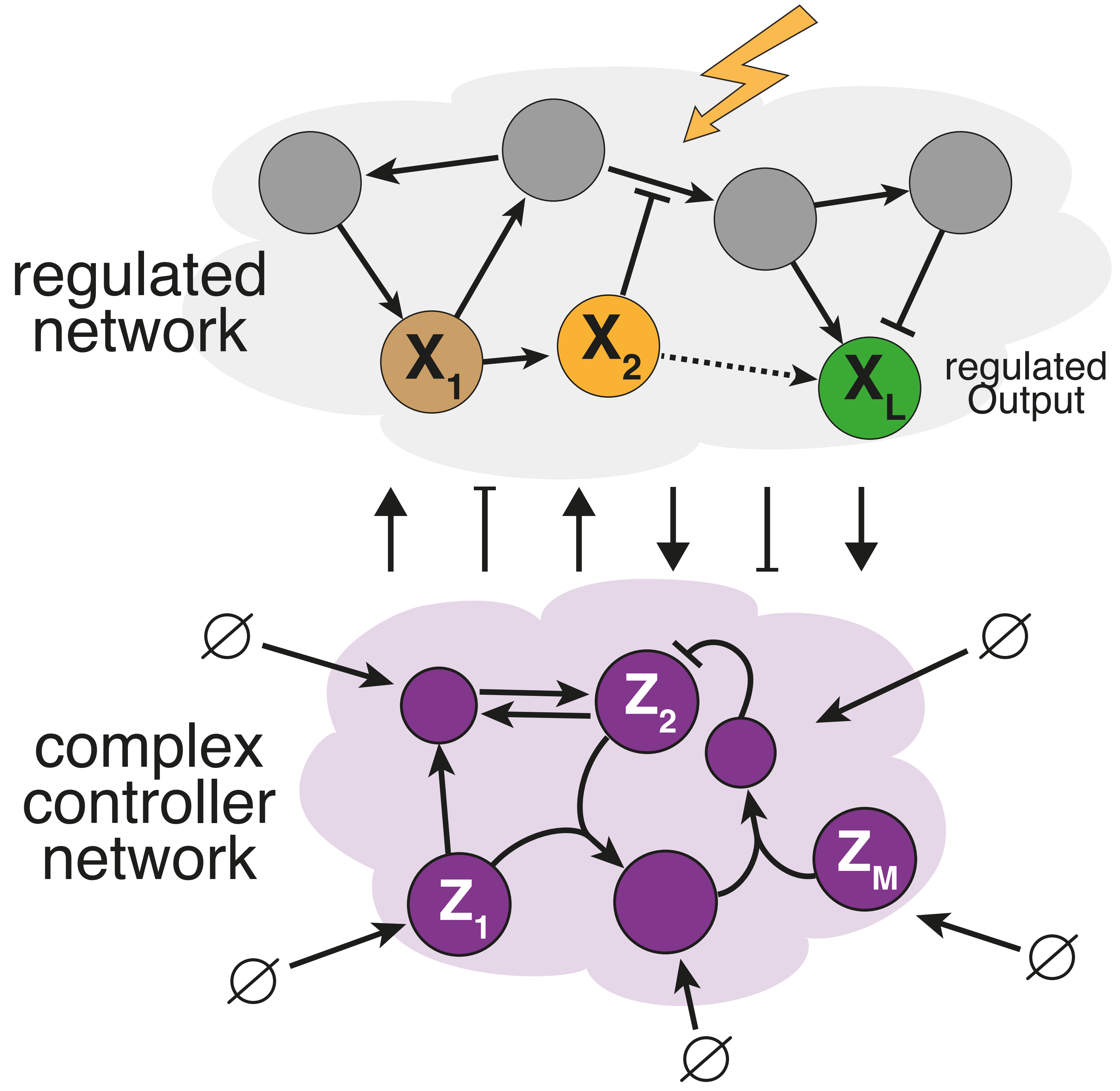
Design & Analysis of Complex Biomolecular Controllers



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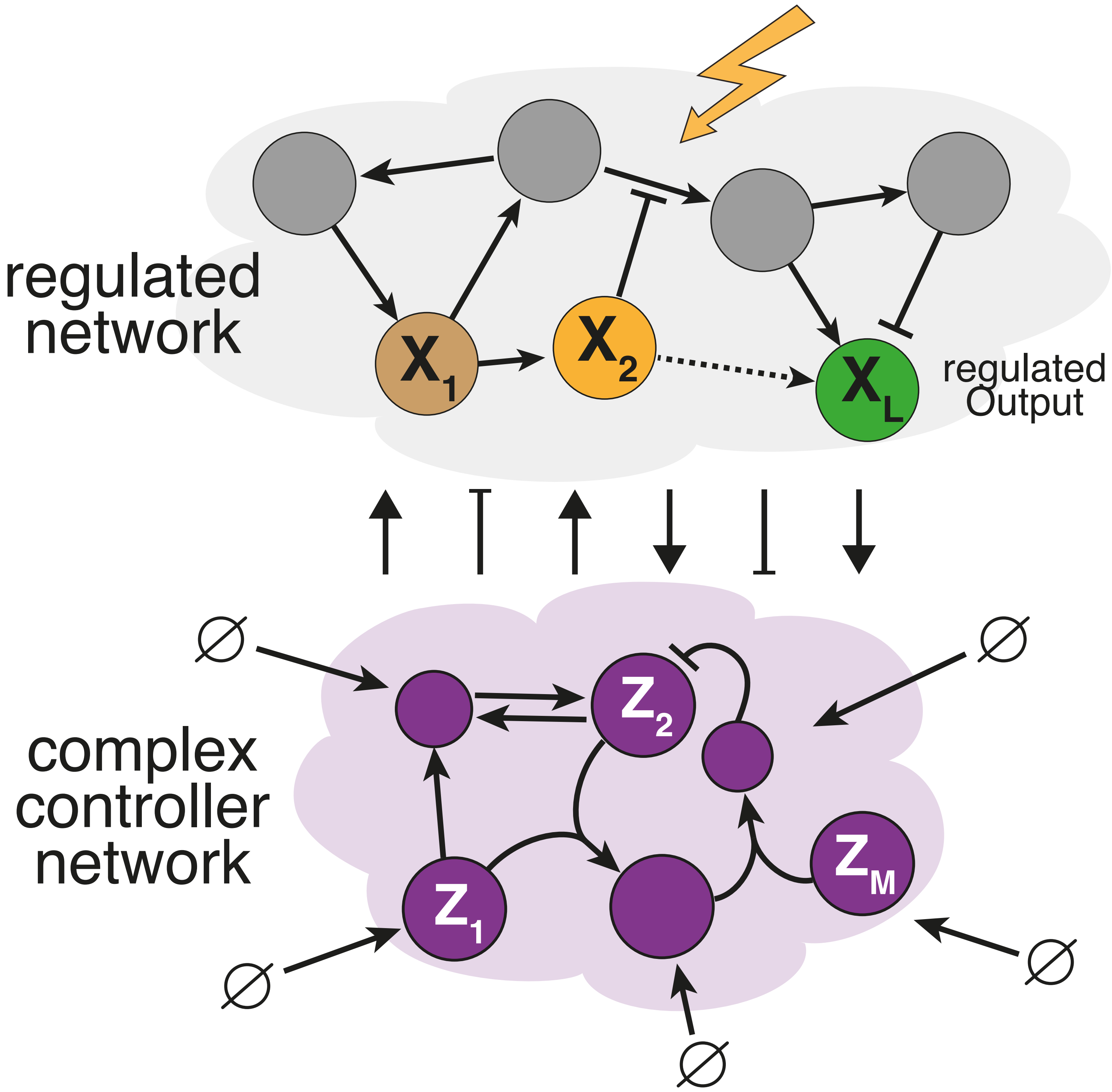

Question 1: Does this controller achieve RPA?

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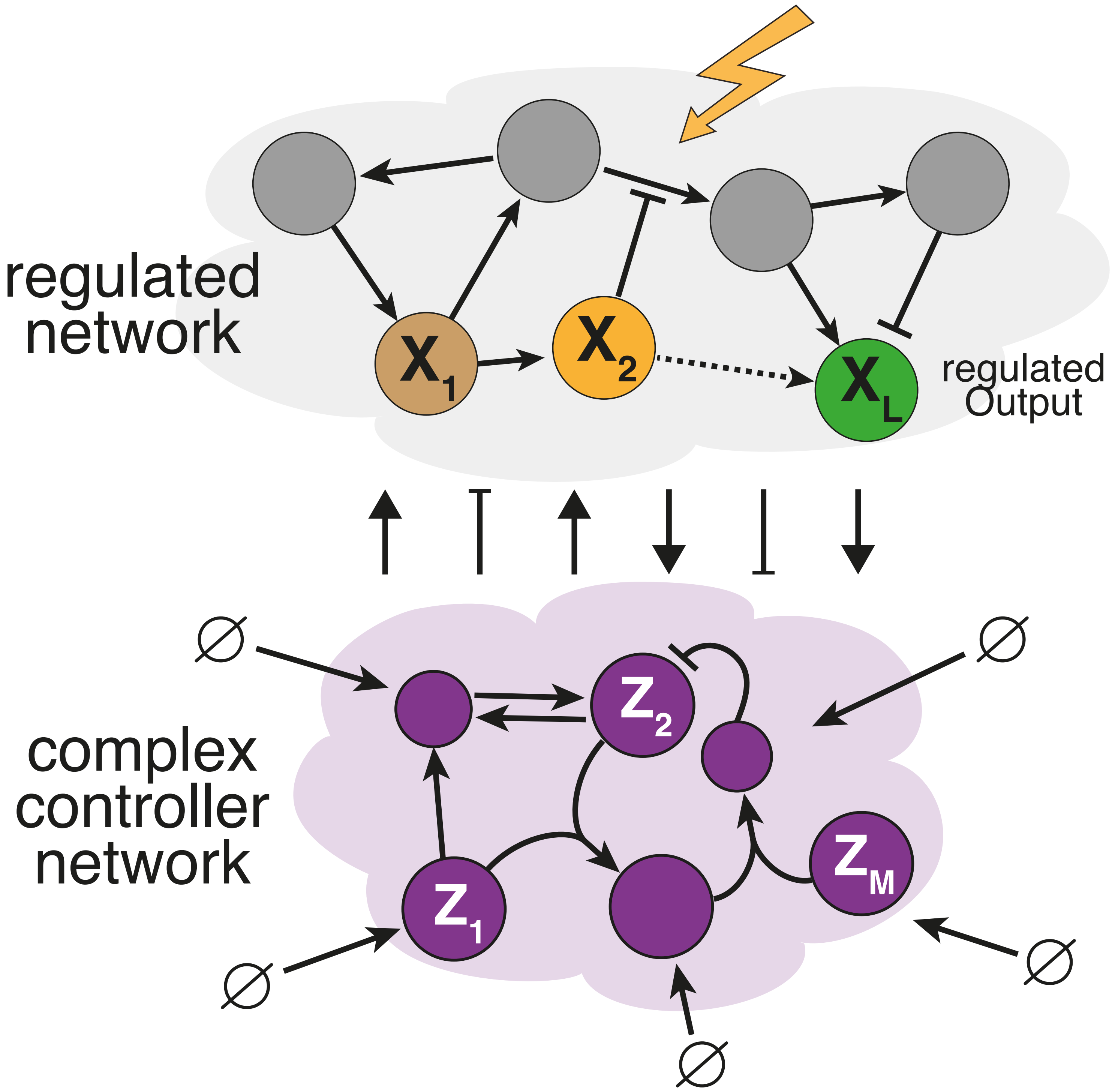

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Answer: Theorem 1 (**RPA Test**)

- Provides sufficient algebraic conditions for **RPA**
- Valid in both deterministic & stochastic settings
- Easy to check graphically

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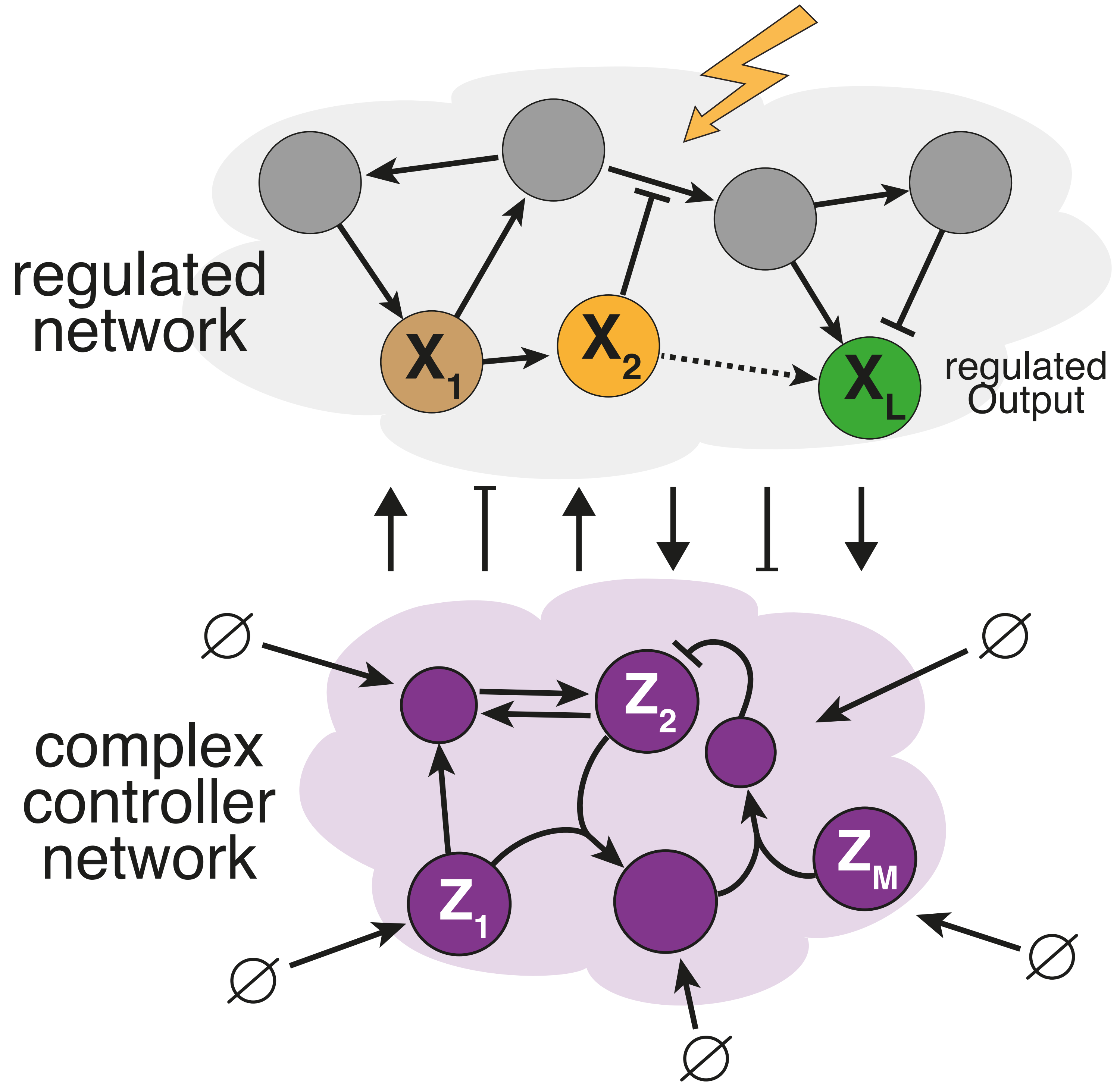

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Question 2: How to analyze such complex controllers?

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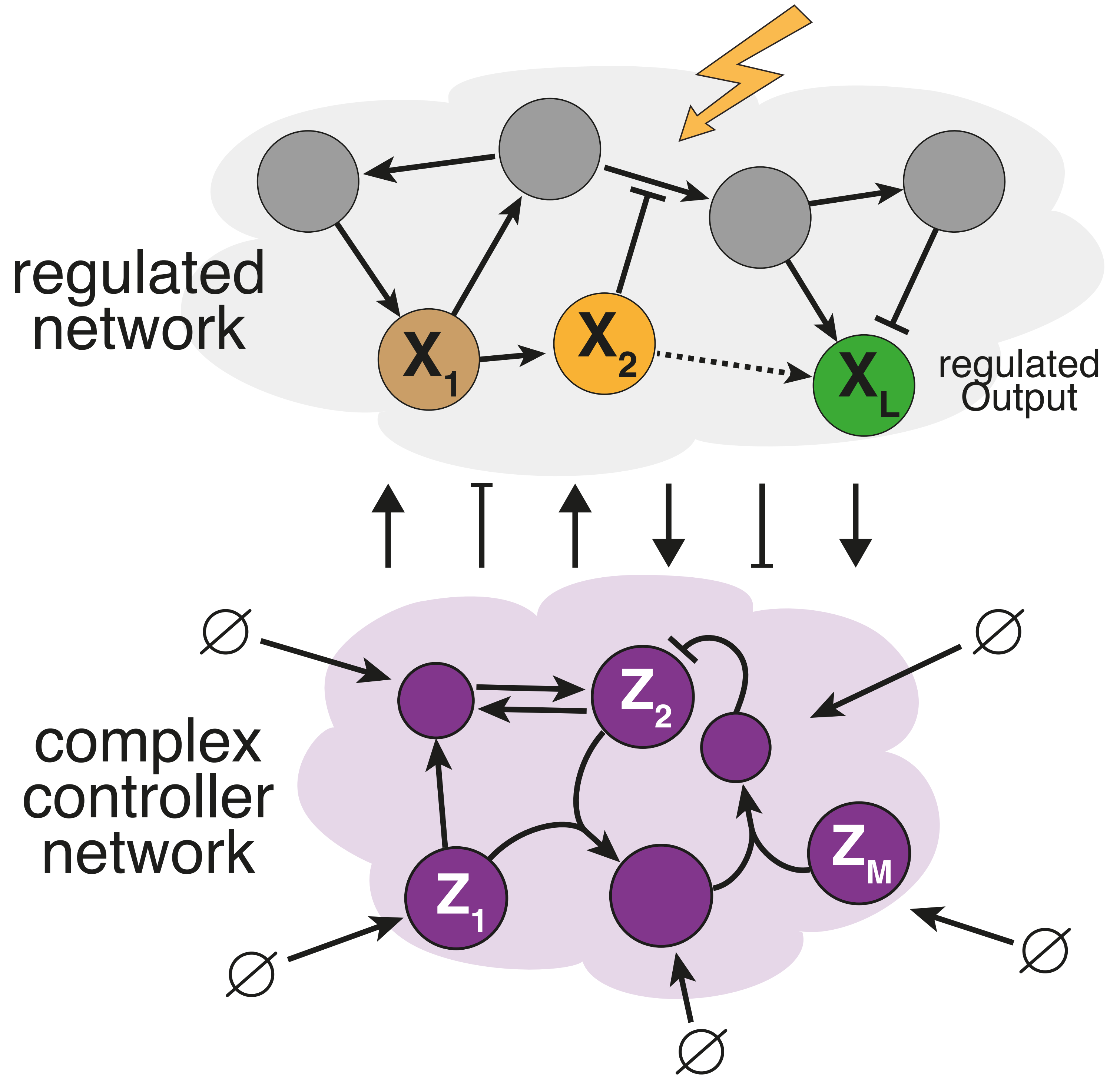
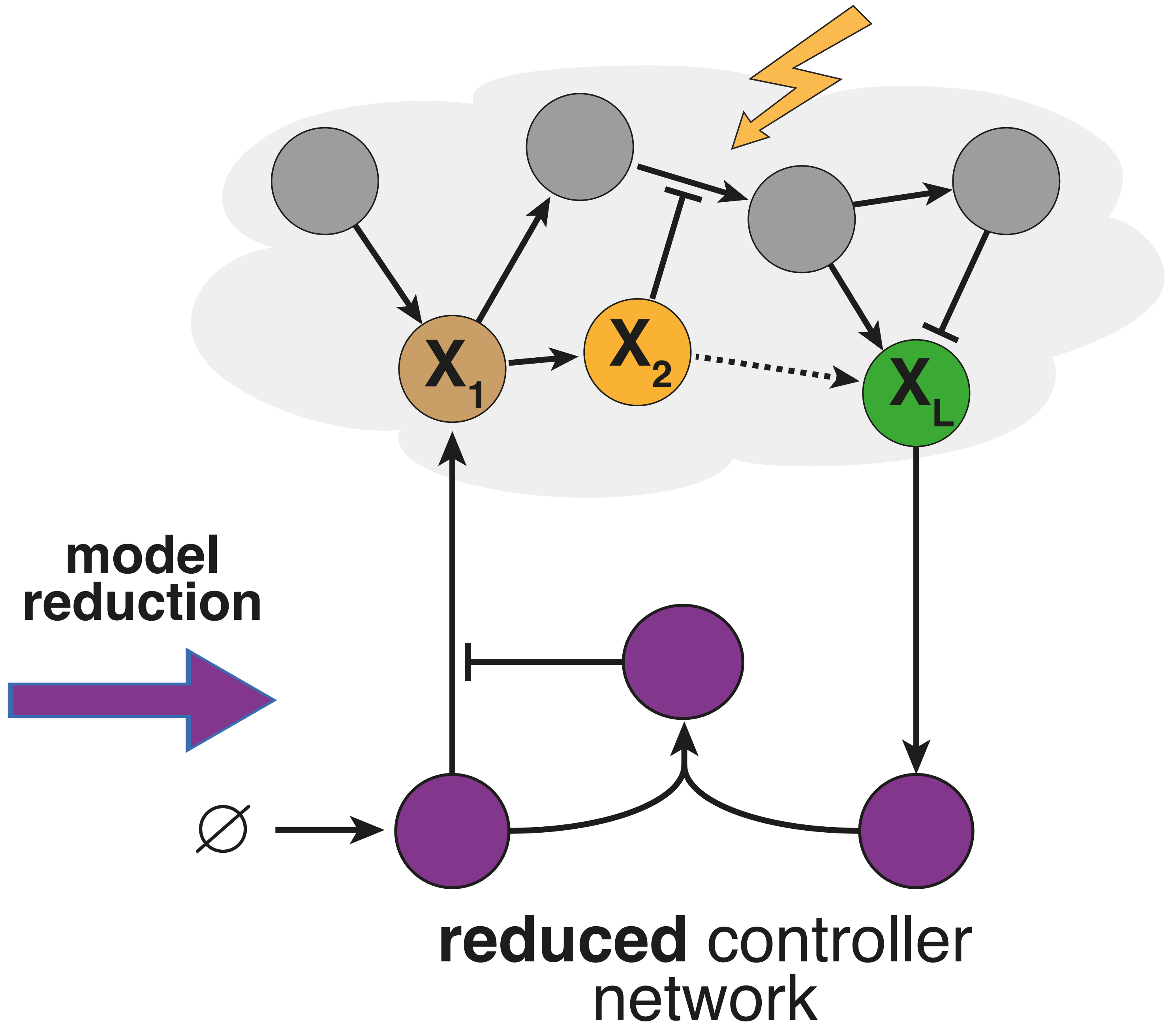
Design & Analysis of Complex Biomolecular Controllers





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Answer: Theorem 1 (**RPA Test**)

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Question 2: How to analyze such complex controllers?

Answer: Theorem 2 (**Model Reduction**)

- Provides an easy-to-use recipe for **model reduction**
- Uncovers the underlying control architectures
- Marries singular perturbation theory to deficiency-zero theorem

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Conclusions

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- Susceptible to poor dynamics and amplified noise
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Looking ahead:

- Control theory will drive synthetic biology forward.
- Next Steps: Develop the theory tailored to applications in cell therapy and bio-production

Thank you

https://maurice-filo.github.io/

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Filo, M., & Khammash, M. (2019, December). Optimal parameter tuning of feedback controllers with application to biomolecular antithetic integral control. In *2019 IEEE 58th Conference on Decision and Control (CDC).*

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