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Frustrated bistability as a means to engineer oscillations in biological systems

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Abstract
Oscillations play an important physiological role in a variety of biological systems. For example, respiration and carbohydrate synthesis are coupled to the circadian clock in cyanobacteria (Ishiura *et al* 1998 *Science* **281** 1519) and ultradian oscillations with time periods of a few hours have been observed in immune response (NF-κB, Hoffmann *et al* 2002 *Science* **298** 1241, Neson *et al* 2004 *Science* **306** 704), apoptosis (p53, Lahav *et al* 2004 *Nat. Genet.* **36** 53), development (Hes, Hirata *et al* 2002 *Science* **298** 840) and growth hormone secretion (Plotsky and Vale 1985 *Science* **230** 461, Zeitler *et al* 1991 *Proc. Natl. Acad. Sci. USA* **88** 8920). Here we discuss how *any* bistable system can be 'frustrated' to produce oscillations of a desired nature—we use the term frustration, in analogy to frustrated spins in antiferromagnets, to refer to the addition of a negative feedback loop that destabilizes the bistable system. We show that the molecular implementation can use a wide variety of methods ranging from translation regulation, using small non-coding RNAs, to targeted protein modification to transcriptional regulation. We also introduce a simple graphical method for determining whether a particular implementation will produce oscillations. The shape of the resulting oscillations can be readily tuned to produce spiky and asymmetric oscillations—quite different from the shapes produced by synthetic oscillators (Elowitz and Leibler 2000 *Nature* **403** 335, Fung *et al* 2005 *Nature* **435** 118). The time period and amplitude can also be manipulated and these oscillators are easy to reset or switch on and off using a tunable external input. The mechanism of frustrated bistability could thus prove to be an easily implementable way to synthesize flexible, designable oscillators.

1. Introduction

Oscillations and bistability in biological systems have been the focus of numerous studies. In order to produce oscillations the system must necessarily contain a negative feedback loop [10]. For specific implementations of a negative feedback loop, such as the repressilator or NF-κB signalling, stability analysis can suggest which parameter values actually give oscillations [8, 11, 10]. However, such analysis is usually cumbersome and the results are hard to generalize to other implementations of negative feedback or more complex networks. Bistable systems are quite common in biological systems, particularly in development where they are used as switches and memory elements [12]. In contrast to oscillators, bistable systems must necessarily contain a positive feedback loop [13, 12]. Several robust bistable systems have been synthetically constructed [14–19]. Figure 1(left) shows the simplest: a self-activating protein [15, 16], A, which can stably exist in either a low or a high concentration state. This bistable system can then be 'frustrated' by adding a negative feedback loop through another protein B (see figure 1, right). When the underlying bistability pushes A towards the high state, more B is produced, thereby decreasing A, and vice versa for the low state.

We have borrowed the term 'frustration' from the physics of antiferromagnets, wherein two adjacent magnetic spins prefer to point in opposite directions: if one is 'up', the other is 'down'—this is a bistable system. Now if another spin interacts with these two (this happens, for instance, in triangular or diamond lattice antiferromagnets) then the third spin cannot decide whether to be up or down, which also

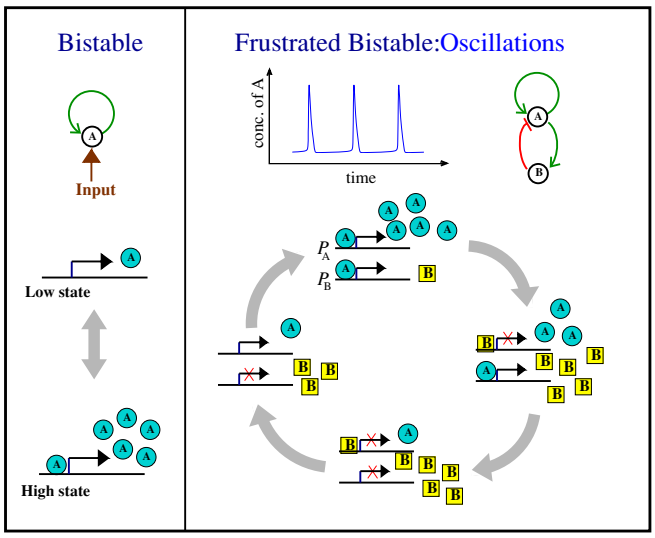

Figure 1. Examples of a one protein system with transcriptional self-activation and a frustrated system based on it. Also shown schematically is their respective behaviour—bistability for the former and oscillations for the latter.

destabilizes the other two spins: they are termed 'frustrated spins' [20]. In analogy to this, we call the motif in figure 1 (right) a frustrated bistable system. Such a combination of a positive and a negative feedback loop forms the core of several models of synthetic and naturally occurring oscillators (in different contexts termed either activator–inhibitor, or relaxation, or hysteresis oscillators) [21–27]. Our analysis adds to this by showing that the general mechanism of frustrated bistability is a very flexible way to make synthetic oscillators with designed properties, and by providing a simple graphical method that can be used to determine the conditions for oscillations based on experimental data alone.

2. Basic model of frustrated bistability

To illustrate frustrated bistability we start with the simplest bistable model system, consisting of a single protein with transcriptional self-activation, describing the dynamics of its concentration A by a differential equation:

$$\frac{\mathrm{d}A}{\mathrm{d}t} = \alpha \frac{b + A^2}{1 + A^2} - A. \tag{1}$$

Here time is measured in units of the lifetime of the protein and the unit of concentration has been chosen such that half-activation occurs at $A = 1$ (see appendix A for the meaning of the terms and parameters). In these units, then, α is the maximum rate of production of A and αb is the basal rate.

Bistability exists for a certain range of values (see appendix A) of the tunable parameter α, which can be considered an external input. For instance, in the bistable lactose transport system, the tunable parameter is the rate of transcription from the *lac* promoter, which is varied by changing the amount of IPTG in the system [17]. We then add frustration by making this input depend on a second protein B which represses transcription of A and is in turn transcriptionally activated by A (see figure 1, right):

$$\begin{aligned} \frac{\mathrm{d}A}{\mathrm{d}t} &= \frac{\alpha}{1 + (B/K)^2} \times \left(\frac{b + A^2}{1 + A^2} \right) - A, \\ \frac{\mathrm{d}B}{\mathrm{d}t} &= \gamma A - \gamma B. \end{aligned} \tag{2}$$

γ is the ratio of the half-life of A to that of B. Note that we have chosen the units of B such that its production rate constant is equal to the degradation constant γ (this is done only for simplicity). K then, in these units, is the Michaelis constant that sets the strength of repression of A by B. (Incidentally, the choice of Hill coefficient 2, used in the repression of A by B, is not essential. See appendix B for oscillations produced with Hill coefficient equal to unity.)

In the absence of this feedback (i.e., if B is held fixed), the system is bistable for a certain range of B values, as shown in figure 2(a) (red lines). When the frustration is introduced the system oscillates, as shown in figure 2(b).

The process producing the oscillations is particularly clear when the protein B has a much longer half-life than A (i.e., when γ is small). In this case, there are two clearly separated timescales: B responds to changes in A slowly, while A responds relatively quickly. Thus, A is always in 'equilibrium', quickly moving to either the high or the low state depending on the value of B. Starting from low B and low A, A quickly increases to the high state. This results in B slowly increasing, and A following the red line marking the high state in figure 2(a). When B becomes sufficiently large, however, the high state ends and A quickly jumps to the low state. Then, B starts to decrease slowly because its production rate drops. As B decreases, A now follows the red line marking the low state, until B goes below the lower threshold. Then A jumps to the high state again and the cycle continues. This trajectory is shown in figures 2(a) (blue curve) and 2(b).

3. Graphical method for determining conditions for oscillations

A simple graphical method can be used to deduce which parameter values give oscillations. For the frustrated bistable system of equation (2), the activation of B by A, as a function of A, can be plotted on the same plot as the bistable solutions, and is shown by the dashed purple line in figure 2(a) (mathematically, it is the 'nullcline' $\mathrm{d}B/\mathrm{d}t = 0$, which in this case is simply the straight line $A = B$ on a linear plot). To get oscillations, *this activation curve must pass between the low and high state curves without intersecting them*. If it does intersect, the system will not oscillate and instead eventually settle into a steady state (see appendix C for more details). Note that both the bistable curves and the activation curve can be determined entirely experimentally. The bistable curves are determined by tuning the external input and measuring the steady-state concentration of the protein as described, for example, in studies of the lactose transport system and various synthetic switches [14–18]. The activation curve can be determined *in vitro* by similarly measuring the steady-state concentration of B when different amounts of A are supplied.

This graphical method is completely general and works for any implementation of frustrated bistability. For instance,

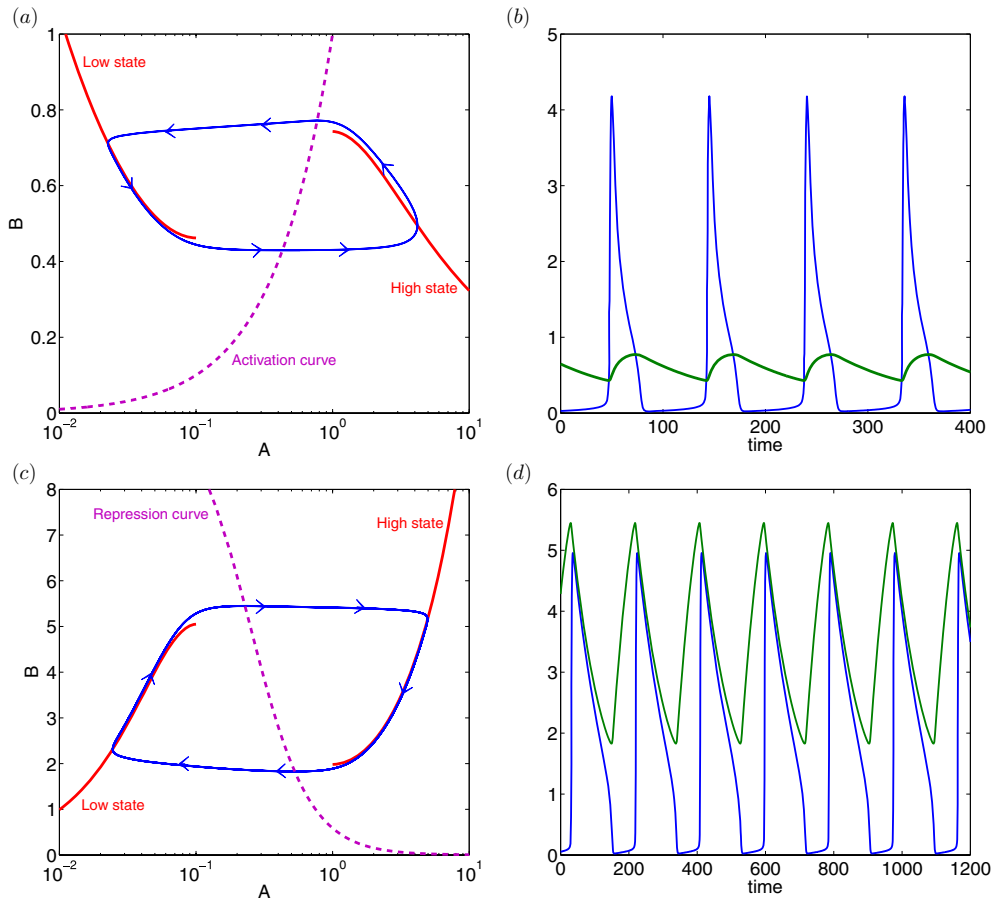

Figure 2. Frustrated bistability in action. (*a*) Solid red lines show the stable steady-state concentrations of the self-activating protein A in the absence of frustration, when B is fixed at different values. The activation of B by A, from equation (2), is shown by the purple dashed line. In order to get oscillations the activation curve must pass between the red low and high state curves without intersecting them. The blue curve with arrows is the oscillating trajectory when frustration is added, when the ratio of half-life of A to half-life of B is $\gamma = 0.01$. Other parameters were set to $K = 0.07$, $\alpha = 225$, $b = 0.01$. (*b*) Time series of A (blue) and B (green) for the oscillating trajectory shown in (*a*). (*c*) Alternative frustrated bistable system, where the frustration is introduced as described by equation (3). With parameter values chosen to be $K = 0.25$, $\alpha = 0.1$, $\gamma = 0.01$, $b = 0.01$, we obtain the blue oscillating trajectory in phase space. For these parameters the $\mathrm{d}B/\mathrm{d}t = 0$ curve (purple dashed line) passes between the low and high state curves. (*d*) Corresponding time series of A (blue) and B (green) for the oscillating trajectory shown in (*c*).

in contrast to the previous example, the frustration could be introduced via a protein B that is repressed by A and in turn activates A:

$$\begin{aligned} \frac{\mathrm{d}A}{\mathrm{d}t} &= B\left(\frac{b + A^2}{1 + A^2}\right) - A. \\ \frac{\mathrm{d}B}{\mathrm{d}t} &= \frac{\alpha}{1 + (A/K)^2} - \gamma B. \end{aligned} \tag{3}$$

Figure 2(*c*) shows how to use the graphical method to find oscillations in this system. A more appropriate name for the $\mathrm{d}B/\mathrm{d}t = 0$ curve, in this case, is 'repression curve' as it decreases as A increases, but the idea is the same—to obtain oscillations the curve must pass between the high and low state curves without intersecting them.

4. Flexibility in the molecular implementation of frustration

The same bistable system can be frustrated by a number of different molecular mechanisms (two of which are demonstrated in figure 2). The only necessity is the existence of a net negative feedback from A to itself, via B. In all cases, the graphical method can be used to determine the requirements for oscillations.

The mechanism of frustrated bistability also works if B acts on A post-transcriptionally. We have examined three broadly different ways of implementing the negative feedback loop, involving (1) transcription regulation, (2) translation regulation and (3) degradation or irreversible modification of A, mediated by B:

(1) Transcription regulation: this is the case discussed in the two examples of figure 2.
(2) Translation regulation can be implemented in several ways. For instance, a small non-coding RNA can be used that is anti-sense to the part of the mRNA sequence of A. In the iron homeostasis system of *E. coli*, an sRNA called RyhB acts like this, and the RyhB–mRNA complex is then rapidly degraded [28, 29]. Other sRNAs may catalyze degradation of target mRNA without

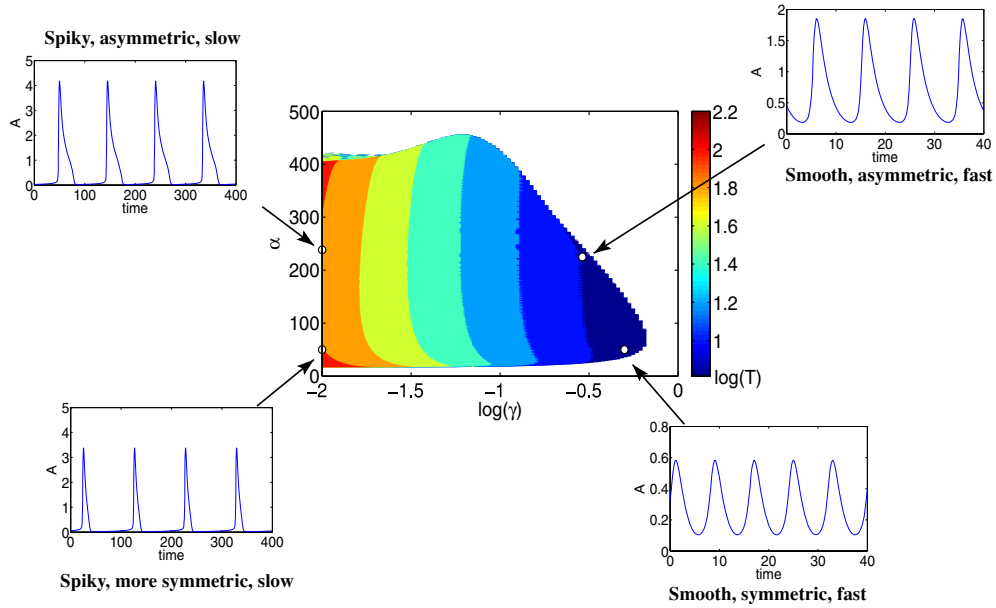

Figure 3. Different shapes of oscillations that can be produced by frustrated bistability. The central colour–contour plot shows how the time period of the oscillations varies as a function of γ, the ratio of half-life of A to B, and α, the maximal production rate of A. Insets show the time series for oscillations with varying levels of spikiness, symmetry, time period and amplitude, produced by choosing γ and α values appropriately.

themselves getting destroyed. Both ways can be used to implement repression of A by B (see appendix D for a specific example of frustrated bistability using translation regulation).

(3) B could directly interact with A and target it for degradation, for instance by tagging it with a ubiquitin molecule, which is how Mdm2 acts on p53 [30]. Alternatively, it could sequester A or simply modify it, for instance by phosphorylation, either way preventing it from acting as a transcription factor. This function could be done with or without recycling of B; both ways provide sufficient frustration.

It is also irrelevant whether the bistability is produced by a direct self-activation, or an indirect one (for instance using two mutually repressing proteins [14], like CI and Cro in lambda phage [19 ,31]). One can also use translation or other post-transcriptional regulation to produce the bistability. Again, what is important is the existence of a positive feedback from A to itself that can produce bistability, not the particular molecular mechanism used.

5. Varying the shape of oscillations

Figure 3 shows a few different shapes of oscillations that can be designed from the basic model by varying two relevant parameters: the ratio of the half-life of A to B, γ, and the maximal production rate of A, α. Based on figure 3 we can list a few guidelines for engineering oscillations of a specific nature:

(1) *Spikiness*: this is mainly controlled by the ratio between the half-lives of A and B. Decreasing the stability of B (increasing the value of γ) makes the oscillations smoother. The value of γ can be readily adjusted by proper tagging of the proteins [32].

(2) *Symmetry*: the maximal production rate of A affects symmetry—increasing α makes the oscillations more asymmetric. α can be increased for example by using a stronger promoter, enhancing the efficiency of translation initiation, or by increasing mRNA stability.

(3) *Time period*: the time period T of the oscillations is strongly affected by the ratio between the half-lives of A and B; deepening colours as γ is decreased indicate the increase of T.

(4) *Amplitude*: the amplitude of the oscillations is also controlled by the ratio of the half-lives of A to B: the larger the value of γ, the smaller the amplitude.

These properties are coupled because a single parameter can affect several properties simultaneously. For instance, symmetry is easy to achieve in combination with smoothness; it is harder to get a symmetric spiky oscillation. However, by modifying combinations of parameters one can get quite a variety of different oscillations, as shown in figure 3. Many of these shapes are quite different from those produced by existing synthetic oscillators. In particular, spiky oscillations are observed in many natural situations (e.g., p53 [4], NFkB [3], growth hormone [6] oscillations) but not in the synthetic repressilator [8] or metabolator [9].

5.1. Modifying the underlying bistable system

Tinkering with the underlying bistable system is another way of changing the shape to the desired one. For instance, the amplitude of the oscillations clearly depends on how far apart the bistable solutions are. Thus, using a stronger promoter or one with a lower basal production level, is a good way of

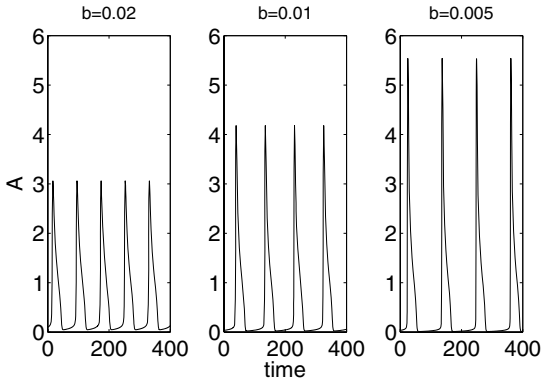

Figure 4. Altering the bistable system. The amplitude of the oscillations increases as the separation between the low and high states is increased, by decreasing the parameter b, which sets the basal level of expression of A.

increasing the amplitude. Figure 4 shows that the amplitude of oscillations increases as the basal level, b, is decreased in equation (2).

Another example: the spikes in the system above are quite sharp because the concentration of A in the high state increases quickly as B increases. To get broader peaks one simply needs to make this dependence weaker. One possibility is to use a protein A that activates itself at low concentrations but represses itself at high concentrations. This could be done, for example, by a dual regulator like CI which behaves exactly like this by using multiple binding sites with different affinities [31].

6. Controlling the oscillator

An important issue for synthetic oscillators is the ability to control them. It should be possible to easily switch on or off the oscillator as well as to reset it. Frustrated bistable systems allow these functions to be easily implemented. An external signal that switches off any of the interactions or the expression of either protein [18] can be used to turn on or off the oscillations. Figure 4(*a*) shows the effect. This could also be done by introducing another protein that binds to and degrades B thereby changing its half-life. With such a set-up, a pulse-like input signal will reset the oscillations, as shown in figure 4(*b*). If it is also necessary to make the switching by a pulse like signal, this can be achieved simply by passing the signal through another bistable module, which would then retain the 'memory' of the pulse in the external input signal even after the signal has decayed.

7. Conclusions and outlook

The main points of this paper are summarized as follows:

- A convenient and flexible way of generating oscillations in genetic circuits is by modifying a bistable system by adding a destabilizing negative feedback loop (see figure 1). We call this 'frustration' in analogy with magnetic spin systems.
- A graphical method can be used to determine parameter values which will result in oscillations, for such frustrated bistable systems (see figure 2). The method is very general and works for many alternative implementations of frustration.
- Finally, such frustrated systems can be easily controlled, and can be tuned to produce a variety of different shapes of oscillations, in particular spiky, asymmetric ones (see figures 3–5).

What makes such frustrated bistable systems particularly attractive for use as synthetic genetic oscillators is the flexibility in implementing the bistability and the frustration. We showed that both transcriptional and translation regulation could be used to implement the interactions that result in the bistability and the frustration. Concerning the latter, Isaacs *et al* [33] has shown how it is possible to insert a short *cis* sequence in a target gene, upstream of the ribosome binding site, and design a small non-coding RNA that can bind to this sequence through base-pair matching, thereby activating translation of the target. Similar techniques could be used to construct small RNAs that repress translation of specific mRNAs (as is the case with many naturally occurring regulatory small RNAs, such as RyhB [28, 29]). Such translation regulation could be used both in the bistable part and in the negative feedback loop of a synthesized frustrated bistable system. The subtle differences in dynamical behaviour produced by using translation regulation [34, 35] could be used to generate oscillations different from those of synthetic oscillators based purely on transcription regulation, such as the repressilator. In addition, using translation regulation achieves specificity of regulation without altering functional properties of the proteins being used in the system. This is very useful when the synthetic oscillator is intended to regulate other downstream genes.

Acknowledgments

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Appendix A. One-protein bistable system

We model the dynamics of the concentration of a self-activating protein, A, using the following differential equation:

$$\frac{\mathrm{d}A}{\mathrm{d}t} = \nu \frac{b + (A/K_A)^h}{1 + (A/K_A)^h} - \frac{A}{\tau}. \tag{A.1}$$

The first term models cooperative transcriptional self-activation with a Michaelis constant K_A and Hill coefficient h, while b determines the basal expression level. ν is the maximal rate of production of A and τ is its average lifetime. Several parameters can be eliminated by measuring time in units of τ, and concentration of A in units of the Michaelis constant K_A:

$$\frac{\mathrm{d}A}{\mathrm{d}t} = \alpha \frac{b + A^h}{1 + A^h} - A. \tag{A.2}$$

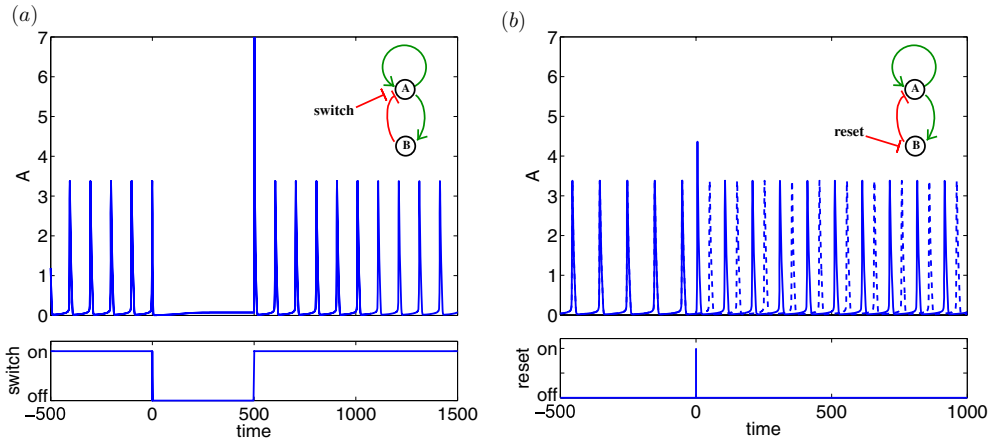

Figure 5. Manipulating the oscillator using external signals. (*a*) One way to switch on or off oscillations is by changing the maximal production rate, α, of A. In the plot, α is reduced from 50 to 10 at time zero, and back to 50 at time 500. (*b*) One way to reset oscillations is by a short pulse of a protein that targets B for degradation. In the plot, the pulse lasts 0.5 units of time, during which B degrades at the same rate as A. Dashed lines show how the oscillations would have continued in the absence of this pulse.

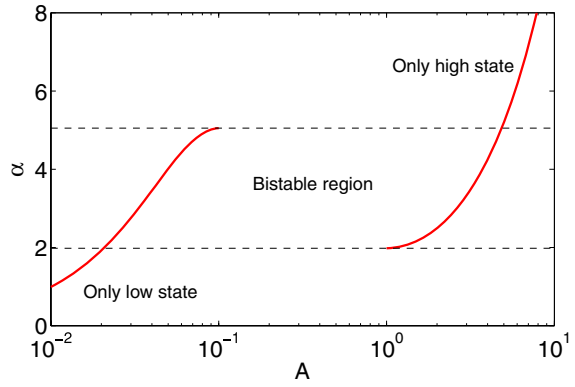

Figure A1. Possible stable steady-state concentrations for a self-activated protein A, as a function of the maximal promoter strength α. For $\alpha < 2$ the system can only be in the low state, where $A \approx 0.01\alpha$. For $\alpha > 5$ the system can only be in the high state, where $A \approx \alpha$. For $2 \leqslant \alpha \leqslant 5$ both states are stable, i.e., the system is bistable.

α is then the maximal rate of production of A, in units of K_A, per unit time (the unit of time is the lifetime τ). Now all parameters and variables are dimensionless.

This system is bistable for some range of α, provided $h \geqslant 2$. For example, as default values, we fix $b = 0.01$, $h = 2$. Then the system is bistable for $2 \leqslant \alpha \leqslant 5$: in the 'low' state, $A \approx b\alpha$, while in the 'high' state $A \approx \alpha$, thus there is a 100-fold separation between the low and high states. For $\alpha < 2$ only the low state exists, and for $\alpha > 5$ only the high state exists (see figure A1).

Appendix B. Frustrated bistable system with Hill coefficient equal to unity

Earlier, we used a Hill coefficient 2, in the repression of A by B, to illustrate frustrated bistability. Figure B1 shows that the system with that Hill coefficient set to 1 also gives oscillations. The equations in that case are

$$\begin{aligned} \frac{\mathrm{d}A}{\mathrm{d}t} &= \frac{\alpha}{1 + B/K} \times \left(\frac{b + A^2}{1 + A^2}\right) - A. \\ \frac{\mathrm{d}B}{\mathrm{d}t} &= \gamma A - \gamma B. \end{aligned} \tag{B.1}$$

Compared to figure 2, the bistable curves are different therefore the same parameter values that worked for the previous case do not give oscillations here. However, the graphical method can be applied in this case also to determine suitable parameter values (see figure B1(*a*)).

Appendix C. Brief proof of the graphical method

When γ is small, the oscillating trajectory of the system follows the bistable solutions closely (see figures 2 and B1). Therefore it is clear that the system will oscillate if

(1) when the trajectory is at the end of the low state, B decreases further (so A is pushed to the high state);
(2) when the trajectory is at the top of the high state, B increases (so A is pushed to the low state).

That is,

(1) $\mathrm{d}B/\mathrm{d}t < 0$ when the trajectory is at the end of the low state;
(2) $\mathrm{d}B/\mathrm{d}t > 0$ when the trajectory is at the end of the high state.

Now, along the activation curve $\mathrm{d}B/\mathrm{d}t$ is exactly zero—this is how the activation curve is defined. In other words, the activation curve separates the region where $\mathrm{d}B/\mathrm{d}t < 0$ (the region above it) from the region where $\mathrm{d}B/\mathrm{d}t > 0$ (below it). Thus, the above criteria for oscillations translate to

(1) the activation curve must pass below the end of the low state;
(2) the activation curve must pass above the end of the high state.

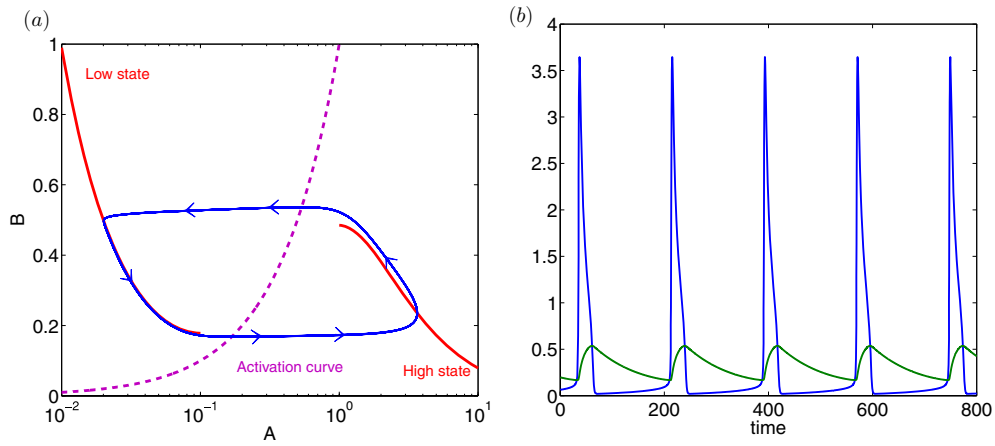

Figure B1. Frustrated bistable system described by equation (B.1), with parameter values chosen to be $K = 0.02$, $\alpha = 50$, $\gamma = 0.01$ and $b = 0.01$. (*a*) Oscillating trajectory (blue) in phase space. For these parameters the $dB/dt = 0$ curve (purple dashed lines) passes between the low and high state curves. (*b*) Corresponding time series of A (blue) and B (green).

For larger γ, these criteria are necessary for obtaining oscillations, but not sufficient—indeed, as seen in figure 3, for large enough γ no oscillations are observed.

Appendix D. Frustration via translation regulation

The basic model of frustrated bistability, described earlier, introduced frustration through a protein B which repressed transcription of A. If instead, B regulated translation of A, for instance by catalyzing the degradation of the mRNA of A, the equations would be

$$\begin{aligned}\frac{dA}{dt} &= \alpha A_m - A.\\ \frac{dA_m}{dt} &= \gamma_m\left(\frac{b+A^2}{1+A^2}\right) - \delta A_m B - \gamma_m A_m.\\ \frac{dB}{dt} &= \gamma A - \gamma B.\end{aligned} \tag{D.1}$$

Here, γ_m is the ratio of the half-life of the mRNA to that of the protein. A_m is the concentration of mRNA of A measured in units such that the production rate of A is α per unit of mRNA, per lifetime of A. The $\delta A_m B$ term models the binding and degradation of the mRNA–B complex. To understand how the frustration works in this case, consider the typical case where the half-life of the mRNA is much less than that of the protein. In that case, the mRNA dynamics occurs on a much faster timescale than the protein dynamics and can be considered to be in equilibrium. That is, $dA_m/dt \approx 0$. The equations then simplify to

$$\begin{aligned}\frac{dA}{dt} &= \frac{\alpha}{1+\delta B/\gamma_m}\times\left(\frac{b+A^2}{1+A^2}\right) - A.\\ \frac{dB}{dt} &= \gamma A - \gamma B.\end{aligned} \tag{D.2}$$

This is identical to the previous example, equation (B.1), with γ_m/δ playing the role of K. To achieve the same oscillations as in figure B1 we could choose, for example, $\gamma_m = 10$ and $\delta = 500$.

Glossary

Bistable system: a bistable system is one that can exist stably, for the same parameter values, in two different states, with different steady-state levels (often, one low and one high) of protein concentration, or gene expression, or whatever variables characterize the system. Whether the system enters one or the other state depends on what initial values these variables start out with. Bistability has been observed, for example, in the lactose transport system in *E. coli* [17] and silencing of the mating-type regions in *S. pombe* [36].

Frustration: in the physics of antiferromagnets, interacting magnetic spins like to align in opposite directions. However, in a triangular lattice, for example, there is no configuration of spin directions that can satisfy this condition for each pair—the third spin of the triangle destabilizes the other two. Such a situation is termed 'frustration' and the spins are called 'frustrated spins' [20].

Synthetic genetic networks: these are artificially constructed networks that combine genes and regulatory elements from cells in combinations that are not known to occur naturally in biological systems. The goal of this is to design and construct networks with a desired behaviour (for example bistability [14–18] or oscillations [8, 9]), and also, thereby, to increase our understanding of naturally occurring gene regulatory networks.

Spiky oscillations: oscillations are often visualized as being smooth and sinusoidal. In biological systems, however, observed oscillations are often quite spiky. That is, they are characterized by short, sharp increases in protein concentration or gene expression, separated by long periods of stasis. Such spiky oscillations have been seen, for example, in hormone production and NF-κB signalling (the term 'spiky oscillations' was introduced to describe the latter in [11]).

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