Supplementary Information

Active degradation of a regulator controls coordination of downstream genes
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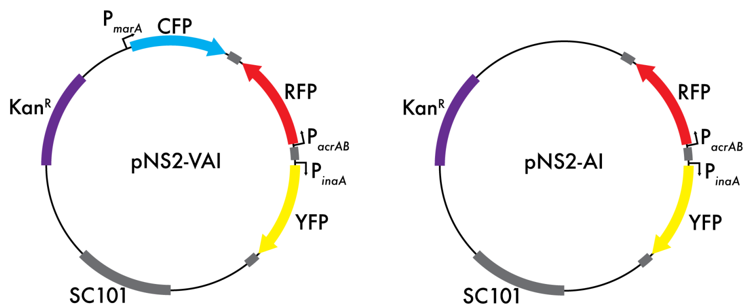
1 Plasmids

1.1 Reporters



Plasmid maps of pNS2-VAI and pNS2-AI. These are the reporter plasmids for the MarA fusion and CRISPRi systems. pNS2-VAI includes three fluorescent protein genes (cerulean CFP, mCherry RFP, and venus YFP) under the control of the promoters as indicated, a low-copy SC101 origin of replication, and a kanamycin resistance marker. pNS2-AI is identical in terms of features with the absence of *cfp* and the corresponding promoter.

Construct	Primers
P_{acrAB}	F: GCCAGTAGATTGCACCGCG R: TCGTGCTATGGTACATACATTCACA
P_{inaA}	F: CAATGCTTTTCAGCGTTAACTCTG R: ACGACAATGACTATAGGTGGT
P_{marA}	F: GGTTGTTATCCTGTGTATCTGG R: ATTAGTTGCCCTGGCAAG

Table 1: Primers for construction of transcriptional reporter plasmids

1.2 CRISPRi knock down

To knock down endogenous Lon protease we used the plasmids and methods from [1]. To target the *lon* gene, we designed the sequence TAAACCACCACATTGCGCAG which binds ~ 100

base-pairs after the transcriptional start site of the *lon* gene. This sequence has a single mutation (underlined letter) at the 14th base-pair. This decreases its repression and causes leaky expression of *lon* to avoid producing off-target phenotypic changes such as increased filamentation (Fig. S1).

1.3 *lon* knockout

To knock out *lon* for Fig. S1 we used the primers from [2]: TCGTGTCATCTGATTACCTGGCG-GAAATTAAACTAAGAGAGAGCTCTATGATTCCGGGGATCCGTCGACC and TTTTTATTA GTGCATTTTGCGCGAGGTCACTATTTTGCAGTCACAACCTGTGTAGGCTGGAGCTGC TTCG along with the pKD13 plasmid [3].

2 Analytic solutions for variance and mutual information over time

Following the methods outlined in [4] we derived the exact analytical solutions for the models described in the text. This allowed us to describe how variance evolves as a function of time for the input X and its two downstream targets Y and Z.

$$Var\{x(t)\} = \frac{1}{2}\tau_x\left(1 - e^{-\frac{2t}{\tau_x}}\right) \tag{1}$$

$$\begin{aligned} Var\{y(t)\} = \frac{\tau_y e^{-2t\left(\frac{1}{\tau_y}+\frac{1}{\tau_x}\right)}}{2\left(\tau_x-\tau_y\right)^2\left(\tau_x+\tau_y\right)} & \left(\frac{g_y^2\tau_x^4}{\tau_y}e^{\frac{2t}{\tau_y}}\left(e^{\frac{2t}{\tau_x}}-1\right)\right. \\ & + \tau_x^3\left(e^{\frac{2t}{\tau_x}}\left(e^{\frac{2t}{\tau_y}}-1\right) - g_y^2\left(e^{\frac{2t}{\tau_x}} - 4e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_x}\right)} + 2e^{2t\left(\frac{1}{\tau_y}+\frac{1}{\tau_x}\right)} + e^{\frac{2t}{\tau_y}}\right)\right) \\ & \left. + \tau_y\tau_x^2\left(g_y^2-1\right)e^{\frac{2t}{\tau_x}}\left(e^{\frac{2t}{\tau_y}}-1\right) - \tau_y^2\tau_x e^{\frac{2t}{\tau_x}}\left(e^{\frac{2t}{\tau_y}}-1\right) + \tau_y^3 e^{\frac{2t}{\tau_x}}\left(e^{\frac{2t}{\tau_y}}-1\right)\right) \end{aligned} \tag{2}$$

$$\begin{aligned} Var\{z(t)\} = \frac{\tau_z e^{-2t\left(\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}}{2\left(\tau_x-\tau_z\right)^2\left(\tau_x+\tau_z\right)} & \left(\frac{g_z^2\tau_x^4}{\tau_z}e^{\frac{2t}{\tau_z}}\left(e^{\frac{2t}{\tau_x}}-1\right)\right. \\ & + \tau_x^3\left(e^{\frac{2t}{\tau_x}}\left(e^{\frac{2t}{\tau_z}}-1\right) - g_z^2\left(e^{\frac{2t}{\tau_x}} - 4e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)} + 2e^{2t\left(\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)} + e^{\frac{2t}{\tau_z}}\right)\right) \\ & \left. + \tau_z\tau_x^2\left(g_z^2-1\right)e^{\frac{2t}{\tau_x}}\left(e^{\frac{2t}{\tau_z}}-1\right) - \tau_z^2\tau_x e^{\frac{2t}{\tau_x}}\left(e^{\frac{2t}{\tau_z}}-1\right) + \tau_z^3 e^{\frac{2t}{\tau_x}}\left(e^{\frac{2t}{\tau_z}}-1\right)\right) \end{aligned} \tag{3}$$

Next, in order to quantify how coordinated diversity of two downstream genes evolves over time we compute the mutual information between the two genes as a function of time. To compute this, we first calculate the covariance over time.

$$Cov\{y(t), z(t)\} = \frac{\tau_x^2 \tau_y \tau_z}{2\left(\tau_x^2 - \tau_y^2\right)(\tau_y + \tau_z)\left(\tau_x^2 - \tau_z^2\right)\left(\tau_x(\tau_y + \tau_z) - 2\tau_y\tau_z\right)} \left(\frac{g_y^2}{\tau_y}(\tau_x + \tau_z)\, e^{-t\left(\frac{2}{\tau_y}+\frac{1}{\tau_z}+\frac{3}{\tau_x}\right)} \left((\tau_y+\tau_z)\,\tau_x^3 e^{t\left(\frac{2}{\tau_y}+\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\left(e^{\frac{2t}{\tau_x}}-1\right) + \tau_x^2 e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_x}\right)}\left(2\tau_y^2 e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)} - \tau_y^2 e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_y}\right)} + 4\tau_z\tau_y e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)} - 2\tau_z^2 e^{\frac{2t}{\tau_x}} + 2\tau_z^2 e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)} + \tau_z^2 e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_y}\right)} - \left(\tau_y^2 + 4\tau_z\tau_y + \tau_z^2\right) e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{2}{\tau_x}\right)}\right) + \tau_y\tau_z(\tau_y+\tau_z)\,\tau_x e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\left(-4e^{\frac{t}{\tau_x}} + 3e^{t\left(\frac{1}{\tau_y}+\frac{2}{\tau_x}\right)} + e^{\frac{t}{\tau_y}}\right) - 2\tau_y^2\tau_z^2 e^{t\left(\frac{1}{\tau_y}+\frac{3}{\tau_x}\right)}\left(e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_y}\right)} - 1\right)\right) + \frac{g_y^2}{\tau_y^2}(\tau_x + \tau_y)\,\tau_z e^{-t\left(\frac{1}{\tau_y}+\frac{2}{\tau_z}+\frac{3}{\tau_x}\right)}\left(e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_y}\right)}\left(2\tau_x(\tau_y+\tau_z)\left(\tau_x(\tau_y+\tau_z)-2\tau_y\tau_z\right)e^{\frac{2t}{\tau_x}} + 2(\tau_x-\tau_y)\, e^{t\left(\frac{1}{\tau_z}+\frac{2}{\tau_x}\right)}\left(\left((\tau_y+\tau_z)\,\tau_x^2 - \tau_y\tau_z\tau_x + \tau_y\tau_z^2\right)\sinh\left(\frac{t}{\tau_x}\right) + \tau_z\left(\tau_y\tau_z - \tau_x(2\tau_y+\tau_z)\right)\cosh\left(\frac{t}{\tau_x}\right)\right)\right) - 2\tau_y^2\left(\tau_x^2 - \tau_z^2\right)e^{t\left(\frac{1}{\tau_z}+\frac{3}{\tau_x}\right)}\right)\right) \tag{4}$$

Next, the correlation of Y and Z is calculated by combining equations 2, 3, and 4 as :

$$Corr\{y(t), z(t)\} = \frac{Cov\{y(t), z(t)\}^2}{Var\{y(t)\}Var\{z(t)\}} \tag{5}$$

This is then converted to mutual information by the following equivalence

$$I\{y(t), z(t)\} = -\frac{1}{2} ln(1 - Corr\{y(t), z(t)\}^2) \tag{6}$$

Note this analytical conversion assumes Gaussian statistics for the underlying stochastic differential equations.

3 Analytic solutions for variance and mutual information over time with scaled variance

The solutions to the equations as illustrated in Figure 3 in the main text are similar to that in Figure 2 except the variance is constrained as a function of correlation time τ.

$$Var\{x(t)\} = 1 - e^{-\frac{2t}{\tau_x}} \tag{7}$$

$$Var\{y(t)\} = \frac{e^{-t\left(\frac{1}{\tau_x}+\frac{1}{\tau_y}\right)}}{(\tau_x-\tau_y)^2(\tau_x+\tau_y)}\left(-g_y^2\tau_x^2(\tau_x+\tau_y)e^{t\left(\frac{1}{\tau_y}-\frac{1}{\tau_x}\right)} + (\tau_x-\tau_y)^2\left(g_y^2\tau_x+\tau_x+\tau_y\right)e^{t\left(\frac{1}{\tau_x}+\frac{1}{\tau_y}\right)} - (\tau_x+\tau_y)\left(\tau_x\tau_y\left(g_y^2-2\right)+\tau_x^2+\tau_y^2\right)e^{t\left(\frac{1}{\tau_x}-\frac{1}{\tau_y}\right)} + 4g_y^2\tau_x^2\tau_y\right) \tag{8}$$

$$Var\{z(t)\} = \frac{e^{-t\left(\frac{1}{\tau_x}+\frac{1}{\tau_z}\right)}}{(\tau_x-\tau_z)^2(\tau_x+\tau_z)}\left(-g_z^2\tau_x^2(\tau_x+\tau_z)e^{t\left(\frac{1}{\tau_z}-\frac{1}{\tau_x}\right)} + (\tau_x-\tau_z)^2\left(g_z^2\tau_x+\tau_x+\tau_z\right)e^{t\left(\frac{1}{\tau_x}+\frac{1}{\tau_z}\right)} - (\tau_x+\tau_z)\left(\tau_x\tau_z\left(g_z^2-2\right)+\tau_x^2+\tau_z^2\right)e^{t\left(\frac{1}{\tau_x}-\frac{1}{\tau_z}\right)} + 4g_z^2\tau_x^2\tau_z\right) \tag{9}$$

$$\begin{aligned} Cov\{y(t),z(t)\} = {} & \frac{e^{-t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{3}{\tau_x}\right)}}{\left(\tau_x^2-\tau_y^2\right)(\tau_y+\tau_z)\left(\tau_x^2-\tau_z^2\right)(\tau_x(\tau_y+\tau_z)-2\tau_y\tau_z)}\tau_x\tau_y\tau_z\Bigg(\tau_y(\tau_x \\ & +\tau_z)\Bigg(e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\left(-1+e^{\frac{2t}{\tau_x}}\right)(\tau_y+\tau_z)\tau_x^3 \\ & +\Bigg(2e^{t\left(\frac{1}{\tau_z}+\frac{2}{\tau_x}\right)}\tau_y^2-e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\tau_y^2+4e^{t\left(\frac{1}{\tau_z}+\frac{2}{\tau_x}\right)}\tau_z\tau_y-2e^{\frac{3t}{\tau_x}}\tau_z^2+2e^{t\left(\frac{1}{\tau_z}+\frac{2}{\tau_x}\right)}\tau_z^2+e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\tau_z^2- \\ & e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{3}{\tau_x}\right)}\left(\tau_y^2+4\tau_z\tau_y+\tau_z^2\right)\Bigg)\tau_x^2 \\ & +e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\left(-4e^{\frac{t}{\tau_x}}+3e^{t\left(\frac{1}{\tau_y}+\frac{2}{\tau_x}\right)}+e^{\frac{t}{\tau_y}}\right)\tau_y\tau_z(\tau_y+\tau_z)\tau_x \\ & -2e^{\frac{3t}{\tau_x}}\left(-1+e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_y}\right)}\right)\tau_y^2\tau_z^2\Bigg)\frac{g_y^2}{\tau_y^2} \\ & +\frac{g_z^2}{\tau_z^2}(\tau_x+\tau_y)\tau_z\Bigg(e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\left(-1+e^{\frac{2t}{\tau_x}}\right)(\tau_y+\tau_z)\tau_x^3 \\ & +\Bigg(-2e^{\frac{3t}{\tau_x}}\tau_y^2+2e^{t\left(\frac{1}{\tau_y}+\frac{2}{\tau_x}\right)}\tau_y^2+e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\tau_y^2+4e^{t\left(\frac{1}{\tau_y}+\frac{2}{\tau_x}\right)}\tau_z\tau_y+2e^{t\left(\frac{1}{\tau_y}+\frac{2}{\tau_x}\right)}\tau_z^2-e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{1}{\tau_x}\right)}\tau_z^2- \\ & e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_z}+\frac{3}{\tau_x}\right)}\left(\tau_y^2+4\tau_z\tau_y+\tau_z^2\right)\Bigg)\tau_x^2+e^{t\left(\frac{1}{\tau_y}+\frac{1}{\tau_x}\right)}\left(-4e^{\frac{t}{\tau_x}}+3e^{t\left(\frac{1}{\tau_z}+\frac{2}{\tau_x}\right)}+e^{\frac{t}{\tau_z}}\right)\tau_y\tau_z(\tau_y+\tau_z)\tau_x \\ & -2e^{\frac{3t}{\tau_x}}\left(-1+e^{t\left(\frac{1}{\tau_z}+\frac{1}{\tau_y}\right)}\right)\tau_y^2\tau_z^2\Bigg)\Bigg) \end{aligned} \tag{10}$$

4 Modified model incorporating growth rate

To consider the effects of growth rate on the system we modified Eqn. 7 to include terms modeling the exponential growth of a bacterial microcolony.

$$Var\{x(t)\} = \left(1 - e^{-\frac{2t}{\tau_x}}\right)\left(1 - \frac{1}{N_{cells}e^{\frac{ln(2)}{t_{div}}t}}\right) \tag{11}$$

where N_{cells} is the starting number of cells, t_{div} is the division time (or length of cell cycle) in minutes. τ_x is the correlation time of the activator, while t is measured in minutes.

5 Parameters

Parameter	**Value**	**Definition**
λ_x	range from 1 to 100	half-life of x
τ_x	$\frac{\lambda_x}{log(2)}$	correlation-time of x
τ_y	$\frac{30.0}{log(2)}$	correlation-time of y
τ_z	$\frac{30.0}{log(2)}$	correlation-time of z
g_y	$0.1\tau_y$	dose response gain of x on y
g_z	$0.1\tau_z$	dose response gain of x on z

Table 2: Parameters used for stochastic simulations and analytical solutions

References

[1] Lei S Qi, Matthew H Larson, Luke A Gilbert, Jennifer A Doudna, Jonathan S Weissman, Adam P Arkin, and Wendell A Lim. Repurposing crispr as an rna-guided platform for sequence-specific control of gene expression. *Cell*, 152(5):1173–1183, 2013.

[2] Tomoya Baba, Takeshi Ara, Miki Hasegawa, Yuki Takai, Yoshiko Okumura, Miki Baba, Kirill A Datsenko, Masaru Tomita, Barry L Wanner, and Hirotada Mori. Construction of escherichia coli k-12 in-frame, single-gene knockout mutants: the keio collection. *Molecular systems biology*, 2(1), 2006.

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