

Supplementary Material for

Coherent activation of a

synthetic mammalian gene network

Diane M. Longo¹, Alexander Hoffmann², Lev S. Tsimring³, Jeff Hasty^{1,3,*}

¹Department of Bioengineering, University of California,
San Diego, La Jolla, CA 92093, USA

²Department of Chemistry and Biochemistry, University of California,
San Diego, La Jolla, CA 92093, USA

³Institute for Nonlinear Science, University of California,
San Diego, La Jolla, CA 92093, USA

*To whom correspondence should be addressed.

Tel: (858)822-3442, Fax: (309)273-0130, E-mail: hasty@ucsd.edu.

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Table S1: Model parameter values

Parameter	Parameter Description	Value
η_A (Raj <i>et al</i> , 2006)	rtTA mRNA degradation rate	.18/hr
η_G (Raj <i>et al</i> , 2006)	GFP mRNA degradation rate	.18/hr
δ_A	rtTA protein degradation rate	.03/hr
δ_G (Corish and Tyler-Smith, 1999)	GFP protein degradation rate	.03/hr
α_A	rtTA basal transcription rate	.1/hr
α_G	GFP basal transcription rate	.1/hr
β_A	rtTA translation rate	3/hr
β_G	GFP translation rate	3/hr
n (Kramer and Fussenegger, 2005)	Hill coefficient	2
f	fold-induction of transcription	30
K_s	Michaelis constant	.08
S	doxycycline concentration	0 to 1 $\mu\text{g/ml}$

Deterministic model of the synthetic positive feedback network

The deterministic model of the synthetic positive feedback network consists of the following four ordinary differential equations describing the levels of rtTA mRNA (m_A), rtTA protein (A), GFP mRNA (m_G), and GFP protein (G):

$$\dot{m}_A = \frac{\alpha_A(1 + f(AK_a)^n)}{1 + (AK_a)^n} - \eta_A m_A \quad (\text{S1})$$

$$\dot{A} = \beta_A m_A - \delta_A A \quad (\text{S2})$$

$$\dot{m}_G = \frac{\alpha_G(1 + f(AK_a)^n)}{1 + (AK_a)^n} - \eta_G m_G \quad (\text{S3})$$

$$\dot{G} = \beta_G m_G - \delta_G G \quad (\text{S4})$$

where α_A and α_G represents the basal synthesis rates of A and G respectively, β_A and β_G represent the protein production rates of A and G respectively, f represents the fold-difference between basal and fully activated synthesis, n represents the Hill coefficient for activator dimers binding to tet operator sites, η_A and η_G represent the mRNA degradation rates of A and G respectively, δ_A and δ_G represent the protein degradation rates of A and G respectively, and K_a represents the association constant of the activator which is determined by the doxycycline concentration (S) via $K_a = S/(K_s + S)$. Note that the region of bistability determined using the deterministic model is in good agreement with the region of bistability determined using the stochastic model (Fig. S1).

For the no-feedback simulation results shown in Fig. S2, the same four ODEs are used but the term for autoregulated rtTA mRNA transcription in Eq. S1 is replaced with the term (α_A) for constitutive rtTA mRNA transcription:

$$\dot{m}_A = \alpha_A - \eta_A m_A \quad (\text{S5})$$

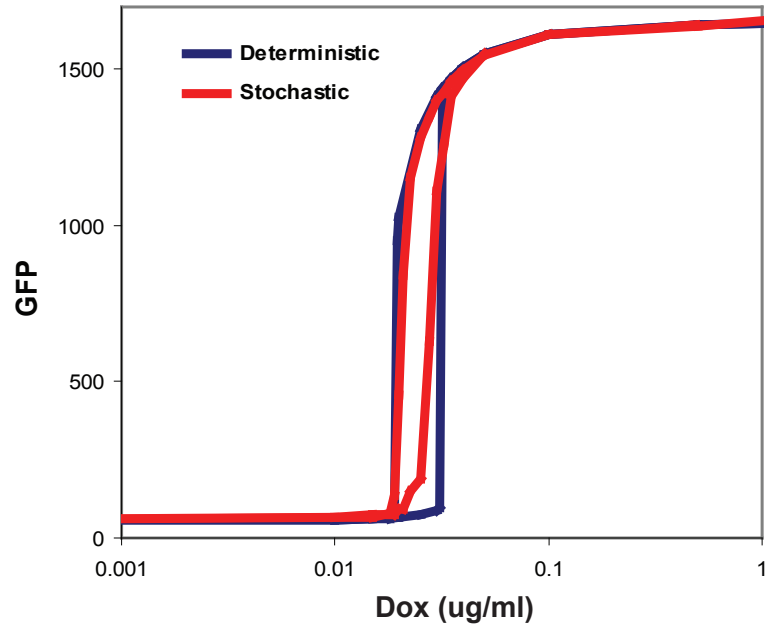


Fig. S1: Steady-state GFP expression levels versus dox concentration determined using the deterministic model (blue) and using the stochastic model (red). Steady-state GFP expression levels for the stochastic model were determined from 50 simulations for each dox concentration

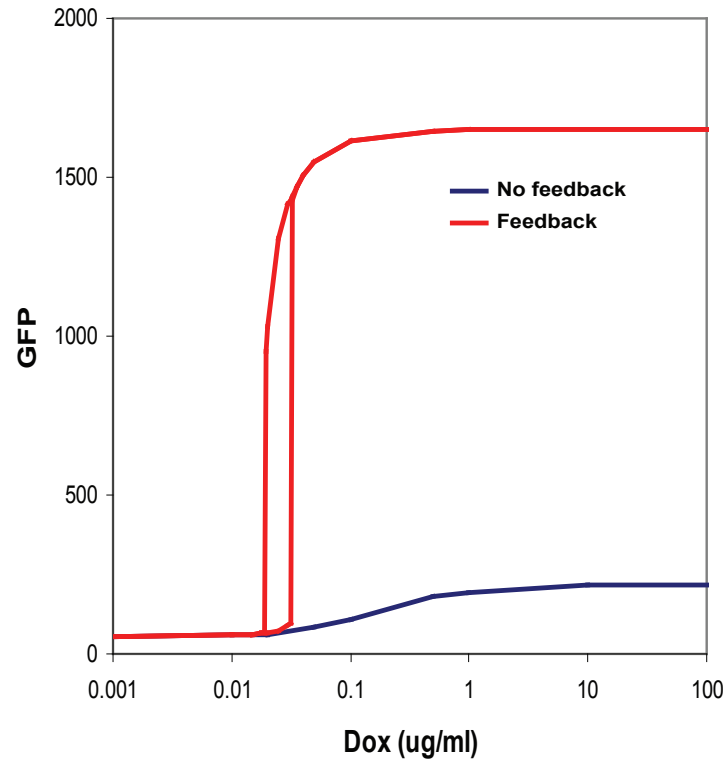


Fig. S2: Steady-state GFP expression levels versus dox concentration for the positive feedback system (red) and for a no-feedback system with constitutive rtTA expression (blue)

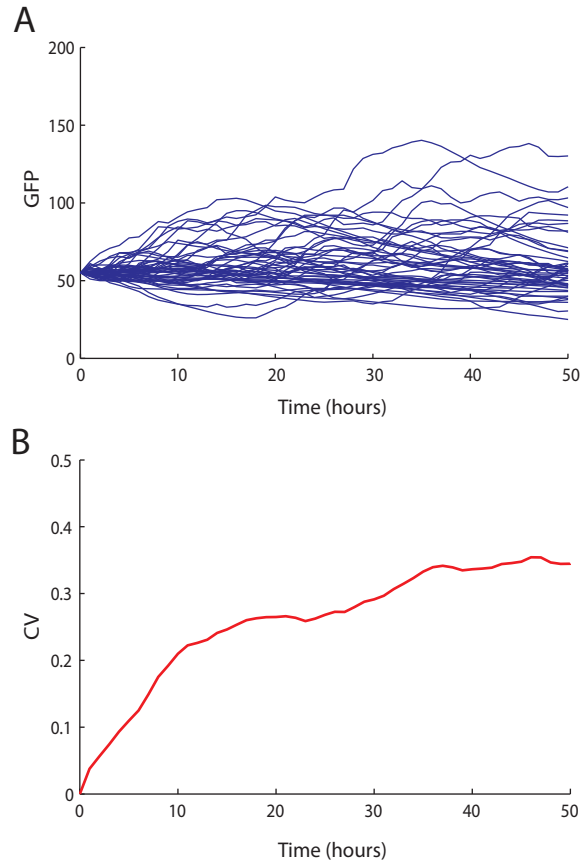


Fig. S3: Model equilibration period. mRNA and protein levels were initialized to their basal steady state values at $t=0$ and the simulations were run for 50 hours with no dox ($S = 0$). **(A)** GFP levels for 50 individual runs of the stochastic simulation during the model equilibration period. **(B)** The coefficient of variation (CV) for 50 simulations

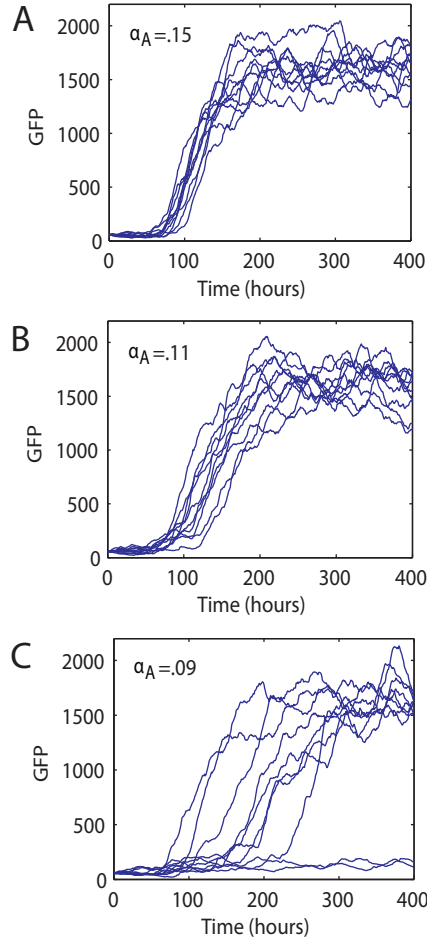


Fig. S4: Dynamic behavior of the synthetic network with different basal rtTA transcription rates. The plot shows ten typical runs of the stochastic simulations with S set to $.1 \mu\text{g/ml}$ dox at $t = 50$ hrs for $\alpha_A = .15$ (A), $\alpha_A = .11$ (B), and $\alpha_A = .09$ (C)

Supplementary References

Corish P, Tyler-Smith C (1999) Attenuation of green fluorescent protein half-life in mammalian cells. *Protein Eng* 12:1035-1040. doi:10.1093/protein/12.12.1035

Kramer BP, Fussenegger M (2005) Hysteresis in a synthetic mammalian gene network. *Proc Natl Acad Sci USA* 102:9517-9522. doi:10.1073/pnas.0500345102

Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S (2006) Stochastic mRNA synthesis in mammalian cells. *PLoS Biol* 4:e309. doi:10.1371/journal.pbio.0040309