Supplementary material

"Design of a bistable switch to control cellular uptake"

By Diego A. Oyarzún[1] and Madalena Chaves[2]

Here we explain: a) how to determine the *bistable regimes* for the uptake circuits (Fig. 2 of the main text); b) how to determine the conditions on the promoter dynamic ranges for bistability (the *design spaces* in Fig. 3–4 of the main text); c) how to determine the conditions for hysteresis in the AR circuit (the inequalities in (19) of the main text). The general model for the uptake circuit is

$$\frac{\mathrm{d}s}{\mathrm{d}t} = g_1(s_0)e_1 - g_2(s)e_2, \tag{S1}$$

$$\begin{aligned} \frac{\mathrm{d}e_1}{\mathrm{d}t} &= \kappa_1^0 + \kappa_1^1\sigma_1(s) - \gamma_1 e_1, \\ \frac{\mathrm{d}e_2}{\mathrm{d}t} &= \kappa_2^0 + \kappa_2^1\sigma_2(s) - \gamma_2 e_2, \end{aligned} \tag{S2}$$

where (s, e_1, e_2) are the concentrations of the metabolite, transport enzyme and utilization enzyme, respectively. The parameters (κ_i^0, κ_i^1) are enzyme expression rates, and γ_i is a first order kinetic rate of protein degradation and dilution by cell growth. We assume that:

- The extracellular metabolite s_0 is constant.
- The enzyme turnover rates satisfy $g_i(0) = 0$, they are monotonically increasing $\mathrm{d}g_i/\mathrm{d}x > 0$, and they saturate at $g_i^{\text{sat}} = \lim_{x\to\infty} g_i(x) = \sup g_i$.
- The promoter response curves satisfy $\mathrm{d}\sigma_i/\mathrm{d}s > 0$ when the metabolite activates gene expression, and $\mathrm{d}\sigma_i/\mathrm{d}s < 0$ when the metabolite represses expression.

In our model, the promoters control enzyme expression between a baseline concentration ("off") and maximal concentration ("on")

$$E_i^{\text{off}} = \frac{\kappa_i^0}{\gamma_i}, \quad E_i^{\text{on}} = \frac{\kappa_i^0 + \kappa_i^1}{\gamma_i}. \tag{S3}$$

[1]Department of Mathematics, Imperial College London, UK; E-mail: d.oyarzun@imperial.ac.uk
[2]BioCore team, INRIA Sophia Antipolis, France.

The promoter dynamic ranges (μ_i in equation (4) in the main text) are defined as:

$$\mu_i = \frac{E_i^{\text{on}}}{E_i^{\text{off}}} = \frac{\kappa_i^0 + \kappa_i^1}{\kappa_i^0}, \tag{S4}$$

and the relative dynamic range (μ_{12} in equation (5) in the main text) as:

$$\mu_{12} = \frac{E_2^{\text{on}}}{E_1^{\text{off}}} = \frac{\kappa_2^0 + \kappa_2^1}{\kappa_1^0}\frac{\gamma_1}{\gamma_2}. \tag{S5}$$

From the definitions in (S3)–(S5), we note the following equivalences:

$$\begin{aligned} E_1^{\text{off}}/E_2^{\text{off}} = \mu_2/\mu_{12}, \quad E_1^{\text{on}}/E_2^{\text{on}} = \mu_1/\mu_{12}, \\ E_1^{\text{on}}/E_2^{\text{off}} = \mu_1\mu_2/\mu_{12}, \quad E_2^{\text{on}}/E_1^{\text{off}} = \mu_{12}. \end{aligned} \tag{S6}$$

Our analysis is based on a separation of time scales and an approximation of the promoter responses σ_i by step functions. In the next sections we detail the general methodology: in Section S1 we show how to recast the model as a 2-dimensional piecewise affine system in conic domains. In Section S2 we explain how to identify the bistable regimes in each circuit. In Section S3 we show how to obtain the conditions for bistability. Finally in Section S4 we derive the conditions for hysteresis in the AR circuit.

S1 Timescale separation and piecewise affine model

Since metabolic dynamics operate in a much shorter time scale than gene expression, we assume that the metabolite is in quasi steady state with respect to the evolution of enzyme concentrations. We can thus take $\mathrm{d}s/\mathrm{d}t \approx 0$ for all t in equation (S1) to get an algebraic equation for the metabolite concentration

$$g_2(s) = g_1(s_0)\frac{e_1}{e_2}. \tag{S7}$$

We can write a reduced version of the complete model (S1)–(S2)

$$\begin{aligned} \frac{\mathrm{d}e_1}{\mathrm{d}t} &= \kappa_1^0 + \kappa_1^1\bar{\sigma}_1(s) - \gamma_1 e_1, \\ \frac{\mathrm{d}e_2}{\mathrm{d}t} &= \kappa_2^0 + \kappa_2^1\bar{\sigma}_2(s) - \gamma_2 e_2, \end{aligned} \tag{S8}$$

where s is the solution of equation (S7) and we have replaced the promoter response curves (σ_i) by

$$\underline{\text{Activation}} \qquad \bar{\sigma}_i(s) = \begin{cases} 0, & s < \theta_i, \\ 1, & s > \theta_i. \end{cases} \qquad\qquad \underline{\text{Repression}} \qquad \bar{\sigma}_i(s) = \begin{cases} 1, & s < \theta_i, \\ 0, & s > \theta_i. \end{cases} \tag{S9}$$

The model in (S8) is 2-dimensional approximation of the original system in (S1)–(S2). It corresponds to a piecewise affine differential equation in which enzyme expression rates change between slow (κ_i^0) and fast rates ($\kappa_i^0 + \kappa_i^1$) depending on whether $s < \theta_i$ or $s > \theta_i$. Using the monotonicity of $g_2(s)$ in (S7), we can find one-to-one correspondences between the concentration s and the ratio e_1/e_2. Since g_2 is an increasing function of s, the inequality $s < \theta_i$ implies that $g_2(s) < g_2(\theta_i)$, which after substituting in (S7) leads to the following equivalences

$$s < \theta_i \iff e_2 > \beta_i e_1, \quad s > \theta_i \iff e_2 < \beta_i e_1, \tag{S10}$$

where $\beta_i = g_1(s_0)/g_2(\theta_i)$. We can use the equivalences in (S10) to recast the reduced model in (S8) as a piecewise affine system defined in three conic domains separated by half-lines of the form $e_2 = \beta_i e_1$ (see Fig. S1).

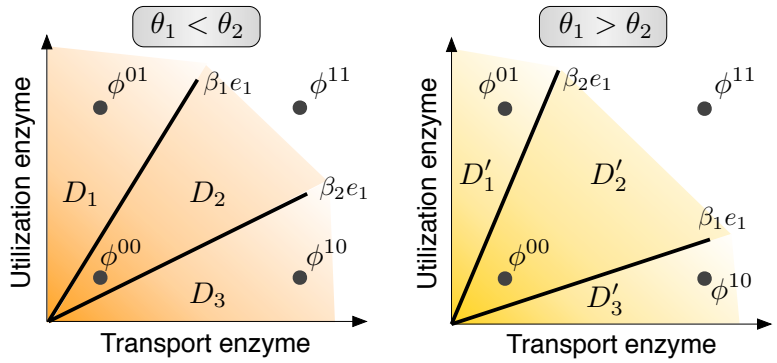

Figure S1 – State space of the reduced piecewise affine model in (S8). The state space is partitioned in three cones (called D_k if $\theta_1 < \theta_2$, or D_k' if $\theta_1 > \theta_2$ for $k = 1, 2, 3$); the β_i parameters are the slopes of the boundary half-lines and defined as $\beta_i = g_1(s_0)/g_2(\theta_i)$. The ϕ^{ij} points are defined in Table S1A.

The general form of the piecewise affine ODEs in (S8) is

$$\frac{\mathrm{d}e}{\mathrm{d}t} = \Gamma \left(\phi^{ij} - e\right), \tag{S11}$$

where we defined the concentration vector as $e = (e_1, e_2)^T$, the matrix $\Gamma = \mathrm{diag}\,\{\gamma_1, \gamma_2\}$, and the ϕ^{ij} vectors are combinations of the baseline and maximal expression levels (E^{off} and E^{on}

given in Table S1A). The vectors ϕ^{ij} take different values in different regions of the state space, givein in Table S1B–C. As an example, next we detail the construction of the piecewise affine model for the (R)epression-(R)epression circuit with thresholds ordered as $\theta_1 < \theta_2$.

Example. RR circuit with $\theta_1 < \theta_2$.

- If $s < \theta_1$ (or equivalently $e_2 > \beta_1 e_1$), both promoters are in the ON state and thus we can write the right hand side of (S8) as

$$\begin{aligned} \frac{\mathrm{d}e_1}{\mathrm{d}t} &= \gamma_1 \left(\frac{\kappa_1^0 + \kappa_1^1}{\gamma_1} - e_1 \right), \\ \frac{\mathrm{d}e_2}{\mathrm{d}t} &= \gamma_2 \left(\frac{\kappa_2^0 + \kappa_2^1}{\gamma_2} - e_2 \right), \end{aligned} \quad \text{if } e_2 > \beta_1 e_1. \tag{S12}$$

- If $\theta_1 < s < \theta_2$ (or equivalently $\beta_2 e_1 < e_2 < \beta_1 e_1$), promoter 1 is in the OFF state, and promoter 2 in the ON state, thus we can write the right hand side of (S8) as

$$\begin{aligned} \frac{\mathrm{d}e_1}{\mathrm{d}t} &= \gamma_1 \left(\frac{\kappa_1^0}{\gamma_1} - e_1 \right), \\ \frac{\mathrm{d}e_2}{\mathrm{d}t} &= \gamma_2 \left(\frac{\kappa_2^0 + \kappa_2^1}{\gamma_2} - e_2 \right), \end{aligned} \quad \text{if } \beta_2 e_1 < e_2 < \beta_1 e_1. \tag{S13}$$

- If $s > \theta_2$ (or equivalently $e_2 < \beta_2 e_1$) both promoters are in the OFF state, and thus we can write the right hand side of (S8) as

$$\begin{aligned} \frac{\mathrm{d}e_1}{\mathrm{d}t} &= \gamma_1 \left(\frac{\kappa_1^0}{\gamma_1} - e_1 \right), \\ \frac{\mathrm{d}e_2}{\mathrm{d}t} &= \gamma_2 \left(\frac{\kappa_2^0}{\gamma_2} - e_2 \right), \end{aligned} \quad \text{if } e_2 < \beta_2 e_1. \tag{S14}$$

We can write equations (S12)–(S14) in vector form and substitute the definitions of E_i^{off} and E_i^{on} (shown in (S3)) to get:

$$\frac{\mathrm{d}e}{\mathrm{d}t} = \begin{cases} \Gamma \left(\phi^{11} - e \right), \text{ if } e \in D_1, \\ \Gamma \left(\phi^{01} - e \right), \text{ if } e \in D_2, \\ \Gamma \left(\phi^{00} - e \right), \text{ if } e \in D_3, \end{cases} \tag{S15}$$

The conic domains D_i are defined in Table S1B and illustrated in Fig. S1; the RR case in (S15) corresponds to the first row of Table S1B.

Table S1 – Piecewise affine description of the timescale-separated model in (S8). (**A**) Possible stable steady states for the transport and utilization enzymes. (**B**) Piecewise affine models with $\theta_1 < \theta_2$; the RR row corresponds to the example in equations (S12)–(S14). (**C**) Piecewise affine models with $\theta_1 > \theta_2$.

A

Steady state	Transport (e_1)	Utilization (e_2)
ϕ^{00}	E_1^{off}	E_2^{off}
ϕ^{01}	E_1^{off}	E_2^{on}
ϕ^{10}	E_1^{on}	E_2^{off}
ϕ^{11}	E_1^{on}	E_2^{on}

B

$\theta_1 < \theta_2$

Circuit	Domain D_1 $e_2 > \beta_1 e_1$	Domain D_2 $\beta_2 e_1 < e_2 < \beta_1 e_1$	Domain D_3 $e_2 < \beta_2 e_1$
RR	$\dot{e} = \Gamma\left(\phi^{11} - e\right)$	$\dot{e} = \Gamma\left(\phi^{01} - e\right)$	$\dot{e} = \Gamma\left(\phi^{00} - e\right)$
AA	$\dot{e} = \Gamma\left(\phi^{00} - e\right)$	$\dot{e} = \Gamma\left(\phi^{10} - e\right)$	$\dot{e} = \Gamma\left(\phi^{11} - e\right)$
AR	$\dot{e} = \Gamma\left(\phi^{01} - e\right)$	$\dot{e} = \Gamma\left(\phi^{11} - e\right)$	$\dot{e} = \Gamma\left(\phi^{10} - e\right)$

C

$\theta_1 > \theta_2$

Circuit	Domain D_1' $e_2 > \beta_2 e_1$	Domain D_2' $\beta_1 e_1 < e_2 < \beta_2 e_1$	Domain D_3' $e_2 < \beta_1 e_1$
RR	$\dot{e} = \Gamma\left(\phi^{11} - e\right)$	$\dot{e} = \Gamma\left(\phi^{10} - e\right)$	$\dot{e} = \Gamma\left(\phi^{00} - e\right)$
AA	$\dot{e} = \Gamma\left(\phi^{00} - e\right)$	$\dot{e} = \Gamma\left(\phi^{01} - e\right)$	$\dot{e} = \Gamma\left(\phi^{11} - e\right)$
AR	$\dot{e} = \Gamma\left(\phi^{01} - e\right)$	$\dot{e} = \Gamma\left(\phi^{00} - e\right)$	$\dot{e} = \Gamma\left(\phi^{10} - e\right)$

S2 Identification of the bistable regimes.

In this section we show how to obtain the bistable regimes in Fig. 2 of the main text. We first show how to obtain the steady state enzyme concentrations and how to guarantee the existence of a steady state metabolite concentration, without computing its value. Later in Section S3 we derive parametric conditions for bistability, which we then use to determine the qualitative value of the metabolite concentration (i.e. the "low", "intermediate" and "high" concentration metabolite levels in Fig. 2 of the main text).

S2.1 Steady state enzyme concentrations

We obtain the stable steady state enzyme concentrations by imposing conditions on the ϕ^{ij} vectors in the piecewise affine models in (S11). The key observation is that a point ϕ^{ij} is a locally stable steady state of the piecewise affine system if and only if it belongs to its corresponding domain. Therefore, for a circuit to have two stable steady states, we need to ensure that at least

two points ϕ^{ij} belong to their conic domain. To guarantee that those steady states lead to a bistable uptake flux, they should have different values for the e_1 coordinate (recall from equation (6) in the main text, that the flux is proportional to the transport enzyme, i.e. $J = g_1(s_0)\bar{e}_1$). We illustrate this idea with an example.

Example. RR circuit with $\theta_1 < \theta_2$.
From Table S1B we see that the RR circuit with $\theta_1 < \theta_2$ can lead to a bistable flux in three cases:

- $\phi^{11} \in D_1$ and $\phi^{00} \in D_3$.
- $\phi^{11} \in D_1$ and $\phi^{01} \in D_2$.
- $\phi^{11} \in D_1$, $\phi^{01} \in D_2$ and $\phi^{00} \in D_3$.

Note that a fourth case, $\phi^{01} \in D_2$ and $\phi^{00} \in D_3$, can be ruled out because e_1 is at a low concentration in both ϕ^{01} and ϕ^{00}, and therefore these two steady states would not lead to a bistable flux.

With the above idea we can single out all the possible bistable regimes for each circuit. In Table S2 we have detailed all the conditions on the ϕ^{ij} vectors for each regime; in particular, the example (RR case with $\theta_1 < \theta_2$) corresponds to regimes RR-0, RR-2 and RR-4 in Table S2. In Table S2 there are a total of 15 possible arrangements of vectors ϕ^{ij} and conic domains that lead to a bistable flux. Note, however, that six of these regimes are infeasible in the sense that the conditions for bistability cannot be met for any combination of positive parameters (marked in red in Table S2). The infeasibility of these regimes can be readily checked from the conditions in Table S2 and the geometry of the state space in Fig. S1. The nine remaining regimes are the ones reported in Fig. 2 of the main text.

S2.2 Existence of the steady state metabolite concentration.

The steady state for the metabolite satisfies the equation in (S7):

$$g_2(\bar{s}) = g_1(s_0)\frac{\bar{e}_1}{\bar{e}_2}. \tag{S16}$$

However, because g_2 saturates at g_2^{sat}, equation (S16) may not have a solution for every $(\bar{e}_1, \bar{e}_2)$ pair. To guarantee that $g_2(\bar{s}) < g_2^{\text{sat}}$, and therefore the existence of a steady state concentration for the metabolite, we need the steady state enzyme concentrations to satisfy

$$\bar{e}_2 > \frac{g_1(s_0)}{g_2^{\text{sat}}}\bar{e}_1 = \check{\beta}\bar{e}_1, \tag{S17}$$

Table S2 – Bistable regimes in each uptake circuit. The regimes in red are infeasible, as the conditions cannot be met with any combination of parameters (due to the geometry of the state space, see Fig. S1). The feasible regimes are those shown in Fig. 2 of the main text. The crosses indicate the threshold-dependent regimes, i.e. those that emerge only under specific orderings of the thresholds.

Regime	$\theta_1 < \theta_2$	$\theta_1 > \theta_2$
RR-0	$\phi^{11} \in D_1, \phi^{00} \in D_3$	$\phi^{11} \in D'_1, \phi^{00} \in D'_3$
RR-1	×	$\phi^{10} \in D'_2, \phi^{00} \in D'_3$
RR-2	$\phi^{11} \in D_1, \phi^{01} \in D_2$	×
RR-3	×	$\phi^{11} \in D'_1, \phi^{10} \in D'_2, \phi^{00} \in D'_3$
RR-4	$\phi^{11} \in D_1, \phi^{01} \in D_2, \phi^{00} \in D_3$	×
AA-0	$\phi^{00} \in D_1, \phi^{11} \in D_3$	$\phi^{00} \in D'_1, \phi^{11} \in D'_3$
AA-1	×	$\phi^{01} \in D'_2, \phi^{11} \in D'_3$
AA-2	$\phi^{00} \in D_1, \phi^{10} \in D_2$	×
AA-3	×	$\phi^{00} \in D'_1, \phi^{01} \in D'_2, \phi^{11} \in D'_3$
AA-4	$\phi^{00} \in D_1, \phi^{10} \in D_2, \phi^{11} \in D_3$	×
AR-0	$\phi^{01} \in D_1, \phi^{10} \in D_3$	$\phi^{01} \in D'_1, \phi^{10} \in D'_3$
AR-1	×	$\phi^{00} \in D'_2, \phi^{10} \in D'_3$
AR-2	×	$\phi^{01} \in D'_1, \phi^{00} \in D'_2, \phi^{10} \in D'_3$
AR-3	$\phi^{01} \in D_1, \phi^{11} \in D_2$	×
AR-4	$\phi^{01} \in D_1, \phi^{11} \in D_2, \phi^{10} \in D_3$	×

where $\check{\beta} = g_1(s_0)/g_2^{\text{sat}}$. Although the exact steady state metabolite concentration can be computed from the equation in (S16), for our purposes it is more useful to determine its concentration relative to the regulatory thresholds θ_1 and θ_2. This allows us to distinguish between different bistable regimes based on the qualitative value of the metabolite concentration. For example, in the case $\theta_1 < \theta_2$, we can classify the metabolite concentration as "low" when $\bar{s} < \theta_1$, "intermediate" when $\theta_1 < \bar{s} < \theta_2$, and "high" when $\bar{s} > \theta_2$. As we show in the next section, we can deduce the qualitative value of the metabolite concentration from the conditions for bistability.

S3 Parametric conditions for bistability

From the ideas in Section S2, we can summarize a general procedure to obtain analytic conditions for bistability:

1. For a given bistable regime in Table S2, impose the conditions for local stability $\phi^{ij} \in D_k$ using the definitions in Table S1.

2. For each stable steady state, impose the condition for existence of the metabolite steady state,

i.e. $e_2 > \check{\beta} e_1$ in (S17).

3. Rewrite the conditions in terms of the promoter dynamic ranges μ_1, μ_2 and μ_{12} using the relations in (S6).

4. Discard any redundant inequalities.

5. Determine the qualitative value of the steady state metabolite concentration by using (S16) for each steady state and combining it with the derived inequalities.

Using the above steps in each of regimes in Table S2 we get the conditions for bistability detailed in Table S3 and Fig. 3–4 of the main text. To illustrate the application of steps 1-5 above, we show the full calculations in detail for two representative cases: the AA-2 regime and the AR-0 regime. These two examples are representative of the general procedure and contain all the elements needed to obtain the conditions for bistability in Table S3.

Example 1: AA-2 regime.

1. Following Table S2, we can guarantee the existence of two stable enzyme steady states by enforcing the following conditions

$$\phi^{00} \in D_1, \text{ and } \phi^{10} \in D_2, \tag{S18}$$

which using the definitions in Table S1A become

$$E_2^{\text{off}} > \beta_1 E_1^{\text{off}}, \text{ and } \beta_2 E_1^{\text{on}} < E_2^{\text{off}} < \beta_1 E_1^{\text{on}}. \tag{S19}$$

2. To guarantee the existence of a steady state for the metabolite, we impose condition (S17) to each steady state in this regime (i.e. ϕ^{00} and ϕ^{10})

$$E_2^{\text{off}} > \check{\beta} E_1^{\text{off}}, \text{ and } E_2^{\text{off}} > \check{\beta} E_1^{\text{on}}. \tag{S20}$$

3. Using the equivalences in (S6), we can rewrite conditions (S19)–(S20) in terms of the dynamic ranges:

$$E_2^{\text{off}} > \beta_1 E_1^{\text{off}} \iff \mu_{12} > \beta_1 \mu_2, \tag{S21}$$

$$\beta_2 E_1^{\text{on}} < E_2^{\text{off}} < \beta_1 E_1^{\text{on}} \iff \beta_2 \mu_1 \mu_2 < \mu_{12} < \beta_1 \mu_1 \mu_2, \tag{S22}$$

$$E_2^{\text{off}} > \check{\beta} E_1^{\text{off}} \iff \mu_{12} > \check{\beta} \mu_2, \tag{S23}$$

$$E_2^{\text{off}} > \check{\beta} E_1^{\text{on}} \iff \mu_{12} > \check{\beta} \mu_1 \mu_2. \tag{S24}$$

4. The conditions (S21)–(S24) can be put together as in Table S3. Note that the inequalities (S23)–(S24) are redundant because $\check{\beta} < \beta_2 < \beta_1$ (recall that $\theta_1 < \theta_2$ in the AA-2 regime) and thus the inequalities in (S21)–(S22) imply that both (S23)–(S24) are automatically satisfied.

5. To determine the location of the metabolite steady state, we substitute ϕ^{00} in equation (S16) to obtain

$$g_2(\bar{s}) = g_1(s_0)\frac{E_1^{\text{off}}}{E_2^{\text{off}}} = g_1(s_0)\frac{\mu_2}{\mu_{12}}, \tag{S25}$$

but from the condition in (S21) we know that $\mu_{12} > \beta_1\mu_2 = (g_1(s_0)/g_2(\theta_1))\,\mu_2$, which after substituting in (S25) leads to

$$g_2(\bar{s}) < g_2(\theta_1). \tag{S26}$$

By monotonicity of g_2 we conclude that that $\bar{s} < \theta_1$, and thus the steady state ϕ^{00} leads to a low steady state concentration for the metabolite.

Conversely, substituting the steady state ϕ^{10} in equation (S16) leads to

$$g_2(\bar{s}) = g_1(s_0)\frac{E_1^{\text{on}}}{E_2^{\text{off}}} = g_1(s_0)\frac{\mu_1\mu_2}{\mu_{12}}, \tag{S27}$$

but from the condition in (S22) we know that $\beta_2\mu_1\mu_2 < \mu_{12} < \beta_1\mu_1\mu_2$, or more explicitly

$$\frac{g_1(s_0)}{g_2(\theta_2)}\mu_1\mu_2 < \mu_{12} < \frac{g_1(s_0)}{g_2(\theta_1)}\mu_1\mu_2, \tag{S28}$$

which after substituting in (S27) leads to

$$g_2(\theta_1) < g_2(\bar{s}) < g_2(\theta_2). \tag{S29}$$

Monotonicity of g_2 implies that $\theta_1 < \bar{s} < \theta_2$ and thus the steady state state ϕ^{10} corresponds to an intermediate metabolite steady state concentration.

Example 2: AR-0 regime.

1. Without loss of generality, here we assume that $\theta_1 > \theta_2$ but the same analysis can be done for the converse case. Following Table S2, we can guarantee the existence of two stable enzyme steady states by enforcing the following conditions

$$\phi^{01} \in D_1', \text{ and } \phi^{10} \in D_3', \tag{S30}$$

which using the definitions in Table S1A become

$$E_2^{\text{on}} > \beta_2 E_1^{\text{off}}, \text{ and } E_2^{\text{off}} < \beta_1 E_1^{\text{on}}. \tag{S31}$$

2. To guarantee the existence of a steady state for the metabolite, we impose condition (S17) to each steady state in this regime (i.e. ϕ^{01} and ϕ^{10})

$$E_2^{\text{on}} > \check{\beta} E_1^{\text{off}}, \text{ and } E_2^{\text{off}} > \check{\beta} E_1^{\text{on}}. \tag{S32}$$

3. Using the equivalences in (S6), we can rewrite conditions (S31)–(S32) in terms of the dynamic ranges:

$$E_2^{\text{on}} > \beta_2 E_1^{\text{off}} \iff \mu_{12} > \beta_2, \tag{S33}$$
$$E_2^{\text{off}} < \beta_1 E_1^{\text{on}} \iff \mu_{12} < \beta_1 \mu_1 \mu_2, \tag{S34}$$
$$E_2^{\text{on}} > \check{\beta} E_1^{\text{off}} \iff \mu_{12} > \check{\beta}, \tag{S35}$$
$$E_2^{\text{off}} > \check{\beta} E_1^{\text{on}} \iff \mu_{12} > \check{\beta} \mu_1 \mu_2. \tag{S36}$$

4. The conditions (S33)–(S36) can be put together as in Table S3. Note that the inequality (S35) is redundant because $\check{\beta} < \beta_2$ and thus (S33) implies that (S35) is automatically satisfied.

5. To determine the location of the metabolite steady state, we substitute ϕ^{01} in equation (S16) we obtain

$$g_2(\bar{s}) = g_1(s_0) \frac{E_1^{\text{off}}}{E_2^{\text{on}}} = \frac{g_1(s_0)}{\mu_{12}}, \tag{S37}$$

but from the condition in (S33) we know that $\mu_{12} > \beta_2 = g_1(s_0)/g_2(\theta_2)$, which after substituting in (S37) leads to

$$g_2(\bar{s}) < g_2(\theta_2). \tag{S38}$$

By monotonicity of g_2 we conclude that that $\bar{s} < \theta_2$, and thus the steady state ϕ^{01} leads to a low steady state concentration for the metabolite.

Conversely, substituting the steady state ϕ^{10} in equation (S16) leads to

$$g_2(\bar{s}) = g_1(s_0) \frac{E_1^{\text{on}}}{E_2^{\text{off}}} = g_1(s_0) \frac{\mu_1 \mu_2}{\mu_{12}}, \tag{S39}$$

but from the condition in (S34) we know that $\mu_{12} < \beta_1 \mu_1 \mu_2 = (g_1(s_0)/g_2(\theta_1))\mu_1 \mu_2$, which

after substituting in (S39) leads to

$$g_2(\bar{s}) > g_2(\theta_1). \tag{S40}$$

which by monotonicity implies that $\bar{s} > \theta_1$ and thus the steady state ϕ^{10} corresponds to a high metabolite steady state concentration.

Table S3 – Conditions for bistability in each regime. The parameters are $\beta_i = g_1(s_0)/g_2(\theta_i)$. The crosses indicate the threshold-dependent regimes, i.e. those that emerge only under specific orderings of the thresholds. The conditions for the threshold-independent regimes are depicted in Fig. 3 of the main text; the conditions for the threshold-dependent regimes are shown in Fig. 4.

Regime	$\theta_1 < \theta_2$	$\theta_1 > \theta_2$
RR-0	$\beta_1\mu_1 < \mu_{12} < \beta_2\mu_2$ $\check{\beta}\mu_2 < \mu_{12}$	$\beta_2\mu_1 < \mu_{12} < \beta_1\mu_2$ $\check{\beta}\mu_2 < \mu_{12}$
AA-0	$\beta_1\mu_2 < \mu_{12} < \beta_2\mu_1$ $\check{\beta}\mu_1 < \mu_{12}$	$\beta_2\mu_2 < \mu_{12} < \beta_1\mu_1$ $\check{\beta}\mu_1 < \mu_{12}$
AA-1	×	$\beta_1 < \mu_{12} < \beta_2$ $\check{\beta}\mu_1 < \mu_{12} < \beta_1\mu_1$
AA-2	$\beta_1\mu_2 < \mu_{12} < \beta_1\mu_1\mu_2$ $\beta_2\mu_1\mu_2 < \mu_{12}$	×
AR-0	$\beta_1 < \mu_{12} < \beta_2\mu_1\mu_2$ $\check{\beta}\mu_1\mu_2 < \mu_{12}$	$\beta_2 < \mu_{12} < \beta_1\mu_1\mu_2$ $\check{\beta}\mu_1\mu_2 < \mu_{12}$
AR-1	×	$\beta_1\mu_2 < \mu_{12} < \beta_2\mu_2$ $\check{\beta}\mu_1\mu_2 < \mu_{12} < \beta_1\mu_1\mu_2$
AR-2	×	$\beta_2 < \mu_{12} < \beta_1\mu_1\mu_2$ $\beta_1\mu_2 < \mu_{12} < \beta_2\mu_2$ $\check{\beta}\mu_1\mu_2 < \mu_{12}$
AR-3	$\beta_2\mu_1 < \mu_{12} < \beta_1\mu_1$ $\beta_1 < \mu_{12}$	×
AR-4	$\beta_1 < \mu_{12} < \beta_1\mu_1$ $\beta_2\mu_1 < \mu_{12} < \beta_2\mu_1\mu_2$ $\check{\beta}\mu_1\mu_2 < \mu_{12}$	×

S4 Conditions for hysteresis in the AR-0 regime

Here we show the derivation of the conditions in (19c)–(19d) for hysteresis in the Activation-Repression circuit operating in the AR-0 bistable regime. The key idea is to guarantee two

saddle-node-like bifurcations for different values of the β_i parameters (and hence different concentrations of extracellular metabolite).

We assume that both promoters have equal regulatory thresholds, i.e. $\theta_1 = \theta_2 = \theta$, as this criterion helps to enlarge the design space for promoter dynamic ranges (recall Fig. 3 in the main text). Note that under equal thresholds, the domains D_2 and D_2' in the piecewise affine models of Table S1 collapse, and moreover $D_1 = D_1'$ and $D_3 = D_3'$. We define the parameter

$$\beta(s_0) = \frac{g_1(s_0)}{g_2(\theta)}, \tag{S41}$$

where with a slight abuse of notation we have made the dependence of β on the metabolite s_0 explicit. Since the transport turnover rate, g_1, is a non-decreasing function, $\beta(s_0)$ increases with the concentration s_0. We can analyze the bifurcations of the piecewise affine model in Table S1 by fixing the location of the ϕ^{ij} points and using the ideas in Example 2 of Section S3 for different values of $\beta(s_0)$. Following the notation in Table S1, to have a bistable hysteretic response we need

$$\text{low flux: } \begin{cases} \phi^{01} \in D_1, \\ \phi^{10} \notin D_3, \end{cases} \quad \text{for } s_0 < s_0^{\text{off}}, \tag{S42}$$

$$\text{bistable flux: } \begin{cases} \phi^{01} \in D_1, \\ \phi^{10} \in D_3, \end{cases} \quad \text{for } s_0^{\text{off}} < s_0 < s_0^{\text{on}}, \tag{S43}$$

$$\text{high flux: } \begin{cases} \phi^{01} \notin D_1, \\ \phi^{10} \in D_3, \end{cases} \quad \text{for } s_0 > s_0^{\text{on}}. \tag{S44}$$

The concentrations s_0^{off} and s_0^{on} in the (S42)–(S44) represent the amount of metabolite needed to switch the circuit from a high to low flux and *vice versa*. Note that condition (S42) is naturally satisfied because $g_1(0) = 0$ and therefore we can always find a sufficiently small s_0^{off} such that $\phi^{10} \notin D_3$ for $s_0 < s_0^{\text{off}}$ (or equivalently $E_2^{\text{off}} > \beta(s_0) E_1^{\text{on}}$ for $s_0 < s_0^{\text{off}}$).

Condition (S43) is identical to the ones in (S30) and therefore it is satisfied provided that the dynamic ranges satisfy the bounds in (S33)–(S34) when $s_0^{\text{off}} < s_0 < s_0^{\text{on}}$.

Condition (S44) can be satisfied if there exists s_0^{on} such that $\phi^{01} \notin D_1$ for $s_0 > s_0^{\text{on}}$, or equivalently

$$E_2^{\text{on}} < \beta(s_0) E_1^{\text{off}}, \tag{S45}$$

for $s_0 > s_0^{\text{on}}$. A sufficient condition for (S45) to hold for $s_0 > s_0^{\text{on}}$ is that in the saturation limit,

i.e. when $s_0 \to \infty$:

$$E_2^{\text{on}} < \hat{\beta} E_1^{\text{off}}, \tag{S46}$$

where $\hat{\beta} = g_1^{\text{sat}}/g_2(\theta)$. The condition (S46) above corresponds to condition (19c) in the main text.

Finally, we need to guarantee that the metabolite steady state $\bar{s}$ exists for all $s_0 > 0$. Recalling the condition in (S36), we need

$$E_2^{\text{off}} > \frac{g_1(s_0)}{g_2^{\text{sat}}} E_1^{\text{on}}, \text{ for all } s_0 > 0, \tag{S47}$$

Since g_1 saturates at g_1^{sat}, a sufficient condition for (S47) to hold for all $s_0 > 0$ is

$$E_2^{\text{off}} > \frac{g_1^{\text{sat}}}{g_2^{\text{sat}}} E_1^{\text{on}}, \tag{S48}$$

which corresponds to condition (19d) in the main text.