Monotone and near-monotone network structure (part II)

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(continued from Part I)

3 I/O Monotone systems

We next describe recent work on monotone input/output systems ("MIOS" from now on). Monotone i/o systems originated in the analysis of mitogen-activated protein kinase cascades and other cell signaling networks, but later proved useful in the study of a broad variety of other biological models. Their surprising breadth of applicability notwithstanding, of course MIOS constitute a restricted class of models, especially when seen in the context of large biochemical networks. Indeed, the original motivation for introducing MIOS, in the 2003 paper [1], was to study an existing non-monotone model of negative feedback in MAPK cascades. The key breakthrough was the realization that this example, and, as it turned out, many others, can be profitably studied by decompositions into MIOS. In other words, a non-monotone system is viewed as an interconnection of monotone subsystems. Based on the architecture of the interconnections between the subsystems ("network structure"), one deduces properties of the original, non-monotone, system. (Later work, starting with [2], showed that even monotone systems can be usefully studied through this decomposition-based approach.)

We review the basic notion from [1]. (For concreteness, we make definitions for systems of ordinary differential equations, but similar definitions can be given for abstract dynamical systems, including in particular reaction-diffusion partial differential equations and delay-differential systems, see e.g. [3].) The basic setup is that of an input/output system in the sense of mathematical systems and control theory [4], that is, sets of equations

\[
\frac{dx}{dt} = f(x,u), \quad y = h(x),
\]

in which states \(x(t)\) evolve on some subset \(X \subseteq \mathbb{R}^n\), and input and output values \(u(t)\) and \(y(t)\) belong to subsets \(U \subseteq \mathbb{R}^m\) and \(Y \subseteq \mathbb{R}^p\) respectively. The coordinates \(x_1, \ldots, x_n\) of states typically represent concentrations of chemical species, such as proteins, mRNA, or metabolites. The input variables, which can be seen as controls, forcing functions, or external signals, act as stimuli. Output variables can be thought of as describing responses, such as movement, or as measurements provided by biological reporter devices like GFP that allow a partial read-out of the system state vector \((x_1, \ldots, x_n)\). The maps \(f : X \times U \to \mathbb{R}^n\) and \(h : X \to Y\) are taken to be continuously differentiable. (Much less can be assumed for many results, so long as local existence and uniqueness of solutions is guaranteed.) An input is a signal \(u : [0, \infty) \to U\) which is locally essentially compact (meaning that images of restrictions to finite intervals are compact), and we write \(\varphi(t, x_0, u)\) for the
solution of the initial value problem \( dx/dt(t) = f(x(t), u(t)) \) with \( x(0) = x_0 \), or just \( x(t) \) if \( x_0 \) and \( u \) are clear from the context, and \( y(t) = h(x(t)) \). See [4] for more on i/o systems. For simplicity of exposition, we make the blanket assumption that solutions do not blow-up on finite time, so \( x(t) \) (and \( y(t) \)) are defined for all \( t \geq 0 \). (In biological problems, almost always conservation laws and/or boundedness of vector fields insure this property. In any event, extensions to local semiflows are possible as well.)

Given three partial orders on \( X, U, Y \) (we use the same symbol \(<\) for all three orders), a monotone I/O system (MIOS), with respect to these partial orders, is a system \( \Pi \) such that \( h \) is a monotone map (it preserves order) and: for all initial states \( x_1, x_2 \) for all inputs \( u_1, u_2 \), the following property holds: if \( x_1 \trianglelefteq x_2 \) and \( u_1 \trianglelefteq u_2 \) (meaning that \( u_1(t) \trianglelefteq u_2(t) \) for all \( t \geq 0 \)), then \( \varphi(t, x_1, u) \trianglelefteq \varphi(t, x_2, u_2) \) for all \( t > 0 \). Here we consider partial orders induced by closed proper cones \( K \subseteq \mathbb{R}^\ell \), in the sense that \( x \preceq y \) iff \( y - x \in K \). The cones \( K \) are assumed to have a nonempty interior and are pointed, i.e. \( K \cap -K = \{0\} \). A strongly monotone system is one which satisfies the following stronger property: if \( x_1 \trianglelefteq x_2 \) and \( u_1 \trianglelefteq u_2 \), then the strict inequality \( \varphi(t, x_1, u) \prec \varphi(t, x_2, u_2) \) holds for all \( t > 0 \), where \( x \prec y \) means that \( y - x \) is in the interior of the cone \( K \).

The most interesting particular case is that in which \( K \) is an orthant cone in \( \mathbb{R}^n \), i.e. a set \( S_\varepsilon \) of the form \( \{ x \in \mathbb{R}^n \mid \varepsilon_i x_i \geq 0 \} \), where \( \varepsilon_i = \pm 1 \) for each \( i \).

When there are no inputs nor outputs, the definition of monotone systems reduces to the classical one of monotone dynamical systems studied by Hirsch, Smith, and others [5]. This is what we discussed earlier, for the case of orthonal cones. When there are no inputs, strongly monotone classical systems have especially nice dynamics. Not only is chaotic or other irregular behavior ruled out, but, in fact, almost all bounded trajectories converge to the set of steady states (Hirsch’s generic convergence theorem [6,7]).

A useful test for monotonicity with respect to orthonal cones, which generalizes Kamke’s condition to the i/o case, is as follows. Let us assume that all the partial derivatives \( \partial f_i / \partial x_j(x, u) \) for \( i \neq j \), \( \partial f_i / \partial u_j(x, u) \) for all \( i, j \), and \( \partial h_i / \partial x_j(x) \) for all \( i, j \) (subscripts indicate components) do not change sign, i.e., they are either always \( \geq 0 \) or always \( \leq 0 \). We also assume that \( X \) is convex (much less is needed.) We then associate a directed graph \( G \) to the given MIOS, with \( n + m + p \) nodes, and edges labeled “+” or “−” (or \( \pm 1 \)), whose labels are determined by the signs of the appropriate partial derivatives (ignoring diagonal elements of \( \partial f / \partial x \)). One may define in an obvious manner undirected loops in \( G \), and the parity of a loop is defined by multiplication of signs along the loop. (See e.g. [2,8] for more details.) Then, it is easy to show that a system is monotone with respect to some orthonal cones in \( X, U, Y \) if and only if there are no negative loops in \( G \). A sufficient condition for strong monotonicity is that, in addition to monotonicity, the partial Jacobians of \( f \) with respect to \( x \) should be everywhere irreducible . (“Almost-everywhere” often suffices; see [5,9]. See these references also for extensions to non-orthant cones in the case of no inputs and outputs, based on work of Schneider and Vidyasagar, Volkmann, and others [10–13]).

In inhibitory feedback, a chemical species \( x_j \) typically affects the rate of formation of another species \( x_i \) through a term like \( h(x_j) = V/(K + x_j) \). The decreasing function \( h(x_j) \) can be seen as the output of an anti-monotone system, i.e. a system which satisfies the conditions for monotonicity, except that the output map reverses order: \( x_1 \geq x_2 \Rightarrow h(x_2) \leq h(x_1) \).

An interconnection of monotone subsystems, that is to say, an entire system made up of monotone components, may or may not be monotone: “positive feedback” (in a sense that can be made precise) preserves monotonicity, while “negative feedback” destroys it. Thus, oscillators such as circadian rhythm generators require negative feedback loops in order for periodic orbits to arise, and hence are not themselves monotone systems, although they can be decomposed into monotone subsystems (cf. [14]). A rich theory is beginning to arise, characterizing the behavior of non-monotone interconnections. For example, [1] shows how to preserve convergence to equilibria; see also the follow-up papers [3,15–18]. Even for monotone interconnections, the decomposition approach is very useful, as it permits locating and characterizing the stability of steady states based upon input/output behaviors of components, as described in [2]; see also the follow-up papers [19–21].
Moreover, a key point brought up in [1, 2, 22, 23] is that new techniques for monotone systems in many situations allow one to characterize the behavior of an entire system, based upon the “qualitative” knowledge represented by general network topology and the inhibitory or activating character of interconnections, combined with only a relatively small amount of quantitative data. The latter data may consist of steady-state responses of components (dose-response curves and so forth), and there is no need to know the precise form of dynamics or parameters such as kinetic constants in order to obtain global stability conclusions and study global bifurcation behavior. We now discuss these issues.

Characteristics

The main results in [1, 2] were built around the study of characteristics, also called step-input steady-state responses or (nonlinear) DC gains. To explain this concept, we study the effect of a constant input \( u(t) \equiv u_0, t \geq 0 \) (in biological terms, the constant input may represent the extracellular concentration of a ligand during a particular experiment, for example). For each such constant input, we study the dynamical system \( \frac{dx}{dt} = f(x, u_0) \). Let us assume that all the solutions of this system approach steady states, and let us call \( K(u_0) \) the set of steady states that arises in this way. To each state \( x \) in this set \( K(u_0) \), one may associate the corresponding output or measured quantity \( h(x_0) \). Let \( k(u_0) \) be the set of all output values that arise in this manner. The graph of the set-valued mapping \( u_0 \mapsto k(u_0) \) is a subset of the cross product space \( \mathbb{R}^m \times \mathbb{R}^p \), which may be thought of as a curve when \( m = p = 1 \), and which describes the possible steady state output values for any given constant input.

Although many results may be given in more generality, we will assume for the remainder of this paper that these mappings are single-valued, not set-valued, in other words that the system is monostable. Thus, a (single-valued) characteristic is said to exist for the system if there is a unique steady state for the dynamical system \( \frac{dx}{dt} = f(x, u_0) \), denoted \( K(u_0) \), and this property is true for all possible constant levels \( u_0 \). We then define the (output) characteristic \( k : U \rightarrow Y \) as the composition \( h \circ K \). Under reasonable assumptions on \( X \) and boundedness, appealing to results from [24, 25] allows one to conclude that \( K(u_0) \) is in fact a globally asymptotically stable (“GAS” from now on) state for \( \frac{dx}{dt} = f(x, u_0) \), so that all trajectories (for this “frozen” value of the input \( u_0 \)), converge to \( K(u_0) \), and the output \( y(t) \) converges to \( k(u_0) \).

Characteristics (dose response curves, activity plots, steady-state expression of a gene in response to an external ligand, etc.) are frequently available from experimental data, especially in molecular biology and pharmacology (for instance, in the modeling of receptor-ligand interactions [26]). A goal of MIOS analysis is to combine the numerical information provided by characteristics with the qualitative information given by (signed) network topology in order to predict global bifurcation behavior. (See [23] for a longer discussion of this “qualitative-quantitative approach” to systems biology.) On the other hand, characteristics are also a very powerful tool for the purely mathematical analysis of existing models, as we show below. Monotone systems with well-defined characteristics constitute a very well-behaved set of building blocks for arbitrary systems. In particular, cascades of such systems inherit the same properties (monotone, monostable response). The original theorems, in the works [1, 2], dealt with systems obtained by interconnecting monotone (or anti-monotone) I/O systems with characteristics in feedback. Let us review them next.

Positive feedback

The first basic theorem refers to a feedback interconnection of two MIOS

\[
\frac{dx_1}{dt} = f_1(x_1, u_1), \quad y_1 = h_1(x_1) \quad (2)
\]

\[
\frac{dx_2}{dt} = f_2(x_2, u_2), \quad y_2 = h_2(x_2) \quad (3)
\]
with characteristics denoted by “k” and “g” respectively. (A degenerate case, in which the second system is memory-free, that is, there are no state variables $x_2$ and $y_2$ is simply a static function $y_2(t) = g(u_2(t))$, is also allowed. In fact, the proof of the general case can be reduced to that of the degenerate case, simply by taking the first system as a cascade connection of the two systems.)

As in [2], we suppose that the inputs and outputs of both systems are scalar: $m_1 = m_2 = p_1 = p_2 = 1$ (see [20] for a generalization to high-dimensional inputs and outputs). The “positive feedback interconnection” of the systems (2) and (3) is defined by letting the output of each of them serve as the input of the other ($u_2 = y_1 = “y”$ and $u_1 = y_2 = “u”$), as depicted in Figure 1(a). Such positive feedback systems may easily be multi-stable, even if the constituent pieces are monostable [27–30]. Let us first discuss how multi-stability may arise in a very intuitive and simple example, and later present the general theorem.

Two typical steady-state responses are as follows. Suppose that $P$ is a protein with Michaelis-Menten production rate and linear degradation: $\frac{dp}{dt} = V_{max}u/(k_m + u) - kp$, where $u$ represents the concentration a substrate that is used in $P$’s formation. The reporter variable is $y(t) = p(t)$. In this case, the steady state when $u(t) \equiv u_0$ is $p_0 = k(u_0) = (V_{max}/k)u_0/(k_m + u_0)$, and we obtain a hyperbolic response, Fig.2(a). The response in this example is graded (“light-dimmer”): it is proportional to the parameter $u_0$ on a large range of values until saturation. In contrast, a sigmoidal (“doorbell”) response, Fig.2(b), which may arise from $\frac{dp}{dt} = V_{max}u^r/(k_m^r + u^r) - kp$ with Hill coefficient (cooperativity index) $r>1$, implies that values of $u_0$ under some threshold will not result in an appreciable activity, while values over the threshold give an abrupt change (respectively, $p_0 \approx 0$ in steady state and $p_0 \approx V_{max}/k$ in steady state). Sigmoidal responses are critical e.g. if a cell must decide in a binary fashion whether a gene should be transcribed or not, depending on an extracellular signal [31–37, 37–39, 39–45]. Cascades of enzymatic reactions can be made to display such ultrasensitive response, if there is a Hill coefficient $r>1$ at each step [46].

Multiple attractors may appear if the output $y$ (for example $y=p$ in the example) is fed-back as input $u$. The mechanism might be an autocatalytic process ($u=y$, e.g. if $p$ helps promote its own transcription) or via a more complicated positive feedback pathway from $p$ to $u$. Formally, substituting $u=p$ into $\frac{dp}{dt} = (V_{max}p^r)/(k_m^r + p^r) - kp$ (where $r=1$ or $r>1$), we obtain the closed-loop equation $\frac{dp}{dt} = (V_{max}p^r)/(k_m^r + p^r) - kp$. We plot in Fig.2(c) both the first term (formation rate) and the second one (degradation), in cases where $r=1$ (left)
or \( r>1 \) (right). For \( r=1 \), for small \( p \) the formation rate is larger than the degradation rate but for large \( p \) the opposite holds, so the concentration \( p(t) \) converges to a unique intermediate value. For \( r>1 \), for small \( p \) the degradation rate is larger, so \( p(t) \) converges to a low value, but for large \( p \) the formation rate is larger and \( p(t) \) converges to a high value instead. Thus, two stable states are created, one low and one high, by this interaction of formation and degradation. (There is also an intermediate, unstable state.) This reasoning is totally elementary, but it provides an intuition for the general result in [2], shown next, which represents a far-reaching generalization. (The result can also be viewed as generalizing aspects of the papers [5,47–55], to arbitrary MIOS.)

We consider Fig. 1(b), where we have plotted together \( k \) and the inverse of \( g \). It is quite obvious that there is a bijective correspondence between the steady states of the feedback system and the intersection points of the two graphs. With some mild technical conditions of transversality and “controllability” and “observability” (the recent papers [56,57] show that even these mild conditions can be largely dispensed with), the following much less obvious facts are true. We first attach labels to the intersection points between the two graphs as follows: a label \( S \) (respectively, \( U \)) if the slope of \( k \) is smaller (respectively, larger) than the slope of \( g^{-1} \) at the intersection point. One can then conclude that “almost all” (in a measure-theoretic sense or in a Baire-category sense) bounded solutions of the feedback system must converge to one of the steady states corresponding to intersection points labeled with an \( S \). The proof reduces ultimately to an application of Hirsch’s generic convergence theorem to the closed-loop system (the technical conditions insure strong monotonicity). However, the value-added is in the fact that stable states can be identified merely from the one-dimensional plot shown in Fig. 1(b). (If each subsystem would have dimension just one, one can also interpret the result in terms of a simple nullcline analysis; see the Supplementary Section of [19].) We remark that the theorems remain true even if arbitrary delays are allowed in the feedback loop and/or if space-dependent models are considered and diffusion is allowed (see [23] for a discussion). A new approach [58], based not on monotone theory but on a notion of “counterclockwise dynamics,” extends in a different direction the range of applicability of this methodology.

We wish to emphasize the potential practical relevance of this result (and others such as [58]). The equations describing each of the systems are often poorly, or not at all, known. But, as long as we can assume that each subsystem is monotone and uni-stable, we can use the information from the planar plots in Fig. 1(b) to completely understand the dynamics of the closed-loop system, no matter how large the number of state variables. It is often said that the field of molecular systems biology is characterized by a data-rich/data-poor paradox: while on the one hand a huge amount of qualitative network (schematic modeling) knowledge is available for signaling, metabolic, and gene regulatory networks, on the other hand little of this knowledge is quantitative, at least at the level of precision demanded by most mathematical tools of analysis. On the other hand, input/output steady state data (from a signal such as a ligand, to a reporter variable such as the expression of a gene monitored by GFP, or the activity of a protein measured by a Western blot) is frequently available. The problem of exploiting qualitative knowledge, and effectively integrating relatively sparse quantitative data, is among the most challenging issues confronting systems biology. The MIOS approach provides one way to combine these two types of data. (For further discussion of this “data-rich/data-poor” issue, see [22,23].)

The theorem from [2] and its generalizations, as well as the negative feedback result discussed below, amount to a model-reduction approach. The bifurcation behavior of the complete closed-loop system is obtained from a low-order reduction (just to two one-dimensional systems, connected in feedback, when \( m = p = 1 \)) and information on the i/o behavior of the components. This model-reduction view is further developed in [20].

More discussion through an example: MAPK cascades

\textit{Mitogen-Activated Protein Kinase (MAPK) cascades} are a ubiquitous “signaling module” in eukaryotes, in-
wolved in proliferation, differentiation, development, movement, apoptosis, and other processes [59–61]. There are several such cascades, sharing the property of being composed of a cascade of three kinases. The basic rule is that two proteins, called generically MAPK and MAPKK (the last K is for “kinase of MAPK,” which is itself a kinase), are active when doubly phosphorylated, and MAPKK phosphorylates MAPK when active. Similarly, a kinase of MAPKK, MAPKKK, is active when phosphorylated. A phosphatase, which acts constitutively (that is, by default it is always active) reverses the phosphorylation. The biological model from [19,59] is in Fig.3(b), were we wrote \( z_i(t), i = 1,2,3 \) for MAPK, MAPK-P, and MAPK-PP concentrations and similarly for the other variables. The input represents an external signal to this subsystem (typically, the concentration of a kinase driving forward the reaction).

We make here the simplest assumptions about the dynamics, amounting basically to a quasi-steady state approximation of enzyme kinetics. (For related results using more realistic, mass-action, models, see [62–64].) For example, take the reaction shown in the square in Fig.3(a). As \( y_3 \) (MAPKK-PP) facilitates the conversion of \( z_1 \) into \( z_2 \) (MAPK to MAPK-P), the rate of change \( dz_2/dt \) should include a term \( \alpha(z_1,y_3) \) (and \( dz_1/dt \) has a term \(-\alpha(z_1,y_3)\)) for some (otherwise unknown) function \( \alpha \) such that \( \alpha(0,y_3) = 0 \) and \( \frac{\partial \alpha}{\partial z_1} > 0, \frac{\partial \alpha}{\partial y_3} > 0 \) when \( z_1 > 0 \). (Nothing happens if there is no substrate, but more enzyme or more substrate results in a faster reaction.) There will also be a term \(+\beta(z_2)\) to reflect the phosphatase action. Similarly for the other species. The system as given would be represented by a set of seven ordinary differential equations (or reaction-diffusion PDE’s, if spatial localization is of interest, or delay-differential equations, if appropriate).

This system is not monotone (at least with respect to any orthant cone), as is easy to verify graphically. However, as with many other examples of biochemical networks, the system is “monotone in disguise”, so to speak, in the sense that a judicious change of variables allows one to apply MIOS tools. (Far more subtle forms of this argument are key to applications to signaling cascades. A substantial research effort, not reviewed here because of lack of space, addresses the search for graph-theoretic conditions that allow one to find such “monotone systems in disguise”; see [22,23,63] for references.)

In this example, which in fact was the one whose study initially led to the definition of MIOS, the following conservation laws: \( y_1(t) + y_2(t) + y_3(t) \equiv y_{tot} \) (total MAPKK) and \( z_1(t) + z_2(t) + z_3(t) \equiv z_{tot} \) (total MAPK) hold true, assuming no protein turn-over. This assumption is standard in most of the literature, because transcription and degradation occur at time scales much slower than signaling. (There is very recent experimental data that suggests that turn-over might be fast for some yeast MAPK species. Adding turn-over would lead to a different mathematical model.) These conservation laws allow us to eliminate variables. The right trick is to eliminate \( y_2 \) and \( z_2 \). Once we do this, and write \( y_2 = y_{tot} - y_1 - y_3 \) and \( z_2 = z_{tot} - z_1 - z_3 \), we are left with the variables \( x, y_1, y_3, z_1, z_3 \). For instance, the equations for \( z_1, z_3 \) look like:

\[
\frac{dz_1}{dt} = -\alpha(z_1,y_3) + \beta(z_{tot} - z_1 - z_3) \\
\frac{dz_3}{dt} = \gamma(z_{tot} - z_1 - z_3, y_3) - \delta(z_3)
\]
for appropriate increasing functions $\alpha, \beta, \gamma, \delta$. The equations for the remaining variables are similar. The graph, ignoring, as usual, self-loops (diagonal of Jacobian), is shown in Figure 4. It is also true that this system is monotone. A consistent spin assignment (including the top input node and the bottom output node) is shown in Figure 4. It is also true that this system has a

![Diagram of a simple MAPK cascade model](image)

Figure 4: Consistent assignment for simple MAPK cascade model

well-defined monostable state space response (characteristic); there is no space to discuss the proof here, so we refer the reader to the original papers [1, 8].

Positive and negative feedback loops around MAPK cascades have been a topic of interest in the biological literature. For example, see [37, 40] for positive feedback and [65, 66] for negative feedback. Since we know that the system is monotone and has a characteristic, MIOS theory as described here can indeed be applied to the example. We study next the effect of a positive feedback $u = gy$ obtained by “feeding back” into the input a scalar multiple $g$ of the output. (This is a somewhat unrealistic model of feedback, since feedbacks act for example by enhancing the activity of a kinase. We pick it merely for illustration of the techniques.)

The theorem does not require actual equations for its applicability. All that is needed is the knowledge that we have a MIOS, and a plot of its characteristic (which, in practice, would be obtained from interpolated experimental data). In order to illustrate the conclusions, on the other hand, it is worth discussing a particular set of equations. We take equations and parameters from [19, 22, 23]:

\[
\begin{align*}
\frac{dx}{dt} &= -\frac{v_2 x}{k_2 + x} + v_0 u + v_1 \\
\frac{dy_1}{dt} &= \frac{v_6 (y_{tot} - y_1 - y_3)}{k_6 + (y_{tot} - y_1 - y_3)} - \frac{v_3 x y_1}{k_3 + y_1} \\
\frac{dy_3}{dt} &= \frac{v_4 x (y_{tot} - y_1 - y_3)}{k_4 + (y_{tot} - y_1 - y_3)} - \frac{v_5 y_3}{k_5 + y_3} \\
\frac{dz_1}{dt} &= \frac{1}{k_{10} + (z_{tot} - z_1 - z_3)} - \frac{v_7 y_3 z_1}{k_7 + z_1} \\
\frac{dz_3}{dt} &= \frac{v_8 y_3 (z_{tot} - z_1 - z_3)}{k_8 + (z_{tot} - z_1 - z_3)} - \frac{v_9 z_3}{k_9 + z_3}
\end{align*}
\]

with output $z_3$. Specifically, we will use the following parameters: $v_0 = 0.0015$, $v_1 = 0.09$, $v_2 = 1.2$, $v_3 = 0.064$, $v_4 = 0.064$, $v_5 = 5$, $v_6 = 5$, $v_7 = 0.06$, $v_8 = 0.06$, $v_9 = 5$, $v_{10} = 5$, $y_{tot} = 1200$, $z_{tot} = 300$, $k_2 = 200$, $k_3 = 1200$, $k_4 = 1200$, $k_5 = 1200$, $k_6 = 1200$, $k_7 = 300$, $k_8 = 300$, $k_9 = 300$, $k_{10} = 300$. (The units are: totals in nM (mol/cm$^3$), v’s in nM·sec$^{-1}$ and sec$^{-1}$, and k’s in nM.)

With these choices, the steady state step response is the sigmoidal curve shown in Figure 3(c), where $y$ is the output $z_3$. We plotted in the same figure the inverse $g^{-1}$ of the characteristic of the feedback system, in this case just the linear mapping $y = (1/g)u$, for three typical “feedback gains” (g=1/0.98, 1/2.1, 1/6).
For $g = 1/0.98$ (line of slope 0.98 when plotting $y$ against $u$), there should be a unique stable state, with a high value of the output $y = z_3$, and trajectories should generically converge to it. Similarly, for $g = 1/2.1$ (line of slope 2.1) there should be two stable states, one with high and one with low $y = z_3$, with trajectories generically converging to one of these two, because the line intersects at three points, corresponding to two stable and one unstable state (exactly as in the discussion concerning the simple protein formation/degradation sigmoidal example in Fig.2(c)). Finally, for $g = 1/6$ (line of slope 6), only the low-$y$ stable state should persist. Fig.5(a-c) shows plots of the hidden variable $y_3(t)$ (MAPKK-PP) for several initial states, confirming the predictions. The same convergence results are predicted if there are delays in the feedback loop, or if concentrations depend on location in a convex spatial domain. Results for reaction-diffusion PDE’s and delay-differential systems are discussed in [23], and simulation results for this example are also provided there.

We may plot the steady state value of $y$, under the feedback $u = gy$, as the gain $g$ is varied, Fig.6(a). This resulting complete bifurcation diagram showing points of saddle-node bifurcation can be also completely determined just from the characteristic, with no need to know the equations of the system. Relaxation oscillations may be expected under such circumstances if a second, slower, feedback loop is used to negatively adapt the gain as a function of the output. Reasons of space preclude describing a very general theorem, which shows that indeed, relaxation oscillations can be guaranteed in this fashion: see [18] for technical details, and [23] for a more informal discussion. Fig.6(b) shows a simulation confirming the theoretical prediction (details in [18, 23]).

**Negative feedback**

A different set of results apply to inhibitory or negative feedback interconnections of two MIOS systems (2)-(3). A convenient mathematical way to define “negative feedback” in the context of monotone systems is to say that the orders on inputs and outputs are inverted (example: an inhibition term of the form $\frac{V}{K+y}$, as usual in biochemistry). Equivalently, we may incorporate the inhibition into the output of the second system (3).
which is then seen as an anti-monotone I/O system, and this is how we proceed from now on. See Fig.1(c).

We emphasize that the closed-loop systems that result are not monotone, at least with respect to any known order.

The original theorem, from [1], is as follows. We assume once more that inputs and outputs are scalar \( m=p=1; \) see [3] for generalizations. We once again plot together \( k \) and \( g^{-1} \), as shown in Fig.1(d). Consider the following discrete iteration:

\[
u_{i+1} = (g \circ k)(u_i).
\]

Then, if solutions of the closed-loop system are bounded and if this iteration has a globally attracting fixed point \( \bar{u} \), as shown in Fig.1(d), then the feedback system has a globally attracting steady state. (An equivalent condition, see [3], is that the iteration have no nontrivial period-two orbits.) We call this result a small gain theorem (“SGT”), because of its analogy to concepts in control theory.

It is easy to see that arbitrary delays may be allowed in the feedback loop. In other words, the feedback could be of the form \( u(t) = y(t-h) \), and such delays (even with \( h = h(t) \) time varying or even state-dependent, as long as \( t-h(t) \to \infty \) as \( t \to \infty \) do not destroy global stability of the closed loop. In [17], we have now shown also that diffusion does not destroy global stability either. In other words, a reaction-diffusion system (Neumann boundary conditions) whose reaction can be modeled in the shown feedback configuration, has the property that all solutions converge to a (unique) uniform in space solution. This is not immediately obvious, since standard parabolic comparison theorems do not immediately apply to the feedback system, which is not monotone.

**Example: MAPK cascade with negative feedback.**

As with the positive feedback theorem, an important feature is applicability to highly uncertain systems. As long as the component systems are known to be MIOS, the knowledge of I/O response curves and a planar analysis are sufficient to conclude GAS of the entire system, which may have an arbitrarily high dimension. For example, suppose we take a feedback like \( u=a+b/(c+z^3) \), with a graph as shown in Fig.7(a), which also shows the characteristic and a convergent discrete 1-d iteration [23]. Then, we are guaranteed that all solutions of the closed-loop system converge to a unique steady state, as confirmed by the simulations in Fig.7(b), which shows the concentrations of the active forms of the kinases.

![Figure 7: inhibition: (a) spiderweb and (b) simulation](image)

**Example: testosterone model.**

This example is intended to show that even for a classical mathematical biology model, a very simple application of the result in [1] gives an interesting conclusion. The concentration of testosterone in the blood of a healthy human male is known to oscillate periodically with a period of a few hours, in response to similar oscillations in the concentrations of the luteinising hormone (LH) secreted by the pituitary gland, and the luteinising hormone releasing hormone (LHRH), normally secreted by the hypothalamus (see [67]). The well-known textbook [68] (and its previous editions) presents this process as an example of a biological oscillator,
and proposes a model to describe it, introducing delays in order to obtain oscillations. (Since the textbook was written, the physiological mechanism has been much further elucidated, and this simple model is now known not to be correct. However, we want merely to illustrate a point about mathematical analysis.) The equations are:

\[
\begin{align*}
\dot{R} &= \frac{A}{K + T} - b_1 R \\
\dot{L} &= g_1 R - b_2 L \\
\dot{T} &= g_2 L(t - \tau) - b_3 T
\end{align*}
\]

\((R, L, T = \text{concentrations of hormones luteinising hormone releasing, luteinising, and testosterone, } \tau = \text{delay; we use } \dot{x} \text{ to denote time derivative). The system may be seen as the feedback connection of the MIOS system}

\[
\begin{align*}
\dot{R} &= u - b_1 R \\
\dot{L} &= g_1 R - b_2 L \\
\dot{T} &= g_2 L - b_3 T
\end{align*}
\]

with the inhibitory feedback \(u(t) = g(T - \tau) = A/(K + T(t - \tau))\) after moving the delay to the loop (without loss of generality). The characteristic is linear, \(T = k(u) = \frac{g_1 A}{b_2 b_3} u\), so \(g \circ k\) is a fractional transformation \(S(u) = \frac{u}{q + u}\). Since such a transformation has no period-two cycles, global stability follows. (For arbitrary, even time-varying, delays.) This contradicts the existence of oscillations claimed in [68] for large enough delays. (See [69], which also explains the error in [68].)

**Example: Lac operon.** The study of *E. Coli* lactose metabolism has been a topic of research in mathematical biology since Jacob and Monod’s classical work which led to their 1995 Nobel Prize. For this example, we look at the subsystem modeled in [70]. The lac operon induces production of permease and \(\beta\)-gal, permease makes the cell membrane more permeable to lactose, and genes are activated if lactose present; lactose is digested by the enzyme \(\beta\)-gal, and the other species are degraded at fixed rates. (In this model from [70], lactose and isolactose are identified, and catabolic repression by glucose via cAMP is ignored.) Delays arise from translation of permease and \(\beta\)-gal. The equations are:

\[
\begin{align*}
\dot{x}_1(t) &= g(x_4(t - \tau)) - b_1 x_1(t) \quad \text{lac operon mRNA} \\
\dot{x}_2(t) &= x_1(t) - b_2 x_2(t) \quad \beta\text{-galactoside permease} \\
\dot{x}_3(t) &= r x_1(t) - b_3 x_3(t) \quad \beta\text{-galactosidase} \\
\dot{x}_4(t) &= S x_2(t) - x_3(t) x_4(t) \quad \text{lactose}
\end{align*}
\]

with \(g(x) := (1 + K x^\rho)/(1 + x^\rho), \ K > 1, \ \text{and the Hill exponent } \rho \ \text{representing a cooperativity effect. (All delays have been lumped into one.) We view this system as a negative feedback loop, where } u = x_1, \ v = x_4, \ \text{of a MIOS system (details in [3]). Since there are two inputs and outputs, now we must study the two-dimensional iteration}

\[
(u, v) \mapsto (g \circ k)(u, v) = \left[\begin{array}{c} g(v) \\ b \end{array} \right].
\]

Based on results on rational difference equations from [71], one concludes that there are no nontrivial 2-periodic orbits, provided that \(\rho < (\sqrt{K} + 1)/(K - 1)\), for arbitrary \(b_1, b_2, b_3, r, S\). Hence, by the theorem, there is a unique steady state of the original system, which is GAS, even when arbitrary delays are present.

These and other conditions are analyzed in [3], where it is also shown that the results from [70] are recovered as a special case. Among other advantages of this approach, besides generalizing the result and giving a conceptually simple proof, we have (because of [17]) the additional conclusion that also for the corresponding
reaction-diffusion system, in which localization is taken account of, the same globally stable behavior can be guaranteed.

**Example: Circadian oscillator.** As a final example of the negative feedback theorem, we pick Goldbeter’s original model of the molecular mechanism underlying circadian rhythms in *Drosophila*. (In this oversimplified model, only *per* protein is considered; other players such as *tim* are ignored.) *PER* protein is synthesized at a rate proportional to its mRNA concentration. Two phosphorylation sites are available, and constitutive phosphorylation and dephosphorylation occur with saturation dynamics, at maximum rate $v_1$’s and with Michaelis constants $K_1$. Doubly phosphorylated *PER* is degraded, also satisfying saturation dynamics (with parameters $v_d, k_d$), and it is translocated to the nucleus with rate constant $k_1$. Nuclear *PER* inhibits transcription of the *per* gene, with a Hill-type reaction of cooperativity degree $n$ and threshold constant $k_I$. mRNA is produced and translocated to the cytoplasm, at a rate determined by a constant $v_s$. Additionally, there is saturated degradation of mRNA (constants $v_m$ and $k_m$). The model is ($P_i = per$ phosphorylated at $i$ sites, $P_N = nuclear per$, $M = per$ mRNA):

\[
\begin{align*}
\dot{M} &= v_s K_1/(K_1 + P_0^k) - v_m M/(k_m + M) \\
\dot{P}_0 &= k_s M - V_1 P_0/(K_1 + P_0) + V_2 P_1/(K_2 + P_1) \\
\dot{P}_1 &= V_1 P_0/(K_1 + P_0) - V_2 P_1/(K_2 + P_1) - V_3 P_1/(K_3 + P_1) + V_4 P_2/(K_4 + P_2) \\
\dot{P}_2 &= V_5 P_1/(K_3 + P_1) - V_4 P_2/(K_4 + P_2) - k_1 P_2 + k_2 P_N - v_d P_2/(k_d + P_2) \\
\dot{P}_N &= k_1 P_2 - k_2 P_N.
\end{align*}
\]

Parameters are chosen exactly as in Goldbeter’s original paper, except that the rate $v_s$ of mRNA translocation to the cytoplasm is taken as a bifurcation parameter. The value $v_s = 0.76$ from [72] gives oscillatory behavior. On the other hand, we may break up the system into the $M$ and $P_i, P_N$ subsystems. Each of these can be shown to be MIOS and have a characteristic. (The existence of a characteristic for the $P$-subsystem is nontrivial, and involves the application of Smillie’s Theorem [74] for strongly monotone tridiagonal systems, and more precisely, repeated application of a proof technique in [74] involving “eventually monotonicity” of state variables.) When $v_s = 0.4$, the discrete iteration is graphically seen to be convergent (see Fig.8(a)), so the theorem guarantees global asymptotic stability even when arbitrary delays are introduced in the feedback. Bifurcation analysis on delay length and $v_s$ indicates that local stability will fail for somewhat larger values. Using again the graphical test, we observe that for $v_s = 0.5$ there appears limit cycle for the *discrete* iteration on characteristics, see Fig.8(b). This suggests that oscillations may exist in the full nonlinear differential equation, at least for appropriate delays lengths. Indeed, the simulation in Fig.8(c) displays such oscillations (see [8, 14]).

![Figure 8: (a) convergent iteration; (b) divergent iteration; (c) oscillations](image)
Multivalued Characteristics

For simplicity, we have not discussed the case when characteristics are set-valued instead of single-valued. This general case can also be productively studied with the toolkit afforded by MIOS interconnection theory, see [56, 57] for positive feedback and [21] for negative feedback.

4 Conclusions

There is a clear need in systems biology to study robust structures and to develop robust analysis tools. The theory of monotone systems provides one such tool. Interesting and nontrivial conclusions can be drawn from (signed) network structure alone, which is associated to purely stoichiometric information about the system, and ignores fluxes.

Associating a graph to a given system, we may define spin assignments and consistency, a notion that may be interpreted also as non-frustration of Ising spin-glass models. Every species in a monotone system (one whose graph is consistent) responds with a consistent sign to perturbations at every other species. This property would appear to be desirable in biological networks, and, indeed, there is some evidence suggesting the near-monotonicity of some natural networks. Moreover, “near”-monotone systems might be “practically” monotone, in the sense of being monotone under disjoint environmental conditions.

Dynamical behavior of monotone systems is ordered and “non chaotic”. Systems close to monotone may be decomposed into a small number of monotone subsystems, and such decompositions may be usefully employed to study non-monotone dynamics as well as to help detect bifurcations even in monotone systems, based only upon sparse numerical data, resulting in a sometimes useful model-reduction approach.

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