Interconnections of Monotone Systems with Steady-State Characteristics*

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Summary. One of the key ideas in control theory is that of viewing a complex dynamical system as an interconnection of simpler subsystems, thus deriving conclusions regarding the complete system from properties of its building blocks. Following this paradigm, and motivated by questions in molecular biology modeling, the authors have recently developed an approach based on components which are monotone systems with respect to partial orders in state and signal spaces. This paper presents a brief exposition of recent results, with an emphasis on small gain theorems for negative feedback, and the emergence of multi-stability and associated hysteresis effects under positive feedback.

1 Introduction

Tools from control theory have long played an important role in the analysis of dynamical systems properties such as stability, hysteresis, and oscillations. Key to the application of control tools, is the idea of viewing a complex dynamical system as feedforward and feedback interconnections of simpler subsystems, thus deriving conclusions regarding the complete system from properties of its building blocks. One may then analyze complicated structures on the basis of the behavior of elementary subsystems, each of which is "nice" in a suitable input/output sense (stable, passive, etc), in conjunction with the use of tools such as the small gain theorem to characterize interconnections.

This paper focuses on a special case of this idea, when the components are *monotone systems with a well-defined steady state response.* Although this work originated in our study of certain particular models in the area of cell signaling, a field of interest in contemporary molecular biology, and we will use such models in order to illustrate our results, the mathematical results

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and techniques that we have developed are of independent interest, and one can easily foresee many other areas of application.

Our framework is that of *monotone systems with inputs and outputs*, a class of systems introduced recently in [2] and further studied in [3]. They provide a natural generalization of classical (no inputs and outputs) monotone dynamical systems, defined by the requirement that trajectories must preserve a partial ordering on states; classical monotone systems include the subclass of *cooperative* systems, in which different state variables reinforce each other (positive feedback) as well as certain more general systems in which each pair of variables may affect each other in either positive or negative, or even mixed, forms. Among the classical references for monotone dynamical systems are the textbook by Smith [29] and the papers [16, 17] by Hirsch, which provide a rich and elegant theory dealing with the precise characterization of omega limit sets and other asymptotic behavior.

The extension to systems with inputs and outputs is by no means a purely academic exercise, but it is a necessary first step in order to analyze interconnections, especially those including feedback loops, built up out of monotone components. It is perhaps remarkable that this 'system-theoretic' view of dynamical systems fits perfectly with one of the main themes and challenges in current molecular biology, namely the understanding of cell behavior in terms of common 'modules' (see e.g. [15]).

In this paper, we provide a brief exposition of several of our results for monotone i/o systems. Proofs are not included, as they can be found in [2, 3]. In addition, in order to make the presentation easier to follow, we often make simplifying assumptions, such as convexity of state spaces and input spaces, which can be substantially relaxed, as discussed in [2, 3]. We first review the basic setup, based upon monotone i/o systems with well-defined steady-state responses. Then we present a very simple yet powerful result for the analysis of global convergence of feedback interconnections in the special case when feedback does not introduces multiple steady states. This result plays the role of a small-gain theorem when applied to negative feedback loops. In general, however, positive feedback may lead to bifurcations of equilibria, and our second main result deals with the analysis of this type of situation, providing a characterization of locations and stability of steady states. A unifying theme to our work is the reduction of the analysis of high-dimensional dynamics to a simple planar graphical test.

In closing this introduction, we wish to comment upon the biological motivations for our work. The study of oscillations in biochemical systems is a major theme in molecular biology modeling; see, for instance, the book [13], which is one of the major references in the field. Our work in monotone systems originated in the analysis of the emergence of oscillations in certain biological inhibitory feedback loops, and specifically in a mathematical model of *mitogen-activated protein kinase (MAPK) cascades*, which represent a 'biological module' or subcircuit which is ubiquitous in eukaryotic cell signal transduction processes. In the paper [32], one of the authors presented a small-gain theorem which provided very tight estimates of parameters at which a Hopf bifurcation occurs, in a model of inhibitory MAPK cascades given in [20], and which had been previously studied numerically in [26]. Analyzing a more realistic model that the one in those references (multiple as opposed to single phosphorylation) led us the results presented in [2] and reviewed here. We will discuss that example, as well as a classical model of circadian oscillators. Multi-stability and associated hysteresis effects form the basis of many models in molecular biology, in areas such as cell differentiation, development, and periodic behavior described by relaxation oscillations. See for instance the classic work by Delbrück [8], who suggested in 1948 that multi-stability could explain cell differentiation, as well as references in the current literature (e.g., [5], [9], [10], [11], [19], [22], [24], [33]). We will discuss a system of differential equations which describes a biochemical network of the type which appears in cell signaling, as an illustration of our theorems on multi-stability.

2 Basic Definitions

We will only consider systems defined by ordinary differential equations. Thus, all state-spaces, as well as input and output signal spaces, are assumed to be subsets of Euclidean space. By an *ordered Euclidean space* we mean here an Euclidean space $\mathbb{R}^n$, for some positive integer n, together with an order $\succeq$ induced by a positivity cone K. That is, $K \subseteq \mathbb{R}^n$ is a nonempty, closed, convex, pointed ($K \bigcap -K = \{0\}$), and solid (K has a nonempty interior) cone, and $x_1 \succeq x_2$ (or "$x_2 \preceq x_1$") means that $x_1 - x_2 \in K$. Given a cone K, two notions of strict ordering are possible. By $x_1 \succ x_2$, one means that $x_1 \succeq x_2$ and $x_1 \neq x_2$, and by $x_1 \gg x_2$ that $x_1 - x_2 \in \mathrm{int}(K)$. For example, with respect to the 'NorthEast' ordering given by the first orthant $K = \mathbb{R}^n_{\geq 0}$, $x_1 \succeq x_2$ means that each coordinate of x_1 is bigger or equal than the corresponding coordinate of x_2, $x_1 \gg x_2$ means that every coordinate of x_1 is strictly larger than the corresponding coordinate of x_2, and $x_1 \succ x_2$ which means that *some* coordinate is strictly larger.

(More general notions of ordered spaces, not necessarily induced by cones, may be considered as well; and indeed some of the results in [2] are proved for more abstract ordered spaces. In addition, one may wish to study monotone I/O systems on infinite-dimensional spaces, particularly when dealing with delay-differential systems or systems defined by partial differential equations.)

By a *state-space* we will mean a subset X of an ordered Euclidean space $(\mathbb{R}^n, K^X)$ such that X is the closure of an open subset of $\mathbb{R}^n$ and it is convex. Similarly, by an *input set* $\mathcal{U}$ we mean a subset of an ordered space $(\mathbb{R}^m, K^{\mathcal{U}})$, and by an *output set* $\mathcal{Y}$ a subset of an $(\mathbb{R}^p, K^{\mathcal{Y}})$. When there is no risk of ambiguity and the meaning is clear from the context, we drop the superscripts and write just 'K' instead of K^X, $K^{\mathcal{U}}$, or $K^{\mathcal{Y}}$. For simplicity, we state many of our results for *single-input single-output* ("SISO") systems, those for which input and output signals are scalar: $m = p = 1$. By an *input* (function) we mean a locally essentially bounded Lebesgue measurable function $u(\cdot) : \mathbb{R}_{\geq 0} \to \mathcal{U}$, and we write $u_1 \succeq u_2$ provided that $u_1(t) \succeq u_2(t)$ for almost all $t \geq 0$.

By a (finite-dimensional continuous-time) *system*, we mean the usual concept from control theory (see e.g. [31]), namely a system with inputs and outputs

$$\dot{x} = f(x,u) , \quad y = h(x) \tag{1}$$

specified by a state space X, an input set $\mathcal{U}$, and an output set $\mathcal{Y}$, such that solutions of (1) that start in X do not leave X (forward invariance of X). We assume that $f : X \times \mathcal{U} \to \mathbb{R}^n$ (which may be thought of as a vector field with parameters) is continuous in (x,u), and is locally Lipschitz continuous in x locally uniformly on u, and that the function $h : X \to \mathcal{Y}$ is continuous. When mentioning derivatives of f, we will implicitly assume that we have in addition required f to be differentiable, meaning that map f extends differentiably to an open subset of $\mathbb{R}^n \times \mathbb{R}^m$ which contains $X \times \mathcal{U}$. We will make the assumption that all solutions with initial states in X must be defined for all $t \geq 0$ (forward completeness), and denote by $x(t) = \phi(t,\xi,u)$ (or just "$x(t,\xi,u)$") the (unique) solution of $\dot{x}(t) = f(x(t),u(t))$ with initial condition $x(0) = \xi$ and input $u(\cdot)$ at time $t \geq 0$. We also sometimes use the notation $y(t,\xi,u)$ as a shorthand for $h(\phi(t,\xi,u))$. For classical dynamical systems $\dot{x} = f(x)$, f does not depend on inputs, and there is no mapping h; they can be thought of as the particular case in which $\mathcal{U}$ and $\mathcal{Y}$ reduce to a single point, so that all definitions to be given will also apply to them.

The system (1) is said to be *monotone* if the following property holds, with respect to the orders on states and inputs:

$$\xi_1 \succeq \xi_2 \quad \& \quad u_1 \succeq u_2 \quad \Rightarrow \quad x(t,\xi_1,u_1) \succeq x(t,\xi_2,u_2) \quad \forall\, t \geq 0$$

and also the mapping h is monotone (with respect to the orders on states and output values). It is an elementary fact (see e.g. [2]) that the above inequality is equivalent to:

$$\xi_1 \gg \xi_2 \quad \& \quad u_1 \succeq u_2 \quad \Rightarrow \quad x(t,\xi_1,u_1) \gg x(t,\xi_2,u_2) \quad \forall\, t \geq 0$$

(this follows from the fact that a set which is the closure of its interior is invariant under a controlled dynamics iff its interior is invariant). A monotone system is *strongly monotone* if the following stronger property holds:

$$\xi_1 \succ \xi_2 \quad \& \quad u_1 \succeq u_2 \quad \Rightarrow \quad x(t,\xi_1,u_1) \gg x(t,\xi_2,u_2) \quad \forall\, t > 0.$$

This is a fairly strong statement, saying that a very strict inequality among states holds immediately after the initial time, provided only that the initial states be weakly comparable to each other.

The basic components to be interconnected will not be merely monotone, but they will satisfy an additional requirement, that they be monostable in the sense of having a well-defined steady-state response for each possible *constant* input. This property, which we define next, *when combined with monotonicity,* will strongly constrain the limiting behavior or arbitrary time-varying inputs.

Definition 1. *The system (1)* admits an input to state (I/S) static characteristic $k^X(\cdot) : \mathcal{U} \to X$ *if, for each constant input* $u(t) \equiv u \in \mathcal{U}$*, there exists a unique globally asymptotically stable equilibrium* $k^X(u)$*. The characteristic is said to be* non-degenerate *if, in addition, the Jacobian* $D_x f(k^X(u), u)$ *is nonsingular, for all such* u*. If (1) admits an I/S characteristic, its* input/output (I/O) characteristic *is by definition the composition* $k^{\mathcal{Y}} := h \circ k^X$*.*

2.1 Infinitesimal Characterizations

It is obviously important to be able to check monotonicity without having to actually solve the differential equations. We quote two results from [2]; in both cases we assume given appropriate positivity cones. The first one uses (contingent) tangent cones, in the sense of nonsmooth analysis, and admits generalizations beyond orders defined by positivity cones:

Theorem 1. *The system (1) is monotone if and only if* h *is monotone and the following property holds:*

$$\xi_1 \succeq \xi_2 \text{ and } u_1 \succeq u_2 \;\Rightarrow\; f(\xi_1, u_1) - f(\xi_2, u_2) \in \mathcal{T}_{\xi_1 - \xi_2} K \,, \tag{2}$$

where $\mathcal{T}_\xi K$ *denotes the (contingent) tangent cone to* K *at the point* ξ*.*

The second one relies upon convex analysis, and is based upon viewing monotone systems with inputs (and outputs) as those for which the vector field f is *quasi-monotone* in the sense of differential equations (see e.g. [25]):

Theorem 2. *The system (1) is monotone if and only if* h *is monotone and the following property holds:*

$$\begin{aligned} \xi_1 \succeq \xi_2,\, u_1 \succeq u_2,\, \zeta \in K^*, \text{ and } \langle \zeta, \xi_1 \rangle = \langle \zeta, \xi_2 \rangle \\ \Rightarrow \langle \zeta, f(\xi_1, u_1) \rangle \;\geq\; \langle \zeta, f(\xi_2, u_2) \rangle \end{aligned} \tag{3}$$

where K^* *is the set of all* $\zeta \in \mathbb{R}^n$ *so that* $\langle \zeta, k \rangle \geq 0$ *for all* $k \in K$*.*

Condition (3) may be separated into the conjunction of: for all ξ and all $u_1 \succeq u_2$, $f(\xi, u_1) - f(\xi, u_2) \in K$, and for all u, $\xi_1 \succeq \xi_2$, and $\langle \zeta, \xi_1 \rangle = \langle \zeta, \xi_2 \rangle$, $\langle \zeta, f(\xi_1, u) \rangle \geq \langle \zeta, f(\xi_2, u) \rangle$. A similar separation is possible in Theorem 1. In both cases, it suffices to check the properties for $\xi_1 - \xi_2 \in \partial K$ instead of arbitrary points.

2.2 Orthant orders

We assume in this section, to simplify the exposition, that both the interior of the state space X and of the input set $\mathcal{U}$ are convex; also, f is supposed to be continuously differentiable.

A special class of positivity cones are orthants. Any orthant K in $\mathbb{R}^n$ has the form

$$K^{(\varepsilon)} \;=\; \{x \in \mathbb{R}^n \mid (-1)^{\varepsilon_i} x_i \geq 0\,,\; i = 1, \ldots, n\}$$

for some binary vector $\varepsilon = (\varepsilon_1, \ldots, \varepsilon_n) \in \{0,1\}^n$. A special case is when states, inputs, and outputs are ordered with $K =$ the main orthant (all $\varepsilon_i = 0$); systems that are monotone with respect to these orthants are called *cooperative systems.*

Proposition 1. *The system (1) is cooperative if and only if the following properties hold:*

$$\frac{\partial f_i}{\partial x_j}(x,u) \geq 0 \quad \forall\, x \in X,\; \forall\, u \in \mathcal{U},\; \forall\, i \neq j$$

$$\frac{\partial f_i}{\partial u_j}(x,u) \geq 0 \quad \forall\, x \in X,\; \forall\, u \in \mathcal{U},\; \forall\, i, j$$

$$\frac{\partial h_i}{\partial x_j}(x) \geq 0 \quad \forall\, x \in X,\; \forall\, i, j\,.$$

The term "cooperative" arises from the fact that the various variables "help" each other (rates of change depend increasingly on the remaining variables).

Under appropriate changes of variables, one may reduce the study of monotone systems with respect to arbitrary orthants to the study of cooperative systems; see [2] for details. The following corollary results from this reduction:

Corollary 1. *The system (1) is monotone with respect to the orders induced from orthants $K^X = K^{(\varepsilon)}$, $K^{\mathcal{U}} = K^{(\delta)}$, and $K^{\mathcal{Y}} = K^{(\mu)}$, if and only if the following properties hold:*

$$(-1)^{\varepsilon_i + \varepsilon_j}\, \frac{\partial f_i}{\partial x_j}(x,u) \geq 0 \quad \forall\, x \in X,\; \forall\, u \in \mathcal{U},\; \forall\, i \neq j$$

$$(-1)^{\varepsilon_i + \delta_j}\, \frac{\partial f_i}{\partial u_j}(x,u) \geq 0 \quad \forall\, x \in X,\; \forall\, u \in \mathcal{U},\; \forall\, i, j$$

$$(-1)^{\varepsilon_i + \mu_j}\, \frac{\partial h_i}{\partial x_j}(x) \geq 0 \quad \forall\, x \in X,\; \forall\, i, j\,.$$

It is important to be able to decide, just from the system equations, if there is *some* orthant K such that a given system is monotone with respect to K. Graphical conditions are very useful for that. It is not difficult to see that the conditions given in [21] for classical monotone systems can be generalized as follows. We associate to a given system (1) a signed digraph, with vertices $x_1, x_2 \dots x_n$, $u_1, u_2, \dots u_m$, $y_1, y_2 \dots y_p$ and edges constructed according to the following set of rules and constraints (if the rules do not all apply, the graph is undefined).

For edges between nodes x_k, the graph is defined only for systems so, that for any pair of integers $1 \le i, j \le n$ with $i \ne j$, either (a) $\partial f_i/\partial x_j(x,u) \equiv 0$, or (b) $\partial f_i/\partial x_j(x,u) \ge 0$ for all x, u and $\partial f_i/\partial x_j(x,u) > 0$ for some x, u, or (c) $\partial f_i/\partial x_j(x,u) \le 0$ for all x, u and $\partial f_i/\partial x_j(x,u) < 0$ for some x, u; in that case, we draw no edges, a positive edge, or a negative edge, respectively, from x_j to x_i. For edges from nodes u_j to nodes x_i, similar rules apply, where we consider the Jacobians $\partial f_i/\partial u_j$. Finally, for edges from nodes x_j to nodes y_i, we proceed analogously using the Jacobians $\partial h_i/\partial x_j$.

A *cycle*, not necessarily directed, is a sequence of vertices $v_{n_0}, v_{n_1} \dots v_{n_L}$ such that $v_{n_0} = v_{n_L}$ and the constraint that, for each $0 \le k \le L-1$, either there is an edge from v_k to v_{k+1} or there is an edge from v_{k+1} to v_k. The sign of a cycle is defined as the product of the signs of the edges comprising it, and the sign of a path is defined to be the product of the signs of its edges.

Proposition 2. *The system (1) which admits an incidence graph according to the above set of rules, is monotone with respect to some orthants K^X, $K^{\mathcal{U}}$ and $K^{\mathcal{Y}}$ if and only if its graph does not contain any negative cycles.*

Graphical conditions for strong monotonicity can be given as well. Suppose that we strengthen the conditions to require that either $\partial f_i/\partial x_j(x,u) \equiv 0$, $\partial f_i/\partial x_j(x,u) > 0$ for all x, u, or $\partial f_i/\partial x_j(x,u) < 0$ for all x, u, and similarly for edges from nodes u_j to nodes x_i and for edges from nodes x_j to nodes y_i. Assuming that a graph in this sense is well-defined, results are provided in [3] which allow one to check if a closure under unity feedback is strongly monotone. The results are stated in terms of "excitability" and "transparency" notions associated to the input/output system. For the purposes of treating the examples in this paper, we only require the more classical version of these results, for systems $\dot{x} = f(x)$ with no inputs:

Proposition 3. *Suppose that the system $\dot{x} = f(x)$ is monotone with respect to an orthant K. If either of these conditions hold:*

1. *the Jacobian $\partial f_i/\partial x_j(x)$ is an irreducible matrix, for each $x \in X$, or*
2. *the Jacobian $\partial f_i/\partial x_j(x)$ is an irreducible matrix, for each $x \in \operatorname{inter} X$ and every trajectory lies in the interior of X for all $t > 0$,*

then the system is strongly monotone with respect to K.

See Theorem 1.1 in [16] or Chapter 4 in [29] for a proof. (These references assume that the system evolves on an open set; however, the same proofs apply when the state space is closed, so part 1 follows immediately. Regarding part 2, one may reduce to case 1 as follows: given $\xi_1 \succ \xi_2$, we know that $x(t/2, \xi_1) \succ x(t/2, \xi_2)$ (monotonicity plus the fact that a flow is always one-to-one); as the interior is forward invariant, one need only consider these states at time $t/2$ as initial states, to conclude that, using the strong monotonicity results for the interior, $x(t,\xi_1) \gg x(t,\xi_2)$. Incidentally, an even weaker condition may be assumed, namely that irreducibility of the Jacobian holds almost everywhere in time along a trajectory.)

3 Feedbacks with Single Steady-States

It is easy to verify that cascade interconnections of monotone systems admitting characteristics are again monotone and admit characteristics. More interesting is the study of feedback, where the output is fed-back to the input of (1) via a static or dynamic feedback law. In this section we study feedback interconnections that lead to global attractivity, and in the next section we study multiple steady states.

We restrict attention to SISO systems, as the results are easier to state in that context. Without loss of generality (otherwise, one may consider $-u$ as an input or $-y$ as an output), we will assume that $K^{\mathcal{U}} = K^{\mathcal{Y}} = \mathbb{R}_{\geq 0}$. Our interest will be in the effect of either "positive" or "activating" feedback (which preserves the orders on inputs and outputs) or or "negative" or "inhibitory" feedback (which inverts the orders). The study of general dynamic feedbacks can be reduced to the study of static feedback, simply by viewing a feedback interconnection

$$\begin{aligned} \dot{x} &= f_x(x,u)\,, \quad y = h_x(x) \\ \dot{z} &= f_z(z,y)\,, \quad u = h_z(z) \end{aligned}$$

as the closure under the static feedback $u = y$ of the composite system

$$\begin{aligned} \dot{x} &= f_x(x,u) \\ \dot{z} &= f_z(z,h_x(x))\,, \quad y = h_z(z) \end{aligned}$$

so we will only state a result for static feedback. (The theorem is proved directly for arbitrary dynamic feedback in [2], and the proof is virtually the same.)

Theorem 3. *Suppose that the system (1) is monotone, with* $K^{\mathcal{U}} = K^{\mathcal{Y}} = \mathbb{R}_{\geq 0}$*, and it has a well-defined I/O characteristic* $k : \mathcal{U} \to \mathcal{Y}$*. Let* $\ell : \mathcal{Y} \to \mathcal{U}$ *be a monotone (increasing or decreasing) map, and denote* $F := \ell \circ k : \mathcal{U} \to \mathcal{U}$*. Suppose that every trajectory of the closed-loop system*

$$\dot{x} = f(x, (\ell \circ h)(x)) \tag{4}$$

is bounded. Then, system (4) has a globally attractive equilibrium provided that the following scalar discrete time iteration on $\mathcal{U}$*:*

$$u_{k+1} = F(u_k) \tag{5}$$

has a unique globally attractive equilibrium $\bar{u}$*.*

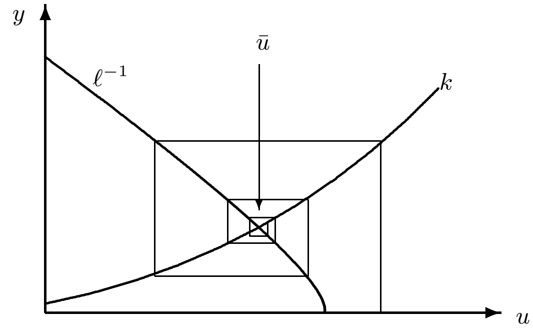

Fig. 1. I/O characteristics in (u, y) plane: negative feedback

For a graphical interpretation of condition (5), for the special case in which ℓ is decreasing ("negative feedback"), see Fig. 1. The figure shows the 'convergent spiderweb' diagram that establishes global convergence to the equilibrium of the discrete-time iteration.

The proof of this theorem is not difficult; let us sketch it here. Consider an arbitrary (bounded, by assumption) solution of the closed-loop system, and the signals $u(t)$ and $y(t)$ that appear as input and output of the original system (1). Note that $u(t) = \ell(y(t))$ for all t. The key is to consider the quantities

$$u_+ := \limsup_{t\to+\infty} u(t),\ u_- := \liminf_{t\to+\infty} u(t),\ y_+ := \limsup_{t\to+\infty} y(t),\ y_- := \liminf_{t\to+\infty} y(t),$$

and to use the fact that monotonicity of (1) implies that

$$k(u_-) \le y_- \le y_+ \le k(u_+) \tag{6}$$

and the fact that (from monotonicity of ℓ) either $\ell(u_-) = y_-$ and $\ell(u_+) = y_+$, or $\ell(u_-) = y_+$ and $\ell(u_+) = y_-$. In the first case, we apply the increasing function ℓ to (6) and obtain $F(u_-) \le u_- \le u_+ \le F(u_+)$, and, inductively using that F is increasing,

$$F^i(u_-) \le u_- \le u_+ \le F^i(u_+)$$

for all $i \ge 1$, so taking limits and using $F^i(u_-) \to \bar{u}$ and $F^i(u_+) \to \bar{u}$, we obtain that $u_- = u_+ = \bar{u}$, and hence $u(t) \to \bar{u}$. Then, from the "convergent input convergent state" property for monotone systems with well-defined characteristics (cf. [2]), one concludes that the state $x(t) \to k^X(\bar{u})$. In the second case, we obtain $F(u_-) \ge u_+ \ge u_- \ge F(u_+)$, and, applying F (now a decreasing function) we have that $F^2(u_-) \le u_- \le u_+ \le F^2(u_+)$, and, inductively using that F^2 is increasing,

$$F^i(u_-) \le u_- \le u_+ \le F^i(u_+)$$

for all *even* i; the proof then is finished as before.

It is worth noting that, in the case of increasing ℓ (positive feedback), the global attractivity condition on (5) is satisfied automatically whenever F has a unique fixed point $\bar{u}$ and it holds that $\bar{u} \leq F(u) \leq u$ for all $u \geq \bar{u}$ and $\bar{u} \geq F(u) \geq u$ for all $u \leq \bar{u}$. This is in turn satisfied whenever the graphs of k and ℓ^{-1} intersect at only one point $\bar{u}$ and there $k'(\bar{u}) < (\ell^{-1})'(\bar{u})$.

Boundedness is assumed in our statement of the theorem. Often in biological applications, boundedness is automatic from conservation of mass and similar assumptions; in any event, we show in ([2]) how the assumption can be weakened provided that some more information is available about the characteristic of the system.

We also remark that arbitrary delays in the feedback loop do not affect the argument. That is, suppose that $u(t) = \ell(y(t - \sigma(t)))$, for some possibly time-varying delay $\sigma(t)$, where σ may be fairly arbitrary, for instance any continuous function such that $t - \sigma(t) \to \infty$ as $t \to \infty$, so that $\limsup y(t - \sigma(t)) = \limsup y(t)$ and similarly for $\liminf$. The same proof then applies. This means that the condition that we gave is a condition for stability under arbitrary delays, as is typically the case with "small-gain" arguments.

A partial converse to this result holds as well: if the feedback ℓ induces a periodic orbit for the discrete iteration, then, for an appropriate delay $\sigma(t)$, an approximate periodic orbit appears. The idea, whose precise statement and proof we leave for a future work, is roughly as follows. Suppose that $k(a) = b$, $\ell(b) = c$, $k(c) = d$, and $\ell(d) = a$. We consider the initial state of the output as $y(t) \equiv d$ for a time long enough that the input $u(t) = \ell(d) = a$ forces the output $y(t)$ to converge to b. Now, for a long enough interval so that convergence to steady state ensues, the input will converge to $\ell(b) = c$, and after a suitable time, the output will converge to $k(c) = d$, after which the pattern will repeat. There results an oscillation of the input between (approximately) the constant values a and c. By adjusting the delay periods, one can achieve sustained oscillations for as long as desired.

4 Multiple Steady States

We now turn to the more general case when several steady states may appear in closed-loop; our result will provide a global analysis tool for systems obtained by positive feedback loops involving monotone systems. In [33], R. Thomas conjectured that the existence of at least one positive loop in the incidence graph is a necessary condition for the existence of multiple steady states. Proofs of this conjecture were given in [14], [23], [30], and [7], under different assumptions on the system (the last reference provides the most general result, using a degree theory argument). However, the existence of positive loops is not sufficient, and our next theorem deals precisely with this question.

Theorem 4. *Suppose that the system (1) is monotone, with $K^{\mathcal{U}} = K^{\mathcal{Y}} = \mathbb{R}_{\geq 0}$, and it has a well-defined nondegenerate characteristic k. Let $\ell : \mathcal{Y} \to \mathcal{U}$ be a monotone increasing map, and denote $F := \ell \circ k : \mathcal{U} \to \mathcal{U}$. Assume that every fixed point of F is non-degenerate ($F'(x) \neq 1$) and that the system (4):*

$$\dot{x} = f(x, (\ell \circ h)(x))$$

is strongly monotone and all of its solutions are bounded. Then, the steady-states of (4) are in 1-1 correspondence with the fixed points of F, and for almost all initial conditions, solutions converge to the set of equilibria of (4) corresponding to inputs for which $F'(u) < 1$.

The proof of this theorem is given in [3]. It relies upon linear control theory arguments for analyzing local stability of the closed loop system, together with the use of a theorem due to Hirsch on almost-everywhere convergence for strongly monotone systems.

Note that the condition $F'(u) < 1$ amounts to local (exponential) stability of the discrete iteration (5). Strong monotonicity of the closed-loop system (4) can be checked in several graph-theoretic ways, based upon conditions on the open-loop system (1), see [2]. Monotonicity of (1) with respect to some orthant and orders $K^{\mathcal{U}} = K^{\mathcal{Y}} = \mathbb{R}_{\geq 0}$ amounts to the incidence graph (defined earlier) having no negative cycles plus the requirement that all paths from the input to the output node must be positive.

5 Example of Multiple Steady States

A typical situation for the application of Theorem 4 is when a monotone system with a well-defined I/O characteristic of sigmoidal shape is closed under unitary feedback: if the sigmoidal function is sufficiently steep, this configuration is known to yield 3 equilibria, 2 stable and 1 unstable. In biological examples, this might arise when a feedback loop comprising any number of positive interactions and an even number of inhibitions is present (no inhibition at all is also a situation which might lead to the same type of behavior). This is a well-known principle in biology. One of its simplest manifestations is the so called "competitive exclusion" principle, in which one of two competing species completely eliminates the other, or more generally, for appropriate parameters the bistable case in which they coexist but the only possible equilibria are those where either one of the species is strongly inhibited. Of course, the interest of our results is in the high-dimensional case in which phase-plane techniques cannot provide the result, and we turn to such an example next. However, let us note that, for the special case of two-dimensional systems, our techniques are very close to those of [6]. In fact, even the 4-dimensional example of a two-repressor system with RNA dynamics, treated in [6] (Appendix I) in an ad-hoc manner, can be shown to be globally bistable as an immediate application of our techniques.

We now turn to a less trivial example where our tools may be applied. (A different example, involving cascades of systems of this type, and with comparisons with experimental data, is treated in [1].) Consider the following chemical reaction, involving various forms of a protein E:

$$\mathrm{E}_1 \overset{\mathrm{U}}{\rightleftarrows} \mathrm{E}_2 \overset{\mathrm{U}}{\rightleftarrows} \dots \overset{\mathrm{U}}{\rightleftarrows} \mathrm{E}_{n-1} \overset{\mathrm{U}}{\rightleftarrows} \mathrm{E}_n$$

being driven forward by an enzyme U, with the different subscripts indicating an additional degree of phosphorylation, and with constitutive dephosphorylation. This is a very general type of "module" that appears in cell signaling. We will be interested in positive feedback from E_n to U.

A typical way to model such a reaction is as follows. We introduce variables $x_i(t)$, $i = 1, \dots, n$ to indicate the fractional concentrations of the various forms of the enzyme E (so that $x_1 + \dots + x_n \equiv 1$, and $x_i \geq 0$, for the solutions of physical interest), and $u(t) \geq 0$ to indicate the concentration of U. The differential equations are then as follows:

$$\begin{aligned}
\dot{x}_1 &= -\sigma_1(u)\alpha_1(x_1) + \beta_2(x_2) \\
\dot{x}_2 &= \sigma_1(u)\alpha_1(x_1) - \beta_2(x_2) - \sigma_2(u)\alpha_2(x_2) + \beta_3(x_3) \\
&\vdots \\
\dot{x}_{n-1} &= \sigma_{n-2}(u)\alpha_{n-2}(x_{n-2}) - \beta_{n-1}(x_{n-1}) - \sigma_{n-1}(u)\alpha_{n-1}(x_{n-1}) + \beta_n(x_n) \\
\dot{x}_n &= \sigma_{n-1}(u)\alpha_{n-1}(x_{n-1}) - \beta_n(x_n)\,.
\end{aligned}$$

We make the assumptions that α_i and β_i (respectively, σ_i) are differentiable functions $[0, \infty) \to [0, \infty)$ with positive (respectively, either positive or identically zero) derivatives, and $\alpha_i(0) = \beta_i(0) = 0$ and $\sigma_i(0) > 0$ for each i. (We allow some of the σ_i to be constant, and in this manner represent steps that are not controlled by U.) Since we are interested in studying the effect of feeding back E_n, we pick $y = x_n$.

We first prove that the characteristic is well-defined. Recall that we are only interested in those solutions that lie in the intersection X of the plane $x_1 + \dots + x_n \equiv 1$ and the nonnegative orthant in $\mathbb{R}^n$. This set is easily seen to be invariant for the dynamics, and it is convex, so the Brower fixed point theorem guarantees the existence of an equilibrium in X, for any constant input $u(t) \equiv a$. We next prove that this steady-state is unique. Redefining if necessary the functions α_i, we will assume without loss of generality that $\sigma_i(a) = 1$ for all i. Let us introduce the nondecreasing functions

$$G_k \;=\; \beta_k^{-1} \circ \alpha_{k-1} \circ \beta_{k-1}^{-1} \circ \dots \circ \beta_2^{-1} \circ \alpha_1$$

for each $k = 2, \dots, n$ and $G(r) := r + G_2(r) + \dots + G_n(r)$. This function is defined on some maximal interval $[0, M]$, consisting of those r such that $\alpha_1(r)$ belongs to the range of β_2, $\alpha_2(\beta_2^{-1}(\alpha_1(r)))$ belongs to the range of β_3, and so forth, and it is strictly increasing. Moreover, for each equilibrium

$x = (x_1, \ldots, x_n)$, it holds that $x_k = G_k(x_1)$, and therefore, recalling that $x_1 + \ldots + x_n = 1$, $G(x_1) = 1$. Thus, if x and $\widetilde{x}$ are two steady states, we have $G(x_1) = G(\widetilde{x}_1)$. Since G is strictly increasing, it follows that $x_1 = \widetilde{x}_1$, and therefore that $x_k = G_k(x_1) = G_k(\widetilde{x}_1) = \widetilde{x}_k$ for all k, so uniqueness is shown.

We must prove stability. For that, we first perform a change of coordinates:

$$z_1 = x_1,\ z_2 = x_1 + x_2,\ \ldots,\ z_{n-1} = x_1 + \ldots + x_{n-1},\ z_n = x_1 + \ldots + x_n$$

so that the equations in these new variables become (using that $\dot{z}_k = (d/dt)(x_1 + \ldots + x_k)$ and $x_k = z_k - z_{k-1}$ for $k > 1$):

$$\begin{aligned}
\dot{z}_1 &= -\sigma_1(u)\alpha_1(z_1) + \beta_2(z_2 - z_1) \\
&\vdots \\
\dot{z}_k &= -\sigma_k(u)\alpha_k(z_k - z_{k-1}) + \beta_{k+1}(z_{k+1} - z_k) \\
&\vdots \\
\dot{z}_{n-1} &= -\sigma_{n-1}(u)\alpha_{n-1}(z_{n-1} - z_{n-2}) + \beta_n(1 - z_{n-1})
\end{aligned}$$

(and $z_n \equiv 1$). When the input $u(t)$ is equal to any given constant, the system described by the first $n-1$ differential equations, seen as evolving in the subset of $\mathbb{R}^{n-1}$ where $0 \leq z_1 \leq z_2 \leq \ldots \leq z_{n-1} \leq 1$, is a *tridiagonal strongly cooperative system*, and thus a theorem due to Smillie (see [27]) insures that all trajectories converge to the set of equilibria. (The proof given in [28] is also valid when the state-space is closed, as here.) Moreover, linearizing at the equilibrium preserves the structure, so applying the same result to the linearized system we know that we have in fact an exponentially stable equilibrium. Thus, characteristics are well defined and nondegenerate.

It is easy to verify from our graph conditions that the system (in the new coordinates) is monotone, since $df_i/dz_j > 0$ for all pairs $i \neq j$, $df_i/du \leq 0$ for all i, and $dh/dz_i = 0$ for all $i < n-1$ and $dh/dz_{n-1} < 0$ (the output is $y = x_n = 1 - z_{n-1}$).

The closed-loop system (4) is obtained in this case (unity feedback) by setting $u = \ell(y) = y$ (and changing to the new coordinates z_i), so, since the output is $1 - z_{n-1}$, we have $u = (\ell \circ h)(z) = 1 - z_{n-1}$. We need to prove strong monotonicity of (4). Closure under positive feedback preserves monotonicity (see [2]). By Proposition 3, it is enough to see that the Jacobian is irreducible in the interior of the state space (where $z_i < z_{i+1}$ strictly) and that solutions exit the boundary instantaneously. In order to prove the irreducibility property, it is enough to note that the size $(n-1) \times (n-1)$ Jacobian matrix A at any interior point has the properties that $a_{i,i-1} = \sigma_i(1 - z_{n-1})\alpha_i'(z_i - z_{i-1}) > 0$ for all $2 \leq i \leq n-1$, $a_{i,i+1} = \beta_i'(z_{i+1} - z_i) > 0$ for all $1 \leq i \leq n-3$, and $a_{n-2,n-1} = \sigma_{n-1}'(1 - z_{n-1})\alpha_{n-2}(z_{n-2} - z_{n-3}) + \beta_{n-1}'(z_{n-1} - z_{n-2}) > 0$. To show that every trajectory lies in the interior of X for all $t > 0$, as the interior of X is itself forward invariant (see e.g. [2]), it is sufficient to prove: for any $T > 0$, if Φ is the set of $t \in [0, T]$ such that $x(t)$ is in the boundary of X (relative to the linear space $x_1+\ldots+x_n=1$), then $\Phi \neq [0,T]$. (It is more convenient to use x coordinates to show this property.) Assume otherwise. For each i, consider the closed set $\Phi_i = \{t \in [0,T] \mid x_i(t)=0\}$, and note that $\bigcup_i \Phi_i = \Phi$. If Φ_i would be nowhere dense for every i, then their union Φ would be nowhere dense, contradicting $\Phi=[0,T]$. Thus there is some i so that Φ_i contains an open interval $(a,b) \subseteq [0,T]$. It follows that, for this i, $\dot{x}_i \equiv x_i \equiv 0$ on (a,b), and (looking at the equations) this implies that $x_{i\pm1} \equiv 0$ and, recursively, we obtain $x_j \equiv 0$ for all j, contradicting $x_1+\ldots+x_n=1$.

As a numerical example, let us pick $\sigma_i(r) = (0.01+r)/(1+r)$, $\alpha_i(r) = 10\,r/(1+r)$, and $\beta_i(r) = r/(1+r)$ for all i, and $n=7$. (The constants have no biological significance, but the functional forms are standard models of saturation kinetics.) A plot of the characteristic is shown in Fig. 2(a). Since the intersection with the diagonal has three points as shown, we know that *the closed-loop system (with $u = x_n$) will have two stable and one unstable equilibrium, and almost all trajectories converge to one of these two stable equilibria.* To illustrate this convergence, we simulated six initial conditions, in each case with $x_2(0) = \ldots = x_6(0) = 0$ and with the following choices of $x_7(0)$: 0.1, 0.2, 0.3, 0.4, 0.5, and 0.8 (and $x_1(0) = 1 - x_7(0)$). A plot of $x_7(t)$ for each of these initial conditions is shown in Fig. 2(b); note the convergence to the two predicted steady states.

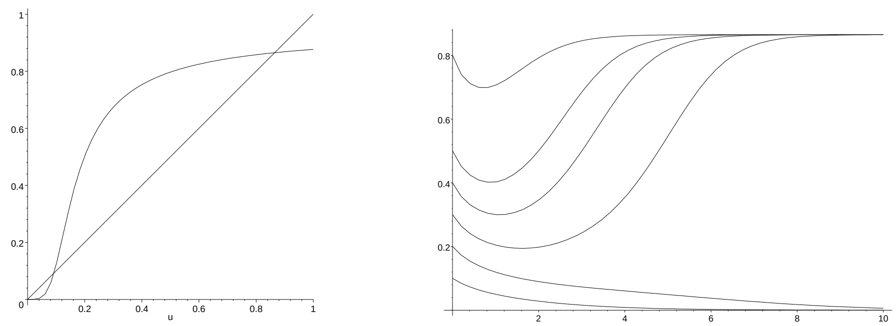

Fig. 2. Enzyme example: (a) Characteristic and (b) Simulations

6 An Example of Negative Feedback

A large variety of eukaryotic cell signal transduction processes operate through "Mitogen-activated protein kinase (MAPK) cascades," which play a role in some of the most fundamental processes of life (cell proliferation and growth, responses to hormones, etc). A MAPK cascade is a cascade connection of three SISO systems, each of which is a system of the type studied in Section 5, the first with with $n = 2$ and the next two with $n = 3$. We already proved that such systems admit I/O characteristics and are monotone, so cascades have the same property; alternatively, a proof can be given directly for this example, see [2]. Thus the complete theory may be applied. We pick now a numerical example in order to illustrate the application of Theorem 3.

Instead of the change of variables used in Section 5, we use the change of variables in [2], in which we eliminate x_2 in the case $n = 3$. The cascade then becomes, in terms of the reduced variables, a system of dimension five. As a concrete illustration, let us consider the open-loop system with these equations:

$$\begin{aligned}
\dot{x}_1 &= \frac{v_2\,(100 - x_1)}{k_2 + (100 - x_1)} - \frac{g_1 x_1}{k_1 + x_1}\frac{g_2 + u}{g_4 + u} \\
\dot{y}_1 &= \frac{v_6\,(300 - y_1 - y_3)}{k_6 + (300 - y_1 - y_3)} - \frac{\kappa_3\,(100 - x_1)\,y_1}{k_3 + y_1} \\
\dot{y}_3 &= \frac{\kappa_4\,(100 - x_1)\,(300 - y_1 - y_3)}{k_4 + (300 - y_1 - y_3)} - \frac{v_5\,y_3}{k_5 + y_3} \\
\dot{z}_1 &= \frac{v_{10}\,(300 - z_1 - z_3)}{k_{10} + (300 - z_1 - z_3)} - \frac{\kappa_7\,y_3\,z_1}{k_7 + z_1} \\
\dot{z}_3 &= \frac{\kappa_8\,y_3\,(300 - z_1 - z_3)}{k_8 + (300 - z_1 - z_3)} - \frac{v_9\,z_3}{k_9 + z_3}.
\end{aligned}$$

This is the model studied in [20], from which we also borrow the values of constants (with a couple of exceptions, see below): $g_1 = 0.22$, $g_2 = 45$, $g_4 = 50$, $k_1 = 10$, $v_2 = 0.25$, $k_2 = 8$, $\kappa_3 = 0.025$, $k_3 = 15$, $\kappa_4 = 0.025$ $k_4 = 15$, $v_5 = 0.75$, $k_5 = 15$, $v_6 = 0.75$, $k_6 = 15$, $\kappa_7 = 0.025$, $k_7 = 15$, $\kappa_8 = 0.025$, $k_8 = 15$, $v_9 = 0.5$, $k_9 = 15$, $v_{10} = 0.5$, $k_{10} = 15$. Units are as follows: concentrations and Michaelis constants (k's) are expressed in nM, catalytic rate constants (κ's) in s^{-1}, and maximal enzyme rates (v's) in $nM.s^{-1}$. The paper [20] showed that oscillations may arise in this system for appropriate values of negative feedback gains. (We have slightly changed the input term, using coefficients g_1, g_2, g_4, because we wish to emphasize the open-loop system before considering the effect of negative feedback.) Figure 3 shows the I/O characteristic of this system, as well as the characteristic corresponding to a feedback $u = K/(1 + y)$, with the gain $K = 30000$. It is evident from this planar plot that the small-gain condition is satisfied - a ‘spiderweb’ diagram shows convergence. Our theorem then guarantees global attraction to a unique equilibrium. Indeed, Figure 4 shows a typical state trajectory.

7 Another Example: Circadian Oscillator

The molecular biology underlying the circadian rhythm in *Drosophila* is the focus of a large amount of both experimental and theoretical work. Goldbeter proposed a simple model for circadian oscillations in [12] (see also his

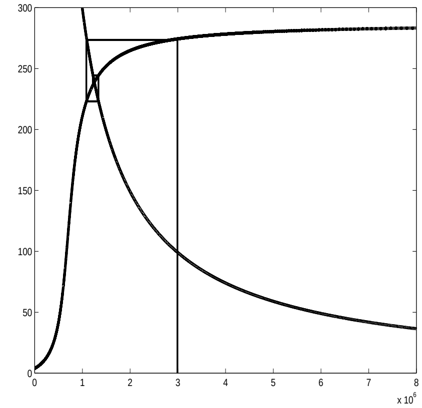

Fig. 3. I/O characteristic and small-gain for MAPK example

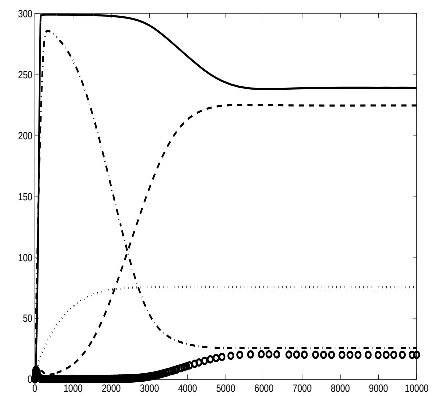

Fig. 4. Simulation of MAPK system under negative feedback satisfying small-gain conditions. Key: x_1 dots, y_1 dashes, y_2 dash-dot, z_1 circles, z_3 solid

book [13]) using a mechanism based on the negative feedback loop of the protein PER inhibiting its own transcription. This (somewhat oversimplified) model is described by the following equations:

$$\dot{M} = v_s K_I^n/(K_I^n + P_N^n) - v_m M/(k_m + M)$$
$$\dot{P}_0 = k_s M - V_1 P_0/(K_1 + P_0) + V_2 P_1/(K_2 + P_1)$$

$$\dot{P}_1 = V_1P_0/(K_1+P_0) - V_2P_1/(K_2+P_1) - V_3P_1/(K_3+P_1) + V_4P_2/(K_4+P_2)$$
$$\dot{P}_2 = V_3P_1/(K_3+P_1) - V_4P_2/(K_4+P_2) - k_1P_2 + k_2P_N - v_dP_2/(k_d+P_2)$$
$$\dot{P}_N = k_1P_2 - k_2P_N$$

where the subscript $i = 0, 1, 2$ in the concentration P_i indicates the degree of phosphorylation of PER protein, P_N is used to indicate the concentration of PER in the nucleus, and M indicates the concentration of *per* mRNA. The parameters (in suitable units μM or h^{-1}) are: $k_2 = 1.3$, $k_1 = 1.9$, $V_1 = 3.2$, $V_2 = 1.58$, $V_3 = 5$, $V_4 = 2.5$, $v_s = 0.76$, $v_m = 0.65$, $k_m = 0.5$, $k_s = 0.38$, $v_d = 0.95$, $k_d = 0.2$, $n = 4$, $K_1 = 2$, $K_2 = 2$, $K_3 = 2$, $K_4 = 2$, $K_I = 1$. The whole point of the model, of course, is that limit cycle oscillations appear. It is important, therefore, to understand to what extent these parameters affect the existence of periodic orbits. We choose to view the system as the feedback closure of the system having M (mRNA) as input and P_N as output (P equations), with the system where P_N negatively regulates the production of M (first equation). The P-subsystem is a monotone tridiagonal system, and characteristics can be analyzed, for small enough inputs M, using techniques as in the previous examples (see [4] for details), while the M subsystem is scalar. We view v_s as a bifurcation parameter. It is remarkable that the conditions for Theorem 3 are satisfied with e.g. $v_s = 0.4$: no oscillations can happen in that case, even under arbitrary delays in the feedback from P_N to M. See Figure 5 for the 'spiderweb diagram' that shows convergence of the discrete iteration. (the dotted and dashed curves are the characteristics). On the other hand,

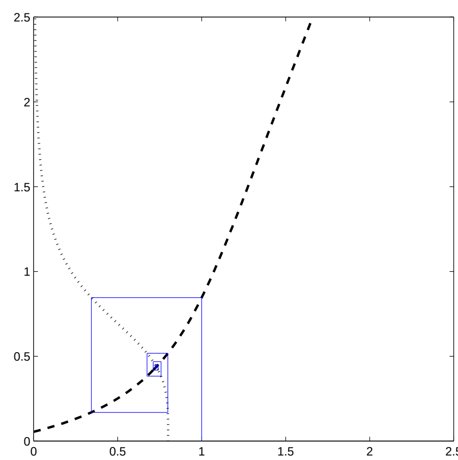

Fig. 5. Stability of spiderweb ($v_s = 0.4$)

for e.g. $v_s = 0.5$, the conditions are violated; see Figure 6 for the 'spiderweb diagram' that shows divergence of the discrete iteration. Thus, and one can

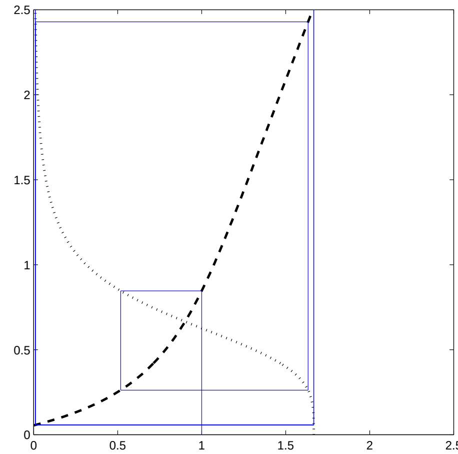

Fig. 6. Instability of spiderweb ($v_s = 0.5$)

expect periodic orbits; and indeed, simulations show that, for large enough delays, such periodic orbits arise, see Figure 7.

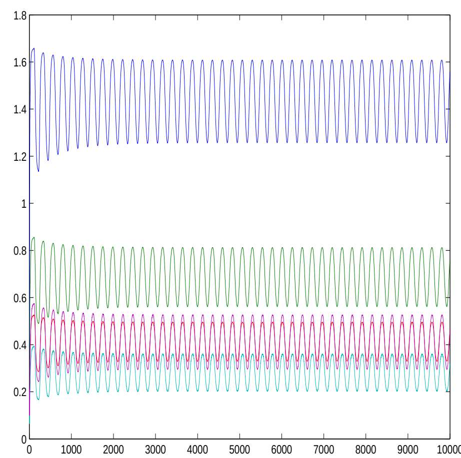

Fig. 7. Oscillations seen in simulations ($v_s = 0.5$, delay of 100, initial conditions all at 0.2), using MATLAB's dde23 package

8 Final Remarks

The theory of monotone systems with well-defined characteristics provide a very powerful method for analyzing both positive and negative feedback interconnections. The theory has only recently started to be developed, but already a large number of nontrivial applications have been handled by it.

For reasons of space, we have omitted a discussion of how complete bifurcation diagrams, with respect to magnitudes of gains, can be immediately derived from the forms of characteristics; see [1, 3] for details.

Another interesting issue, not included here, regards the necessity of the monotonicity assumption in Theorem 4. We show by examples in [2] how relying upon a well-defined characteristic may lead to erroneous conclusions, if monotonicity is not checked first.

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