The heterodimer auto-repression loop: a robust and flexible pulse-generating genetic module

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We investigate the dynamics of the heterodimer autorepression loop (HAL), a small genetic module in which a protein A acts as an auto-repressor and binds to a second protein B to form an AB dimer. For suitable values of the rate constants the HAL produces pulses of A alternating with pulses of B. By means of analytical and numerical calculations, we show that the duration of A-pulses is extremely robust against variation of the rate constants while the duration of the B-pulses can be flexibly adjusted. The HAL is thus a minimal genetic module generating robust pulses with tunable duration an interesting property for cellular signalling.

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Living cells regulate their response to stimuli through biochemical reaction networks where genes, messenger RNAs (mRNAs) and proteins interact with each other [1]. Genes control the synthesis of proteins via mRNAs, while their activities are regulated by specific DNA-binding proteins called transcription factors (TF). Proteins bind to each other to regulate their properties. These multiple interactions are organized in entangled feedback loops, which generate a complex and collective dynamics. Despite the high complexity of biological networks, many specific dynamical mechanisms can be attributed to small genetic modules comprising a few genes, their mRNAs and proteins [2, 3]. Thus, many studies have aimed to uncover the dynamical design principles of such modules, viewed as building blocks for larger systems or as devices for synthetic biology. For example, the appearance of oscillations has been linked to negative feedback and time delays [2], and the importance of mechanisms such as complexation [4] or saturated degradation [5, 6] for oscillations has been highlighted.

While much effort has been devoted to assess the robustness of biochemical oscillations, it has generally been quantified only by the constancy of the total period. The latter is an important criterion for oscillations whose purpose is time keeping, as in circadian clocks, but it is not always relevant. Recent studies (see [5] for a review) revealed that also signaling proteins, which detect and deliver cellular signals, can display oscillating dynamics. In some systems, oscillations appear as discrete pulses separated by constant time intervals [9], while in others the intensity of upstream signals determines the time interval between pulses [10, 11], which may thus be used to encode information [2]. A natural question is then whether we can identify simple model systems that display similar behavior. In this Letter, we investigate the dynamical properties of such a minimal genetic module, the Heterodimer Autorepression Loop (HAL). The HAL generates a periodic “pulsating” output in the concentrations of two different proteins where the pulses of one protein alternate with the pulses of the other one. We will use the term “pulses” rather than “oscillations” to emphasize that we think primarily to the model as a genetic device for cellular signalling, rather than for time keeping. Remarkably, the duration of the pulses of one protein is robust against variations in the rate constants, while the time interval between two pulses, where the other protein is dominant, is tunable.

The HAL consists of a self-repressing TF protein A that can bind to its own gene to inhibit mRNA synthesis, or to another protein B, then becoming inactive (Fig. 1). Self-repression is a pervasive motif in transcriptional networks [12–14], and protein-protein interactions modifying TF activity are also ubiquitous [15], making the HAL very plausible biologically. Accordingly, the HAL appeared with high frequency in evolutionary algorithm calculations searching for oscillating modules [16]. The HAL can be described by the following deterministic differential equations, obtained from the reactions in Supplemental Fig. 1 using mass action kinetics:

\[
\begin{align*}
\frac{d[G]}{dt} &= \omega(1 - [G]) - \alpha[G][A] \\
\frac{d[M]}{dt} &= \mu_M[G] + \mu_A^B(1 - [G]) - \delta_M[M] \\
\frac{d[A]}{dt} &= \mu_A[M] - \delta_A[A] - \gamma_{AB}[A][B] + \lambda_{AB}[AB] \\
\frac{d[B]}{dt} &= \mu_B - \delta_B[B] - \gamma_{AB}[A][B] + \lambda_{AB}[AB] \\
\frac{d[AB]}{dt} &= \gamma_{AB}[A][B] - \lambda_{AB}[AB] - \delta_{AB}[AB]
\end{align*}
\]

where [A], [B], [AB] and [M] are the concentrations of A, B, AB and of the mRNA produced by the gene G_A, respectively (since G_B is unregulated, the concentration of its mRNA is not a variable). The first equation in [1]
The gene dynamics of gene $G_A$ activity, which is a continuous variable $0 \leq [G] \leq 1$, with $[G] = 0$ (resp., $[G] = 1$) when the gene is permanently protein-bound and repressed (resp., unbound and active) [4] [17]. Such an average activity appears naturally in rate equations derived from a moment expansion of the chemical master equation [15]. It takes into account that due to transcriptional bursting [19] [23], gene activity is out of equilibrium and lags variations in TF concentration. The equation used here is valid only when the gene response is not too slow compared to mRNA and protein lifetimes [13], thus the predictions of our deterministic approach will be carefully checked with stochastic simulations of the HAL.

To explore the dynamics of the HAL, the rate constant values were randomly sampled in typical biological ranges obtained from the literature [24] [28], as shown in Table I. Robust pulses were found in a significant domain of parameter space (Supplemental Figure 2). As a general rule, pulses are observed if $\gamma_{AB}$ is large while $\lambda_{AB}$ is small, so that the complex is irreversibly formed (large or small meaning close to the upper or lower bound in Table I). Also, the protein production rates $\mu_A$ and $\mu_B$ need to be sufficiently large and to verify $\mu_B \lesssim \mu_A \mu_M/\delta_M$. The latter condition expresses that the productions of $A$ and $B$ should be balanced, with $A$ synthesized faster than $B$ for a fully active gene ($[G] = 1$, with mRNA concentration $[M] = \mu_M/\delta_M$), and more slowly for an inactive gene. The average period was $T_{tot} \approx 100$ min.

Figure 1 shows a typical pulsating solution of (1), with a period $T_{tot} = 64$ min. The mutual "sequestration" of $A$ and $B$ induced by the dimerization leads to an alternation of pulses where either $A$ or $B$ is predominant (referred to as the $A$- and $B$-phase), the other protein remaining at low levels. Inside each pulse, the dominant protein first accumulates as it is synthesized faster than the other while complexation removes the two proteins in equal quantities. Then, it decreases to almost zero when the situation is reversed. During the $B$-phase, the gene is unpressed, and $A$ synthesis rate increases as mRNA builds up. During the $A$-phase, the gene is repressed and $A$ synthesis rate decreases as mRNA is degraded. The key for cycling is thus that during each phase, there is a time where $A$ and $B$ synthesis rates become equal, which is at the peak of the pulse.

Thus, mRNA life time plays the role of a time delay, a crucial ingredient for oscillations [29]. The sequestration of the TF A also plays an important role by inducing an ultrasensitive response in gene activity [30], a strong nonlinear effect [31] which favors oscillations like a high transcriptional cooperativity would do. This ultrasensitivity is presumably also important in other gene circuits where sequestration induces oscillations [4].

To get an estimate of the pulses period, we make some simplifications. We assume perfect repression ($\mu_M = 0$) and irreversible complex formation ($\lambda_{AB} = 0$). With the latter assumption, we do not need to track dimer $AB$, leading from Eqs. (1) to a system of four differen-

![FIG. 1. A schematic representation of the HAL module. The gene $G_A$ is repressed by its own protein $A$, which forms an inactive dimer $AB$ with a second protein/molecule $B$. Proteins and mRNA degrade with rates $\delta_A$, $\delta_B$, $\delta_{AB}$ and $\delta_M$ (reactions not shown).](image-url)

| Parameter | Value | Reference |
|-----------|-------|-----------|
| $1/\mu_M$ | [0.1, 100] | [23] |
| $1/\delta_M$ | [3, 60] | [24] |
| $1/\mu_A$ | $[10^{-4}, 10]$ | [29] |
| $1/\mu_B$ | $[10^{-3}, 10]$ | [26] |
| $1/\delta_A$, $1/\delta_B$, $1/\delta_{AB}$ | $[4, 2000]$ | [27] |
| $1/\omega$ | $[1, 60]$ | [29] |
| $1/\gamma_{AB}$ | $[0.02, 20]$ | [1] |
| $1/\lambda_{AB}$ | 100 | - |
| $1/\mu_M$ | $10^3$ | - |
| $[A_0]$ | $[1, 100]$ | - |

$^a$ Obtained from the value of $\mu_A$, and assuming a typical number of 10 mRNA’s in the cell.

$^b$ Assuming that the formation of the AB complex is diffusion limited and $D = 1 \mu m^2 \cdot s^{-1}$.

$^c$ This choice implies a small dissociation rate, so that the complex is irreversibly formed.

$^d$ This is the transcription rate from a gene with the protein a bound. For an ideal repressor $\mu_M = 0$, we assume here that there is a weak transcription even with the protein bound. This rate is however at least 10 smaller that the transcription rate from a free gene (see value of $1/\mu_M$ above).

$^e$ Here it is assumed that one needs from 1 to 100 proteins in the volume at threshold to bind to the gene.
A detailed analysis of Eqs. (3) is presented in the Supplementary Material. Here we give simple arguments explaining the main features observed. During the A-phase, \( m(\tau) \) decays exponentially [set \( g = 0 \) in Eqs. (2)]. Denoting by \( m_A \) and \( m_B \) the mRNA concentrations at the beginnings of the A- and B-phases (Fig. 2), we have \( m_B = m_A e^{-T_a} \). To get pulses, A synthesis must be faster than B synthesis when A-phase starts \((k_a m_A > k_b)\), and slower when B-phase starts \((k_a m_B < k_b)\), which yields \( m_B < k_b / k_a < m_A \). Assuming stationarity of the B protein \((db/d\tau \sim 0)\) in the A-phase, we get

\[
\frac{da}{d\tau} = k_a(m(\tau) - k_b) = k_a m_A e^{-\tau} - k_b \tag{5}
\]

The solution of (5) is a pulse with a peak \((da/d\tau = 0)\) at mRNA concentration \( m^* = k_b / k_a \) (Fig. 2). The pulse duration \( T_a \) is found by setting \( a(T_a) = 0 \):

\[
\frac{T_a}{1 - e^{-T_a}} = \frac{k_a m_A}{k_b} \tag{6}
\]

Hence, \( T_a \) depends only on the ratio \( k_a m_A / k_b \). Since pulses require \( k_a m_A / k_b > 1 \), \( T_a \) cannot become too small. Eq. (6) might suggest that large values of \( k_a / k_b \) lead to arbitrarily large \( T_a \). However, this is not true because the B-phase shrinks as \( k_a / k_b \) gets larger, since B synthesis is then faster than A synthesis only for a short time. Hence the variations of \( m \) during the B-phase become smaller and smaller as \( k_a / k_b \) increases, since the mRNA characteristic time is 1. Consequently, \( m_B / m_A = e^{-T_a} \) remains...
close to 1, thus bounding $T_a$. In simple words, changes in the rate constants which could affect $T_a$ are compensated by a associated change in the mRNA maximum concentration $m_A$. Thus, there is a natural negative feedback loop stabilizing A-pulse duration.

To corroborate these results, based on the reduced model (2) and further approximations, we numerically computed $T_a$ and $T_b$ using the full equations (1) for parameter sets $\{k_i\}$ centered around the set $\{k_0^0\}$ used in Fig. 2. Each $k_i$ was selected randomly and uniformly on a logarithmic scale in the interval $[\frac{1}{2}k_0^0, 2k_0^0]$. In total $10^3$ sets were generated, of which 98% had a pulsating output, showing that the parameter set of Fig. 2 is well inside the pulsating domain in parameter space. Although the data span a wide range of values of $\beta$, the computed values of $T_a$, $T_b$ and $T_{tot}$ are in close agreement with the analytical approximation (Fig. 4).

A legitimate question is then whether our findings still hold true when the stochastic nature of biochemical networks cannot be ignored, especially since a slow promoter dynamics may be needed to obtain long intervals between A-pulses. We therefore carried out stochastic simulations of the reaction network of Fig. 1 using the Gillespie algorithm [32]. Pulses are observed for both high and low values of $\beta$, with a stable time interval between A-pulses (Fig. 5 and Supplemental Material), which confirms the relevance of our analysis.

Summarizing, we have investigated the dynamics of the HAL, a pulse generator based on the competing effects of self-repression and complexation. Self-repression alone does not typically induce oscillations, unless time delays [33] or strong nonlinearities are introduced. Protein complexation generates an effective ultrasensitive response [34], which can induce oscillations as in other examples [31], including the mixed-feedback loop [4] or the monomer-dimer oscillator [16]. Since the only role of $B$ is to sequester $A$, $B$ does not need to be a protein but could be any inhibitor molecule binding to $A$ to block its transcriptional activity.

A striking feature of the HAL is that the duration of the A-pulses is robust against variation of the rate constants, whereas the duration of the B-pulses is tuneable. It has been suggested that biological signals may be encoded in time interval between pulses [7–11]. Since the HAL is a robust and flexible pulse generator, it would perfectly fit into this design.

The self-repression motif is highly represented in genetic networks [3]. It would be interesting to see if the HAL, a simple extension of this motif, is also ubiquitous. Known examples of oscillations based on a self-repressing protein $A$ have been attributed to delay or high cooperativity, perhaps sometimes obscuring the implication of a binding partner $B$. A closely related oscillator is the Mixed-feedback loop (MFL) [4], which is also based on a $AB$ dimer formation, but the protein $A$ activates the transcription of gene $G_B$ instead of repressing itself. Interestingly, an analysis of E. coli motifs involving both transcriptional and protein-protein interactions led to the discovery of the MFL but since it excluded self-repression, was not able to detect the HAL [35]. The MFL network motif is overrepresented in Yeast cells [36] and is also at the core of circadian clocks in Mammals, Neurospora or Drosophila [4]. It is natural to expect that the HAL, being closely related to the MFL, is also the core component of some natural biochemical oscillators. Its simplicity, and interesting dynamical properties also make the HAL a promising module for synthetic biology.

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[1] B. Alberts, A. Johnson, J. Lewis, K. Roberts, and P. Walter, *Molecular Biology of the Cell* (Garland Science, New York, 2002).
[2] J. J. Tyson, K. C. Chen, and B. Novak, *Curr. Opin. Cell Biol.* 15, 221 (2003).
[3] U. Alon, *An Introduction to Systems Biology: Design Principles of Biological Circuits* (Chapman and Hall, 2006).
[4] P. Francois and V. Hakim, *Phys. Rev. E* 72, 031908 (2005).
[5] S. Krishna, M. H. Jensen, and K. Sneppen, *Proc. Nat. Acad. Sci. USA* 103, 10840 (2006).
[6] W. Mather, M. R. Bennett, J. Hasty, and L. S. Tsimring, *Phys. Rev. Lett.* 102, 068105 (2009).
[7] B. Mengel, A. Hunziker, L. Pedersen, A. Trusina, M. H. Jensen, and S. Krishna, *Curr. Opin. Genet. Dev.* 20, 656 (2010).
[8] J. E. Purvis and G. Lahav, *Cell* 152, 945 (2013).
[9] G. Lahav, *Adv Exp Med Biol* 641, 28 (2008).
[10] N. Hao and E. K. O'Shea, *Nat. Struct. and Mol. Biology* 19, 31 (2012).
[11] J. Locke, J. Young, M. Fontes, M. Hernández, and M. Elowitz, *Science* 334, 366 (2011).
[12] R. Hermesen, B. Ursem, and P. R. ten Wolde, *PLoS Comput. Biol* 6, e1000813 (2010).
[13] H. Salgado, A. Santos-Zavaleta, S. Gama-Castro, D. Millan-Zarate, E. Diaz-Peredo, F. Sanchez-Solano, E. Perez-Rueda, C. Bonavides-Martinez, and J. Collado-Vides, *Nucleic Acids Research* 29, 72 (2001).
[14] I. M. Keseler, J. Collado-Vides, S. Gama-Castro, J. Ingraham, S. Paley, I. T. Paulsen, M. Peralta-Gil, and P. D. Karp, *Nucleic Acids Research* 33, D334 (2005).
[15] D. Szklarczyk, A. Franceschini, M. Kuhn, M. Simonovic, A. Roth, P. Mingeux, T. Doerks, M. Stark, J. Muller, P. Bork, L. J. Jensen, and C. v. Mering, *Nucleic Acids Res.* 39, D561 (2010).
[16] M. van Dorp, B. Lannoo, and E. Carlon, *Phys. Rev. E* 88, 052722 (2013).
[17] P. E. Morant, Q. Thommen, F. Lemaire, C. Vandermoore, B. Parent, and M. Lefranc, *Phys. Rev. Lett.* 102, 068104 (2009).
[18] W. Mather, M. R. Bennett, J. Hasty, and L. S. Tsimring, *Phys. Rev. Lett.* 102, 068105 (2009).
[19] X. Darzacq, Y. Shav-Tal, V. de Turris, Y. Brody, S. M. Shenoy, R. D. Phair, and R. H. Singer, *Nat. Struct. and Mol. Biology* 14, 796 (2007).
[20] J. R. Chubb, T. Trecq, S. M. Shenoy, and R. H. Singer, *Curr. Biol.* 16, 1018 (2006).
[21] D. M. Suter, N. Molina, D. Gatfield, K. Schneider, U. Schibler, and F. Naef, *Science* 332, 472 (2011).
[22] C. V. Harper, B. Finkenstädter, D. J. Woodcock, S. Friedrichsen, S. Semprini, L. Ashall, D. G. Spiller, J. J. Mullins, D. A. Rand, J. R. E. Davis, and M. R. H. White, *PLoS Biol* 9, e1000607 (2011).
SUPPLEMENTAL MATERIAL

In this document we provide a detailed analysis of various properties of the HAL module.

ANALYSIS OF FULL MODEL

We first consider the full model, which is given by:

$$\begin{aligned}
\frac{d[G]}{dt} &= \omega(1 - [G]) - \alpha[G][A] \\
\frac{d[M]}{dt} &= \mu_M[G] + \mu_M^2(1 - [G]) - \delta_M[M] \\
\frac{d[A]}{dt} &= \mu_A[M] - \delta_A[A] - \gamma_{AB}[A][B] \\
&+ \lambda_{AB}[AB] + \omega(1 - [G]) - \alpha[G][A] \quad (7) \\
\frac{d[B]}{dt} &= \mu_B - \delta_B[B] - \gamma_{AB}[A][B] + \lambda_{AB}[AB] \\
\frac{d[AB]}{dt} &= \gamma_{AB}[A][B] - \lambda_{AB}[AB] - \delta_{AB}[AB]
\end{aligned}$$

Table 6 lists all the reactions of the HAL module shown in Fig. 1 of the main text and the corresponding mass action terms. There are 12 rate constants.

The first equation governs the time evolution of a variable [G] which represents an average gene activity. Even in the cases where gene activity is considered as a stochastic variable alternating between two values (active and inactive), such equations can be derived from moment expansions of the chemical master equation [13]. The form used here is valid when the variances of the stochastic variables can be neglected. If the results of [13] can be transposed here, this would be the case when $\omega/\delta_M \geq 1$.

However, we have checked with stochastic simulations that this heuristic bound is too pessimistic, because the main discrepancy observed for lower values of $\omega/\delta_M$ is only a slightly higher variability in interpulse time intervals. Thus, Equations (7) are adequate for most parameter sets considered in our analysis.

In the limit of fast gene dynamics, the quasi-steady-state approximation $\frac{d[G]}{dt} = 0$ yields $[G] = (1 + \alpha[A]/\omega)^{-1}$. Substituting this in (7), we get a system of four equations:

$$\begin{aligned}
\frac{d[M]}{dt} &= \omega_M + \omega_M^2[A] - \delta_M[M] \\
\frac{d[A]}{dt} &= \mu_A[M] - \delta_A[A] - \gamma_{AB}[A][B] + \lambda_{AB}[AB] \\
\frac{d[B]}{dt} &= \mu_B - \delta_B[B] - \gamma_{AB}[A][B] + \lambda_{AB}[AB] \\
\frac{d[AB]}{dt} &= \gamma_{AB}[A][B] - \lambda_{AB}[AB] - \delta_{AB}[AB]
\end{aligned}$$

which recovers the standard Michaelis-Menten form for the mRNA synthesis.

Figure 7 shows a plot of “phase diagrams” of the system. To compute it we fixed the parameters to the following values $\mu_M^{-1} = 0.5$, $\delta_M^{-1} = 20$, $\mu_A^{-1} = 0.04$, $\mu_B^{-1} = 10^{-2}$, $\delta_A^{-1} = 0.1$, $\delta_{AB}^{-1} = 1000$, $\gamma_{AB} = 10$, $\gamma_{AB}^{-1} = 10$, $\omega^{-1} = 100$.

| Reaction | Reactants | rate $\rightarrow$ | Products | Term |
|----------|-----------|-------------------|----------|------|
| G transcription: | $G$ | $\mu_M$ | $G + M$ | $\mu_M[G]$ |
| M degradation: | $M$ | $\delta_M$ | $\varnothing$ | $\delta_M[M]$ |
| M translation: | $M$ | $\mu_A$ | $M + A$ | $\mu_A[M]$ |
| A degradation: | $A$ | $\delta_A$ | $\varnothing$ | $\delta_A[A]$ |
| B production: | $\varnothing$ | $\mu_B$ | $B$ | $\mu_B$ |
| B degradation: | $B$ | $\delta_B$ | $\varnothing$ | $\delta_B[B]$ |
| G repression: | $G + A$ | $\alpha$ | $G_A$ | $\alpha[G][A]$ |
| G_A deregulation: | $G_A$ | $\omega$ | $G + A$ | $\omega(1 - [G])$ |
| G_A transcription: | $G_A$ | $\mu_M$ | $G_A + M$ | $\mu_M(1 - [G])$ |
| AB complexation: | $A + B$ | $\gamma_{AB}$ | $AB$ | $\gamma_{AB}[A][B]$ |
| AB dissociation: | $AB$ | $\delta_{AB}$ | $A + B$ | $\lambda_{AB}[AB]$ |
| AB degradation: | $AB$ | $\delta_{AB}$ | $\varnothing$ | $\delta_{AB}[AB]$ |

FIG. 6. List of all the biochemical reactions which define the HAL module. By convention rates are denoted by: $\mu$ for production rates, $\delta$ for degradation rates, $\alpha$ for binding rates, $\omega$ for unbinding rates, $\gamma$ for complexation rates and $\lambda$ for dissociation rates. The rightmost column gives the corresponding rates in the differential equations as obtained from mass action kinetics.

$[A]_0 = 1$. Six of these parameters $\alpha$, $\omega$, $\mu_M$, $\mu_A$, $\mu_B$, $\delta_A$ and $\delta_B$ were varied two at a time while keeping four of them fixed (recall that $\alpha = \omega/[A]_0$). This procedure generates $6 \cdot 5/2 = 15$ two dimensional slices of the phase diagram. The analysis consists in numerically integrating Eqs. (7) for every set of input rates while identifying if the solution is pulsating or stationary. The pulsating domain is show as black in Fig. 7. The axes in the phase diagrams in Fig. 7 are in logarithmic scale and each axes covers a variation of two orders of magnitude centered around the values of rates given above. Hence the selected point is rather far from the phase boundaries. Apart from the pulsating solution we distinguish two types of stationary solutions with high A (high B) shown as red (green) in Fig. 7. In these phases one of the two proteins has typically much higher concentration than the other.
FIG. 7. Slices of phase diagrams of the model (7) depicting various phases as a function of the six “most relevant” parameters $\alpha$, $\omega$, $\mu_M$, $\mu_A$, $\mu_B$, $\delta_A$ and $\delta_B$. The black area corresponds to the pulsating domain, while the red (resp. green) to steady states characterized by high concentration of A (resp. B).
Figure 8 (a-d) shows the effect on change of rates on the concentrations of the proteins and mRNA in the pulsating regime. Fig. 8(a) reproduces the same rates as in Fig. 2 of the main paper, while the cases (b), (c) and (d) correspond to variations of a single rate with respect to the case (a). The duration of the A peaks is quite robust against the parameters variation, while $T_b$ varies: (a) $T_a = 2.01$, $T_b = 2.13$ (b) $T_a = 1.71$, $T_b = 3.81$ (c) $T_a = 1.93$, $T_b = 3.48$ and (d) $T_a = 1.76$, $T_b = 3.76$. In the case (b) the $B$ protein synthesis rate $\mu_B$ is doubled with respect of (a). This has a strong effect in the height and duration of the peaks of $B$, but a milder effect on the duration of the peaks of $A$. Halving the value of (c) $\omega$ and of (d) $\mu_A$ is also affecting strongly the peaks of $B$. While the duration of the peaks of $A$ is robust, their height is not. This appears to be mostly affected by a change in $\mu_B$, in agreement with the analysis of the reduced model in the next section.

**ANALYSIS OF THE REDUCED MODEL**

We present here the details of the analytical calculations for the durations of the A and B phases for the reduced model:

\[
\begin{align*}
\frac{dg}{d\tau} &= \Omega(1 - g) - \sigma ga \quad (9a) \\
\frac{dm}{d\tau} &= g - m \quad (9b) \\
\frac{da}{d\tau} &= k_a m - ab \quad (9c) \\
\frac{db}{d\tau} &= k_b - ab \quad (9d)
\end{align*}
\]

As shown in Fig. 2 of the main text two phases can be identified in the pulsating domain: in one phase, $[A]$ is peaked and $[B]$ is small, while in the other phase $[B]$ is peaked and $[A]$ small. We refer to these as to the A-phase and to the B-phase, respectively. The two phases are due to the mutual sequestration of $A$ and $B$. In the A-phase the $G_A$ gene is strongly repressed, the mRNA synthesis is stopped and the mRNA concentration $m(t)$ decreases due to degradation. As long as $k_a m > k_b$ (rescaled variables and parameters, see Eqs. (9c) and (9d)) the production of $a$ dominates over the production of $b$. Once the mRNA concentration drops and $k_a m < k_b$, the production of $B$ becomes dominant and the concentration of $A$ starts decreasing till $A$ is completely sequestered out of the system and the transition to the B-phase is made.

In the B-phase the protein $A$ is released from its gene $G_A$ promoter site. The absence of repression produces a rise in the mRNA concentration; when the mRNA concentration reaches the threshold value $k_a m = k_b$, the concentration of $B$ starts decreasing and one is back to the A-phase again.

We compute now the duration of the two phases. Let us start from the A-phase. A first assumption is that the gene is constantly repressed when the concentration of $A$ is high, hence $g = 0$. We can thus eliminate the variable $g$ from Eq. (9b) to obtain the solution:

\[
m(\tau) = m_A e^{-\tau} \quad (10)
\]

where we set the origin of time $\tau = 0$ at the beginning of the A-phase. Using the same notation as the paper $m_A$ and $m_B$ indicate the mRNA concentrations at the beginning of the A- and of the B-phases. A second assumption is that in the A-phase the concentration of $B$ is stationary hence $db/d\tau = 0$, which implies $k_b = ab$ from Eq. (9d). Substituting this into Eq. (9c) and using (10) we get the following equation for the evolution of $a$:

\[
\frac{da}{d\tau} = k_a m_A e^{-\tau} - k_b \quad (11)
\]

As shown in Fig. 2 of the main text two phases can be identified in the pulsating domain: in one phase, $[A]$ is peaked and $[B]$ is small, while in the other phase $[B]$ is peaked and $[A]$ small. We refer to these as to the A-phase and to the B-phase, respectively. The two phases are due to the mutual sequestration of $A$ and $B$. In the A-phase the $G_A$ gene is strongly repressed, the mRNA synthesis is stopped and the mRNA concentration $m(t)$ decreases due to degradation. As long as $k_a m > k_b$ (rescaled variables and parameters, see Eqs. (9c) and (9d)) the production of $a$ dominates over the production of $b$. Once the mRNA concentration drops and $k_a m < k_b$, the production of $B$ becomes dominant and the concentration of $A$ starts decreasing till $A$ is completely sequestered out of the system and the transition to the B-phase is made.

In the B-phase the protein $A$ is released from its gene $G_A$ promoter site. The absence of repression produces a rise in the mRNA concentration; when the mRNA concentration reaches the threshold value $k_a m = k_b$, the concentration of $B$ starts decreasing and one is back to the A-phase again.

We compute now the duration of the two phases. Let us start from the A-phase. A first assumption is that the gene is constantly repressed when the concentration of $A$ is high, hence $g = 0$. We can thus eliminate the variable $g$ from Eq. (9b) to obtain the solution:

\[
m(\tau) = m_A e^{-\tau} \quad (10)
\]

where we set the origin of time $\tau = 0$ at the beginning of the A-phase. Using the same notation as the paper $m_A$ and $m_B$ indicate the mRNA concentrations at the beginning of the A- and of the B-phases. A second assumption is that in the A-phase the concentration of $B$ is stationary hence $db/d\tau = 0$, which implies $k_b = ab$ from Eq. (9d). Substituting this into Eq. (9c) and using (10) we get the following equation for the evolution of $a$:

\[
\frac{da}{d\tau} = k_a m_A e^{-\tau} - k_b \quad (11)
\]
The solution of the previous equation with initial condition \( a(0) = 0 \) is
\[
a(\tau) = k_a m_A \left( 1 - e^{-\tau} \right) - k_b \tau \tag{12}
\]
which is a function with a single maximum beyond which it decreases monotonically and it becomes negative at long times, which is obviously an unphysical result. We can estimate the duration of the A-phase from the requirement \( a(T_a) = 0 \), which gives:
\[
\frac{T_a}{1 - e^{-T_a}} = \frac{k_a m_A}{k_b} \tag{13}
\]
For the B-phase we assume that the concentration of free \( A \) in solution is very small so that the binding rate to the gene promoter site is negligible. We can approximate Eq. (9a) with \( \frac{dg}{dt} \approx \Omega (1 - g) \), from which we get the following solution:
\[
g(\tau) = 1 - e^{-\Omega (\tau - T_a)} \tag{14}
\]
where we used the initial condition \( g(T_a) = 0 \) in the B-phase, \( T_a \leq \tau \leq T_a + T_b \). We approximate further the previous expression to the first order in the exponential:
\[
g(\tau) \approx \Omega (\tau - T_a) \tag{15}
\]
and which is valid for \( \tau - T_a \ll \Omega^{-1} \). We now plug in the previous expression into Eq. (9b) and solve it to get for the mRNA concentration in the B-phase (\( T_a \leq \tau \leq T_a + T_b \)):
\[
m(\tau) = \Omega \left( \tau - T_a - 1 \right) + e^{-(\tau - T_a)} (m(T_a) + \Omega)
= \Omega \left( \tau - T_a - 1 \right) + e^{-(\tau - T_a)} (m_A e^{-T_a} + \Omega) \tag{16}
\]
where we have used Eq. (10): \( m(T_a) = m_A e^{-T_a} \). We proceed as done for the A-phase. We assume that \( a \) is stationary in the B-phase, i.e. \( da/d\tau = 0 \) which yields \( k_a m = ab \) (Eq. 9c). Substituting this result in Eq. (9d) we get the following Equation for the growth of \( b \):
\[
\frac{db}{d\tau} = k_b - k_a m \tag{17}
\]
with \( m(\tau) \) given by Eq. (16). Using the initial condition \( b(T_a) = 0 \) we get:
\[
b(\tau) = k_b \left( \tau - T_a \right) - k_a \Omega \left[ \frac{1}{2} (\tau - T_a)^2 - (\tau - T_a) \right]
- k_a \left( 1 - e^{-(\tau - T_a)} \right) (m_A e^{-T_a} + \Omega) \tag{18}
\]
We obtain the length of the B-phase from the requirement that \( b(T_a + T_b) = 0 \), which leads to the following relation
\[
(k_b + k_a \Omega) T_b = \frac{1}{2} k_a \Omega T_b^2 + k_a \left( 1 - e^{-T_b} \right) (m_A e^{-T_a} + \Omega) \tag{19}
\]
An additional relation is obtained by requiring that at the end of B-phase: \( m(T_a + T_b) = m_A \) which yields from Eq. (16):
\[
m(0) = \Omega \frac{-1 + T_b + e^{-T_b}}{1 - e^{-T_a - T_b}} \tag{20}
\]
Inserting the previous equation in Eq. (13) we get:
\[
\frac{T_a}{e^{T_a} - 1} = \beta \frac{-1 + T_b + e^{-T_b}}{1 - e^{-T_a}} \tag{21}
\]
where we defined \( \beta = k_a \Omega/k_b \). We now use Eq. (13) to get an expression for \( m(0) \) which we substitute in (19) to get:
\[
\frac{T_a}{e^{T_a} - 1} = \beta \left( \frac{T_b - T_b^2}{2} \right) + T_b \frac{1 - e^{-T_b}}{e^{T_a} - 1} - \beta \tag{22}
\]
We also note that for \( \beta = 2 \) the exact solution of Eqs. (21) and (22) is \( T_a = T_b = 2 \), i.e. the two phases have equal duration. For \( \beta > 2 \) (\( \beta < 2 \)) one has \( T_a > T_b \) (\( T_a < T_b \)). In terms of the original kinetic constants, the parameter \( \beta \) reads:
\[
\beta = \frac{\omega \mu_M \mu_A}{\mu_B \delta_M} \tag{23}
\]
It characterizes the relative importances of the A-phase and B-phase. The A-phase dominates if \( \omega \) (unbinding rate of the repressor A from its gene), \( \mu_M \) (mRNA synthesis rate) or \( \mu_A \) (protein A synthesis rate) are large. The B-phase is favored when \( \mu_B \) (protein B synthesis rate) or \( \delta_M \) (mRNA degradation rate) are large.

**On the robustness of \( T_a \)**

We analyze now the dependence of \( T_a \) and \( T_b \) on \( \beta \). Eq. (22) is of the form
\[
f_1(T_a) = f_2(T_b, \beta) \tag{24}
\]
where \( f_1 \) and \( f_2 \) are the following functions:
\[
f_1(x) = \frac{x}{e^x - 1} \tag{25}
\]
and
\[
f_2(x, \beta) = \beta \frac{(x - x^2/2) + x}{1 - e^{-x}} - \beta \tag{26}
\]
For any \( x > 0 \), the function \( f_1(x) \) satisfies \( 0 < f_1(x) < 1 \). This implies that \( T_b, \) solution of (21) and (22) must be such that \( 0 < f_2(T_b, \beta) < 1 \).

Figure 9 shows a plot of \( f_2(x, \beta) \) for two values of \( \beta \). For large values of \( x \), the function becomes negative and its value satisfies \( 0 < f_2(T_b, \beta) < 1 \) only for a limited
range of $x$. This range varies strongly with $\beta$, which implies a variation of $T_b$ with $\beta$. The analysis of $f_2(x, \beta)$ shows that $\lim_{\beta \to 0} T_b = \infty$ and $\lim_{\beta \to \infty} T_b = 0$.

To proceed further we combine (21) and (22) to eliminate $\beta$. We obtain:

$$\frac{T_a}{e^{T_a} - 1} = \frac{T_b}{1 - e^{-T_b} - \frac{T_b - 1}{-1 + e^{-T_b}} (e^{T_a} - e^{-T_b})}$$  \hspace{1cm} (27)

In the limit $T_b \to 0$ (large $\beta$) the previous relation becomes:

$$\frac{T_a}{e^{T_a} - 1} = \frac{3}{e^{T_a} + 2}$$  \hspace{1cm} (28)

which has as unique solution $T_a \approx 2.149$. In the opposite limit $T_b \to \infty$ we get from (27):

$$\frac{T_a}{e^{T_a} - 1} = 2 e^{-T_a}$$  \hspace{1cm} (29)

which has as solution $T_a \approx 1.594$. Hence this analysis shows that while $T_b$ is unbounded and assumes any positive values when $\beta$ is varied, $T_a$ is bounded in the interval $[1.594, 2.149]$. As discussed in the paper, the changes in rate constants which could potentially affect $T_a$ are compensated by a change in $m_A$, the mRNA concentration at the beginning of the A-phase, such that the ratio

$$c \equiv \frac{k_a m_A}{k_b}$$  \hspace{1cm} (30)

remains constant. Using Eq. (13), we can compute a range for $c$ using the estimated range of values of $T_a$. The result is $2 \leq c \leq 2.4$.

**On the amplitude of $A$**

One can get some insights on the amplitude of $A$ from the analysis of the simplified model. The maximum of $\alpha$ is obtained from Eq. (12):

$$\max_{\tau} \alpha = k_b \left( \frac{k_a m_A}{k_b} - 1 - \log \frac{k_a m_A}{k_b} \right)$$  \hspace{1cm} (31)

which shows that this quantity is not robust. Indeed, we have shown that the solution of Eqs. (21) and (22) are such that the ratio $\beta$ is is bounded and assumes any positive values when $\beta$ is varied, $T_a$ is bounded in the interval $[1.594, 2.149]$. As discussed in the paper, the changes in rate constants which could potentially affect $T_a$ are compensated by a change in $m_A$, the mRNA concentration at the beginning of the A-phase, such that the ratio

$$c \equiv \frac{k_a m_A}{k_b}$$  \hspace{1cm} (30)

remains constant. Using Eq. (13), we can compute a range for $c$ using the estimated range of values of $T_a$. The result is $2 \leq c \leq 2.4$.

**STOCHASTIC ANALYSIS**

We extended the analysis of the HAL to the stochastic regime, performing simulations using the Gillespie algorithm [32]. Typical outputs of these simulations are given in Fig. 5 of the main text, which shows that the protein concentrations evolve through peaks of variable duration and height due to stochastic fluctuations. To quantify the variability in the dimensionless durations of the two phases, we studied their probability distribution for two different parameter sets, as shown in Figure 10. The two top graphs are obtained using the parameter values $\mu_M^1 = 1.11$, $\delta_M^1 = 16.67$, $\mu_A^{-1} = 0.59$, $\mu_B^{-1} = 0.05$, $\delta_A^{-1} = \delta_B^{-1} = 10^3$, $\delta_A^0 = 10$, $\gamma_A^0 = 0.02$, $\omega^{-1} = 100$, $[A]_0 = 1$ (and $\lambda_A$ and $\mu_A$ fixed as in Table I). These constants correspond to a value of $\beta = 0.21$. The two bottom graphs are generated using the same rates except...
TABLE II. Average durations obtained from (i) stochastic simulations of the chemical reactions in Fig. 6, (ii) deterministic simulation of Eqs. (7) and (iii) Eqs. (21) and (22) (analytical approximation).

| β       | Quantity | Stochastic | Deterministic | Analytical |
|---------|----------|------------|---------------|------------|
| 0.21    | ⟨Ts⟩    | 1.47       | 1.30          | 1.70       |
|         | cv(Ts)  | 0.49       |               |            |
|         | ⟨Tb⟩    | 1.68       | 1.48          | 10.00      |
|         | cv(Tb)  | 0.60       |               |            |
| 1.91    | ⟨Ts⟩    | 1.71       | 1.44          | 1.98       |
|         | cv(Ts)  | 0.44       |               |            |
|         | ⟨Tb⟩    | 0.51       | 0.35          | 0.50       |
|         | cv(Tb)  | 0.83       |               |            |

for δ_M⁻¹ = 50, which corresponds to β = 1.91. We note that β strongly influences the duration of the peaks of B; conversely the distribution of Ts is only weakly affected while β is varied of almost an order of magnitude. This is consistent with the deterministic analysis developed in the main text.

The average values ⟨Ts⟩ and coefficients of variation cv(Ts) = σ(Ts)/⟨Ts⟩, where σ(Ts) is the standard deviation of Ts, are given in Table II which compares them to the values of Ta and Tb obtained from the deterministic simulations of the full model [Eqs. (7)], as well as from the analytical approximation [Eqs. (21) and (22)]. The values obtained confirm that Ta is much less sensitive than Tb to β. For each value of β and each average duration, the agreement between the three estimates is reasonable except for ⟨Tb⟩ at low β. This seems to indicate that for some parameter sets with a low value of β, the analytical approximation severely overestimates Tb, perhaps because it misses an ingredient leading to a faster dynamics. However, this does not affect our conclusion that Ta is relatively constant, nor that Tb is largely tunable. Table II also shows that the stochastic variability affects more the duration of the B phase than that of the A phase.

Summarizing, the stochastic analysis of the system supports the conclusions drawn from the study of the deterministic model: while the A-phase appears to be rather robust, the duration of the B-phase is tunable and more subject to stochastic fluctuations. One interesting issue to be left for future investigations is whether it is possible to find simple extensions of this genetic module for which the stochastic fluctuations in Ta can be further reduced.