Bell-shaped dose response for a system with no IFFLs

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Abstract

It is well known that the presence of an incoherent feedforward loop (IFFL) in a network may give rise to a steady state non-monotonic dose response. This note shows that the converse implication does not hold. It gives an example of a three-dimensional system that has no IFFLs, yet its dose response is bell-shaped. It also studies under what conditions the result is true for two-dimensional systems, in the process recovering, in far more generality, a result given in the T-cell activation literature.

1 Introduction

An "incoherent feedforward loop" (IFFL) is a pair of directed paths between two nodes which have opposite signs. IFFLs are a ubiquitous motif in systems biology [1]. It is well-known that the presence of IFFLs may result in steady state dose responses which are non-monotonic see e.g. [4] or the references in [7]. Negative feedbacks *or* IFFLs are necesssary, as otherwise the theory of monotone systems implies that the dose response ("input to state characteristic") will be monotone on input values [2, 3]. (In fact, for monotone systems, even transient responses at any given time also behave monotonically on input magnitude.)

It has been argued that IFFLs are also *necessary*. For example, in [5] the authors state that *"models without an incoherent feed-forward loop but with negative feedback... cannot produce a bell-shaped dose-response"* (main text as well as section "Negative feedback cannot produce a bell-shaped dose-response" in SI). This conclusion was based on a computational screen of 58,905 network architectures, as well as explicit examples in the SI. We show here that this converse is false, i.e. one may have non-monotonic dose-responses even in systems that include no IFFLs (but, of course, include negative feedback loops, as these are necessary if there are no IFFLs). Our counterexample is not included in the class of reaction formulas systems computationally explored in [5], thus explaining the discrepancy.

We also study under what conditions the result is true for two-dimensional systems, in the process recovering, in far more generality, a result given in [5] in the context of T-cell activation.

2 A counterexample for three-dimensional systems

Consider the following system:

$$\begin{aligned}\dot{x}_1 &= -ax_1 + f(x_2) + x_3\\ \dot{x}_2 &= g(x_1) - dx_2\\ \dot{x}_3 &= x_2 - x_3 + u\end{aligned}$$

where the functions f and g are decreasing and non-negative functions of x_1 and x_2 respectively, and $a, , b, c$ are positive constants. The system evolves on states $x_i(t) \geq 0$, and the input u is taken as a constant in some interval $I \subseteq [0, \infty)$. The output is $y = h(x_1, x_2, x_3) = x_3$. Note that the system is positive, meaning that when starting from nonnegative x_1, x_2, x_3 the solutions remain nonnegative.

The Jacobian matrix (with respect to the variables x_i), evaluated at any state, has the following sign structure:

$$J = \begin{pmatrix} - & - & + \\ - & - & 0 \\ 0 & + & - \end{pmatrix}$$

which we represent graphically as in Fig. 1.

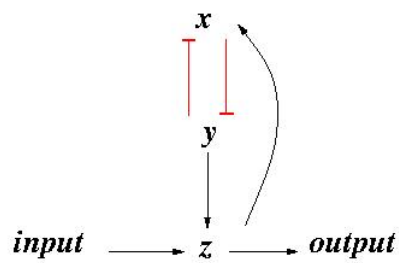


Figure 1: Signed digraph representing the three-dimensional example. (Using x, y, z instead of x_i's.)

Suppose that for each constant input value $u \in I$ there is an asymptotically stable steady state X_u. The output at this steady state is $y = h(X_u)$, and the mapping $u \mapsto h(X_u)$ is defined as the *steady state dose response* of the system.

Clearly, there and no IFFLs in our network. We will now provide functions f and g for which the dose response is non-monotonic.

We define the functions f and g as follows:

$$\begin{array}{rcl} f(s) & := & \alpha e^{-b\sigma(s)} \\ g(s) & := & \beta e^{-c\sigma(s)} \\ \sigma(s) & := & \left\{ \begin{array}{ll} 0.8 & 0 \leq s < 0.8 \\ s & 0.8 \leq s \leq 1.2 \\ 1.2 & 1.2 \leq s \end{array} \right. \end{array}$$

and the positive parameters a, α, b, β, c are:

$$a = 20/e^2\,, \quad b = 2\,, \quad c = 1\,, \quad d = 1/e\,, \quad \alpha = 10\,, \quad \beta = 1\,.$$

Finally, the input interval is:

$$I \;:=\; [0.25, 0.45]\,.$$

There is a stable steady state for each $u \in I$, and simulations from random initial states indicate that the state X_u, $u \in I$ is globally asymptotically stable. For example, when $u = 10/e^2 - 1 \approx 0.3534$, the corresponding steady state is

$$X_{u_0} = (x_1, x_2, x_3) \;=\; (1, 1, 10/e^2)$$

and the output is $y = 10/e^2$. The steady-state dose response has a bell-shape plot (i.e., it is not monotonic) as a function u. The plots in Fig. 2 show the dose-response and maximum real part of eigenvalues at these steady states (showing that all eigenvalues have negative real part).

Remark. A small technical issue is that the saturation σ is not differentiable at those states for which either x_1 or x_2 equals exactly 0.8 or 1.2, which happens only a set of measure zero. Thus the Jacobian is not well-defined at every state in the classical sense. However, it is well-defined in the sense of nonsmooth analysis, and in any event the signs of interactions reflect the increasing or decreasing influence of each variable on each other variable, and this can be defined with no need to take derivatives. The saturation function σ could be replaced by an approximating differentiable function, but use of σ makes calculations somewhat simpler. ∎

This example was derived as follows. Ignore first the saturations, and consider the Jacobian (with respect to the x variables):

$$J = \begin{pmatrix} -20e^{-2} & -20e^{-2x_2} & 1 \\ -e^{-x_1} & -e^{-1} & 0 \\ 0 & 1 & -1 \end{pmatrix}$$

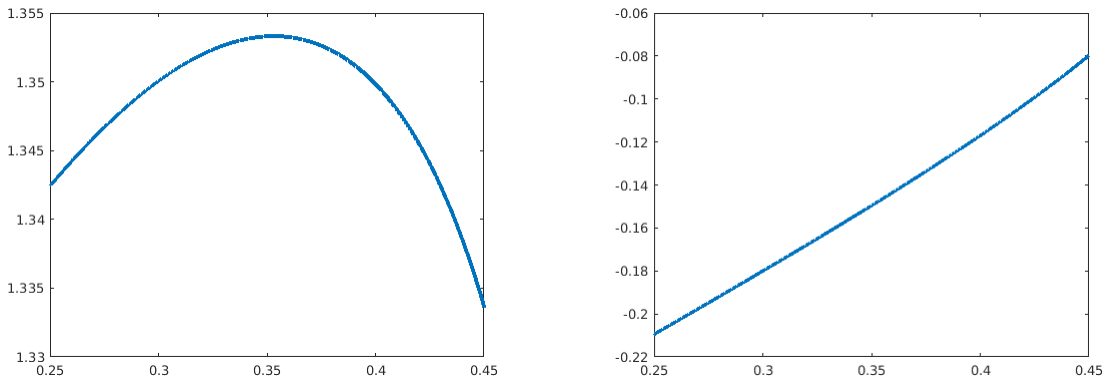


Figure 2: (Left) Dose response. (Right) Maximum real part of eigenvalues, as a function of u, showing stability.

which has characteristic polynomial $P(\lambda) = \lambda^3 + a_2\lambda^2 + a_3\lambda + a_4$. The Routh-Hurwitz criterion requires that $a_2, a_3, a_4 > 0$ and $\Delta := a_2a_3 - a_4 > 0$ for stability. We have:

$$\begin{aligned}
a_2 &= e^{-1} + 20e^{-2} + 1 \\
a_3 &= e^{-1} + 20e^{-2} + 20e^{-x_1}e^{-2x_2} + 20e^{-3} \\
a_4 &= 20e^{-x_1}e^{-2x_2} - e^{-x_1} + 20e^{-3} \\
\Delta &= e^{-1} + 20e^{-2} + e^{-x_1} + 400e^{-5} + 20e^{-4} + e^{-2} \\
&\qquad + 400e^{-4} + 40e^{-3} + 20e^{-1}e^{-x_1}e^{-2x_2} + 400e^{-2}e^{-x_1}e^{-2x_2}\,.
\end{aligned}$$

When $x_1 = 1$ and $x_2 = 1$ (as in the equilibrium X_u corresponding to $u = 10/e^2 - 1$),

$$P(\lambda) = \lambda^3 + (e^{-1} + 20e^{-2} + 1)\lambda^2 + (e^{-1} + 20e^{-2})\lambda + e^{-1}$$

and

$$\Delta = e^{-2} + 40e^{-3} + 400e^{-4} + 20e^{-2}$$

so that this equilibrium is stable.

It follows that, in particular, the Jacobian is nonsingular at such an equilibrium, so that, by the implicit function theorem, we know that there is a local curve X_u of equilibria for each u near $u = 10/e^2 - 1$. We then guessed a range where stability will be preserved. Note that the expression for a_4 is increasing on x_1 and x_2 and it is positive when $x_1 = 0.9$ and $x_2 = 0.88$, so the matrix J is Hurwitz for values $x_1 \geq 0.9$ and $x_2 \geq 0.88$, which include all the steady states X_u for $u \in I$. This suggests the range to be used, and the eigenvalue plot confirms the results.

The saturation function was added in order to provide global stability; without it, we found that there is bistability.

The fact that the dose response is bell-shaped follows by a perturbation argument. In terms of the derivatives X_u' of X_u with respect to u, we have an implicit equation

$$JX_u' + b \;=\; 0$$

where $b = \mathrm{col}(0, 0, 1)$. Thus the derivative of the output $y = x_3$ is, as a function of u:

$$-cJ^{-1}b \;=\; -\det(J)^{-1}\gamma$$

where γ is the $(3, 3)$ entry of the adjugate matrix, which is the principal 2×2 minor of J, namely zero. Thus we have a point at which the output may have a local minimum or maximum. One could check the second derivative to see that this is strict, but at this point a simulation completes the proof.

Remark. In any dimension, for systems $\dot{x} = f(x, u)$ with output $y = h(x)$, one may similarly check by a perturbation argument if the determinant of the Jacobian of f and a suitable minor do not change sign

along the state space. A sufficient condition for this can be given in terms of regular interval matrices [6]. We omit the easy details.

Remark. The bell-shaped dose response in Fig 2 spans only a small range of output values. One could rescale this example to have a more drammatic effect. There are at least two ways to change this while still having the same result (no IFFL):

- We can add a new variable, let us say x_4, with an equation $\dot{x}_4 = \varphi(x_3) - x_4$, where now the output is x_4, and the function φ is strictly increasing. The network structure, still not containing any IFFL subcircuits, is shown in 3. Now the dose-response is $\varphi(h(X_u))$, where $h(X_u)$ was the previous

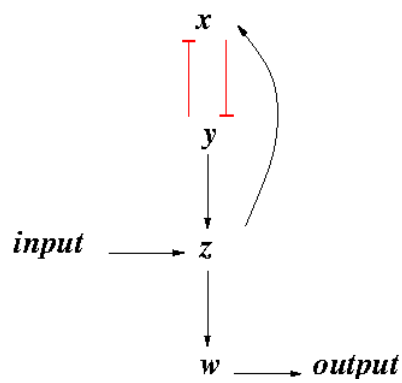


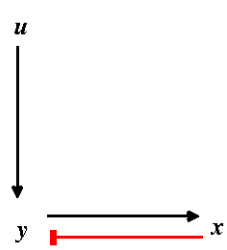
Figure 3: Signed digraph representing the four-dimensional example. (Using x, y, z, w instead of x_i's.)

  dose response. We can take a Hill function for φ, with a K_m of about 1.35, rendering the new dose response initially almost 0, changing to 1, and finally back to near zero.

- A less elegant alternative, if we want to insist on three-dimensional example, is to make a change of variables $x_3 = \varphi(x_3)$, with the same function φ as above. Now the equation for $\dot{x}_1$ will have a term $\varphi^{-1}(x_3)$, which is still increasing on x_3, and the equation for $\dot{x}_3$ witll have the term $\varphi'(\varphi^{-1}(x_3))$ multiplying the previous left hand side, so all Jacobians still have the same sign.

3 The case of two-dimensional systems

Consider this diagram for a system, containing one negative feedback loop (and no IFFL):



Here u is the input, y the output, and x the intermediate state that provides the negative feedback loop. We model this as

$$\begin{aligned} \dot{x} &= f(x, y) \\ \dot{y} &= g(x, y, u) \end{aligned}$$

on $x(t) \geq 0$, $y(t) \geq 0$ (with $u \geq 0$ seen as a constant in this problem), and we impose these conditions (subscripts indicate partial derivatives):

1. $f_y(x,y) > 0$ for all x, y (arrow from y to x is activating),
2. $g_x(x,y,u) \leq 0$ for all x, y, u (arrow from x to y is repressing),
3. $g_u(x,y,u) \geq 0$ for all x, y, u (arrow from u to y is activating),
4. $f(0,y) \geq 0$, $g(x,0,u) \geq 0$ for all x, y, u (invariance of nonnegative orthant).

   In addition, we impose these constraints to make the problem non-trivial:

5. for each non-negative constant input u, there is a unique (non-negative) steady state, denoted as (x_u, y_u)
6. (x_u, y_u) depends smoothly on u (this is done for purely technical reasons, to be able to compute the sensitivity on u),
7. this steady state is exponentially asymptotically stable.

We call y_u as a function of u the *dose response*.

We will show:

- The *dose response need not be monotone* (it may be bell-shaped).
- However, *if we impose the additional requirement* that $f_x(x,y) \leq 0$ for all x, y, then the dose response *is monotone*.

This last requirement says, in effect, that x can degrade but there is no auto-catalysis on x.

We prove the second statement and give an example for the first one.

The proof of is immediate by the arguments given above - now the adjugate matrix is just a single element that is always negative. We prove it in detail.

We compute the Jacobian:

$$J_{x,y} = \begin{pmatrix} f_x & f_y \\ g_x & g_y \end{pmatrix}$$

The assumed stability condition means that the trace is negative and the determinant is positive (at the equilibrium (x_u, y_u), for each u):

$$T := f_x + g_y < 0 \quad D := f_x g_y - f_y g_x > 0\,.$$

(Observe that, if we assume that both $f_x \leq 0$ and $g_y < 0$, then we automatically have $T \leq 0$ and $D \geq 0$, but we do not need these additional assumptions, since we are assuming stability.)

The steady states satisfy

$$f(x_u, y_u) = 0, \quad g(x_u, y_u, u) = 0$$

at all u, and are differentiable in u, so we differentiate:

$$f_x \frac{\partial x_u}{\partial u} + f_y \frac{\partial y_u}{\partial u} = 0 \quad g_x \frac{\partial x_u}{\partial u} + g_y \frac{\partial y_u}{\partial u} + g_u \equiv 0\,.$$

Thus

$$\frac{\partial y_u}{\partial u} = -\frac{f_x}{f_y}\frac{\partial x_u}{\partial u}$$

and

$$\frac{-D}{f_y}\frac{\partial x_u}{\partial u} + g_u \;=\; \frac{f_y g_x - f_x g_y}{f_y}\frac{\partial x_u}{\partial u} + g_u \;=\; (g_x - g_y f_x/f_y)\frac{\partial x_u}{\partial u} + g_u \;=\; g_x \frac{\partial x_u}{\partial u} + g_y\left(-\frac{f_x}{f_y}\frac{\partial x_u}{\partial u}\right) + g_u \;=\; 0$$

which gives

$$\frac{\partial x_u}{\partial u} \;=\; \frac{f_y}{D} g_u$$

and thus substituting back in the formula for $\frac{\partial y_u}{\partial u}$:

$$\frac{\partial y_u}{\partial u} \;=\; -\frac{f_x}{f_y}\frac{f_y}{D}g_u \;=\; -\frac{f_x}{D}g_u\,.$$

Therefore, if we also have $f_x \leq 0$ then $\frac{\partial y_u}{\partial u} \geq 0$ and we conclude that y_u is increasing on u. (Strictly if $f_x < 0$ and $g_u > 0$.)

This ends the proof. ∎

Remark. Note that not all the properties 1-7 are used in the proof. Also, the same result holds if we have an invariant set $\mathcal{V}$ for the solutions of the differential equations and we are only interested in solutions that remain in $\mathcal{V}$. Of course, the conditions are then imposed on all $(x, y) \in \mathcal{V}$. ∎

Remark. In the paper [5] the authors prove the same result for the particular system evolving on $[0, 1]^2$ in which:

$$\begin{aligned} f(x,y) &= \alpha(y)(1-x) - \delta x \\ g(x,y,u) &= u(1-y) - (\beta + \gamma x)y \end{aligned}$$

and

$$\alpha(y) = c + \frac{a}{a + \left(\frac{b}{y}\right)^n}$$

for certain (positive) parameters a, b, c. (For simplicity, we redefined the input in that paper, to absorb an additive and a multiplicative constant.) All conditions for our result are satisfied. (Note that $f_x = -\alpha(y) - \delta < 0$.) The existence of a unique steady state follows from the fact that one can solve the first equation to obtain $x = \theta(y) := \alpha(y)/(\delta + \alpha(y))$ and when substituted into $u(1-y) = (\beta + \gamma\theta(y))y$ we have that the left hand side decreases from u to zero and the right had side increases from zero, so there is a unique equilibrium (and it is smoothly dependent on u by the implicit function theorem). ∎

This construction also hints at the counterexample. We need to find f and g so that: 1-7 hold and g_x changes sign along the curve (x_u, y_u).

In order to obtain such a counterexample, we will take special forms for f and g:

$$\begin{aligned} f(x,y) &= -\alpha(x) + y \\ g(x,y,u) &= \beta(x) - y + u + \kappa \end{aligned}$$

where $\kappa > 0$ is a constant (this could be though of either as constitutive production of y or as a lower bound on a redefined input $\kappa + u$) and the smooth functions α and β are defined below.

We need the following properties:

1. $f_y(x, y) > 0$ (satisfied because $f_y = 1 > 0$)
2. $g_x(x, y, u) \leq 0$, i.e. $\alpha' \geq 0$ (here prime is derivative w.r.t. x)
3. $g_u(x, y, u) \geq 0$ (satisfied because $g_u = 1$)
4. $f(0, y) \geq 0$, $g(x, 0, u) \geq 0$: we'll take $\alpha(0) = 0$ and $\beta(x) \geq 0$

and additional properties as follows.

The steady state for constant input u solves these two equations:

$$y = \alpha(x)$$

and

$$\beta(x) - \alpha(x) + u + \kappa = 0$$

or equivalently

$$\gamma(x) \;:=\; \alpha(x) - \beta(x) = u + \kappa\,.$$

We will require that, for some $\delta > 0$, $\gamma(\delta) = \kappa$ and $\gamma(x) > 0$, $\gamma'(x) \geq 0$ for all $x \in [\delta, \infty)$. By the implicit mapping theorem, this says that there is a unique (and differentiable) solution $x \in [\delta, \infty)$ of $\gamma(x) = u + \kappa$, for each $u \geq \kappa$, and this solution depends smoothly on u. This will be x_u. We will also require $\alpha(x) \geq 0$ for all $x \in [\delta, \infty]$, which then gives $y_u = \alpha(x_u)$. Thus, there is a unique steady state for each u, and (x_u, y_u) depends smoothly on y, guaranteeing gives properties 5 and 6. There remains to assure stability: $D > 0$ and $T < 0$ at all equilibria, Since $f_x = -\alpha$, $f_y = 1$, $g_x = \beta'$, $g_y = -1$:

$$D = f_x g_y - f_y g_x = \alpha' - \beta' = \gamma', \quad T = f_x + g_y = -\alpha - 1$$

we need to require $\alpha(x) > -1$ for all $x \in [\delta, \infty)$ (we already required $\gamma' > 0$). To summarize, we need:

- smooth functions $\alpha, \beta : [0, \infty) \to \mathbb{R}$,
- $\alpha(0) = 0$,
- $\beta(x) \geq 0$ for all $x \in [0, \infty)$,
- numbers $\delta > 0$, $\kappa > 0$ so that $\gamma := \alpha - \beta$ has $\gamma(\delta) = \kappa$,
- $\gamma'(x) > 0$ for all $x \in [\delta, \infty)$ (this implies that $\gamma(x) \geq \gamma(\delta) > 0$)
- $\alpha(x) > -1$ for all $x \in [\delta, \infty)$.

Our objective is to get a sign change in $\frac{\partial y_u}{\partial u}$, so we need that f_x changes sign, i.e. α' should admit both a positive and a negative sign on $x \in x \in [\delta, \infty)$.

There are many possible choices for such α and β. We pick a rather artificial one merely to show the existence of such functions, not for any practical significance. We will pick

$$0 < \varepsilon < \frac{1}{(1+\pi)^2} \approx 0.0583$$

and any

$$0 < \delta \leq \pi$$

and these functions:

$$\begin{aligned} \beta(x) &:= \frac{1}{1+x}, \\ \widetilde{\alpha}(x) &:= \frac{1}{1+x} + \sin x + (1+\varepsilon)x, \\ \alpha(x) &:= \theta(x)\,\widetilde{\alpha}(x), \end{aligned}$$

where θ is a smooth function which is 0 at $x = 0$ (thus assuring $\alpha(0) = 0$) and $\theta(x) \equiv 1$ for $x \geq \delta$. Observe that this definition assures that $\widetilde{\alpha}(x) = \alpha(x)$ for all $x \geq \delta$. Such functions θ can be obtained by an abstract partition of unity argument. However, the same argument can be adapted to work with a Hill function $\theta(x) = x^n/(K^n + x^n)$ with $0 \ll K \ll 1$ and $n \gg 1$.

Note that $-\frac{1}{(1+x)^2} \geq -1$ and $\cos x \geq -1$, so the function $\widetilde{\alpha}(x)$ has derivative

$$\widetilde{\alpha}'(x) \;=\; -\frac{1}{(1+x)^2} + \cos x + 1 + \varepsilon \;\geq\; -1 - 1 + 1 + \varepsilon = -1 + \varepsilon$$

which shows that $\alpha'(x) = \widetilde{\alpha}'(x) > -1$ for $x \geq \delta$.

Let $\widetilde{g}(x) = \widetilde{a}(x) - \beta(x) = \sin x + (1+\varepsilon)x$, and that $\widetilde{\gamma}(x) = \gamma(x)$ for all $x \geq \delta$. Observe that

$$\widetilde{g}'(x) = = \;\cos x + 1 + \varepsilon \geq -1 + 1 + \varepsilon = \varepsilon > 0$$

for all x and that $\gamma(0) = 0$, so it follows that $\widetilde{\gamma}(x) > 0$ for all x (and thus in particular for $x \geq \delta$).

When $x = \pi$, $\alpha'(\pi) = -\frac{1}{(1+\pi)^2} + \varepsilon < 0$ because of the choice of ε. On the other hand, for $x = 2\pi$, $\alpha(x) = \widetilde{\alpha}'(x) = -\frac{1}{(1+2\pi)^2} + 2 + \varepsilon > 1 + \varepsilon > 0$. This shows that α' changes sign.

We now define

$$\kappa := \widetilde{\alpha}(\delta) - \beta(\delta) = \alpha(\delta) - \beta(\delta) = \sin\delta + 1.001\delta,$$

so that $\gamma(\kappa) = \delta$ is satisfied.

For example, if we take $\varepsilon = 0.001$ and $\delta = \pi/2$, $\kappa = 1 + 1.001\pi/2$.

As a numerical example, with $\varepsilon = 0.001$ and

$$u + \kappa \approx 3.1378, 3.1447, 4.0461 \qquad (u \approx 0.5654, 0.5723, 1.4737)$$

which were obtained by evaluating $\gamma(x)$ at the respective points below:

$$x_u = 2.8000, 3.1000, 5.0000$$

and the does response is not monotone, since we have these respective values for y:

$$y_u = 3.4009, 3.3886, 4.2127$$

(we built up this example by starting from the fact that $\frac{\partial y_u}{\partial u} < 0$ in an interval around an u for which $x_u = \pi$, since the sign of $\frac{\partial y_u}{\partial u}$ is the sign of α' at that point). See Fig. 4 for part of the plot of y_u vs u.

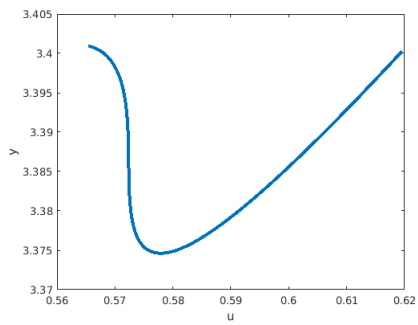


Figure 4: Non-monotone dose-response for two-dimensional example

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