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## Supplemental Data

# The Incoherent Feedforward Loop Can Provide Fold-Change Detection in Gene Regulation

**Lea Goentoro, Oren Shoval, Marc Kirschner, and Uri Alon**

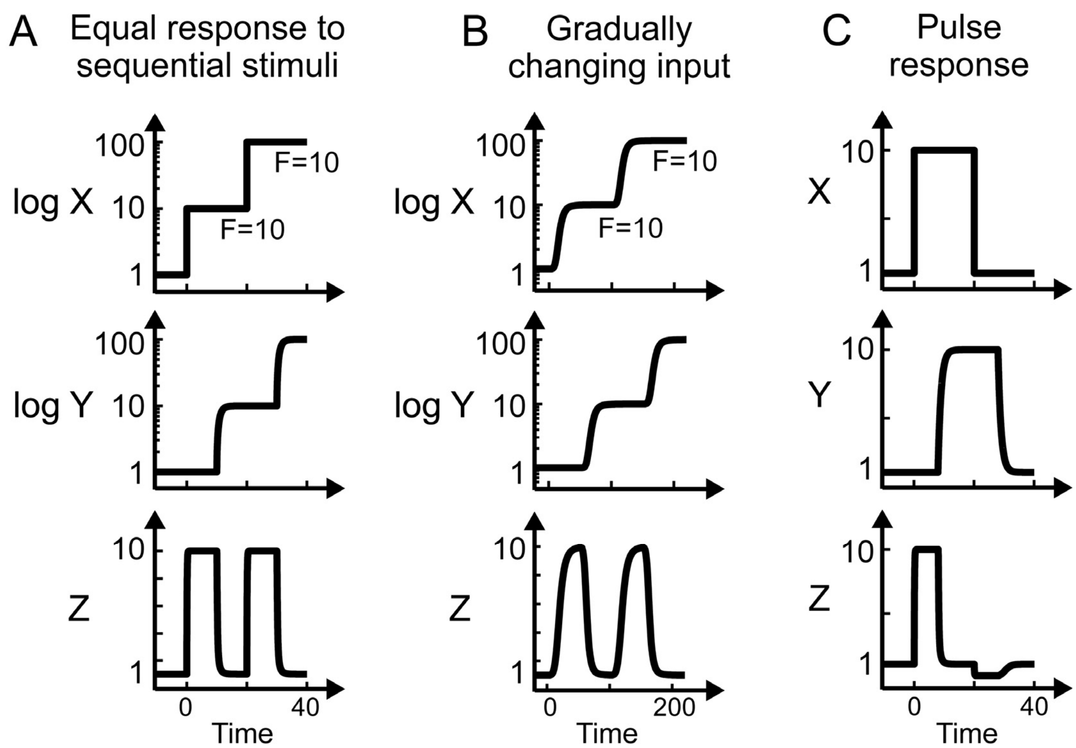


**Figure S1. I1-FFL Shows Fold-Change Detection to Sequential Steps, Gradually Changing Input, and Pulses**

**(A)** The I1-FFL generates identical responses to sequential signal steps with the same fold-change. **(B)** The fold-change detection feature is maintained for any time varying form of input X(t). **(C)** The I1-FFL response to a pulse shows an undershoot of Z dynamics. Pulse signals are often found in biological systems. A pulse-like signal also mimics a situation when after a period of signaling, signal degrades away or is actively removed to prepare cells for the next round of signaling. Removal of signal resets the memory Y (Y returns to the basal level).

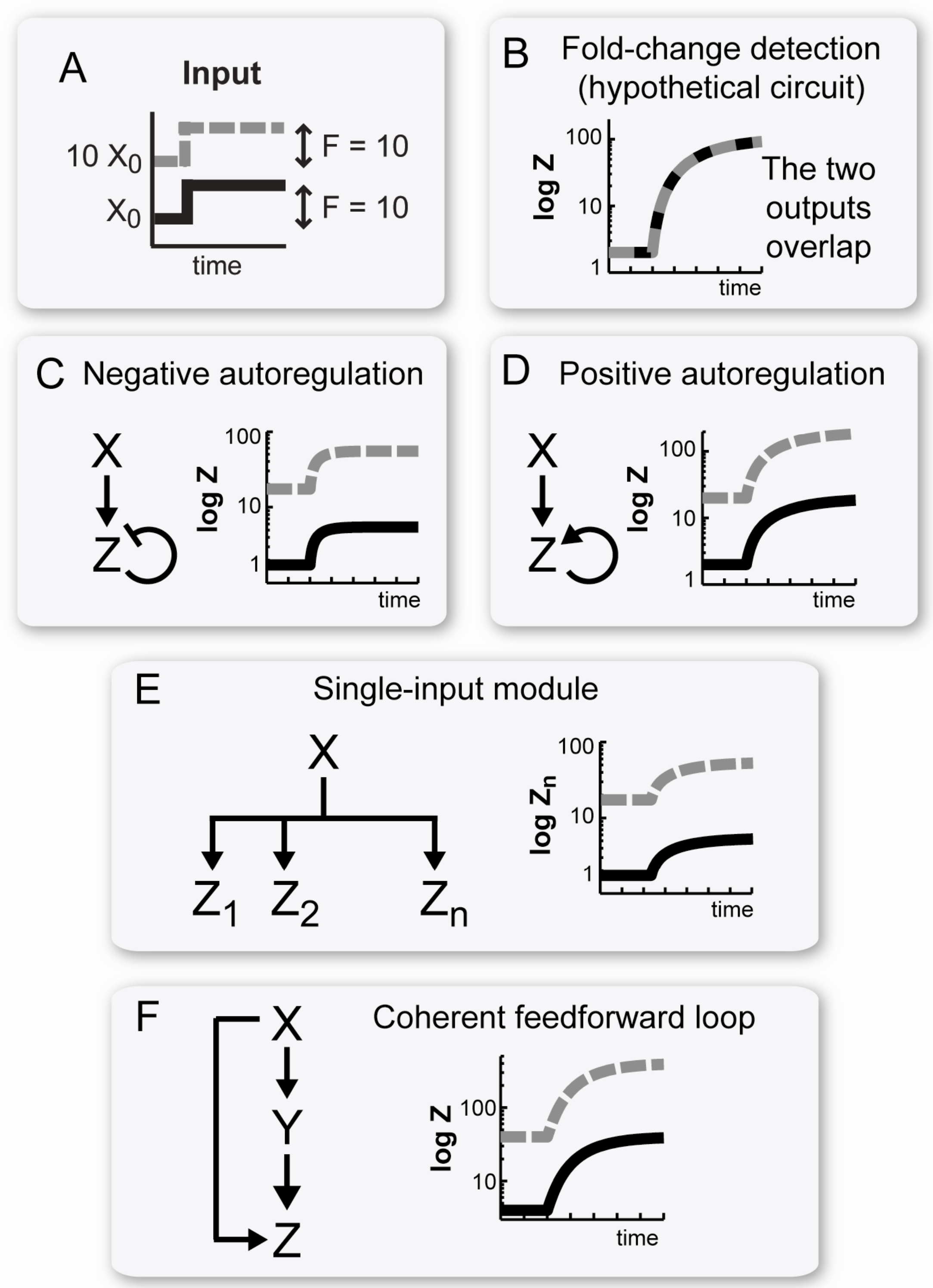


**Figure S2. Other Common Transcriptional Network Motifs Show Response that Depends on the Absolute Levels of the Input**

For illustration purposes, we stimulated network motifs with two step inputs of different absolute levels, but identical fold-changes **(A)**. If a circuit shows a response that depends on the fold-changes in the input, and not the absolute levels of the input, then the outputs to the two step stimuli would perfectly overlap **(B)**. All motifs, except for the incoherent FFLs, do not show fold-change detection: the dynamics and level of Z depends on the absolute level of the activator X.

Intuitively, fold-change detection requires a memory of the basal level of the activator X. Recurrent network motifs such as positive autoregulation, negative autoregulation, and single-input module do not have a distinct component that can act as a memory of X. Consequently, they cannot provide fold-change detection **(C-E)**.

Another motif, the coherent feedforward loop **(F)**, resembles the incoherent feedforward loop, with one difference: the effect of Y on the output Z is of the same sign as the effect of X on Z (*i.e.*, Y activates Z instead of repressing it). This difference abolishes the fold-change detection behavior. Even though Y mimics the level of the activator X (hence acting as a memory of X), it no longer facilitates a temporal comparison between the basal level of X and the new level of X (because Y amplifies, and not opposes, the effects of X).

Mathematically, fold-change detection translates into a structural requirement of how Y appears in the differential equation describing the dynamics of Z.

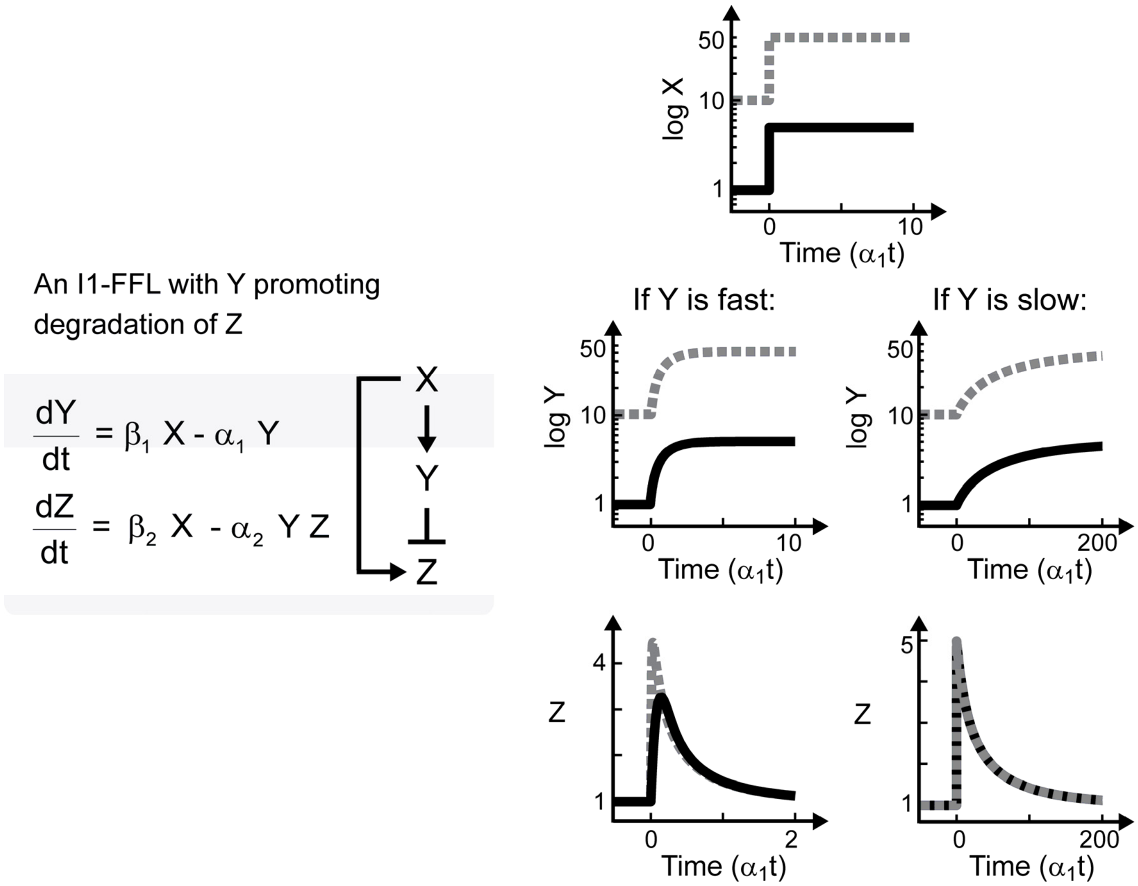


**Figure S3. I1-FFL Connected in a Different Way Can Provide Fold-Change Detection**

An I1-FFL where Y acts by degrading Z, rather than repressing the production of Z, called a “shifter” (Levchenko and Iglesias, 2002; Ma et al., 2009; Tyson et al., 2003) can provide fold-change detection in the limit where the dynamics of Y is very slow or delayed relative to the dynamics of Z. Under this condition, a quasi steady-state prevails: Z is at quasi steady-state, relative to the slow dynamics of Y,

$Z_{st} = \dfrac{\beta_2 X}{\alpha_2 Y(t,X)}$. Since Y also depends on X, this cancels out the X-dependence of Z. In this limit, at all times, Z responds only to fold-changes in the activator X.

# Analytical solution for the I1-FFL

As derived in Figure 3 in the main text, the dynamic equations for Y and Z are:

$$\frac{dY}{dt} = \beta_1 X - \alpha_1 Y \tag{1}$$

$$\frac{dZ}{dt} = \frac{\beta_2 X}{Y} - \alpha_2 Z \tag{2}$$

Let us define the following dimensionless variables,

$$y = \frac{Y}{\beta_1 X_0 / \alpha_1} \tag{3}$$

$$z = \frac{Z}{\beta_2 \alpha_1 / \beta_1 \alpha_2} \tag{4}$$

$$\tau = \frac{t}{1/\alpha_1} \tag{5}$$

$$f = \frac{X}{X_0} \tag{6}$$

where $X_0$ is the basal level of X, as depicted in Figure S4. Introducing the dimensionless variables into equations 1-2, we obtain the following dimensionless dynamic equations describing the I1-FFL,

$$\frac{dy}{d\tau} = f - y \tag{7}$$

$$r\frac{dz}{d\tau} = \frac{f}{y} - z \tag{8}$$

where the dimensionless group r is the ratio of Z and Y lifetimes,

$$r = \frac{\alpha_1}{\alpha_2} \tag{9}$$

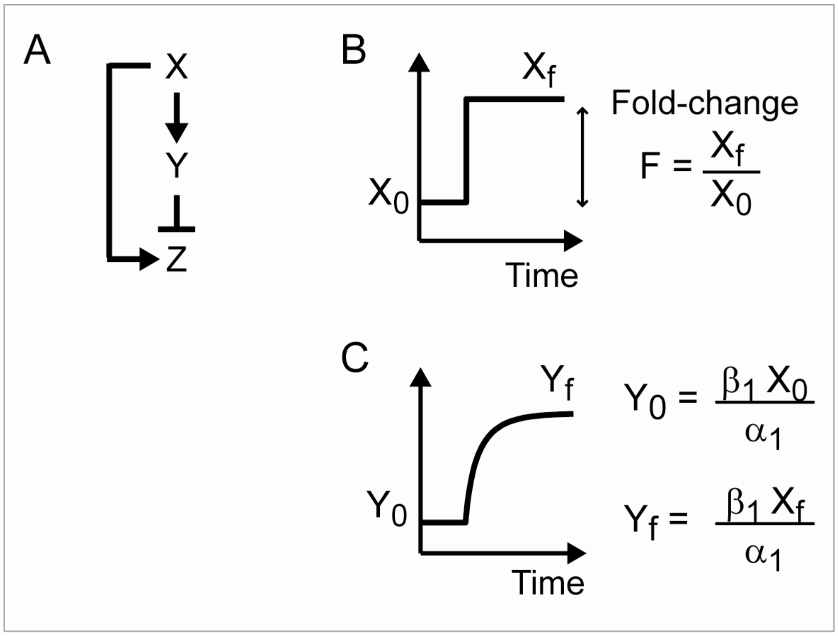


**Figure S4. (A)** The type-1 incoherent feedforward loop (I1-FFL). **(B)** The input to the circuit is the activator X. The **fold-change** in X is defined as the ratio of the new level of X to the previous level of X (**$F = X_f / X_0$**). **(C)** Y increases to a new steady-state level in response to a step change in the level of X.

Integrating equation 7 with the initial condition y(0)=1,

$$y = 1 + (f-1)\left(1 - e^{-\tau}\right) \tag{10}$$

Equation 10 describes how Y changes in time in response to a step increase in the activator X (Figure S4).

Substituting equation 10 into equation 8, we obtain a differential equation describing the dynamics of Z,

$$r\frac{dz}{d\tau} = \frac{f}{1 + (f-1)\left(1 - e^{-\tau}\right)} - z \tag{11}$$

Equation 11 can be solved analytically when r=1 ($\alpha_1=\alpha_2$),

$$\frac{dz}{d\tau}=\frac{f}{1+(f-1)\left(1-e^{-\tau}\right)}-z \tag{12}$$

Integrating equation 12 with the initial condition z(0)=1,

$$z=\left[e^{\tau}+\ln\left(f\,e^{\tau}-f+1\right)-\frac{1}{f}\ln\left(f\,e^{\tau}-f+1\right)\right]e^{-\tau} \tag{13}$$

Equation 13 shows that the dynamics of Z, both during the transient time and steady state, responds only to the fold-changes in X (f), and not on the absolute level of X. The profile of Z is shown in Figure S5.

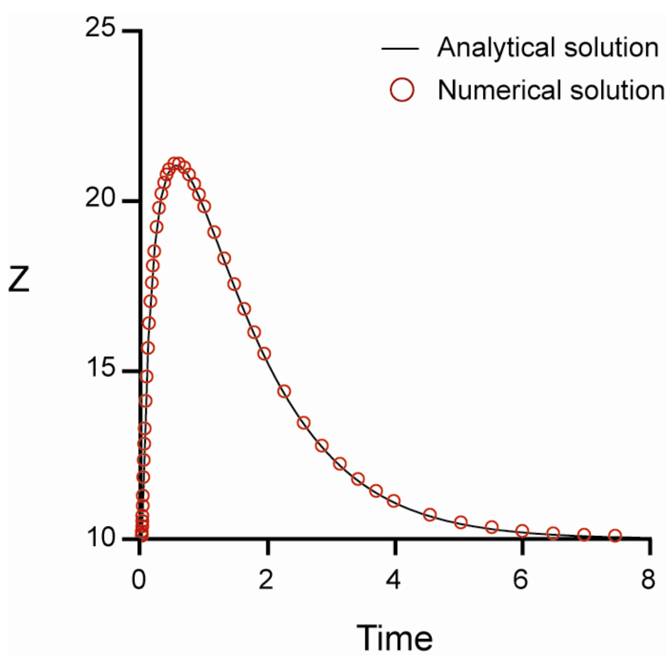


**Figure S5.** The dynamic of Z in response to a step increase in the activator X. The solid line depicts the analytical solution (equation 13); the circles depict the numerical solution (equation 2). The parameters used in this plot were: $\beta_1=1$, $\beta 2=10$, $\alpha_1=\alpha_2=1$,

## References

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