**Dimer dissociation constant *in vitro* and *in vivo***

The dimer dissociation constant $K_d$ is defined as the ratio of the concentrations of the monomers ($p_1$) and dimers ($p_2$) in steady state, i.e., $K_d = p_1^2/p_2$. In terms of the basic kinetic parameters (the association rate $k_a$ and the dissociation rate $k_d$) the *in vitro* dissociation constant is simply $K_d^{(0)} = k_d/k_a$. However, the effective value of $K_d$ is modified *in vivo* due to a variety of processes, including those that change the rate constants $k$. Even if the rate constants do not change, $K_d$ will also be affected by proteolysis or dilution, which alter the steady-state cellular protein concentrations. In the latter case, the combined effects of all these processes can be described at the level of chemical kinetics by the equations

$$\frac{dp_1}{dt} = S - \lambda_{p_1} \cdot p_1 - 2k_a p_1^2 + 2k_d p_2$$  \[5\]

$$\frac{dp_2}{dt} = -\lambda_{p_2} \cdot p_2 + k_a p_1^2 - k_d p_2$$  \[6\]

where $S$ is the protein synthesis rate and $\lambda_{p_1}, \lambda_{p_2}$ are the monomer, dimer degradation rates respectively; see the main text. The steady-state solution of Eq. 6 is $(\lambda_{p_2} + k_d) \cdot p_2 = k_a p_1^2$, which according to the definition of the dissociation constant given above, yields an expression for the *in vivo* dissociation constant

$$K_d = \frac{k_d + \lambda_{p_2}}{k_a} = K_d^{(0)} + \frac{\lambda_{p_2}}{k_a}.$$  \[7\]

Note that the *in vivo* $K_d$ is independent of $\lambda_{p_1}$, hence it does not depend on whether the protein is cooperatively stable (i.e., whether $\lambda_{p_1}$ and $\lambda_{p_2}$ are equal or different). As shown in Eq. 7, $K_d$ is larger than the corresponding *in vitro* value $K_d^{(0)}$. Assuming that many dimers are diluted by cell division in the rapid exponential growth phase ($\sim$ 50 min half-life), then we can use typical monomer-monomer association rate of $k_a^{-1} \sim 20$ nM-min for small proteins (60,61) to estimate a typical order of magnitude for the offset $\lambda_{p_2}/k_a$ to be $\sim 0.3$ nM. Shifts of this magnitude in $K_d$ are
marginally relevant for typical TFs with $K_d^{(0)} \sim 10 \text{nM}$, but they can become quite significant for larger proteins (e.g., enzymes) that have smaller $k_a$ or proteins that are rapidly degraded by proteolysis, see Table 2.

**Thermodynamic models of transcriptional control**

In the text, we used the effective Hill functions

$$g_a([TF]) \approx \frac{f^{-1} + ([TF]/\kappa)^n}{1 + ([TF]/\kappa)^n}$$  \[8\]

$$g_a([TF]) \approx \frac{1 + ([TF]/\kappa)^n/f}{1 + ([TF]/\kappa)^n}$$  \[9\]

to describe the activities of the promoters shown in Fig. 1c-f. In this description, promoter activity is effectively characterized by three numbers, see Fig. 3: (i) $f$ which defines the maximum fold-change in promoter activity, (ii) $\kappa$ which indicates the concentration that separates the HIGH plateau from the transition region, and (iii) the “Hill coefficient” $n$ which describes the transcriptional cooperativity in the transition region. Here, we use a more realistic description of the promoter activity based on the thermodynamic models of transcriptional initiation (11-14), and relate the effective parameters used in Eqs. 8 and 9 to the biochemical parameters controlling protein-DNA and protein-protein interactions. We stress that our results which follow all implicitly presume that the promoters of interest are sufficiently weak (13,14).

**Simple activation and repression.** In the thermodynamic description, the promoter activity is assumed to be proportional to the equilibrium probability $P$ that the RNA polymerase (RNAP) binds to the core promoter. The dependence of $P$ on cellular TF concentrations ($TF$) for the four cis-regulatory architectures shown in Fig. 1 has been calculated elsewhere (13,14). The single activator (Fig. 1c) and repressor (Fig. 1e) promoters have the forms

$$P_{act}([TF]) \propto \frac{1 + \omega_{a-p} \cdot [TF]/K_a}{1 + [TF]/K_a}$$  \[10\]
\[ P_{\text{rep1}}([TF]) \propto \frac{1}{1 + [TF] / K_R + L} \]  \[11\]

In the above equations, \( K_A \) and \( K_R \) refer to the dissociation constant between the TF and the respective operator sequence in vivo, \( \omega_{A-P} = \exp(-\Delta G_{A-P} / RT) \) is the Boltzmann weight of the activator-RNAP interaction, and \( L \) describes the effect of “promoter leakage” in repression which is expected to occur even for \([TF] \rightarrow \infty^*\). Since the effective promoter activity functions \((g_A, g_R)\) shown in Eqs. 8 and 9 are simply \( P_{\text{act1}} \), \( P_{\text{rep1}} \) normalized by their maximum values, one can compare Eqs. 8, 9 with 10, 11 to yield \( n=1 \) for both cases, with \( f = \omega_{A-P} \) and \( \kappa = K_A \) for the activator, and \( f = L^{-1} \) and \( \kappa = K_R \) for the repressor.

An important feature of the promoter activity function in the context of genetic circuits is the sensitivity \( s \), which is defined as the absolute value of the log-log slope of \( g([TF]) \), i.e.,

\[
s_A = \frac{d \ln g_A}{d \ln [TF]}. \tag{12}
\]

\[
s_R = -\frac{d \ln g_R}{d \ln [TF]}. \tag{13}
\]

They are plotted in Fig. 5a as a function of \([TF] / \kappa\) for different values of \( f=10, 100, 1000 \) for the activator and the repressor. Note that the maximum value of \( s([TF]) \), referred to here as \( s^* \), occurs at the mid point of the transition region (see Fig. 3) and is strongly dependent on the magnitude of the fold-change \( f \). As shown in Fig. 5b, even at \( f = 100 \), \( s^*=0.82 \) is still below the theoretical maximum set by \( n=1 \). Since large \( f \) is more readily available for repressors (due to the strong exclusion interaction between RNAP and the promoter-bound repressor), it is easier to attain large sensitivity using repressor-controlled promoters.

**Cooperative activation.** For the promoter shown in Fig. 1d involving two operators for the activator, the corresponding promoter occupancy probability (13,14) is:

* For instance, this may be due to the collision of the replication fork with the repressed promoter which occurs at least once per cell cycle.
$P_{act2}([TF]) \propto \frac{1 + [TF]/K_H + \omega_{A-P} \cdot [TF]/K_A + \omega_{A-A} \cdot \omega_{A-P} \cdot [TF]^2 / (K_A \cdot K_H)}{1 + [TF]/K_A + [TF]/K_H + \omega_{A-A} \cdot [TF]^2 / (K_A \cdot K_H)}$ \[14\]

where $K_H$ refers to the in vivo dissociation constant of the TF to upstream “helper” operator, and $\omega_{A-A}$ is the Boltzmann weight associated with activator-activator interaction. The form (Eq. 14) is clearly different from the Hill form (Eq. 8), and the values of the effective Hill parameters $n$ and $\kappa$ will necessarily depend on the actual values of the parameters $K_A$, $K_H$, $\omega_{A-P}$, and $\omega_{A-A}$. In the main text, this promoter was used to model the $P_{RM}$ promoter of phage lambda studied experimentally by Issacs et al (8). For this case, the primary and helper activator sites are the operators $O_{R2}$ and $O_{R1}$ respectively whereas we neglected the very weak repressive site $O_{R3}$ (62,63). The TF, lambda repressor protein CI, can stimulate transcription while bound at $O_{R2}$†; the same TF can interact cooperatively with an adjacent TF bound upstream at $O_{R1}$ (62).

It is well known that the transcriptional stimulation provided by CI at $P_{RM}$ is weak, with $\omega_{A-P} \approx 11$ (64). The in vivo values of the CI-operator dissociation constants $K_A$ and $K_H$ are not known, but their ratio is expected to be equivalent to the ratio of the in vitro dissociation constants. The latter have been measured, together with the CI-CI affinity $\omega_{A-A}$. There are two sets of parameter values commonly used in the phage lambda literature: (i) Shea & Ackers (1985) (11) where $K_A/K_H \approx 17$ and $\omega_{A-A} \approx 25$, and (ii) Koblan & Ackers (1992) (65) where $K_A/K_H \approx 25$ and $\omega_{A-A} \approx 100$. In Fig. 6a, we plot the function $P_{act2}$ (normalized by its maximum value) against $[TF]/K_H$ for the two sets of parameters (the solid black and grey lines). The two curves are then fit to the effective Hill form (Eq. 8) to extract the effective parameters: $n \approx 1.53$ and $\kappa \approx 1.22K_H$ for SA85; and $n \approx 1.69$ and $\kappa \approx 0.64K_H$ for KA92; see the dashed lines in Fig. 6a. In Fig. 6b, we plot the sensitivity function $s_A([TF])$ for the two cases. We see that despite the improved Hill cooperativity for this promoter $(n \approx 1.5 - 1.7)$, the marginal fold-change $f = \omega_{A-P} = 11$ in activation limits the maximum sensitivity to $s_A^* < 1$.

† The stimulation of transcription by TFs (i.e. activation) typically described in thermodynamic models involves the “thermodynamic recruitment” of RNAP by the activator via a mutual attractive interaction (25). Transcriptional stimulation by CI involves instead catalyzing the rate of RNAP-promoter isomerization from a closed inactive form to an open active form (64). This change in isomerization rate, $\Delta k_{iso}$, is mathematically equivalent to “thermodynamic recruitment” with $\omega_{A-P} \approx \Delta k_{iso}$.
Dual repression. For the promoters controlled by two repressor sites (Fig. 1f), the corresponding activity function (13,14) is given by

\[ P_{\text{rep}}(\mathcal{T}F) \propto \frac{1}{(1 + [\mathcal{T}F] / K_{R1}) \cdot (1 + [\mathcal{T}F] / K_{R2}) + L} \]  \[ \text{[15]} \]

where \( K_{R1} \) and \( K_{R2} \) refer to the in vivo dissociation constants for the two operators, and \( L \) again describes promoter leakage. Examples of such cis-regulatory constructs are the very strongly repressively controlled promoters constructed by Lutz & Bujard (27) with very low leakiness. In Fig. 7a, we plot the relative activity function for \( K_{R1} = K_{R2} \), and \( L = 10^{-3} \) (solid line). The dashed line is the best fit to the Hill form (Eq. 9), with the effective parameters \( n = 1.63, \kappa \approx 0.52 K_{R1} \), and \( f = L^{-1} \). The sensitivity function \( s_R([\mathcal{T}F]) \) plays an important role in the ring-oscillator circuit (as will be shown below); it is plotted in Fig. 7b. Owing to the large \( f \), \( s_R^* \) is in this case close to its maximal theoretical value set by the Hill coefficient \( n = 1.63 \).

Bistability

This genetic circuit shown in Fig. 1a consists of a single gene, which encodes a protein that homodimerizes and activates its own transcription. The steady-state occurs when degradation and synthesis rates are balanced, and is given by Eq. 4 or more conveniently by

\[ \left( \frac{\gamma}{\kappa \cdot \lambda_{p2}} \right) \cdot g_A(p_2^*) = \frac{\lambda_{p1}}{\lambda_{p2}} \cdot \sqrt{\frac{K_d \cdot p_2^*}{\kappa}} + 2 \frac{p_2^*}{\kappa} \]  \[ \text{[16]} \]

where \( p_2^* \) is the steady-state TF dimer concentration. Given the form of the promoter activity function \( g_A \), the above equation can be solved to yield the stable solutions \( p_2^*/\kappa \) as a function of the dimensionless, programmable parameters, \( \gamma/\kappa \cdot \lambda_{p2} \) and \( K_d/\kappa \) for different choices of \( \lambda_{p1}/\lambda_{p2} \).

For the promoter containing a single operator site (Fig. 1c), \( g_A(p_2^*) \) is given by Eqn. 8 with \( n = 1 \) as discussed above. Solving Eqn. 16 using Mathematica 4.2 (66), we obtain various regimes of parameter space supporting bistability. Such regimes are plotted in Fig. 4a for \( f = 100 \) and \( \lambda_{p1}/\lambda_{p2} = 1,3,10 \).
The origin of the beneficial effects of cooperative stability (see “Benefit” section in Supp. Info.) is that it extends the regime of protein concentrations where cooperativity is obtained through dimerization from \( p < K_d \) where most of the proteins are monomers, to \( p < K_d \cdot \left( \frac{\lambda_{p1}}{\lambda_{p2}} \right)^2 \), see Eq. 16. This effectively decreases the value of \( K_d \) needed for bistability by a factor of \( \left( \frac{\lambda_{p1}}{\lambda_{p2}} \right)^2 \). The grey and hatched regions in Fig. 4a can be viewed as the black region with an appropriate reduction in \( K_d \). Moreover, for the case shown in Fig. 4d with \( K_d = 10 \text{ nM} \) and 10-fold cooperative stability, the dimer concentrations (black curves) are identical to those in Fig. 4c, which were obtained for \( K_d = 1000 \text{ nM} \) and \( \lambda_{p1} = \lambda_{p2} \). The same black curve could also be obtained for \( K_d = 100 \text{ nM} \) and approximately a 3-fold effect in cooperative stability (not shown). Due to this large \( \left( \frac{\lambda_{p1}}{\lambda_{p2}} \right)^2 \)-fold reduction in \( K_d \), we expect a few-fold effect in cooperative stability to exert a large impact on circuit function.

The above procedure is repeated for the promoter with double operators (Fig. 1d) which we use as a model of the P_{RM} promoter studied in the experiment of Issacs et al (8). The corresponding promoter activity function is \( g_A \) as sketched in Fig. 6a and described by the approximate Hill form (Eq. 8) with \( f \approx 11 \) and \( n \approx 1.7 \) (i.e. [KA92]). In Fig. 8, we again plot the bistable region in the space of the two programmable parameters \( \frac{\gamma}{\kappa} \cdot \frac{1}{\lambda_{p2}} , \frac{K_d}{2\kappa} \) for \( \lambda_{p1}/\lambda_{p2} = 1 \). Despite the improved Hill coefficient for this promoter when compared to the single operator promoter discussed above, the regime of bistability for both cases are similarly limited (compare the black band in Fig. 8 and Fig. 4a). This is a consequence of the small fold-change \( (f) \) involved in auto-activation of the P_{RM} promoter studied by Isaacs et al. (8).

**Oscillation**

For the repressillator circuit of Fig. 1b, we have 3 genes whose mRNA and (total) protein concentrations are denoted by \( m^{(i)} \) and \( p^{(i)} \) with \( i \in \{1,2,3\} \). An approximate picture of the circuit behavior can be obtained by assuming that the 3 genes have the same properties, e.g., the same promoter structure and the same synthesis/turnover rates (9). The circuit topology of Fig. 1b then leads to the following kinetic equations.
\[
\frac{d}{dt} m^{(i)} = \alpha g_R \left( p_2^{(i-1)} \right) - \lambda_m m^{(i)} \tag{17}
\]

\[
\frac{d}{dt} p^{(i)} = \nu m^{(i)} - \Delta \left( p_2^{(i)} \right) \tag{18}
\]

In Eq. 17, the promoter activity function \( g_R \left( p_2^{(i-1)} \right) \) is given by Eq. 9 and the functional TFs are again dimers (of concentration \( p_2^{(i)} \) for each species \( i \)), with \( p_2^{(0)} = p_2^{(3)} \) completing the circuit loop.

We assume rapid equilibration between the monomers and dimers, such that the dimer and total protein concentrations are related by the condition

\[
p = \sqrt{K_d \cdot p_2} + 2p_2 \tag{19}
\]

for each species. According to the spirit of our approximation, these dimers all have the same \( K_d \). In Eq. 18, we introduced the protein degradation function

\[
\Delta = \lambda_p 1 \sqrt{K_d \cdot p_2} + 2\lambda_{p2} p_2 \tag{20}
\]

which gives the total protein degradation rate.

To find the condition for oscillation for the system defined by Eqs. 17-20, we follow the analysis of Elowitz (67) and first solve for the steady-state concentrations \( \{ m^{(i)} = m^*, p_2^{(i)} = p_2^* \} \) such that the left-hand side of Eqs. 17-18 are zero. This is given by the condition

\[
\gamma \cdot g_R \left( p_2^* / \kappa \right) = \Delta \left( p_2^* \right) \tag{21}
\]
where \( \gamma = \frac{\alpha \cdot V}{\lambda_m} \). We then analyze small perturbations about this steady state and find the condition where undamped oscillatory solutions emerge (68). This amounts to finding the purely imaginary eigenvalues of the following Jacobian \(^{1}\):

\[
J = \begin{pmatrix}
-1 & 0 & 0 & 0 & -x \\
1 & -y & 0 & 0 & 0 \\
0 & -x & -1 & 0 & 0 \\
0 & 0 & 1 & -y & 0 \\
0 & 0 & 0 & -x & -1 \\
0 & 0 & 0 & 0 & 1 -y
\end{pmatrix}
\]  

[22]

where \( x = -\frac{\gamma}{\lambda_m} \cdot \frac{d}{dp} g_r \left( p_2(p) \right) \bigg|_{p^*} \), \( y = \lambda_m^{-1} \cdot \frac{d}{dp} \Delta \left( p_2(p) \right) \bigg|_{p^*} \), and \( p_2(p) \) is given by Eq. 19. The only acceptable solution of this system is given by the condition

\[
\frac{(y+1)^2}{y} = \frac{3(x/y)^2}{4 - 2(x/y)}
\]  

[23]

which can be alternatively expressed as \( x/y = \psi(y) \) where

\[
\psi(y) = \frac{(1+y)^2}{3y} \left( \sqrt{1 + \frac{12y}{(1+y)^2}} - 1 \right).
\]  

[24]

It is straightforward to show that the steady-state solution is stable whenever \( x/y < \psi(y) \). Thus oscillation is not possible in this regime. For \( x/y > \psi(y) \), we verified that the system remains in an oscillatory state by direct numerical integration of the kinetic Eqs. 17 and 18. The condition \( x/y = \psi(y) \) defines the boundary of the oscillatory phase. The function \( \psi(y) \) is plotted in Fig. 9 and the oscillatory region is hatched.

\(^1\) We have rewritten Eqs. 17-18 where the mRNA concentration and time have been rescaled to \( m \cdot \sqrt[\lambda_m]{} \) and \( t \cdot \lambda_m \).
From the form of $\psi(y)$, it is clear that no oscillation is possible if $x/y < 4/3$. For $x/y > 2$, oscillation will occur independently of $y$. For the intermediate range $4/3 < x/y < 2$, oscillation is most favorable for $y \approx 1$, i.e. the minimum of $\psi(y)$. To interpret the physical meaning of these conditions, it is useful to note the relation

$$\frac{x}{y} = \frac{s_r(p_2^*)}{r(p_2^*)}$$

[25]

where $s_r \equiv -\frac{d \ln g_r}{d \ln p_2|_{p_1^*}}$ is the sensitivity function, and $r \equiv \frac{d \ln \Delta}{d \ln p_2|_{p_1^*}}$ is the log-log slope of the degradation function defined in Eq. 20. Eq. 25 shows that the function (i.e. oscillation) of the circuit is intimately related to the sensitivity involved in transcription control and degradation. Since $1/2 \leq r \leq 1$, $y$-independent oscillation will always occur if $s_r(p_2^*) > 2$. However, this is hardly satisfied for typical promoters. In the regime where $r \approx 1/2$ (i.e., the degradation flux in Eq. (20) is predominantly through monomer loss such that the nonlinearity in dimerization is harnessed), $y$-independent oscillation will always occur if $s_r(p_2^*) > 1$. This sensitivity is achievable by a promoter with two repressor sites (see Fig. 7b), but not for a promoter with a single repressor site since $s_r < n = 1$ (Fig. 5). For the latter promoter, $y$-dependent oscillation can still occur but now $s_r(p_2^*) > 2/3$ and $y$ must be close to 1 (see Fig. 9).

The value of $y$ is given explicitly by the model parameters as

$$y = \left(\frac{\lambda_{p1}}{\lambda_m \sqrt{\frac{K_d}{p_2^*} + 2 \frac{\lambda_{p2}}{\lambda_m}}}\right) / \left(\sqrt{\frac{K_d}{p_2^*} + 2}\right)$$

[26]

There are three regimes for $y$ (see next section, “Benefit”): (i) when $p_2 \ll K_d$, then $y = \lambda_{p1}/\lambda_m$, and oscillation is most favorable in this regime if the mRNA and monomer degradation rates are

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8 This relation can be derived starting from the definition of $x$ and $y$, by applying the chain-rule of differentiation and invoking the steady-state condition (Eqn. 21).
comparable (where $y \approx 1$). (ii) when $K_d \ll p_2^* \ll \left(\frac{\lambda_{p1}}{\lambda_{p2}}\right)^2 K_d$, then $y \approx \frac{\lambda_{p1}}{\lambda_m} \sqrt{K_d/p_2^*}$, and depending on the steady-state dimer concentration $p_2^*$, $y$ spans the range $\lambda_{p2}/\lambda_m < y < \lambda_{p1}/\lambda_m$. (iii) when $p_2^* \gg \left(\frac{\lambda_{p1}}{\lambda_{p2}}\right)^2 K_d$, then $y = \lambda_{p2}/\lambda_m$.

From our analysis, oscillation is favored when (1) there is a large cooperativity/nonlinearity in synthesis ($s_R > 1$) and/or degradation ($r < 1$), (2) the fold-change $f$ is large, so that $s_R$ is close to the Hill coefficient $n$, and (3) the protein monomer and mRNA turnover rates are comparable ($y \approx 1$).

From the above analysis, we see that cooperative stability (i.e., $\lambda_{p1}/\lambda_{p2} > 1$) improves the condition for oscillation in two ways: first, by increasing the regime of parameter space that is governed by $r \approx 1/2$, and second, by bringing the monomer degradation rate closer to mRNA degradation, where $y \approx 1$.

**Circuit without cooperative stability.** We examine in detail promoters with a single repressive operator site with $n = 1$ (see Fig. 1e) and no cooperative stability ($\lambda_p \equiv \lambda_{p1} = \lambda_{p2}$). We plot the region of spontaneous oscillation as a function of the programmable parameters ($y/\kappa \cdot \lambda_{p2}^{-}, K_d/\kappa$) for different fixed ($\lambda_{p1}/\lambda_{p2}, \lambda_{p2}/\lambda_m$) using $s_R$ for the promoter containing a single repressor site with $n = 1$ and $f' = 100,1000$. The contours of oscillatory parameter space shown in Fig. 10 were generated using Mathematica 4.2 (66) by first numerically solving Eq. 21 to obtain $p_2^*$ for each set of parameters, then determining $y$ and $x/y$ according to their definitions, e.g., see Eq. 22, and finally comparing to the oscillatory condition $x/y > \psi(y)$.

For $\lambda_p \equiv \lambda_{p1} = \lambda_{p2}$, we show in Fig. 10a the oscillatory parameter regime for typical ($f = 100$) and exceptionally strong ($f = 1000$) repressors, paired with either typical ($\lambda_p / \lambda_m = 0.1$) or rapid ($\lambda_p / \lambda_m = 1$) protein turnover. Even for the most favorable combination, i.e. $\lambda_p / \lambda_m = 1$ and $f = 1000$, oscillation is not possible until $K_d / \kappa > 100$. Thus, for typical $K_d \sim 10$ nM and typical $\kappa$ of $1 \sim 1000$ nM, this system cannot sustain oscillations. In the experiment of Elowitz & Leibler (9), oscillation
was obtained by (i) adding ssrA-tags to the TFs so that they degrade much faster to make the protein
and mRNA turnover rates comparable, and (ii) using some of the strongest repressive promoters
known (27). The latter not only makes \( f > 1000 \), but the multiple repressive operator sites (shown in
Fig. 1f) increase the value of the Hill-like coefficient associated with transcriptional initiation to
\( n \approx 1.63 \) (see Fig. 7b), thereby further broadening the accessible parameter space. While this
“extreme” solution found by Elowitz & Leibler (9) is a triumph of synthetic engineering, it would be
desirable to obtain a functional circuit already with typical components, e.g. for TFs with
\( \frac{\lambda_p}{\lambda_m} \sim 0.1 \) and promoters consisting of a single repressive operator site with \( f \leq 100 \).

**Circuit with cooperative stability.** By destabilizing the monomers with respect to the dimer species,
cooperative stability has two effects: First, as in the case of the self-activating one-gene switch, it
extends the regime where cooperativity is obtained through dimerization from \( p < K_d \)
to \( p < K_d \cdot \left( \frac{\lambda_{p1}}{\lambda_{p2}} \right)^3 \) (see next section), thereby making the oscillatory regime more accessible to
TFs with smaller \( K_d \). Second, the condition for oscillation favors the *monomer* degradation rate be
closer to the mRNA degradation rate; see Eq. 26. For typical gene products with \( \frac{\lambda_{p2}}{\lambda_m} \sim 0.1 \), a 10-
fold effect in cooperative stability puts \( y \approx 1 \), thereby further extending the oscillatory regime. This
is illustrated in Fig. 10b, where we specifically plotted the oscillatory regime for \( \lambda_{p1} = \lambda_m = 10\lambda_{p2} \)
with both \( f = 100 \) and \( f = 1000 \). In comparison to the circuit without cooperative stability (Fig.
10a), oscillation is now possible with typical molecular components.

**The benefit of cooperative stability**

We now seek to further clarify the mechanism by which cooperative stability can improve the
function of genetic circuits. To this end, let us consider the steady-state protein abundances of both
monomers, \( p_1^* \), and dimers, \( p_2^* \), as a function of the protein synthesis rate \( S \) (which is
proportional to the underlying mRNA concentration). In the steady state, the fluxes of protein
synthesis and protein degradation are equal,

\[
S = (\lambda_{p1} \cdot p_1^* + 2\lambda_{p2} \cdot p_2^*) ,
\]  
[27]
and the dimerization reaction is in equilibrium, \((p_1^*)^2 = K_d \cdot p_2^*\). In the presence of cooperative stability \((\lambda_{p1} > \lambda_{p2})\), there are then three regimes:

(i) When \(S \ll \frac{K_d \lambda_{p1}}{2}\), monomer degradation is dominant \((\lambda_{p1} \cdot p_1^* \gg 2\lambda_{p2} \cdot p_2^*)\) and the total protein abundance is proportional to the monomer abundance \((p^* \approx p_1^*)\).

(ii) When \(\frac{K_d \lambda_{p1}}{2} \ll S \ll \frac{K_d \lambda_{p1}}{2} \left(\frac{\lambda_{p1}}{\lambda_{p2}}\right)\), monomer degradation is still dominant, but the total protein abundance is now proportional to the dimer concentration \((p^* \approx 2p_2^*)\).

(iii) When \(S \gg \frac{K_d \lambda_{p1}}{2} \left(\frac{\lambda_{p1}}{\lambda_{p2}}\right)\), degradation occurs predominantly through dimers \((2\lambda_{p2} \cdot p_2^* \gg \lambda_{p1} \cdot p_1^*)\) and the total protein abundance is proportional to the dimer concentration.

Note that regime (ii) is absent when there is no cooperative stability \((\lambda_{p1} = \lambda_{p2})\). In Fig. 11, we plot \(p_1^*, p_2^*, \) and \(p^*\) as a function of \(S\) on a double-logarithmic scale, both without (Fig. 11a) and with cooperative stability (Fig. 11b). In these plots, the sensitivity of the protein concentrations can be read off as the log-log slope, and the regimes (i - iii) are labelled above the plots. First, let us focus on the dimer concentration (red curve), which is relevant for the case of obligate dimers (non-functional monomers) considered in the main text. For small rates of protein synthesis, \(p_2^*\) has a sensitivity of two, which is due to the nonlinearity of the dimerization reaction, both with and without cooperative stability. However, without cooperative stability, this sensitivity is limited to regime (i), whereas it extends to include regime (ii) with cooperative stability, see Fig. 11b. This is a consequence of the fact that the nonlinearity in dimerization can only be harnessed when monomer degradation is dominant.
Now let us consider a protein that can function both as monomer or dimer. Without cooperative stability ($\lambda_{p1} = \lambda_{p2}$), the total protein concentration (black curves) is linear in protein synthesis, i.e. it has a log-log slope of one in Fig. 11a. Consequently, a given fold-change on the mRNA level will always yield the same fold-change on the protein level. In contrast, with cooperative stability ($\lambda_{p1} > \lambda_{p2}$), the total protein concentration $p^*$ acquires a sensitivity that is greater than one in regime (ii). Hence, the fold-change on the protein level can be amplified over that on the mRNA level, even when the protein is equally functional as monomer and dimer. One may regard these two effects as the essence of cooperative stability: an extension of the multimerization-induced nonlinearity into the regime of larger protein synthesis rates, and an amplification of the fold-change in the total protein concentration.

**Potential costs of cooperative stability**

If a cell demands a particular protein abundance at steady state, an elevated rate of degradation has to be compensated by an increase in the rate of protein synthesis. In principle, this elevated protein synthesis rate might be considered “wasted” protein. In Fig. 12, we choose an example where a cell requires a target LOW (~3 nM) and HIGH level (~300 nM) of total protein with no cooperative stability ($\lambda_{p1} = \lambda_{p2}$, see Fig. 12a) and with cooperative stability ($\lambda_{p1} > \lambda_{p2}$, see Fig. 12b). For purposes of comparison, the fold-change in synthesis rates between the two cases is shown in Fig. 12b. The larger fold-change in synthesis rate for the LOW state (compared to the HIGH state) is a consequence of cooperative stability: Most of the protein is monomer in the LOW state, so a much larger change in protein synthesis is needed to compensate for the increased monomer degradation rate ($\lambda_{p1} > \lambda_{p2}$). However, since the protein synthesis rate in the LOW state is small, the amount of “wasted” protein in the LOW state is expected to be small in practice.

Another potential cost of cooperative stability is a load on the proteolytic machinery. For example, compare the monomer degradative flux (solid, gray line) in the HIGH state with no cooperative stability (Fig. 12a) and with cooperative stability (Fig. 12b). The monomer degradative flux has increased 10-fold from 0.5 nM min$^{-1}$ (“degradation” through dilution with no cooperative stability) to 5 nM min$^{-1}$ (degradation through active proteolysis with cooperative stability). This
increased load suggests that there will be a biological limit as to how many proteins can use cooperative stability, since extensive use will saturate the proteases.
References:


Table 2: *In vitro* values for $K_d^{(0)}$, $k_d^{-1}$, $k_a^{-1}$ were taken from the literature for a few exemplary cases. Presuming that dimer “turnover” *in vivo* occurs primarily through dilution $\lambda_{p2}^{-1} \approx 70$ min, we can estimate the *in vivo* dimer dissociation constant $K_d$. Note that two-state dimers such as the Arc repressor tend to have smaller $k_a^{-1}$ than the typical value given above ($k_a^{-1} \sim 20 \text{nM-min}$), due presumably to the lack of orientation constraints in the association process (74). On the other hand, large proteins such as β-galactosidase will tend to have larger $k_a^{-1}$, which can lead to large shifts in $K_d$. 

<table>
<thead>
<tr>
<th>Protein</th>
<th>$K_d^{(0)}$ (nM)</th>
<th>$k_d^{-1}$ (min)</th>
<th>$k_a^{-1}$ (nM-min)</th>
<th>$K_d$ (nM)</th>
<th>References</th>
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<tbody>
<tr>
<td>Arc</td>
<td>10</td>
<td>0.2</td>
<td>2</td>
<td>10</td>
<td>(69)</td>
</tr>
<tr>
<td>HIV-1 protease</td>
<td>4</td>
<td>8</td>
<td>32</td>
<td>4.5</td>
<td>(70)</td>
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<tr>
<td>CRP</td>
<td>0.1-1</td>
<td>330</td>
<td>30 - 300</td>
<td>0.5 – 5</td>
<td>(71,72)</td>
</tr>
<tr>
<td>β-galactosidase</td>
<td>0</td>
<td>0</td>
<td>3900</td>
<td>56</td>
<td>(73)</td>
</tr>
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</table>
Figure 5: (a) Plot of the sensitivity for simple activation ($s_a$) and repression ($s_R$) at $n=1$ for different $f = 10, 100, 1000$. The legend to these curves is located in the adjacent figure. (b) Plot of the maximum sensitivity ($s^*$) as a function of promoter strength $f$. The shape of the curve is independent of $n$ (i.e. the Hill coefficient simply scales the height or $s^*$) and whether the Hill function is repressive ($g_R$) or activating ($g_A$).
Figure 6: (a) Log-log plot of the promoter activity $P_{act2}$ for the Isaacs promoter (8) (see Fig. 1d) as a function of relative concentration $[TF]/K_H$ using two different sets of experimental parameters: SA85 (11) and KA92 (65). Each set of parameters is fit to $g_A([TF])$, an approximate Hill function described in Eq. 8. We obtain: $n \approx 1.53$, $\kappa \approx 1.22 K_H$, and $f \approx 11$ for SA85; and $n \approx 1.69$, $\kappa \approx 0.64 K_H$, and $f \approx 11$ for KA92. (b) The log-log slope of the activity function (the sensitivity $s_A$) as a function of relative protein concentration $[TF]/K_H$ is plotted for both sets of parameters for $P_{act2}$ and the approximate Hill function $g_A([TF])$. 
Figure 7: (a) Log-log plot of the promoter activity $P_{rep2}$ that describes the promoters engineered by Lutz et al. (27) (see Fig. 1f) as a function of relative concentration $[TF]/K_{R1}$ using the parameters $K_{R1} = K_{R2}$ and $L = 10^{-3}$. The promoter activity $P_{rep2}$ is fit to $g_R([TF])$, an approximate Hill function described in Eq. 9. We obtain parameters $n = 1.63, \kappa \approx 0.52K_{R1}$. (b) The log-log slope (sensitivity $s_R$) of the promoter activity as a function of relative protein concentration $[TF]/K_{R1}$ is plotted for $P_{rep2}$ and the approximate Hill function $g_R([TF])$. 
Figure 8: The regime of bistability (shaded region) in the parameter space \((\gamma/\kappa \cdot \lambda_p, K_d/\kappa)\) for linear degradation \((\lambda_{p1} = \lambda_{p2})\) using the KA92 parameters \(f = 11, n = 1.7\) (see Fig. 6) that best describe the P_{RM} promoter used by Isaacs et al. (8).
Figure 9: Plot of $\psi(y)$ where the unstable solutions (oscillatory) that satisfy $x/y > \psi(y)$ are in the hatched region. All $x/y > 2$ are oscillatory, independent of the relative rates of protein and mRNA degradation encapsulated by the parameter $y$. 
Figure 10: Quantitative characteristics of the three-gene oscillator involving repressor binding to a single operator site ($n=1$). (a) Oscillatory regime (shaded regions) in the parameter space for the case of linear degradation ($\lambda_{p1} = \lambda_{p2}$), using $\lambda_{p2} / \lambda_{m} = \{0.1, 1\}$ and $f = \{100, 1000\}$, representing \{typical, rare\} values, respectively. Our axes have the same parameter combinations as in Fig. 4a. For $(\lambda_{p2} / \lambda_{m}, f) = (0.1, 100)$, sustained oscillation is not possible within the physiological parameter range being shown. (b) For the circuit with cooperative stability ($\lambda_{p1} = 10 \lambda_{p2}$), we plot in the same parameter space the oscillatory regime with typical dimer/mRNA degradation rates ($\lambda_{p2} / \lambda_{m} = 0.1$), at fixed repression strengths $f = 100$ (black) and $f = 1000$ (grey). A significant part of the physiological parameter space now displays oscillatory behavior.
Figure 11: Log-log plot of the steady-state abundances of monomers, $p_1^*$ (green, solid curve), dimers, $p_2^*$ (red, solid curve), and total protein, $p^*$ (black, solid curve) as a function of protein synthesis rate $S$. All concentrations are in units of nM, and the dimer dissociation constant is kept fixed ($K_d = 10$ nM). We compare (a) the case with no cooperative stability ($\lambda_{p_1} = \lambda_{p_2}$) and (b) the case with cooperative stability ($\lambda_{p_1} = 10\lambda_{p_2}$). The case without cooperative stability corresponds to the situation where both monomers and dimers are passively diluted through cell growth (50-minute half-life). The case with cooperative stability corresponds to the situation where monomers are actively degraded (5-minute half-life). The sensitivities (log-log slope) of the protein concentrations as a function of protein synthesis rate are indicated by the arrows with the dashed lines as guides for the eye. The three regimes (i - iii) described in the text are labelled above the respective plots.
Figure 12: Log-log plot of steady-state protein abundances $p_1^*$ (green, solid curve), $p_2^*$ (red, solid curve), and $p^*$ (black, solid curve) as a function of protein synthesis rate $S$ with (a) no cooperative stability ($\lambda_{p_1} = \lambda_{p_2}$) and (b) with cooperative stability ($\lambda_{p_1} > \lambda_{p_2}$). The curves and parameters are identical to Figure 11. We are interested in the protein synthesis rate $S$ necessary to maintain a LOW (3 nM) and HIGH total protein concentration (300 nM). These rates are highlighted for the case of no cooperative stability (dashed, vertical bars) and with cooperative stability (solid, vertical bars). Shown on the same plot (in different units) is the monomer degradative flux (nM min$^{-1}$) at steady state for monomer (grey, solid curve). The three regimes (i - iii) described in the text are labelled above the respective plots.