Supplemental Material

1 In-vitro Transcriptional Oscillator

The transcriptional oscillator that we simulate in conjunction with the comparator was developed by Franco et al. using a composite network motif wherein two different proteins interact through genelet switches (genelets) [1]. A schematic representation of this network motif is shown in Fig. S. 1. Here, \( SW_{12} \) and \( SW_{21} \) are synthetic genes that produce RNA molecules \( rA_1 \) and \( rI_2 \) respectively. \( rA_1 \) activates \( SW_{21} \) while \( rI_2 \) inhibits \( SW_{12} \). The right arrow from \( rI_2 \) illustrates that \( rI_2 \) is used as the input to the biomolecular comparator.

\[
[rA_1] = k^{osc}_{p}[SW_{12}] - k^{osc}_{d}[rA_1], \tag{S1}
\]
\[
\tau[SW_{21}] = [SW_{21}^{tot}] \frac{[rA_1]^m}{K_A^m + [rA_1]^m} - [SW_{21}], \tag{S2}
\]
\[
[rI_2] = k^{osc}_{p}[SW_{21}] - k^{osc}_{d}[rI_2], \tag{S3}
\]
\[
\tau[SW_{12}] = [SW_{12}^{tot}] \frac{K_I^n}{K_I^n + [rI_2]^n} - [SW_{12}]. \tag{S4}
\]

Eqs. S1-S4 are first order coupled differential equations which can be solved numerically while sweeping various parameters to explore the possibility of achieving a sustained oscillatory response. Such analysis can be found in [1]. The response of the output \( rI_2 \) species is shown in Fig. S. 2, where parameters were chosen as in [1]. In this plot, an oscillating temporal behavior of \( rI_2 \) species can be seen.
2 Stochastic Simulation Results

To conduct the stochastic kinetic simulation, we used Lotka-Volterra equations to produce an oscillatory species [2]. These equations are often defined as

\[
\dot{x} = k_1 x - k_2 x y, \quad \text{(S5)}
\]

\[
\dot{y} = k_3 x y - k_4 y. \quad \text{(S6)}
\]

Here, \(x\) and \(y\) are two different species which can result in an oscillatory response given appropriate values of the rate constants \((k_1, k_2, k_3\) and \(k_4\)). An example oscillatory response is shown in Fig. S. 3(a). Considering \(x\) as a source species, a deterministic response of the comparator is shown in Fig. S. 3(b). The results of the same systems simulated using Gillespie’s algorithm for sampling stochastic kinetic time trajectories are shown in Fig. S. 4(a) and Fig. S. 4(b). The stochastic response (solid line) of \(I, T, A\) and \(O\) follows the deterministic solution (dotted line) closely (Fig. S. 4(a)). Moreover, even though there is variation in the amplitude of the source species, the comparator’s output remains consistent (Fig. S. 4(b)). These simulation results suggest that the comparator’s response is robust even when the number of molecules in the system is relatively small and intrinsic noise is considered via stochastic chemical kinetic simulations.

![Graphs showing stochastic and deterministic responses](image)

Fig. S. 3: (a) Mass-action simulated response of Eqs. S5-S6 for \(k_1 = 0.017 \text{ s}^{-1}, k_2 = 0.0008 \text{ s}^{-1}, k_3 = 0.0003 \text{ s}^{-1}\) and \(k_4 = 0.0083 \text{ s}^{-1}\). The initial concentrations of \([x]\) and \([y]\) are 10 nM. (b) Deterministic mass action response of the comparator while using \(x\) as a source species. The threshold and the high output concentration of the comparator are set to be 30 nM and 50 nM respectively. Here, \(k_p^p = k_d^p = k_d^T = k_d^A = k_d^O = 0.03 \text{ s}^{-1}\), \([P_T] = [P_A] = 20 \text{ nM}, k_p^T = 0.044 \text{ s}^{-1}, k_p^A = 0.073 \text{ s}^{-1} k_r^3 = 0.15 \text{ mM}^{-1}\text{s}^{-1}\) and \(k_r^f = 2.92 \text{ mM}^{-1}\text{s}^{-1}\).
3 Contraction Theory

Here, we show that the comparator output converges to a periodic function for a periodic input. This property can be demonstrated using contraction theory [3]. We apply the following Theorem from [3, 4]:

**Theorem 3.1** Consider a system \( \dot{x} = f(x, u) \). If the system is defined on a closed convex subset of \( \mathbb{R}^n \), if \( f(\cdot, \cdot) \) is infinitesimally contractive, and if \( f(\cdot, \cdot) \) is \( T \)-periodic, then the system admits a unique periodic solution \( \phi(t) \) having period \( T \), and every solution \( x(t) \) converges to \( \phi(t) \) for \( t \to \infty \).

First, if the input \( S(t) \) is periodic with period \( T \), then the dynamics of the comparator \( \dot{x} = f(x, S(t)) \) are \( T \)-periodic, i.e. they satisfy:

\[
\dot{x} = f(x, S(t)) = \hat{x} = f(x, S(t+T)). \tag{S7}
\]

We now show that the comparator trajectories evolve on the positive orthant of \( \mathbb{R}^4 \), and they are bounded for bounded inputs, so that they are defined on a closed convex set of \( \mathbb{R}^n \). For example, we consider the dynamics of \( [I] \), which can be upper bounded as:

\[
\frac{d[I]}{dt} \leq k_p^f[S] - k_d^f[I], \tag{S8}
\]

Therefore, the solution \( [I](t) \) is upper bounded by the solution of \( \frac{d[I]}{dt} = k_p^f[S] - k_d^f[I] \), which is an asymptotically stable system whose solution is bounded for bounded input \( [S] \). We can apply analogous reasoning for the other comparator species. Thus, the comparator solutions are defined on a compact, convex subset of \( \mathbb{R}^5_{\geq 0} \) if the input \([S]\) is bounded. A conservative compact set that is forward invariant for the system is:

\[
C = \{(I), [T], [A], [O] \in \mathbb{R}^5_{\geq 0} : [I] \leq [S]_{max}, [T] \leq k_p^f[Pr]/k_d^fT, [A] \leq k_p^d [P_A]/k_d^d, [O] \leq k_p^d (k_p^d [P_A]/k_d^d) ([S]_{max}/k_d^d) \},
\]

where again we assume that the input \([S]\) is a bounded signal that does not go above \([S]_{max} \). Finally, we check under what conditions the comparator is infinitesimally contractive [4], i.e. for a chosen matrix measure \( \mu \) we check if:

\[
\mu(J_s) \leq -c^2, \tag{S9}
\]
where $J_x$ is the Jacobian matrix evaluated at every $x \in C$, and $c$ is a positive constant. We begin with the matrix measure $\mu_1$ $[5, 3]$, induced by the $\|x\|_1$ vector norm in $\mathbb{R}^n$ that is defined as:

$$
\mu_1(J_x) = \max_{1 \leq j \leq n} \left\{ J_x(j, j) + \sum_{i \neq j} |J_x(i, j)| \right\}, \quad (S10)
$$

We take the modified matrix measure $\mu_{P,1}$ induced by the vector 1-norm $\|Px\|_1$, where $P = \text{diag}(p_1, p_2, p_3, p_4)$ is a nonsingular matrix and $p_i > 0$ are weights that can be chosen depending on the parameters of the system. This weighted measure and $\mu_1$ are related as: $\mu_{P,1}(J_x) = \mu_1(P J_x P^{-1})$. The weighted Jacobian matrix is:

$$
J_{x,P} = P J_x P^{-1} = \begin{bmatrix}
-k_d I - k_r T [I] & -p_3 k_r T [I] & 0 & 0 \\
-p_2 k_r T [I] & -k_d I - k_r T [T] & 0 & 0 \\
-p_1 k_r k_A [A] & 0 & -k_d I - k_r T [I] & 0 \\
-p_1 k_r k_A [A] & 0 & -p_4 k_r k_A [I] & -k_d O
\end{bmatrix}. \quad (S11)
$$

We now check condition Eq. S9 using the measure $\mu_{P,1}$. We should note that we are not interested in finding the value of the measure $\mu_{P,1}(J_x)$; we just want to ensure that it is negative. Condition Eq. S9 yields the following inequalities:

$$
-k_d I - k_r T [I] + \frac{p_2}{p_1} k_r T [I] + \frac{p_3}{p_1} k_r k_A [A] + \frac{p_4}{p_1} k_r k_A [A] \leq -c_1^2, \quad (S12)
$$

$$
-k_d T - k_r T [T] + \frac{p_2}{p_1} k_r T [T] \leq -c_2^2, \quad (S13)
$$

$$
-k_d A - k_r A [I] + \frac{p_4}{p_3} k_r k_A [I] \leq -c_3^2, \quad (S14)
$$

$$
-k_d O \leq -c_4^2. \quad (S15)
$$

Choosing $p_1 = p_2 = 1$, we find $c_2 = \sqrt{k_d^T}$; also, $c_4 = \sqrt{k_d O}$. As for Eq. S14, picking $p_3 \geq p_4$ we ensure that the left hand side of the inequality is negative, and we choose $c_3 = \sqrt{k_d A}$ as well. We still need to find $c_1$; based on our choice $p_1 = p_2 = 1$, and defining $\varepsilon = (p_3 + p_4)$, inequality Eq. S12 becomes:

$$
-k_d I + \varepsilon k_r A [A] \leq -c_1^2. \quad (S16)
$$

Because $[A] \leq \frac{k_A [P_A]}{k_d}$, we have:

$$
-k_d I + \varepsilon k_r A \frac{k_A [P_A]}{k_d} \leq -c_1^2. \quad (S17)
$$

To ensure contractivity, we pick:

$$
\varepsilon = (p_3 + p_4) = \frac{1}{2} k_d^I k_r \frac{k_A [P_A]}{k_d}, \quad (S18)
$$

and $c_1 = \sqrt{k_d^I/2}$. This concludes our analysis, and our contractivity rate is $c = \min\{c_i\}$. Note that the rate $c$ depends on the binding rates and thresholds designed for the comparator and on the weights chosen for our matrix measure.
4 Equilibrium Point Analysis

To determine whether the steady state of the comparator (given a constant input $[S]$) is stable, we used linear perturbation analysis about the equilibrium point. We start by determining the equilibria from the governing Eqs. S19-S22 of the biomolecular comparator

\[
\frac{d[I]}{dt} = k^I_p[S] - k^I_d[I] - k^T_d[T][I], \quad (S19)
\]

\[
\frac{d[T]}{dt} = k^T_p[P_T] - k^T_d[T] - k^T_r[I][T], \quad (S20)
\]

\[
\frac{d[A]}{dt} = k^A_p[P_A] - k^A_d[A] - k^A_r[I][A], \quad (S21)
\]

\[
\frac{d[O]}{dt} = k^A_r[I][A] - k^O_d[O], \quad (S22)
\]

by equating the derivatives of various species in Eqs. S19-S22 to zero (for a constant input $S$),

\[
[I] = \frac{k^I_p[S]}{k^I_d + k^T_d[T]}, \quad (S23)
\]

\[
[T] = \frac{k^T_p[P_T]}{k^T_d + k^T_r[I][T]}, \quad (S24)
\]

\[
[A] = \frac{k^A_p[P_A]}{k^A_d + k^A_r[I][T]}, \quad (S25)
\]

\[
[O] = \frac{k^A_r[I][A]}{k^O_d[T][A]}, \quad (S26)
\]

In order to linearize the differential equations around this equilibrium point, we define state ($x$) and input ($u$) variables as

\[
x = \begin{bmatrix} [I] & [T] & [A] & [O] \end{bmatrix}^T, \quad u = \begin{bmatrix} [S] & \begin{bmatrix} k^T_p[P_T] \\ k^A_p[P_A] \end{bmatrix} \end{bmatrix}^T. \quad (S27)
\]

It should be noted that these variables depend on time ($t$), to simplify the notation, we do not explicitly denote this dependence. Here, the dynamics of $x$ and $u$ are related by a nonlinear function:

\[
x = f(x, u). \quad (S28)
\]

Assuming the equilibrium point is $(\tau, \varpi)$, at this point we know that $f(\tau, \varpi) = 0$. Now, suppose a small perturbation is added at the equilibrium:

\[
x = \tau + \delta x, \quad (S29)
\]

\[
u = \varpi + \delta u. \quad (S30)
\]

Using the Taylor expansion and Eqs. S29 and S30, the nonlinear system (Eq. S28) can be linearized while neglecting higher order terms:

\[
\frac{d\delta x}{dt} = J_x \delta x + J_u \delta u, \quad (S31)
\]

where $J$ is the Jacobian matrix and determined using Eqs. S19-S22:

\[
J_x = \begin{bmatrix}
-k^I_d & -k^T_d[T] & 0 & 0 \\
-k^T_d[T] & -k^T_d & 0 & 0 \\
-k^A_d[A] & 0 & -k^A_d & 0 \\
k^A_d[A] & 0 & k^A_d[T] & -k_d^O
\end{bmatrix}, \quad (S32)
\]

and
The solution of Eq. S31 is well known:

\[
\delta x = e^{J_x t} \delta x_0 + \int_0^t e^{J_x(t-\tau)} J_u u(\tau) \, d\tau.
\]  
(S34)

By calculating the eigenvalues (\(\lambda\)) of \(J_x\), an insight on how the perturbation affect the equilibrium of the system can be obtained:

\[
det(\lambda I_4 - J_x) = 0,
\]  
(S35)

where \(I_4\) is a 4\(\times\)4 identity matrix. Using Eqs. S32 and S33, Eq. S35 can be solved:

\[
\lambda_1,2 = -k_d^I - k_d^T \underline{T} - k_d^T \underline{T} \pm \sqrt{(-k_d^I - k_d^T \underline{T} + k_d^T \underline{T})^2 - 4(k_d^T \underline{T})^2},
\]  
(S36)

\[
\lambda_3 = -k_d^A - k_d^A \underline{T},
\]  
(S37)

\[
\lambda_4 = -k_d^O.
\]  
(S38)

Here, \(\lambda_{1,2}\) will have complex part for the cases when \(4(k_d^T \underline{T})^2\) term (inside the square root of Eq. S36) dominates over the others terms. This may result in damped oscillations around the steady state response. However, due to the fact that \(I\) and \(T\) species are output-of-phase and their minimum steady state values are close to zero, so for a steady state response, the product of \(\underline{T}\) and \(\underline{T}\) will always be close to zero. When assuming \(\underline{T}\) term approximates zero, Eq. S36 can be simplified to:

\[
\lambda_1 = -k_d^I - k_d^T \underline{T}; \lambda_2 = -k_d^T - k_d^T \underline{T}.
\]  
(S39)

When this is the case, the negative eigenvalues demonstrate that the exponential term in Eq. S34 decays with time and therefore so does the perturbation. This means that the comparator operates stably around the equilibrium when a local perturbation is applied.

5 \([S] - [O]\) curve

The steady state response of comparator is shown here with respect to \(S\). The shape of the \([S] - [O]\) curve is a hill function like response wherein \(O\) changes from low to high quickly when \([S]\) is more than \([\beta]\) and remains at a constant high value (\(\alpha\)) with increasing \(S\).
In a system containing only Reactions 2.1 and 2.2 from the main text, species $I$ would reach the same steady state as $S$ when $k^I_d$ and $k^I_d$ are same and these rates are fast enough to follow $S$. Thus, under these conditions we would expect that $I_{\text{max}}$ would be $[S]_{\text{max}}$, where $[S]_{\text{max}}$ is the maximum concentration obtained by the oscillatory input signal $S$. Likewise we can observe that in a system containing only Reaction 2.5, $T$ reaches steady state $[\beta]$.

Fig. S. 5: Transfer characteristic of the comparator, plotted using Eqs. 14 and 15. The threshold and the high output concentration of the comparator are set to be 10 $\mu$M and 25 $\mu$M respectively. Here, $k^I_p = k^I_P = 0.0031 \text{ s}^{-1}$, $[P_T] = [P_A] = 20 \mu$M, $k^T_p = 0.0015 \text{ s}^{-1}$, $k^T_d = 0.004 \text{ s}^{-1}$, $k^A_p = 0.0067 \text{ s}^{-1}$, $k^A_d = 0.0064 \text{ s}^{-1}$, $k^O_p = 0.0054 \text{ s}^{-1}$, $k^O_d = 0.06 \mu$M$^{-1}\text{s}^{-1}$ and $k^T_r = 2.9 \mu$M$^{-1}\text{s}^{-1}$.

### 6 Constraints on the Degradation Rates

In a system containing only Reactions 2.1 and 2.2 from the main text, species $I$ would reach the same steady state as $S$ when $k^I_d$ and $k^I_d$ are same and these rates are fast enough to follow $S$. Thus, under these conditions we would expect that $I_{\text{max}}$ would be $[S]_{\text{max}}$, where $[S]_{\text{max}}$ is the maximum concentration obtained by the oscillatory input signal $S$. Likewise we can observe that in a system containing only Reaction 2.5, $T$ reaches steady state $[\beta]$. In the full comparator system, $I$ would never go higher than $[S]_{\text{max}}$ because the other reactions deplete $I$, and $S$ is not changed by any of the reactions in the system. Likewise, $T$ would never go higher than $[\beta]$ because it is produced only in Reaction 2.5 and its production rate is not dependent on the concentration of any species in the system. Further, in this system, Reaction 2.3 depletes both $I$ and $T$. Because we have designed the comparator to operate when $k^I_d[I]_{\text{max}} \gg k^A_d[I]_{\text{max}} \gg \max\{k^I_d, k^I_p, k^T_d\}$, this depletion happens very rapidly compared to the production of $I$ or $T$, so we can assume it always goes to completion. When $I$ and $T$ both are at their maximum values, this depletion consumes up to $\max\{S_{\text{max}}, \beta\}$ of $I$ so that $[I]$ goes no higher than $[S]_{\text{max}} − [\beta]$. We must set $\beta$ to be smaller than $[S]_{\text{max}}$, or $[I]$ will remain at zero. Therefore, Eq. S19 can be simplified to a proportional controller in the absence of $T$. Here, the subscripts max and min correspond to the maximum and minimum concentration values of the respective species.

\[
\frac{d[I]_{0\rightarrow([S]_{\text{max}}−\beta)}}{dt} = k^I_d[S] − k^I_d[I].
\]  

(S40)

For zero initial concentration of $[I]$, the solution of Eq. S40 is

\[
[I]_{0\rightarrow([S]_{\text{max}}−\beta)} = ([S]_{\text{max}} − \beta)(1 − e^{-k^I_d t}).
\]  

(S41)

While $S$ goes from $[S]_{\text{min}}$ to $[S]_{\text{max}}$ in time $T_{sr}$, $I$ should approach its steady state value. We can therefore approximate the value of $k^I_d$ determining the time $I$ takes to increase from its initial value (0) to its high steady state value $[S]_{\text{max}} − [\beta]$

\[
k^I_d = \frac{1}{T_{sr}} \ln \left( \frac{([S]_{\text{max}} − \beta)}{([S]_{\text{max}} − \beta) − [I]_{\text{max}}} \right).
\]  

(S42)

Similarly, while $S$ is degrading from $[S]_{\text{max}} \rightarrow [S]_{\text{min}}$, $I$ should degrade quickly to its lowest concentration $([I]_{\text{max}})$ from its maximum value $([S]_{\text{max}} − [\beta])$. $[I]_{\text{min}}$ should be close to zero because $T$ degrades $\beta$ amount of $I$ much faster than the rate at $I$ is produced so we can rewrite Eq. S19 as

\[
\frac{d[I]_{([S]_{\text{min}}−\beta)\rightarrow[I]_{\text{min}}}}{dt} = −k^I_d[I].
\]  

(S43)
Therefore

\[ k_d^I = \frac{1}{T_{sf}} \ln \left( \frac{([S]_{\text{max}} - \beta)}{[I]_{\text{min}}} \right). \]  

(S44)

Thus, for \( I \) to follow \( S \) closely, Eq. S42 and S44 imply that \( k_d^I \gg \max \{1/T_{sr}, 1/T_d \} \).

When \( I \) is produced and degraded, \( O \) should be able to switch from low to high and high to low states quickly respectively. While \( O \) is starting to get produced from its initial zero concentration, the product of \( k_r^A \), \([I] \) and \([A] \) should be constant so that \( k_r^A[I][A]/k_d^O \) should be equal to \([\alpha] \) and so the high steady state of \( O \) will be \([\alpha] \). Therefore assuming \( I \) and \( A \) reach their maximum and minimum concentration respectively before \( O \) is produced, Eq. S22 can be simplified to

\[ \frac{d[O]_{[\alpha]-[\alpha]}}{dt} = k_d^O [\alpha] - k_d^O [O]. \]  

(S45)

For \( O \) to reach its high steady state quickly while \( S \) changes from its minimum to maximum value,

\[ k_d^O \geq \frac{1}{T_{sr}} \ln \left( \frac{[\alpha]}{[\alpha] - [O]_{\text{max}}} \right). \]  

(S46)

Because we require that \( k_r^A[I]_{\text{max}} \gg k_d^A \), we assume that \( O \) is degraded only when \([I] \approx 0 \). This allows us to rewrite Eq. S22 for the case when \( O \) changes from its high steady state value \([\alpha] \) to low steady state value \([0] \).

\[ \frac{d[O]_{[\alpha]-[0]}}{dt} = -k_d^O [O]. \]  

(S47)

For \([O] \) to transition from \([\alpha] \) to \([0]_{\text{min}} \) in time \( T_d \) (as \([S]\) transitions from \([S]_{\text{max}} \) to \([S]_{\text{min}} \)), we must have

\[ k_d^O = \frac{1}{T_d} \ln \left( \frac{[\alpha]}{[O]_{\text{min}}} \right). \]  

(S48)

Therefore both \( k_r^I \) and \( k_d^O \) need to be much larger than \( \max \{1/T_{sr}, 1/T_d \} \) so that \( I \) and \( O \) produced and degraded quickly. Even though we assumed that \( k_r^I \) and \( k_d^I \) are same, they can be different and the comparator can still produce on and off response as shown in Fig. 6(a). Moreover, when the degradation rate of \( O \) is faster than the degradation rate of \( A \), \([O] \) can initially rise above its target value, producing a transient “spike.” This is because the production rate of \( O \) is proportional to the product of \( k_d^A \) and \([I][A]\) (Eq. S22) and so while \( O \) starts switching from its minimum value to maximum, the product \([I][A]\) should reach a constant steady low value. However, when \( k_d^A \) is smaller than \( k_d^O \), \( A \) takes longer to reach to its minimum concentration, so even a small amount of \( I \) is amplified because \( k_r^A[I]_{\text{max}} \gg k_d^A \). Once \( A \) reaches \([A]_{\text{min}} \), the output quickly converges to its designed on value \([\alpha] \). Therefore, \( k_d^A \) should be at least as large as \( k_d^O \). \( k_d \) must also be small enough to satisfy the time scale separation \( k_d^A \ll k_d^A \) (Eq. 20).
Concentration ($M$)
0 10 20 30 40 50

Fig. S. 7: The effect of variation in $k_p^I$ with respect to $k_d^I$, and $k_p^A$ with respect to $k_d^O$ are shown here. (a) The comparator’s output can still produce an on and off response for the cases when $k_p^I \geq k_d^I$. When $k_p^I < k_d^I$, the high steady state of $I$ will be smaller than $[S]_{\text{max}} - [\beta]$, and so much $O$ will be not produced enough to reach its high steady state. (b) When $k_p^A \geq k_d^O$ the comparator does not produce any spikes in the output response. Unless stated otherwise, $k_p^i = k_d^i = 0.0031 \text{ s}^{-1}$, $[P_T] = [P_A] = 20 \mu\text{M}$, $k_p^T = 0.0015 \text{ s}^{-1}$, $k_d^T = 0.004 \text{ s}^{-1}$, $k_p^A = 0.0067 \text{ s}^{-1}$, $k_d^A = 0.0064 \text{ s}^{-1}$, $k_d^O = 0.0054 \text{ s}^{-1}$, $k_T^A = 0.06 \mu\text{M}^{-1}\text{s}^{-1}$ and $k_T^O = 2.9 \mu\text{M}^{-1}\text{s}^{-1}$.

7 Numerical Simulation Results

We used numerical simulations of the comparator to verify that our analysis of how the values of the reaction rates and concentrations of the components affect the operation of the comparator. In these simulations we varied degradation rates, $k_r^A / k_r^A$ and $k_f^T / k_r^A$. For the case where the comparator acts on the transcriptional oscillator described in Section 1 with input $S$ being species $rI2$, results are shown in Figs. S. 7, S. 8 and S. 9 respectively. Unless stated otherwise, $[P_A] = [\alpha] = 25 \mu\text{M}$, $[P_T] = [\beta] = 10 \mu\text{M}$, $k_p^I = k_d^I = k_p^A = k_d^A = 0.006 \text{ s}^{-1}$, $k_p^T = k_d^T = k_p^O = k_d^O = 0.06 \mu\text{M}^{-1}\text{s}^{-1}$ and $k_T^A = 3 \mu\text{M}^{-1}\text{s}^{-1}$. In each of the simulations, the initial concentrations of $I$, $P$, $A$ and $O$ are set at zero. In Fig. S. 7, we examine how changing the value of the degradation rate changes the response of the comparator. As we describe in the main text, different degradation rates need to be large enough so that the transition time of $O$ from its low concentration to its high concentration and vice-versa can be as short. Fig. S. 7 shows what happens when the degradation rates do not meet these requirements: the output signal of the comparator fails to attain its high and low state quickly.

Fig. S. 8 shows the effect of setting $k_p^A$ to be too small. Here, a small $k_p^A$ value slows the production of $O$ so that the the comparator’s output may not attain the pre-defined high states. We then examined what happens when $k_f^T$ is not set to be much faster than $k_p^A$, which we suggest is important in the main paper (Fig. S. 9). The comparator is designed so that Reaction 2.5 faster than Reaction 2.6. When this condition is satisfied, Reaction 2.5 has the effect of destroying quantity $\beta$ of $I$ before $I$ can react with $A$ to produce the output signal. This destruction controls the amount of the output that is produced. If $I$ is not destroyed quickly, it will remain high even as $S$ drops. This effect can be seen for small $k_f^T$ values in Fig. S. 9.
Fig. S. 7: Simulated response of Eqs. S19-S22 at different values of the degradation rate while selecting \( k_{A^r}^T \) and \( k_{T^r}^T \) such that the ratios of \( k_{A^r}^T / k_{A^d} \) and \( k_{T^r}^T / k_{A^d} \) both remain constant. If the degradation rate is too low compared to \( 1/ \min(T_{w^r}, T_{d^r}) \), \( O \) will not switch rapidly between two defined concentration limits.

Fig. S. 8: The effect of different values of \( k_{A^r}^T \) on the temporal response of the output. \( k_{d^r}^T \) and \( k_{A^d}^T \) are the same in each simulation. For \( O \) to reach its designed maximum value, \( k_{A^r}^T \) must remain high as compared with \( k_{d^r}^T \). \( k_{d^r}^T \) has units s\(^{-1}\) while \( k_{A^r}^T \) and \( k_{T^r}^T \) have units of M\(^{-1}\)s\(^{-1}\).

8 Comparator output sensitivity to parameter variations

In order to determine the sensitivity of the comparator response to parameters variations, we conducted ode based numerical simulation wherein initially, for each iteration, degradations rates are varied randomly by 20% from a mean value (0.006 s\(^{-1}\)) while fixing the values of \( \beta, \alpha, P_T, P_A, k_{T^P}^T, k_{A^P}^T, k_{A^r}^T \) and \( k_{T^r}^T \) (Fig. S. 10). We then perturbed all the rates randomly including \( k_{d^r}^T \) and \( k_{T^r}^T \) by 20% from their original values for a fixed value of \( \beta, \alpha, P_T, P_A, k_{T^P}^T \) and \( k_{A^P}^T \), (Fig. S. 11). In these plots, we observe that for each iteration, the response of \( O \) is close to the case when the rates were unperturbed. As a source species, we used r12 species of the transcriptional oscillator described in Section 1.

we also checked the effect of large variations in the rates by randomly perturbing different parameters in such a way that the conditions for the separations of time scales between different processes (Eqs. 22, 25, 29 and 30 in the main text) may be violated. To do so, we randomly reduced some parameters up to a factor of 2 from their unperturbed values. For most of the iterations, the comparator’s output still achieves the desired amplitude
Concentration (µ M)

Fig. S. 9: The effect of different values of $k_T^r$ on the temporal response of the output. When $k_T^r$ is too small, the input is not destroyed as quickly as the output signal decreases. As a result, too much output is produced.

Fig. S. 10: Simulated response of the comparator while considering random variations in the degradation rates for different values of $\alpha$ and $\beta$. Here, for each iteration we randomized $k_d^A$ and $k_d^O$ such that $k_d^A \geq k_d^O$ is always true.

Fig. S. 11: Simulated response of the comparator while considering random variations in the degradation rates $k_T^A$ and $k_T^r$ for different values of $\alpha$ and $\beta$. Here, for each iteration we randomized $k_d^A$ and $k_d^O$ such that $k_d^A \geq k_d^O$ is always true.
9 Frequency Analysis of the Comparator Response

We numerically computed the empirical frequency response of the comparator, shown in Fig. S. 13(a). In this simulation we measured the amplitude of the comparator output for oscillatory inputs of variable frequency and identical amplitude equal to 5 µM (FFT analysis showed that the principal frequency of the output signal is always equal to the frequency of the input). For the same degradation rates \( k^A_d = k^T_d = k^O_d = 0.006 \text{ s}^{-1} \), the amplitude of the output remains constant and close to \( \alpha = 25 \text{ µM} \) up to frequencies around \( 10^{-3} \text{ Hz} \) (period of about 10 minutes). Fig. S. 13(b) shows the Bode plot for the linearized differential equations (Eqs. S19-S22) at the steady state values (Eq. S23-S26). This linearized model presents a behavior similar to the nonlinear system: the amplitude of the output signal is flat and close to \( \alpha \), and tapers off at high frequencies. When the degradation rate decreases we observe that the cutoff frequency becomes smaller and smaller, which means that the comparator maintains its amplitude tracking property only if the input is sufficiently slow. Our analytical predictions on the nonlinear system are verified also by the linearized system: for a range of low frequencies where the tracking properties of the comparator are satisfied, we find that \( [T] \) is in phase opposition relative to \( [S] \) (Fig. S. 13(c)), while \( [O] \) is perfectly in phase with \( [S] \) (Fig. S. 13(d)).

Second, we considered the comparator linearized equations, defined by matrices \( J \) at Eq. S32 (Jacobian) and \( J_u \) at Eq. S33 (linearized input matrix). For the linearized system, we derived the transfer functions between input \( [S] \) and outputs \( \delta[T] \) and \( \delta[O] \). The transfer functions are obtained using the Laplace transform method, assuming zero initial conditions. When we consider \( \delta[O] \) as the output, the system is:

\[
\frac{d\delta x}{dt} = J\delta x + J_u\delta u
\]

\[
y = [0 \ 0 \ 0 \ 1] \delta x = C\delta x
\]

Applying the Laplace transform method we obtain, where \( X, Y, \) and \( U \) are the state, output, and input functions in the Laplace domain, we find the transfer function:

\[
Y = [C(sId - J)^{-1} J_u] U = G(s) U,
\]

where \( Id \) is a 4x4 identity matrix. The first component of \( G(s) \) is the transfer function between \( \delta[S] \) and \( \delta[O] \). The transfer function between \( \delta[S] \) and \( \delta[T] \) can be found in a similar way, defining \( C = [0 \ 1 \ 0 \ 0] \). Transfer functions can be evaluated numerically using the commands \( \text{ss} \) and \( \text{tf} \) of MATLAB’s Linear Systems Toolbox. The frequency response of the linearized system is computed using MATLAB’s \text{bode} \ command, which returns the magnitude and phase responses of \( Y \), also known as the system’s Bode plot. The resulting magnitude plot of \( O \) is shown in Fig. S. 13(b) while the phase plots of \( T \) and \( O \) are shown in Figs. S. 13(c) and 13(d) respectively.
Fig. S.13: Frequency analysis of the comparator. (a) Empirical frequency response of $O$ (determined numerically) to a sinusoidal signal $[S] = a_o + a \sin(2\pi/T_p)$ where $a_o = 10 \mu$M and $a = 5 \mu$M. (b) Frequency response of the output $O$ of the linearized comparator equations (Magnitude Bode plot). Phase response of species (c) $T$ and (d) $O$. The legend parameter $k_X^{N2}$ is the value of $k_p^{N1}$, $k_d^{N1}$, $k_p^{N2}$, $k_d^{N2}$, $k_{A}^{N1}$ and $k_{A}^{N2}$ which here are all assumed to be the same.

References


