Supporting Online Material for

Robust, Tunable Biological Oscillations from Interlinked Positive and Negative Feedback Loops

Tony Yu-Chen Tsai, Yoon Sup Choi, Wenzhe Ma, Joseph R. Pomerening, Chao Tang, James E. Ferrell Jr.*

*To whom correspondence should be addressed. E-mail: james.ferrell@stanford.edu

Published 4 July 2008, *Science* **321**, 126 (2008)
DOI: 10.1126/science.1156951

This PDF file includes:

Supporting Online Material

Robust, tunable biological oscillations from interlinked positive and negative feedback loops

Tony Yu-Chen Tsai, Yoon Sup Choi, Wenzhe Ma, Joseph R. Pomerening, Chao Tang, and James E. Ferrell, Jr.

Computational modeling

For each ordinary differential equation (ODE) model analyzed, we solved the differential equations numerically using a Runge-Kutta method. To construct amplitude vs. frequency curves, we first chose a bifurcation parameter and then identified the range of the parameter over which the system exhibited limit cycle oscillations by locating the Hopf bifurcations at which oscillations were born and extinguished. We then used an iterative algorithm to march along the chosen parameter between the two bifurcations. For each set of parameters we calculated the limit cycle solution of the system, keeping track of the amplitude and frequency of the limit cycle solution. We made use of Mathematica (Wolfram Research, Inc.) and MatCONT (*S1*), a public toolbox for bifurcation analysis in Matlab (The MathWorks Inc.), for these computations.

Below we describe the ODE models, parameter sets, and bifurcation parameters we examined.

1. Negative feedback and positive-plus-negative feedback cell cycle oscillators. This is a nine ODE model of the embryonic mitotic oscillator in *Xenopus laevi*s (*S2*). Depending upon parameter choice, the model can function as a negative feedback circuit or as a positive-plus-negative feedback circuit. Parameters were chosen so that at a realistic feedback strength of r=10, the model would reproduce the experimental curves for the steady-state activity of CDK1 as a function of constant cyclin concentration, and for the time course of CDK1 activation when driven by a constant rate of cyclin synthesis (*S2, S3*).

To make it easier to distinguish between complexes (e.g. CDK1-cyclin) and products of individual species (e.g. CDK1 × cyclin), for this model we have written all time-dependent species as explicit functions of time. Thus, *cdk1cyclin*[*t*] represents the concentration of the CDK1-cyclin complex, whereas *cdk1*[*t*]*cyclin*[*t*] represents the product of the free CDK1 and cyclin concentrations.

ODEs:

$$\frac{dcyclin[t]}{dt} = k_{synth} - k_{dest} apc_{act}[t] cyclin[t] -$$
$$k_a\left(cdk1_{tot} - cdk1cyclin[t] - cdk1cyclinyp[t] - cdk1cyclinyptp[t] - cdk1cyclintp[t]\right)cyclin[t] +$$
$$k_d cdk1cyclin[t]$$

$$\frac{dcdk1cyclin[t]}{dt} =$$
$$k_a\left(cdk1_{tot} - cdk1cyclin[t] - cdk1cyclinyp[t] - cdk1cyclinyptp[t] - cdk1cyclintp[t]\right)cyclin[t] -$$
$$k_d cdk1cyclin[t] - k_{dest} apc_{act}[t] cdk1cyclin[t] - k_{wee1} wee1_{act}[t] cdk1cyclin[t] -$$
$$k_{wee1basal}\left(wee1_{tot} - wee1_{act}[t]\right)cdk1cyclin[t] + k_{cdc25} cdc25_{act}[t] cdk1cyclinyp[t] +$$
$$k_{cdc25basal}\left(cdc25_{tot} - cdc25_{act}[t]\right)cdk1cyclinyp[t]$$

$$\frac{dcdk1cyclinyp[t]}{dt} = k_{wee1} wee1_{act}[t] cdk1cyclin[t] + k_{wee1basal}\left(wee1_{tot} - wee1_{act}[t]\right)cdk1cyclin[t] -$$
$$k_{cdc25} cdc25_{act}[t] cdk1cyclinyp[t] - k_{cdc25basal}\left(cdc25_{tot} - cdc25_{act}[t]\right)cdk1cyclinyp[t] -$$
$$k_{cak} cdk1cyclinyp[t] + k_{pp2c} cdk1cyclinyptp[t] - k_{dest} apc_{act}[t] cdk1cyclinyp[t]$$

$$\frac{dcdk1cyclinyptp[t]}{dt} = k_{cak} cdk1cyclinyp[t] - k_{pp2c} cdk1cyclinyptp[t] -$$
$$k_{cdc25} cdc25_{act}[t] cdk1cyclinyptp[t] - k_{cdc25basal}\left(cdc25_{tot} - cdc25_{act}[t]\right)cdk1cyclinyptp[t] +$$
$$k_{wee1} wee1_{act}[t] cdk1cyclintp[t] + k_{wee1basal}\left(wee1_{tot} - wee1_{act}[t]\right)cdk1cyclintp[t] -$$
$$k_{dest} apc_{act}[t] cdk1cyclinyptp[t]$$

$$\frac{dcdk1cylintp[t]}{dt} = k_{cdc25} cdc25_{act}[t] cdk1cyclinyptp[t] +$$
$$k_{cdc25basal}\left(cdc25_{tot} - cdc25_{act}[t]\right)cdk1cyclinyptp[t] - k_{wee1} wee1_{act}[t] cdk1cyclintp[t] -$$
$$k_{wee1basal}\left(wee1_{tot} - wee1_{act}[t]\right)cdk1cyclintp[t] - k_{dest} apc_{act}[t] cdk1cyclintp[t]$$

$$\frac{dcdc25_{act}[t]}{dt} = k_{cdc25on}\left(\frac{cdk1cyclintp[t]^{ncdc25}}{ec50_{cdc25}^{ncdc25} + cdk1cyclintp[t]^{ncdc25}}\right)\left(cdc25_{tot} - cdc25_{act}[t]\right) -$$
$$k_{cdc25off} cdc25_{act}[t]$$

$$\frac{dwee1_{act}[t]}{dt} = -k_{wee1off}\left(\frac{cdk1cyclintp[t]^{nwee1}}{ec50_{wee1}^{nwee1} + cdk1cyclintp[t]^{nwee1}}\right)wee1_{act}[t] + k_{wee1on}\left(wee1_{tot} - wee1_{act}[t]\right)$$

$$\frac{dplx1}{dt} = k_{plx1on}\left(\frac{cdk1cyclintp[t]^{nplx1}}{ec50_{plx1}^{nplx1} + cdk1cyclintp[t]^{nplx1}}\right)\left(plx1_{tot} - plx1_{act}[t]\right) - k_{plx1off} plx1_{act}[t]$$

$$\frac{dapc_{act}[t]}{dt} = k_{apcon}\left(\frac{plx1_{act}[t]^{napc}}{ec50_{plx1}^{napc} + plx1_{act}[t]^{napc}}\right)\left(apc_{tot} - apc_{act}[t]\right) - k_{apcoff} apc_{act}[t]$$

Initial conditions:

$$cyclin[0] = 0$$

$$cdk1cyclin[0] = 0$$

$$cdk1cyclinyp[0] = 0$$

$cdk1cyclinyptp[0] = 0$

$cdk1cyclintp[0] = 0$

$cdc25_{act}[0] = 0$

$wee1_{act}[0] = wee1_{tot}$

$plx_{act}[0] = 0$

$apc_{act}[0] = 0$

Parameters:

$k_{dest} = 0.01$

$k_a = 0.1$

$k_d = 0.001$

$k_{wee1} = 0.05$

$k_{wee1basal} = k_{wee1} / r$

$k_{cdc25} = 0.1$

$k_{cdc25basal} = k_{cdc25} / r$

$cdc2_{tot} = 230$

$cdc25_{tot} = 15$

$wee1_{tot} = 15$

$apc_{tot} = 50$

$plx1_{tot} = 50$

$nwee1 = 4$

$ncdc25 = 4$

$napc = 4$

$nplx1 = 4$

$ec50_{plx1} = 40$

$ec50_{wee1} = 40$

$ec50_{cdc25} = 40$

$ec50_{apc} = 40$

$k_{cdc25on} = 1.75$

$k_{cdc25off} = 0.2$

$k_{apcon} = 1$

$k_{apcoff} = 0.15$

$k_{plx1on} = 1$

$k_{plxoff} = 0.15$

$k_{wee1on} = 0.2$

$k_{wee1off} = 1.75$

$k_{cak} = 0.8$

$k_{pp2c} = 0.008$

For the negative feedback model, we took $r = 1$, which is equivalent to assuming that the activities of Wee1 and Cdc25 are not affected by the activity of CDK1. For the positive-plus-negative feedback model, we took $r = 5$, 10, or 15 to compare the effects of different feedback strengths. In all cases, the oscillations were found to be born and extinguished through supercritical Hopf bifurcations. Thus a change in the type of bifurcation was not responsible for the change in the shape of the amplitude vs. frequency curves that positive feedback produced.

The following five models are negative-feedback-only oscillators:

2. Repressilator. Elowitz and Leibler used a six variable ODE model to guide their construction of the Repressilator, an artificial negative feedback oscillator, in *E. coli* (*S4*). The model consists of three mRNAs, $m1$, $m2$, and $m3$, which give rise to three proteins, $p1$, $p2$, and $p3$. Each protein inhibits the transcription of the next message (e.g. $p1$ represses $m2$). We varied the frequency of the oscillator by varying one of the translation rates by a factor ϕ. Oscillations were born and extinguished through supercritical Hopf bifurcations. The parameters for the model are as given by Elowitz and Leibler (*S4*).

ODEs:

$$\frac{dm_1}{dt} = -m_1 + \frac{\alpha}{1 + p_3^n} + \alpha_0$$

$$\frac{dm_2}{dt} = -m_2 + \frac{\alpha}{1 + p_1^n} + \alpha_0$$

$$\frac{dm_3}{dt} = -m_3 + \frac{\alpha}{1 + p_2^n} + \alpha_0$$

$$\frac{dp_1}{dt} = \beta\left(m_1 - \phi p_1\right)$$

$$\frac{dp_2}{dt} = \beta\left(m_2 - p_2\right)$$

$$\frac{dp_3}{dt} = \beta\left(m_3 - p_3\right)$$

Initial conditions:

$$m_1[0] = 1$$
$$m_2[0] = m_3[0] = p_1[0] = p_2[0] = p_3[0] = 0$$

Parameters:

$$n = 2$$
$$\alpha = 300$$
$$\alpha_0 = 0$$
$$\beta = 0.2$$

3. Pentilator. If the time scales of transcription and translation are separable, the Repressilator can be reduced to three ODEs. Since a two-ODE negative feedback loop cannot generate sustained oscillations, it seemed reasonable that a three ODE system would require careful balancing of kinetic parameters to oscillate, and would therefore oscillate over a limited range of frequencies. We were therefore curious to see if a five-ODE analog of the repressilator (the 'Pentilator') might have a qualitatively different amplitude/frequency relationship compared to the Repressilator and the other three ODE negative feedback oscillators being examined here. However, as shown in Figure 2, it turned out to behave very similarly to the Repressilator: oscillations were born and extinguished through supercritical Hopf bifurcations, and the operational frequency range of the model was small.

ODEs:

$$\frac{dm_1}{dt} = -m_1 + \frac{\alpha}{1 + p_5^n} + \alpha_0$$
$$\frac{dm_2}{dt} = -m_2 + \frac{\alpha}{1 + p_1^n} + \alpha_0$$
$$\frac{dm_3}{dt} = -m_3 + \frac{\alpha}{1 + p_2^n} + \alpha_0$$
$$\frac{dm_4}{dt} = -m_4 + \frac{\alpha}{1 + p_3^n} + \alpha_0$$
$$\frac{dm_5}{dt} = -m_5 + \frac{\alpha}{1 + p_4^n} + \alpha_0$$
$$\frac{dp_1}{dt} = \beta\left(m_1 - \phi p_1\right)$$
$$\frac{dp_2}{dt} = \beta\left(m_2 - p_2\right)$$

$$\frac{dp_3}{dt} = \beta(m_3 - p_3)$$

$$\frac{dp_4}{dt} = \beta(m_4 - p_4)$$

$$\frac{dp_5}{dt} = \beta(m_5 - p_5)$$

Initial conditions:

$$m_1[0] = 1$$
$$m_2[0] = m_3[0] = m_4[0] = m_5[0] = p_1[0] = p_2[0] = p_3[0] = p_4[0] = p_5[0] = 0$$

Parameters:

$$n = 2$$
$$\alpha = 300$$
$$\alpha_0 = 0$$
$$\beta = 0.2$$

4. Goodwin oscillator. The Goodwin model is a three-variable ODE system proposed forty years ago (*S5*), which has been applied to the analysis of circadian oscillations (*S6-S10*). The parameters used here were taken from Ruoff and co-workers (*S8*). Because they have emphasized that the frequency of the oscillator is a relatively sensitive function of the degradation rates, we chose the degradation rate constant to be the bifurcation parameter ϕ.

Note that for some of the parameter sets used by Ruoff and co-workers, the Goodwin model produces damped oscillations that approach a stable steady state (or stable spiral) rather than a stable limit cycle (*S8*). Here we examined only limit cycle oscillations.

ODEs:

$$\frac{dx}{dt} = \frac{k_1}{1 + z^9} - k_4 x$$

$$\frac{dy}{dt} = k_2 x - \phi y$$

$$\frac{dz}{dt} = k_3 y - k_6 z$$

Initial conditions:

$$x[0] = 0.0348$$
$$y[0] = 0.347$$
$$z[0] = 1.735$$

Parameters:

$$k_1 = 1$$
$$k_2 = 1$$
$$k_3 = 1$$
$$k_4 = 0.2$$
$$k_6 = 0.1$$

5. Frzilator. The Frzilator was proposed by Igoshin and co-workers to account for oscillatory swarming motions in *Myxococcus* (*S11*). The model consists of three ODEs and assumes that all of the activating and inactivating enzymes are highly saturated by their substrates. This model differs from the other negative feedback models considered here in that oscillations are born and extinguished through subcritical Hopf bifurcations rather than supercritical Hopf bifurcations. This means that there is a non-zero amplitude below which the model cannot generate sustained oscillations. It also means that near the bifurcations, a locally stable steady-state (or spiral) coexists with limit cycle oscillations, and the initial conditions determine which behavior is exhibited by the model.

ODEs:

$$\frac{df}{dt} = \phi\left(\frac{1-f}{0.01+(1-f)}\right) - d_f\left(\frac{f}{0.005+f}\right)e$$
$$\frac{dc}{dt} = k_c\left(\frac{1-c}{0.005+(1-c)}\right) - d_c\left(\frac{c}{0.005+c}\right)$$
$$\frac{de}{dt} = k_e\left(\frac{1-e}{0.005+(1-e)}\right) - d_e\left(\frac{e}{0.005+e}\right)$$

Initial conditions:

$$f[0] = 0.503$$
$$c[0] = 0.623$$
$$e[0] = 0.980$$

Parameters:

$k_c = 4$

$k_e = 4$

$d_f = 1$

$d_c = 2$

$d_e = 2$

6. Metabolator. The Metabolator is a synthetic gene-metabolic oscillator that rewires the glycolytic flux to drive an oscillation between two pools of metabolites in acetate pathway (*S12*). The design is based on two negative feedback loops. When acetyl phosphate (AcP) level increases, acetyl-CoA synthetase (Acs) is activated to drive the flux away from AcP, and phosphate acetyltransferase (Pta) is repressed to decrease the production of AcP. Here we vary the enzyme copy number ϕ in the model to change the frequency of the oscillations.

ODEs:

$$\frac{d[AcCoA]}{dt} = V_{Acs} - V_{pta} + V_{gly} - k_{TCA}[AcCoA]$$

$$\frac{d[AcP]}{dt} = V_{pta} - V_{Ack}$$

$$\frac{d[OAc^-]}{dt} = V_{Ack} - k_3[HOAc]$$

$$\frac{d[HOAc]}{dt} = V_{AcE} - k_3[HOAc]$$

$$\frac{d[lacI]}{dt} = \frac{\phi\left([AcP]/K_{g,1}\right)^n}{1+\left([AcP]/K_{g,1}\right)^n} + \alpha_0 - d[lacI]$$

$$\frac{d[Aca]}{dt} = \frac{a_1\phi\left([AcP]/K_{g,2}\right)^n}{1+\left([AcP]/K_{g,2}\right)^n} + a_1\alpha_0 - d[Acs]$$

$$\frac{d[pta]}{dt} = \frac{a_2\phi}{1+\left([lacI]/K_{g,3}\right)^n} + a_2\alpha_0 - d[pta]$$

where

$$V_{pta} = \frac{k_1[pta][AcCoA]}{K_{m1} + [AcCoA]}$$

$$V_{Acs} = \frac{k_2[Acs][OAc^-]}{K_{m2} + [OAc^-]}$$

$$V_{Ack} = k_{Ack,f}[AcP] - k_{Ack,r}[OAc^-]$$

$$V_{AcE} = 100\left([OAc^-][H^+] - K_{eq}[HOAc]\right)$$

$$V_{out} = k_3\left([HOAc] - [HOAc_E]\right)$$

Initial conditions:

$$AcCoA[0] = 0$$
$$AcP[0] = 2$$
$$OAc^-[0] = 0$$
$$HOAc[0] = 0.0009$$
$$lacI[0] = 0.00055$$
$$Acs[0] = 0.011$$
$$pta[0] = 0.00844$$

Parameters:

$$k_{TCA} = 10$$
$$k_1 = 80$$
$$k_2 = 0.8$$
$$K_{m1} = 0.06$$
$$K_{m2} = 0.1$$
$$k_{Ack,f} = k_{Ack,r} = 1$$
$$[H^+] = 10^{-7.6}$$
$$K_{eq} = 10^{-4.5}$$
$$k_3 = 0.01$$
$$a_0 = 0$$
$$a_1 = a_2 = 20$$
$$K_{g,1} = K_{g,2} = 10$$
$$K_{g,3} = 0.001$$
$$d = 0.06$$

The following four models are positive-plus-negative feedback oscillators:

7. Meyer and Stryer model of calcium oscillations. This is a three ODE model that accounts for many of the observed characteristics of cytosolic calcium oscillations (*S13*). The variable x represents cytoplasmic Ca^{2+}; y represents IP_3; and z represents ER Ca^{2+}. The parameter

R, which represents the fractional activation of a calcium-mobilizing G-protein coupled receptor, is used to vary the oscillator's frequency.

ODEs:

$$\frac{dx}{dt} = c_1 \frac{y^3}{(K_1 + y)^3} z - c_2 \frac{x^2}{(x + K_2)^2} + c_3 z^2 - c_6 (x / c_7)^{3.3} + c_6$$

$$\frac{dy}{dt} = c_4 R \frac{x}{x + K_3} - c_5 y$$

$$\frac{dz}{dt} = -c_1 \frac{y^3}{(K_1 + y)^3} z + c_2 \frac{x^2}{(x + K_2)^2} - c_3 z^2$$

Initial conditions:

$$x[0] = 0.1$$
$$y[0] = 0.01$$
$$z[0] = 25$$

Parameters:

$$c_1 = 6.64$$
$$c_2 = 5$$
$$c_3 = 0.0000313$$
$$c_4 = 1$$
$$c_5 = 2$$
$$c_6 = 0.5$$
$$c_7 = 0.6$$
$$K_1 = 0.1$$
$$K_2 = 0.15$$
$$K_3 = 1$$

8. van der Pol oscillator. The van der Pol oscillator model is a one-variable, second order differential equation, which can also be written as a pair of first order differential equations. The model has one parameter, ϕ, which was used to vary the frequency of oscillations.

ODEs:

$$\frac{dx}{dt} = y$$

$$\frac{dy}{dt} = \phi(1 - x^2) y - x$$

Initial conditions:

$$x[0] = 0.1$$
$$y[0] = 0$$

9. Fitzhugh-Nagumo oscillator. Parameters for the Fitzhugh-Nagumo relaxation oscillator (*S14, S15*) were taken from the Vibe website (*S16*). The coupling parameter ϕ was varied to change the frequency of the oscillations.

ODEs:

$$\frac{dv}{dt} = v(v-\theta)(1-v) - w + \omega$$
$$\frac{dw}{dt} = \phi(v - \gamma w)$$

Initial conditions:

$$v[0] = 0$$
$$w[0] = 0$$

Parameters:

$$\theta = 0.2$$
$$\omega = 0.112$$
$$\gamma = 2.5$$

10. Cyanobacteria circadian oscillator. The cyanobacterial circadian oscillator has been reconstituted in vitro using three purified proteins, KaiA, KaiB, and KaiC (*S17*). One of these proteins, KaiC, undergoes a sequence of phosphorylation and dephosphorylation reactions that produces the circadian cycles. Rust and co-workers have modeled these oscillations as a set of three experimentally-constrained ODEs that describe the interconversion of KaiC between a threonine-phosphorylated form (*T*), a doubly-phosphorylated form (*D*), and a serine-phosphorylated form (*S*) (*S18*). The remaining unphosphorylated form of KaiC can be calculated from these three forms and a conservation equation.

ODEs:

$$\frac{dT}{dt} = k_{UT}(S)U + k_{DT}(S)D - k_{TU}(S)T - k_{TD}(S)T$$
$$\frac{dD}{dt} = k_{TD}(S)T + k_{SD}(S)S - k_{DT}(S)D - k_{DS}(S)D$$

$$\frac{dS}{dt} = k_{US}(S)U + k_{DS}(S)D - k_{SU}(S)S - k_{SD}(S)S$$

where

$$k_{XY}(S) \equiv k_{XY}^{0} + \frac{k_{XY}^{A} A(S)}{K_{1/2} + A(S)}$$
$$A(S) \equiv \max\{0, [KaiA] - 2S\}$$

Initial conditions:

$$T[0] = 0.68$$
$$D[0] = 1.36$$
$$S[0] = 0.34$$

Parameters:

$$[KaiA] = 1.3$$
$$[KaiC]_{total} = U + T + D + S = 3.4$$
$$K_{1/2} = 0.43$$
$$k_{UT}^{0} = k_{TD}^{0} = k_{SD}^{0} = k_{US}^{0} = k_{DT}^{0} = 0$$
$$k_{TU}^{0} = 0.21$$
$$k_{DS}^{0} = 0.31$$
$$k_{SU}^{0} = 0.11$$
$$k_{UT}^{A} = 0.479$$
$$k_{TD}^{A} = 0.213$$
$$k_{SD}^{A} = 0.506$$
$$k_{US}^{A} = 0.053$$
$$k_{TU}^{A} = 0.0798$$
$$k_{DT}^{A} = 0.173$$
$$k_{DS}^{A} = \phi$$
$$k_{SU}^{A} = -0.133$$

Supplementary Figure S1:

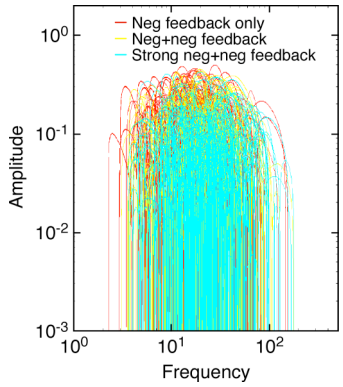

Fig. S1. Amplitude vs. frequency curves for randomly-parameterized oscillator models. Each curve corresponds to a different random parameter set. The red curves are from the negative-feedback-only model (Fig. 4A). The same curves are also included in Fig. 4F. The yellow and cyan curves are from the negative-plus-negative feedback model (Fig. 4C). The yellow curves correspond to weaker feedback (k_7=0-100) and the cyan curves to stronger feedback (k_7=500-600).

Table S1: Ranges for the random parameter sets

Parameter	Units	Range		Distribution
k_1	dimensionless	0 – 10		Uniform
k_2	dimensionless	0 – 1000		Uniform
k_3	dimensionless	0 – 10		Uniform
k_4	dimensionless	0 – 1000		Uniform
k_5	dimensionless	1		Fixed
k_6	dimensionless	0 – 1000		Uniform
k_7	dimensionless	Neg	0	Fixed
		Pos+neg, Neg+neg	0 – 100	Uniform
		Stronger pos+neg, Stronger neg+neg	500 – 600	Uniform
n_1	dimensionless	1 – 4		Uniform
n_2	dimensionless	1 – 4		Uniform
n_3	dimensionless	1 – 4		Uniform
n_4	dimensionless	1 – 4		Uniform
K_1	concentration	0 – 4		Uniform
K_2	concentration	0 – 4		Uniform
K_3	concentration	0 – 4		Uniform
K_4	concentration	0 – 4		Uniform

The parameters refer to the ordinary differential equation models shown in Figure 4A-C. The seven rate constants (k_1 through k_7) were non-dimensionalized by dividing by k_5.

References and Notes

S1. A. Dhooge, W. Govaerts, Y. A. Kuznetwov, *ACM Transactions on Mathematical Software* **29**, 141 (2003).
S2. J. R. Pomerening, S. Y. Kim, J. E. Ferrell, Jr., *Cell* **122**, 565 (2005).
S3. J. R. Pomerening, E. D. Sontag, J. E. Ferrell, Jr., *Nature Cell Biol*. **5**, 346 (2003).
S4. M. B. Elowitz, S. Leibler, *Nature* **403**, 335 (2000).
S5. B. C. Goodwin, Ed., *Oscillatory behavior in enzymatic control processes*, vol. 3 (Permagon Press, Oxford UK, 1965), vol. 3, pp. 425-438.
S6. P. Ruoff, S. Mohsenzadeh, L. Rensing, *Naturwissenschaften* **83**, 514 (1996).
S7. P. Ruoff, M. Vinsjevik, C. Monnerjahn, L. Rensing, *J. Theoret. Biol*. **209**, 29 (2001).
S8. P. Ruoff, M. Vinsjevik, C. Monnerjahn, L. Rensing, *J Biol. Rhythms* **14**, 469 (1999).
S9. P. Ruoff, M. Vinsjevik, S. Mohsenzadeh, L. Rensing, *J. Theoret. Biol*. **196**, 483 (1999).
S10. P. Ruoff, L. Rensing, R. Kommedal, S. Mohsenzadeh, *Chronobiol. Int*. **14**, 499 (1997).
S11. O. A. Igoshin, A. Goldbeter, D. Kaiser, G. Oster, *Proc. Natl. Acad. Sci. U.S.A*. **101**, 15760 (2004).
S12. E. Fung *et al*., *Nature* **435**, 118 (2005).
S13. T. Meyer, L. Stryer, *Proc. Natl. Acad. Sci. U.S.A*. **85**, 5051 (1988).
S14. R. FitzHugh, *Biophys. J*. **1**, 445 (1961).
S15. J. Nagumo, S. Arimoto, S. Yoshizawa, *Proc. IRE* **50**, 2061 (1964).
S16. D. S. Eck. *Vibe* (www.iro.umontreal.ca/~eckdoug/vibe/, 2003).
S17. M. Nakajima *et al*., *Science* **308**, 414 (2005).
S18. M. J. Rust, J. S. Markson, W. S. Lane, D. S. Fisher, E. K. O'Shea, *Science* **318**, 809 (2007).