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A new mechanism for bistability in chemical reaction networks

D. Angeli
Dip. di Sistemi e Informatica
Università di Firenze
Via di S. Marta 3, 50139 Firenze, Italy
angeli@dsi.unifi.it

Abstract. Systems with counter-clockwise Input-Output dynamics were recently introduced in order to study convergence of positive feedback loops (possibly to many different equilibrium states). The paper shows how this notion can be used in order to perform bifurcation analysis and globally predict multistability of a closed-loop feedback interconnetion just by using the knowledge of steady-state Input-Output responses of the systems. To illustrate the theory our method is then applied to a recently published model of MAPK cascade. Furthermore, a library of examples (mainly motivated by molecular biology) of systems which are guaranteed to enjoy the property is presented and discussed.

I. INTRODUCTION AND MOTIVATIONS

Since the pioneering works by Delbrück, [4], bistability and more in general multistability, have been recognized as a fundamental dynamical behaviour associated to many models in molecular biology and playing a crucial role in phenomena such as cell differentiation, development, and even in the insurgence of periodic behaviours due to the combination of an hysteretic system interacting with a (typically much slower) negative feedback loop around it, thus giving rise to what is usually named "relaxation oscillator". One common class of models in molecular biology, which also have the potential for multistability, is that of nonlinear (positive) systems with sign-definite jacobian, viz. systems of differential equations $\dot{x} = f(x)$ evolving in a subset of euclidean space ($X \subset \mathbb{R}^n$, typically the positive orthant) and such that the associated jacobian matrix $Df(x)$ is entry-wise sign-definite, throughout X. It was first conjectured by Thomas, [14], and later proved by several authors with different techniques and under slightly different technical assumptions, [3], that for the class of systems described above multiple equilibria are only allowed provided that a positive feedback loop exists, viz. a cycle in the graph associated to the system comprising an even number of negative edges, where the sign of an edge is determined according to the sign of the corresponding entry of the Jacobian matrix. This condition, however, is only necessary, and actually leaves completely aside the problem of the dynamics of the system, in other words it is rather a necessary condition for multistationariety (viz. the presence of multiple steady states) rather than a condition for multistability, for which not only the existence of multiple steady states is required, but also the fact that the stable states must be attractors for the whole state space X, except possibly a set of measure zero which typically would be at the boundary between the two (o more) basins of attractions.

In this respect, it comes as no surprise that also the quest for sufficient (possibly checkable) conditions for multistability poses a challenging and interesting question in the long term quest for a "reverse engineering" methodology. In other words, understanding the basic principles behind a specific observed dynamical behaviour, could potentially open the way for the "synthesis" of that particular behaviour in situations where it normally does not arise or to restore the conditions which due to some pathology had prevented that behaviour from showing up, [6].

Recently we developed a simple graphical method, based on steady-state quantitative measures performed on an open-loop system, which guarantees, under certain graph-theoretical assumptions on the sign patterns of the jacobian, that the corresponding unitary feedback system exhibit bistability, [2] (see [9], [12] for related approaches). The graph theoretical assumptions are needed in order to restrict the class of dynamical behaviours potentially exhibited by the system and basically amount to requiring that the resulting flow be monotone with respect to initial conditions according to some partial order sitting in the state space X. In words this is saying that the more of certain chemical compounds are present at time 0, the more will be there at all future times (where the notion of "more" really needs to be made precise mathematically). The class of monotone system is however not as general as one would like; first of all it is a subclass of sign-definite systems (which already poses too strong a requirement in many situations), secondarily, especially for high order random graphs with $+$ or $-$ labels attached to edges, it is rather the exception than the rule. The aim of this note is to illustrate a new class of systems, introduced in [1], independent of monotone systems, yet for which analysis can be performed along the same lines as in the case of monotone systems and dynamical behaviour inferred just by exploiting static Input-

Output measuraments of the systems responses . In other words, and modulo some technicalities, the same methods applied in [2] to infer multistability of monotone systems, would apply to this class of systems. These are the so called *systems with Counter Clockwise Input-Output Dynamics*, which will be illustrated in more detail in the next section.

II. Systems with Counter-clockwise Input-Output dynamics

One of the main stumbling blocks when analyzing nonlinear dynamical systems and their interconnections is the lack of a notion of frequency response and phase-lag introduced by the system. In this respect, it is useful to recall that in the linear world, the “principle of superposition of effects” allows to analyze Input-Output maps on a frequency-by-frequency basis and this in turn leads to a notion of frequency-dependent gain (the amplification produced by the system for a sinusoidal input at that specific frequency) and of phase-lag (viz. the delay expressed in radians) introduced by the system. When superposition principles do not hold (as is the case of nonlinear systems) one can still try to define notions of Input-Output gains; typically not on a frequency by frequency basis, however, but with respect to some norm of interest in input and output space. This has many useful applications and leads to a very rich and deep theory (see for instance [13]), but does not allow to generalize the notion of phase-lag introduced by the system. One remarkable exception to this situation is the property called “passivity”; this property in fact has a precise frequency-domain characterization for linear systems and also a very meaningful and physically appealing interpretation in the time-domain, which therefore can be adopted also for nonlinear systems. This property, born in the context of electrical (nonlinear) networks and Hamiltonian systems, is very useful in analyzing “negative feedback” interconnections. It is in fact preserved under parallel and negative feedback. In this way, complex interconnected systems built out of passive components are still passive.

Roughly speaking, the property of counter-clockwise Input-Output dynamics, plays for positive feedback interconnections the same role that passivity does for negative feedbacks. It is therefore likely to be a very useful and powerful tool; moreover, it seems more appropriate for the study of multistable systems, which typically arise when, at least locally in state-space, destabilization is introduced around a certain equilibrium by means of positive feedback loops. The intuitive idea behind the property is very simple; a wide range of nonlinear systems when excited by some time-varying input signal (say for simplicity a periodic input) tend to produce an output signal which, after a transient and modulo some “delay” and typically some distortion and some smoothing which is going on due to the dynamics of the system, will reproduce the same qualitative behavior of the corresponding input

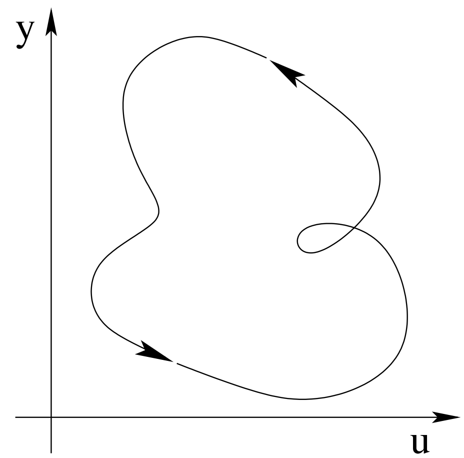

Fig. 1. The Input-Output plot for a System with CCW dynamics

(for instance it will be a periodic output signal of the same period as the input). The amount of delay may vary a lot between different systems; a way to quantify this “delay” is to look at the curve described by $u(t)$ and $y(t)$ and ask that the evolution happens in a counter-clockwise direction in the (u, y)-plane. In other words that the area integral $\int_0^T \dot{y}u\,dt$ be positive when computed over one period. Now, this is not yet a precise mathematical definition, as we only considered periodic inputs and disregarded the fact that there might be transients due to the effect of initial conditions, before the system settles to a periodic output. However, this definition is good enough to understand the basic idea behind the “positive feedback” convergence theorem which will be illustrated later. In particular, a positive feedback interconnection of two systems Σ_1 and Σ_2, with inputs (respectively) u_1, u_2 and outputs y_1, y_2 is characterized by the interconnections: $u_2 = y_1$ and $u_1 = y_2$. This means that the (u_1, y_1)-plane of Σ_1 maps to the (y_2, u_2)-plane of Σ_2 and in particular that counter-clockwise rotations in the (u_2, y_2) plane, are seen as clockwise when plotted in the (u_1, y_1) axis. This is why persistent oscillations cannot arise in the closed-loop system. In fact, they need to happen in a counter-clockwise direction in the (u_1, y_1)-plane (by the counter-clockwise I-O dynamics assumption on Σ_1) and at the same time in a clockwise direction, because of the same assumption on Σ_2. Precise definitions follow:

Definition 2.1: We say that a system has Counter-Clockwise (CCW) Input-Output dynamics if, for any initial condition ξ and any differentiable and uniformly bounded input-output pair $u - y$, (with $y(\cdot) := h(x(\cdot, \xi, u), u(\cdot))$) the following inequality holds:

$$\liminf_{T \to +\infty} \int_0^T \dot{y}(t)' u(t)\, dt > -\infty. \tag{1}$$

□

Restricting the attention at periodic input-output pairs condition (1) really amounts to asking that the area encircled by the curve $\gamma(t) := (u(t), y(t))$ in the (u, y)-plane be positive. For technical reasons it is of interest to consider a slightly stronger notion of counter-clockwise I-O dynamics (similarly to what is usually done in the passivity literature).

Definition 2.2: We say that a system has *Strictly* CCW Input-Output dynamics if, for any initial condition ξ and any differentiable and uniformly bounded input-output pair $u-y$, with $y(\cdot) = h(x(\cdot, \xi, u), u(\cdot))$ the following inequality holds:

$$\liminf_{T\to+\infty} \int_0^T \dot{y}(t)' u(t) - \delta(|\dot{y}(t)|)/[1 + \gamma(|x(t)|)dt > -\infty. \tag{2}$$

for some positive definite function δ and some class $\mathcal{K}$ function γ. □

It is worth pointing out that the notion of counter-clockwise I-O dynamics is, unlike standard passivity, invariant with respect to translations in u and y. This is already a very interesting fact, as there is no pre-fixed zero input (or output) value. The convergence theorem will only imply $\dot{u}, \dot{y} \to 0$, but, a priori, there is not a specific value to which inputs and outputs need to converge. This is important as in uncertain systems the equilibria may vary according to the uncertainty; moreover, in biological applications, the position of the equilibrium typically depends upon the strength and shape of the steady-state response curves of the system in feedback. In traditional nonlinear control this problem is usually disregarded as both the system and the controller have or are designed so that the zero-output steady-state response corresponds to a zero-input signal.

Several other versions of the property are possible; for instance, in a nonlinear set-up, there is no special reason for considering inputs and outputs measurements with respect to a linear scale, (in particular measures could be in logarithmic scale); more in general one could think of measuring the area in the (u, y)-plane with respect to a certain density function $\rho(u, y) > 0$. In this respect taking a separable density function $\rho(u, y) = \rho_1(u) \cdot \rho_2(y)$ is in fact equivalent to working in an auxiliary plane $\tilde{u} = \int_0^u \rho_1(\mu)\, d\mu$ and $\tilde{y} = \int_0^y \rho_2(\eta)\, d\eta$. The precise definitions are reported below:

Definition 2.3: We say that a system has Counter Clock-Wise Input-Output dynamics with respect to all (some) density function $\rho(u, y) > 0$ if, for any initial condition ξ and any differentiable and uniformly bounded input-output pair $u - y$, (with $y(\cdot) := h(x(\cdot, \xi, u), u(\cdot))$) the following inequality holds:

$$\liminf_{T\to+\infty} \int_0^T \dot{y}(t)' \int_0^{u(t)} \rho(\mu, y(t))\, dt > -\infty. \tag{3}$$

□

Strict versions of the property are also possible, while the convergence Theorem keeps its validity in the present set-up provided that the two systems are CCW with respect to specular density functions ($\rho_1(u, y) = \rho_2(y, u)$): i. e. if the first one is CCW for all ρ and the second one is CCW with respect to some ρ.

III. How to check the property

Checking counter-clockwise I/O dynamics, without actually knowing the explicit expressions of the input u and of the corresponding solution x is not always an easy task. Similarly to passivity we need to consider a Lyapunov-like characterization of the property. A system is [strictly] CCW provided that there exists a $\mathcal{C}^1$ function $V : X \to \mathbb{R}$ so differentiating along trajectories of the system $\dot{x} = f(x, u)$, $y = h(x)$ it holds for all $x \in X$ and all u:

$$\begin{array}{c} D_x V(x)\, f(x, u) \leq D_x h(x)\, f(x, u)\, u \\ {[-\delta(|D_x h(x)\, f(x, u)|)/\{1 + \gamma(|x|)\}]} \end{array} \tag{4}$$

Finding Lyapunov functions as in (4) is unfortunately a very difficult task and there is no general algorithm for performing such a choice of a function, especially if we are dealing with large uncertainties on parameter values as is often the case in biological systems. It is therefore important to have a catalog of systems (for instance characterized by the sign of the entries of the corresponding jacobians) for which the property holds regardless of the uncertainties involved. In this Section we will provide a library of "low-dimensional" building blocks which enjoy the CCW property together with a couple of rules which allow to compose the simple blocks and build higher dimensional examples with the same property.

A. Scalar static nonlinearities

A nonlinear static function $y = h(u)$ can of course be interpreted as a dynamical systems which associates to any input signal $u(\cdot)$ the corresponding output $y(\cdot) = h(u(\cdot))$. Any SISO piecewise differentiable function $h(\cdot)$ enjoys the CCW I-O dynamics property with respect to arbitrary density functions ρ. The case of multiple inputs and multiple outputs is more complex.

B. One-dimensional systems

Systems of the following type:

$$\dot{x} = f(x, u) \qquad y = h(x)$$

with $x \in X \subset \mathbb{R}$, $u \in U \subset \mathbb{R}$ and $D_u f(x, u) > 0$ for all $u \in U$ and all $x \in X$ and $D_x h > 0$ have strictly counter-clockwise dynamics with respect to arbitrary density functions ρ.

C. Two-dimensional systems

The following nonlinear planar system:

$$\begin{array}{rcl} \dot{x} &=& f_1(x, y, u) \\ \dot{y} &=& f_2(x, y) \end{array} \tag{5}$$

with output y and input u has counter-clockwise dynamics with respect to some density function $\rho(u, y) > 0$. We

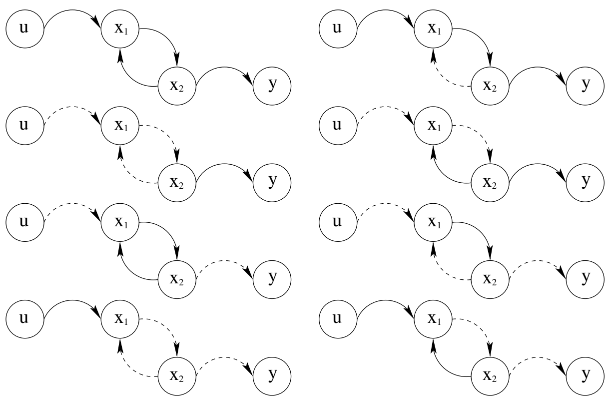

Fig. 2. Relative degree 2 systems with counter-clockwise dynamics: Positive edges (solid line), Negative edges (dashed line)

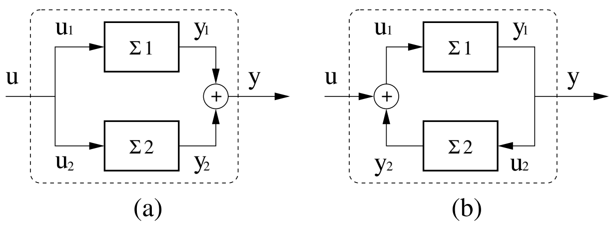

Fig. 3. Interconnection rules: (a) parallel; (b) feedback.

assume that f_1 and f_2 are $\mathcal{C}^1$ functions and that the following sign pattern holds for the jacobian:

$$\frac{\partial f_1}{\partial u} > 0 \quad \frac{\partial f_2}{\partial x} < 0 \tag{6}$$

Moreover if the system is linear in u, then ρ can be taken equal to 1. Standard changes of coordinates lead to the catalog of relative degree 2 planar systems which have counter-clockwise Input-Output dynamics among those with sign definite jacobians (see Figure 2).

D. Interconnection rules

Very little is known as for interconnections of systems with counter-clockwise Input-Output dynamics with respect to some or all density functions ρ. If $\rho = 1$, however the following composition rules apply:

1) The parallel interconnection of systems with CCW dynamics is itself a system with CCW dynamics. Parallel interconnections are characterized by the following equations:

$$u = u_1 = u_2 \qquad y = y_1 + y_2$$

2) The positive feedback interconnection of CCW systems is itself a system with CCW dynamics. Positive feedback interconnections are characterized by the following equations:

$$u_1 = u + y_2 \qquad y = y_1 = u_2$$

These interconnection rules are illustrated in Figure 3.

IV. Detecting multistability in feedback interconnections

A. Convergence theorem

Consider the following positive feedback interconnection:

$$\begin{array}{rcl} \dot{x}_1 & = & f_1(x_1, u_1) \\ y_1 & = & h_1(x_1) \\ & & \\ \dot{x}_2 & = & f_2(x_2, u_2) \\ y_2 & = & h_2(x_2) \\ & & \\ u_1 = y_2 & & y_2 = u_1 \end{array} \tag{7}$$

Assume that:

1) Each of the individual subsystems has strictly Counter-Clockwise I-O dynamics
2) Each of the subsystem admits a well-defined I-O characteristic

Then, if trajectories are bounded, they converge to equilibria. Moreover if characteristics are hyperbolic and the intersections of $k_1 \circ k_2$ transversal to the diagonal, almost all trajectories converge to the asymptotically stable equilibria corresponding to the intersections for which $[k_1 \circ k_2]' < 1$.

B. Practical significance

An I-O characteristic $k(\cdot)$ is the map, $k : \mathcal{U} \to \mathcal{Y}$ that associates to each constant input, the corresponding steady-state value of the output. We say that a system admits an I-O characteristic if for all us there exists a unique globally asymptotically stable equilibrium. The characteristic is hyperbolic if for each equilibrium point the Jacobian matrix computed at the equilibrium has all eigenvalues outside the imaginary axis (hence, by the stability assumption, in the open left half plane). Plotting in the same plane the characteristic k_1 and k_2^{-1}, the stable equilibria can be identified, by virtue of the previous theorem, by looking at the intersection points in which the slope of k_1 is strictly less than the slope of k_2^{-1}. If some parameter is varied, "saddle-node" bifurcation occur at the tangency point of the two characteristics. Some of the assumptions of the Convergence theorem can be relaxed; for instance, one of the two subsystems could enjoy the property in a non-strict sense (for instance being a static nonlinearity). We illustrate its application by means of the example in the following Section.

V. A new mechanism for bistability in MAPK cascades

A. Kholodenko's model: the qualitative picture

Recently a new model for MAPK cascades was presented by Kholodenko and coworkers in [11]. In its simplest version the model takes into account 3 forms of different MAPK, these are the dephosphorylated MAPK denoted by M_1, the MAPK phosphorylated at one site denoted M_2 and the MAPK phosphorylated on both residues M_2. Since no distinction is made between phosphorylation on threonine or on tyrosine, one can regard this model as a first approximation in which the phosphorylation follows a strictly ordered mechanism. Interestingly this model allows for multistability without a positive feedback loop from stage $n > 1$ to stage 1 of the cascade. We discuss here a method for analyzing Kholodenko and possibly higher dimensional such examples. The equations introduced by Kholodenko read as follow:

$$\begin{aligned} \dot{M_1} &= \theta_1(M_1, M_2, M_3) - \theta_2(M_1, M_2) \\ \dot{M_2} &= -\theta_1(M_1, M_2, M_3) + \theta_2(M_1, M_2) \\ &- \theta_3(M_1, M_2) + \theta_4(M_1, M_2, M_3) \\ \dot{M_3} &= \theta_3(M_1, M_2) - \theta_4(M_1, M_2, M_3) \end{aligned} \tag{8}$$

where θ_is are $\mathcal{C}^1$ functions with the following monotonicity properties:

$$\begin{array}{ccc} \frac{\partial \theta_1}{\partial M_1} < 0 & \frac{\partial \theta_1}{\partial M_2} > 0 & \frac{\partial \theta_1}{\partial M_3} < 0 \\ \frac{\partial \theta_2}{\partial M_1} > 0 & \frac{\partial \theta_2}{\partial M_2} < 0 & \frac{\partial \theta_2}{\partial M_3} = 0 \\ \frac{\partial \theta_3}{\partial M_1} < 0 & \frac{\partial \theta_3}{\partial M_2} > 0 & \frac{\partial \theta_3}{\partial M_3} = 0 \\ \frac{\partial \theta_4}{\partial M_1} < 0 & \frac{\partial \theta_4}{\partial M_2} < 0 & \frac{\partial \theta_4}{\partial M_3} > 0 \end{array} \tag{9}$$

Actually in [11], explicit expressions of the functions θ_is are provided, as well as parameters value which fit experimental data. To our purposes, at least at this stage, it is enough to concentrate on the qualitative shape of the functions only. Notice, for instance, that $\frac{\partial \theta_4}{\partial M_1}$ and $\frac{\partial \theta_3}{\partial M_1}$ have the same sign; therefore the system jacobian is not sign-definite, or at least is not such for all possible choices of θ_is (nor, in the case of parameterized θ_is, for all possible choices of kinetic constants). In particular, then, we cannot apply the analysis machinery developed in [2].

Hereby we are interested in applying the machinery of counter-clockwise dynamical systems (recently developed in [1]) in order to understand, by means of a simple graphical test involving quantitative static information on the system, precise conditions which give rise to multi-stable behaviour. Going back to the equations in (8), we notice that $M_{tot} = M_1(t)+M_2(t)+M_3(t)$ is constant along trajectories, and therefore we may replace M_2 everywhere in the equations by $M_{tot} - M_1 - M_3$, thus bringing down dimension by 1:

$$\begin{aligned} \dot{M_1} &= \theta_1(M_1, M_{tot} - M_1 - M_3, M_3) \\ &\quad -\theta_2(M_1, M_{tot} - M_1 - M_3) \\ \dot{M_3} &= \theta_3(M_1, M_{tot} - M_1 - M_3) \\ &\quad -\theta_4(M_1, M_{tot} - M_1 - M_3, M_3). \end{aligned}$$

We may regard this system as the closed-loop unitary feedback interconnection ($u = y$) of the following system:

$$\begin{aligned} \dot{M_1} &= \theta_1(M_1, M_{tot} - M_1 - M_3, M_3) \\ &\quad -\theta_2(M_1, M_{tot} - M_1 - M_3) \\ \dot{M_3} &= \theta_3(u, M_{tot} - u - M_3) \\ &\quad -\theta_4(M_1, M_{tot} - u - M_3, M_3) \\ y &= M_1. \end{aligned} \tag{10}$$

Notice that system (10) falls into the class of systems considered in Section 3; in particular it has strictly counter-clockwise input/output dynamics with respect to some density function ρ. Since scalar static maps also exhibit counter-clockwise I/O dynamics with respect to any density function, though not strictly (basically they encircle a zero area , regardless of the density function ρ), static feedback interconnections of (10) can be analyzed by exploiting the main convergence theorem in [1].

B. Kholodenko's model: the actual parameters

More in detail, the following numerical set-up is considered in [11]:

$$\begin{aligned} \theta_1(M_1, M_2, M_3) &= \frac{k_4^{cat} MKP3_{tot} M_2}{K_{m4}(1+\frac{M_3}{K_{m3}}+\frac{M_2}{K_{m4}}+\frac{M_1}{K_{m5}})}; \\ \theta_2(M_1, M_2) &= \frac{k_1^{cat} MAPKK_{tot} M_1}{K_{m1}(1+\frac{M_1}{K_{m1}}+\frac{M_2}{K_{m2}})}; \\ \theta_3(M_1, M_2) &= \frac{k_2^{cat} MAPKK_{tot} M_2}{K_{m2}(1+\frac{M_1}{K_{m1}}+\frac{M_2}{K_{m2}})}; \\ \theta_4(M_1, M_2, M_3) &= \frac{k_3^{cat} MKP3_{tot} M_3}{K_{m3}(1+\frac{M_3}{K_{m3}}+\frac{M_2}{K_{m4}}+\frac{M_1}{K_{m5}})}. \end{aligned} \tag{11}$$

where the kinetic constants are given in the table below:

Par.	Value	Par.	Value	Par.	Value
k_1	0.02	k_{-1}	1	k_2	0.01
k_3	0.032	k_{-3}	1	k_4	15
h_1	0.045	h_{-1}	1	h_2	0.092
h_3	1	h_{-3}	0.01	h_4	0.01
h_{-4}	1	h_5	0.5	h_6	0.086
h_{-6}	0.0011				

and the following expressions hold:

$$\begin{aligned} k_1^{cat} &= k_2 \\ k_2^{cat} &= k_4 \\ k_3^{cat} &= h_2/(1 + h_2/h_3) \\ k_4^{cat} &= h_5/(1 + h_5/h_6 + h_{-3}(h_{-4} + h_5)/(h_3\, h_4)) \\ K_{m1} &= (k_{-1} + k_2)/k_1 \\ K_{m2} &= (k{-3} + k_4)/k_3 \\ K_{m3} &= (h_{-1} + h_2)/(h_1 + h_1 h_2/h_3); \\ K_{m4} &= \frac{h_{-4}+h_5}{h_4(1+h_5/h_6+h_{-3}(h_{-4}+h_5)/(h_3\, h_4))} \\ K_{m5} &= h_6/h_{-6}. \end{aligned} \tag{12}$$

Moreover, $MAPKK_{tot} = 50$ and $MKP3_{tot} = 100$ and $M_{tot} = 500$. For the considered parameters values the system admits a well-defined I/O characteristics. Solving the equations, it turns out that the σ-shaped curve in Fig. 4 represents the input-output static characteristic $u \rightarrow M(u)$. This can be obtained by solving symbolically the system of equations:

$$\begin{aligned} &\theta_1(M_1, M_{tot} - M_1 - M_3, M_3) = \\ &= \theta_2(M_1, M_{tot} - M_1 - M_3) \\ &\theta_3(M_1, M_{tot} - M_1 - M_3) = \\ &= \theta_4(M_1, M_{tot} - M_1 - M_3, M_3). \end{aligned} \tag{13}$$

The next step for the application of the method is to make sure that the equilibria on the characteristic are globally

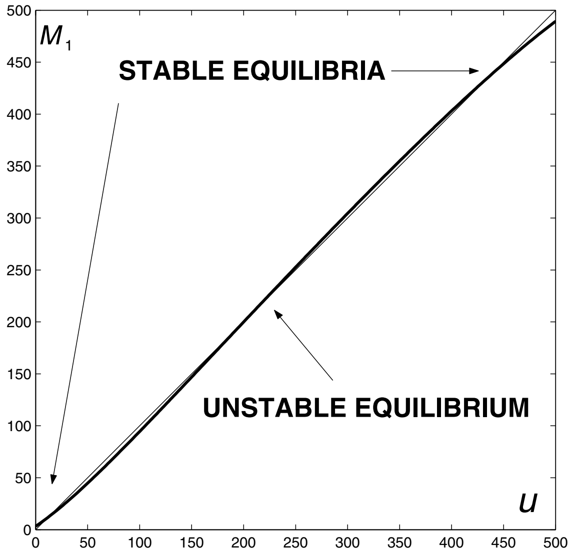

Fig. 4. Static Input-Output characteristic

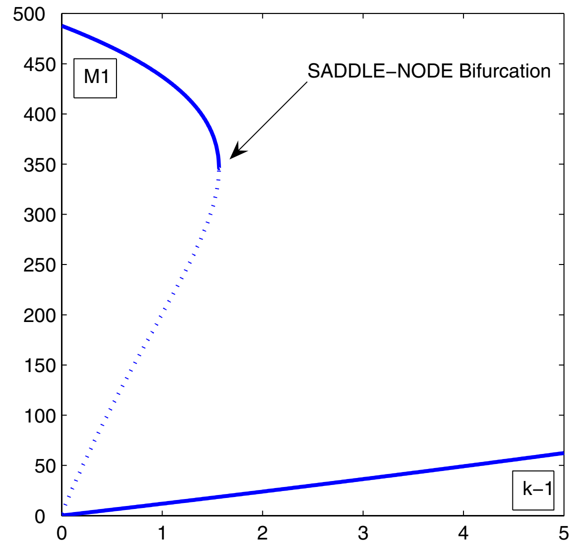

Fig. 5. Equilibria of the closed-loop system (M_1 coordinate) as k_{-1} ranges in $[0, 5]$

asymptotically stable. Local asymptotic stability is straightforward by results on sign-stability, in fact the jacobian has the following sign-pattern:

$$D_x f(x, u) = \begin{bmatrix} - & + \\ - & - \end{bmatrix}$$

which is a stable configuration no matter what the entries of the matrix are, [8]. Moreover, since in original coordinates $M_1(t) + M_2(t) + M_3(t) = M_{tot}$, by forward invariance of the positive orthant, solutions are uniformly bounded. In order to conclude GAS at the unique equilibrium we only need to rule out the possibility of periodic solutions. Since the system is planar, this trivially follows from the fact that $\text{div} f(s, u) = \text{tr}(D_x f(x, u)) < 0$. Thus the closed-loop system has three equilibria, each corresponding to one intersection of the diagonal with the I/O static characteristic, two of which are stable (those for which the slope $k'(u) < 1$ and the middle one is exponentially unstable. Almost all solutions, except possibly for a zero measure set of initial conditions, converge to the asymptotically stable equilibria. Performing the above analysis for different values of the parameters allow to obtain the bifurcation diagram reported in Fig. 5, where we let the parameter k_{-1} vary.

VI. CONCLUSIONS

This paper illustrates how the recently developed notion of system with counter-clockwise I-O dynamics can be used in order to investigate convergence and multistability in dynamical systems. Few qualitative informations (relative for instance to the sign pattern of the jacobian), combined with static I-O measurements performed on a pair of open-loop systems with counter-clockwise I-O dynamics allow to conclude convergent (and possibly multistable) behaviour of their closed-loop feedback interconnection. Applications to MAPK models are illustrated as an example.

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