Supporting Appendix

Fixed Point Analysis

In this section, we study the fixed point properties of a feedback loop composed of an arbitrary number, N, of nodes whose dynamics is given by Eq. (1) in the main text, which we repeat here

$$\frac{dx_i}{dt} = g_i^{(A,R)}(x_i, x_{i-1}) \qquad i = 1 \ldots N. \qquad [1]$$

Our analysis proceeds by noting that, using the monotonicity condition, we can write explicit functional relations between neighboring variables in the steady state (when $dx_i/dt = 0$)

$$g_i^{(A,R)}(x_i^*, x_{i-1}^*) = 0 \quad \Rightarrow \quad x_i^* = f_i^{(A,R)}(x_{i-1}^*) \qquad [2]$$

Notice that the functions f_i have the same monotonicity properties as the g_is with respect to the second argument (for this it is necessary that $g_i(x,y)$ be a monotonically *decreasing* function of x). By iterative substitution, we obtain

$$x_i^* = f_i(x_{i-1}^*) = f_i(f_{i-1}(x_{i-2}^*)) = \ldots = $$
$$= f_i \circ f_{i-1} \circ f_{i-2} \circ \ldots \circ f_{i+1}(x_i^*) \equiv F_i(x_i^*) \qquad [3]$$

where $\circ$ denotes convolution of functions. Here, we introduced the function $F_i(x)$, which quantifies how the species i interacts with itself by transmitting signals along the loop. Notice also that if Eq.(3) holds for one value of i, then it holds for any i, since it is sufficient to apply $f_{i+1}()$ on both sides to obtain the equation for x_{i+1}^* and so on. For feedback loops, much useful information can be obtained from the properties of $F_i(x)$. Firstly, by applying the chain rule, we obtain the slope of $F_i(x)$ at x: $F_i'(x) = \prod_j f_j'(x_j)|_{x_i=x}$. The r.h.s is always greater (less) than zero if the number of repressors present in the loop is even (odd). In the former case, there can be multiple fixed points, i.e., this is a necessary condition for multistability. On the other hand, when there are an odd number of repressors, then $F_i(x)$ is positive and monotonically decreasing, meaning that there is one and only one solution to the fixed point equation $x_i^* = F_i(x_i^*)$. The system Eq. **1** has one unique fixed point, which we denote $\mathbf{x}^*$. To perform the stability analysis, we write the characteristic polynomial evaluated at this point

$$\prod_i [\lambda - \partial_x g_i(x,y)|_{x=x^*}] = \prod_i \partial_y g_i(x,y)|_{x=x^*}. \qquad [4]$$

The above equation can be greatly simplified using the relation $F'(x) = \prod_i \partial_y g_i(x,y)/\partial_x g_i(x,y)$, which is a consequence of the implicit function theorem and the chain rule. One then obtains the following equation

$$\prod_{i=1}^{N} \left(\frac{\lambda}{h_i} + 1\right) = F'(x^*) \qquad [5]$$

where the $h_i = -\partial_x g_i(x_i, x_{i=1})|_{x^*}$ are the degradation rates at the fixed point. Notice that, since $F'(x)$ is always negative in a negative feedback loop, all coefficients of the characteristic polynomial are non-negative, hence it can not have real positive roots.

This means that the destabilization of the fixed point can only occur via a Hopf bifurcation, i.e. with two complex conjugate eigenvalues crossing into the positive real half-plane.

In the simple case in which all the degradation rates are equal and unchanging (i.e, $h_i = \gamma$, a constant) the roots of the polynomial (5) in the complex plane are the vertices of a polygon centered on $-\gamma$ with a radius $|F'|$ as sketched in Fig. 5. Therefore,
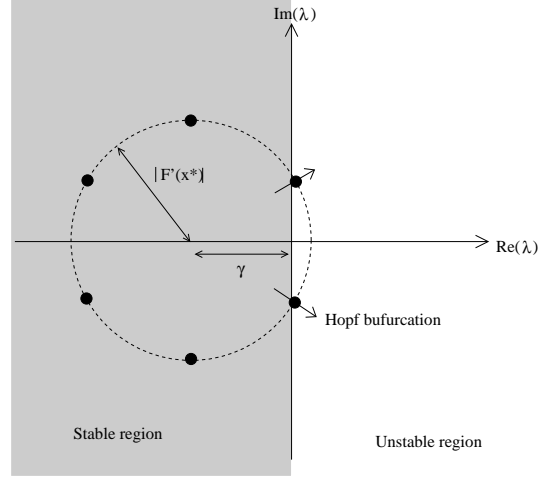

Fig. 5: Sketch of the Hopf bifurcation in the eigenvalue complex plane, in the case in which all the degradation rates are equal to a constant γ.

the fixed point will remain stable as long as

$$|F'(x^*)| \cos(\pi/N) < \gamma. \qquad [6]$$

In this case, Hopf's theorem (see e.g. [1]) ensures the existence of a periodic orbit close to the transition value, whose period is

$$T = 2\pi/Im(\lambda) \qquad [7]$$

which, in the simple case of equal degradation timescales, becomes $T = 2\pi/[|F'(x^*)| \cdot \sin(\pi/N)]$. Notice that the Hopf theorem does not ensure that the orbit is stable; however, since the system is bounded and there are no other fixed points, we expect the orbit to be attracting, at least close to the transition point.

Now we apply these ideas to the 3-repressor example discussed in the main text:

$$\frac{dx_i}{dt} = c - \gamma x_i + \alpha \frac{1}{1 + (x_{i-1}/K_i)^h} \qquad i = 1 \ldots 3. \qquad [8]$$

The coordinates of the fixed point are all equal due to symmetry. We denote by x^* the solution to the equation $\gamma x = c + \alpha/(1 + (x/K)^h)$. Then the characteristic polynomial is simply

$$(\lambda + \gamma)^3 = -\left(\frac{\alpha}{1 + (x^*/K_i)^h}\right)^3 \qquad [9]$$

Notice, that this can be written as

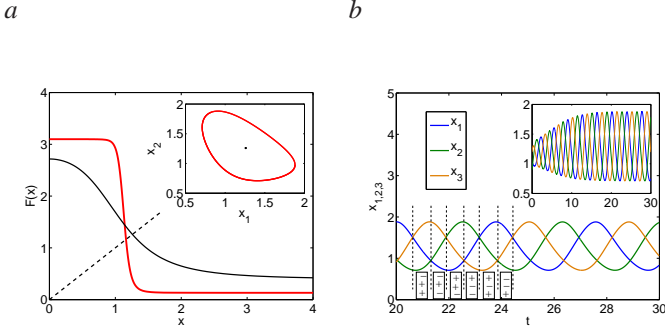$$\left(\frac{\lambda}{\gamma} + 1\right)^3 = F'(x^*) \qquad [10]$$

Fig. 6: A simple genetic oscillator described by Eq. **8**. (*a*) Plot of $F_1(x)$ for $h = 2$ (black solid curve) and $h = 4$ (red curve). Other parameters are kept fixed: $\alpha = 3.0$, $c = 0.1$, $K = 1$, $\gamma = 1$. The fixed point value of x_1 in each case lies at the intersection of the curves with the dotted line. Inset shows the corresponding trajectories in the x_1x_2 plane. (*b*) Time evolution of $x_{1,2,3}$ for the $h = 4$ case after a stable limit cycle is reached (*Inset* shows a longer time plot including the transient). Also shown is the symbolic dynamics for this time evolution.

The stability condition is then

$$|F'(x^*)| \cos(\pi/3) < \gamma. \qquad [11]$$

Fig. 6*a* shows that $|F'|$ satisfies this condition for $h = 2$ (black curve) but not for $h = 4$ (red curve), when the other parameters are kept fixed at the values $\alpha = 3.0$, $c = 0.1$, $K = 1$, $\gamma = 1$. Consequently, for $h = 2$ the trajectory converges to a stable fixed point, whereas it converges to a stable limit cycle for $h = 4$, as shown in the inset of Fig. 6*a*.

Symbolic Dynamics

In this section we give the mathematical details of the section "Symbolic dynamics" in the paper. This section is organized in the same order as in the main text and we will repeat the statements made there in a mathematically more rigorous way.

Let us use Γ to denote the phase space, i.e. the positive orthant

$$\Gamma = \{x_i > 0\} \qquad \forall i = 1 \dots N. \qquad [12]$$

In the systems in which trajectories are bounded only when the concentrations do not grow more than some maximum value (this is the case of saturated degradation), all the following considerations still hold, with the prescription of taking Γ to be the subset of the positive orthant in which the concentrations are bounded.

Our goal is to describe how the space Γ is partitioned by the N nullclines μ_i defined by $g_i(x_i, x_{i-1}) = 0$. The properties we are about to state are all consequences of the monotonicity of the functions $g_i(x_i, x_{i-1}) = 0$, the constraint of having bounded and persistent orbits and the existence of a unique fixed point $\mathbf{x}^*$ [1].

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[1] It is worth remarking here that the existence of a fixed point is more a consequence of the boundedness condition, rather than the monotonicity condition. Indeed, if the functions $f_i(x_{i-1})$ and $f_{i-1}^{-1}(x_{i-1})$ (see Eq. **2**) have independent support, they will obviously have no intersection. But in this case the system will

Returning to the partitioning of Γ, the first important property is that any nullcline divides Γ into two simply connected sets, one in which $g_i(\mathbf{x}) > 0$ and one in which $g_i(\mathbf{x}) < 0$. Notice also that these manifolds cannot be tangent at the fixed point because of monotonicity and since they can depend on at most one common variable. All these properties imply that the Γ space is partitioned by the nullclines into 2^N simply connected subsets, which we called "sectors" in the main text. In each of these sectors every component of the vector field has a definite, unchanging, sign. We use here the same notation as the main text and denote each of these sectors with a sign vector like $(+, -, -, \dots +)$. As stated in the paper, the uniqueness of the fixed point and the fact that the field has a constant sign inside a sector allows one to exclude the possibility of an attractor entirely contained within a sector. In the rest of this section, we discuss the case in which the fixed point is unstable. Since we assume that trajectories are bounded, starting from a sector the trajectory has to leave it by crossing one of the N boundaries [2]. We show in Fig. 3 in the main text that a given boundary $g_i = 0$ can be crossed in just one direction using a simple, two dimensional example. We show here a three dimensional example of how a stable periodic orbit crosses the nullclines. We consider the following model, consisting of one repressor and two activators (this is similar to one of the 3-variable models of the p53 system discussed in ref. 3):

$$
\begin{aligned}
dx_1/dt &= s - x_3 x_1/(K + x_1) \\
dx_2/dt &= x_1^2 - x_2 \\
dx_3/dt &= x_2 - x_3 \qquad [13]
\end{aligned}
$$

This system of equations has a stable periodic orbit as an attractor for parameters values $s = 30$, $K = .1$. The phase space portrait, together with a plot of the nullclines, is shown in Fig.(7).

Indeed, due to the fact that a given nullcline $g_i = 0$ is "flat" in all directions perpendicular to x_i and x_{i-1}, no new topological features appear in the higher dimensional cases; in particular the direction in which this nullcline can be crossed depends only on the sign of g_i and g_{i-1}, and never on any other nullcline. This directly follows from the fact that all the manifolds $\mu_i \cap \mu_j$ (intersections of a pair of nullclines) are simply connected; it is easy use the function $f_i(x)$ defined in Eq. **2** to write an explicit and continuous parameterization of these manifolds. It should be clear at this point that the following fact is true in any dimension N: the portion of the nullcline that forms the boundary between two adjacent sectors can only be crossed in one direction.

In conclusion, since the direction in which the nullcline i can be crossed depends only on the sign of g_i and g_{i-1}, this direction can be extrapolated directly from a qualitative analysis of

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violate our condition 1: persistence requires that $\lim_{x\to 0} g(x, y) > 0 \; \forall y$ and boundedness requires that $\lim_{x\to\infty} g(x, y) < 0 \; \forall y$; in this case, the nullclines have to cross. This fact is crucial also for the other considerations in this section on the phase space portrait. Notice that if the system is permanent (a slightly stronger condition than bounded+persistent), the existence of a fixed point can be directly demonstrated by means of Brouwer's fixed point theorem (see e.g., ref. 2).

[2] Strictly speaking, one has to exclude the possibility that the trajectory leaves the sector by crossing at the intersection between two nullclines, i.e. one of the sets $\mu_i \cap \mu_j$. This would correspond to two components of the field changing sign at the same time. We will not consider this case here since it not robust, occurring only for a set of parameter values that is of measure zero.
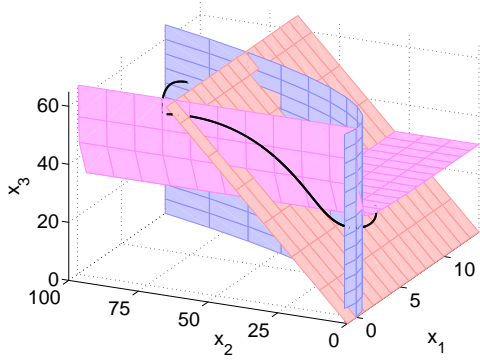
Fig. 7: Stable periodic orbit and plot of the nullclines for a model of the p53 system, defined by the system of equations (13).

the 2-dimensional case, as done in Fig. 3. In other words, all the nullclines behave qualitatively like one of the two nullclines sketched in Fig. 7. depending on whether they correspond to an activating or a repressing interaction, when seen in the (x_i, x_{i-1}) plane. From this it follows that the general rules can be extrapolated directly from the two dimensional case:

- If the variable $(i-1)$ represses i, the nullcline i can be crossed if g_i and g_{i-1} have the same sign.

- If the variable $(i-1)$ activates i, the nullcline i can be crossed if g_i and g_{i-1} have opposite signs.

We associate to a given symbol, or sector, the quantity H defined as the number of boundaries that can be crossed from that sector. Notice that $H = 0$ is impossible. For this to happen, the above rules must be violated by every adjacent pair of signs, i.e., the two signs on either end of an activation arrow must be the same, while the signs on both ends of a repression arrow must be different. As there are an odd number of repressors, this is impossible. Therefore, $1 \leq H \leq N$. When the trajectory crosses a nullcline $g_i = 0$, as a simple consequence of the transition rules, H either stays constant if the nullcline g_{i+1} can be crossed from the new sector, or decreases by two if g_{i+1} cannot be crossed (see Fig. 8). Physically, if we think of a crossable boundary as an unsatisfied bond between sign i and $i-1$ (termed "mismatches" in the main text), H is the number of such mismatches, hence it quantifies the level of "frustration" in the system. The time evolution can then (i) solve two neighboring unsatisfied bonds, or (ii) shift an unsatisfied bond one place to the right, i.e. from i to $i+1$. As a consequence: H can never increase, and it must always be an odd number.

We expect the system to end up in a state in which $H = 1$ and the unsatisfied bond keeps on moving around the loop in the direction of the arrows. This represents, at the level of symbolic dynamics, a single "signal" traveling around the loop. A direct implication is that the extremal points (maxima or minima) of all variables should appear in the time series in the order in which the species are arranged in the cycle.

This is the simplest scenario and is the only one for $N < 4$. What can actually happen for $N \geq 4$ is that $H = 3$ can become a
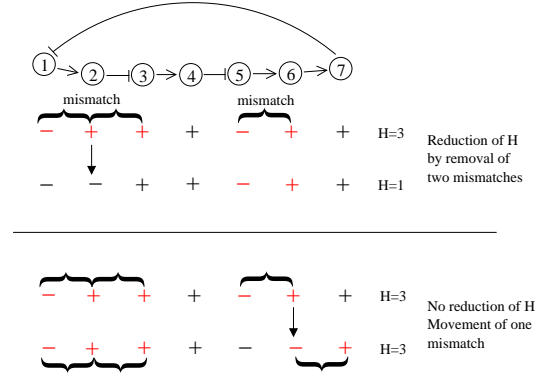

Fig. 8: Consequences of crossing a nullcline for the sign vector and the quantity H. (*Upper*) A sign is changed between two mismatches and, consequently, H decreases by 2. (*Lower*) A sign is changed next to just one mismatch. In this case, the mismatch simply moves one step ahead in the loop; the value of H does not change.

stable state, with three different signals traveling along the feedback loop. However, even if there is an attractor with $H = 3$, it must anyway coexist with an $H = 1$ attractor, since if a trajectory ever starts from, or enters, a sector with $H = 1$, it can never return to an $H = 3$ sector. Furthermore, the $H = 3$ attractor is likely to require some fine tuning of parameters to avoid one mismatch traveling "faster", catching up and annihilating another one. It is thus likely that any perturbation or noise would bring the system to the "ground state" attractor characterized by $H = 1$, and that is the one we expect to observe in biological systems.

Unobserved Variables

We conclude by demonstrating that the presence of unobserved variables does not alter our conclusions. Essentially the rules we stated depend only on the overall sign of the interaction between two variables and it is irrelevant if there are species in between mediating their interaction. We will clarify what we mean with an example and then give an argument for the general case. The example is the three-repressor loop of the previous section, in which one of the species is unobserved. We write its symbolic dynamics (as in Fig. 6b) and simply cancel the second row:

$$
\begin{matrix} x_1 \\ x_2 \\ x_3 \end{matrix}
\begin{pmatrix} - \\ + \\ - \end{pmatrix}
\begin{pmatrix} + \\ + \\ - \end{pmatrix}
\begin{pmatrix} + \\ - \\ - \end{pmatrix}
\begin{pmatrix} + \\ - \\ + \end{pmatrix}
\begin{pmatrix} - \\ - \\ + \end{pmatrix}
\begin{pmatrix} - \\ + \\ + \end{pmatrix}
$$

$$
\longrightarrow
\begin{pmatrix} - \\ - \end{pmatrix}
\begin{pmatrix} + \\ - \end{pmatrix}
\begin{pmatrix} + \\ + \end{pmatrix}
\begin{pmatrix} - \\ + \end{pmatrix}
\begin{matrix} x_1 \\ x_3 \end{matrix}
\qquad [14]
$$

The resulting symbolic dynamics is that of a two species loop with one activator and one repressor (like the p53-Mdm2 oscillations in Fig. 1). Here, as expected, two repressing links become one activating link if the intermediate species, x_2, is unobserved.

In general, let us consider two chemical species A and B belonging to a negative feedback loop, and consider the case in which all the intermediate chemical species going from A to B

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are unobserved. We showed that the stationary symbolic dynamics is characterized by a single mismatch moving in the direction of the loop.

First, suppose that there are an *even* number of repressors between A and B. Then, it follows that when the mismatch is outside the segment of the loop going from A to B then both A and B will be increasing or decreasing together. The reason is that, if there is no mismatch, two adjacent species have the same increasing/decreasing character if one is activating the other and opposite if one is repressing the other. On the other hand, when the mismatch lies within this segment the behavior of A and B will be opposite, i.e., they will have opposite signs in the symbolic dynamics. The sign of B flips when the mismatch passes from within the segment to outside it. If the intermediate species are unobserved, just before the sign of B flips, the signs of A and B in the symbolic dynamics will be opposite. Thus, our algorithm will show that A activates B.

Exactly the converse of the above happens when there are an *odd* number of repressors between A and B. In this case, A and B have opposite signs in the symbolic dynamics when the mismatch is outside the segment, and same signs when the mismatch is inside the segment. Thus, just before the sign of B flips, the signs of A and B will be the same, implying that A represses B.

The net effect is that when the intermediate species are unobserved our algorithm will show that: (i) A activates B, when there are an even number of repressors between them, and (ii) A represses B, when there are an odd number of repressors between them.

References

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[2] Hofbauer J, Sigmund K (1998) *Evolutionary Games and Population Dynamics* (Cambridge Univ., Cambrigde, UK)

[3] Geva-Zatorsky N, Rosenfeld N, Itzkovitz S, Milo R, Sigal A, Dekel E, Yarnitzky T, Liron Y, Polak P, Lahav G, *et al.* (2006) *Mol Sys Biol* 2:2006.0033.