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# Molecular Systems Biology and Control: A Qualitative-Quantitative Approach

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*Abstract*— **This paper is a complement to the author's presentation at the session *Molecular Systems Biology & Control*. it focuses specifically on an approach to biological network analysis which combines qualitative with quantitative data in order to characterize global dynamics.**

## I. Introduction

Within the last few years, the field of "molecular systems biology" has taken shape, having as its goal the unraveling of the basic dynamic processes, feedback control loops, and signal processing mechanisms underlying life. Leading biologists have recognized that new systems-level knowledge is urgently required in order to conceptualize and organize the revolutionary developments taking place in the biological sciences, and new educational programs are being established at major universities.

The mini-tutorial "Molecular Systems Biology and Control" provides an introduction to the field of systems biology. The paper accompanying the author's presentation is published as [26], and it provides a brief introduction to some of the main molecular biology concepts and terminology, as well as illustrations of systems-theoretic opportunities and challenges afforded by the field. It also briefly describes an approach, due to the author and collaborators, that combines qualitative (graph-theoretic) knowledge with a relatively small amount of quantitative (steady-state step response) data for components in order to provide an understanding of global dynamics. The goal of this short conference paper is to provide some details and additional discussion regarding this last approach. However, we first very briefly review some basic notions. **The full paper [26] should be consulted for a detailed discussion of this tutorial material.**

**Cells.** The fundamental unit of life is the cell. One may view cell life as a collection of "wireless networks" of interactions among proteins, RNA, DNA, and small molecules involved in signaling and energy transfer, that process environmental signals, induce appropriate cellular responses, and sequence internal events such as gene expression, thus allowing cells and entire organisms to perform their basic functions. These control and communication networks may be incredibly sophisticated, involving multiple *signal transduction pathways* in which information is relayed among enzymes through chemical reactions (for instance, phosphorylation).

As an illustration, the diagram in Fig.1 shows the top-level schematics of a wiring diagram of signaling circuitry in the mammalian cell. It shows the main signaling pathways

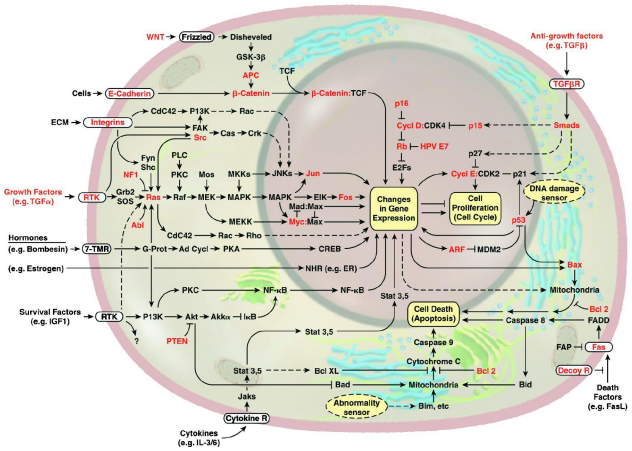


Fig. 1. Signaling circuitry in mammalian cells, with permission from [20]

for growth, differentiation, and apoptosis (commands which instruct the cell to die). Highlighted in red are some of the genes known to be functionally altered in cancer cells.

Research in molecular biology, genomics, and proteomics has produced, and will continue to produce, a wealth of data describing the elementary components of intracellular networks as well as detailed mappings of their pathways and environmental conditions required for activation.

The *genome* is the genetic information of an individual, encoded in DNA molecules, which are arranged into chromosomes. It provides a "parts list" which describes all the proteins that are potentially present in every cell of a given organism. The read-out of genetic information —bringing-in the instructions into working memory for execution, in a computer analogy— begins when DNA information is *transcribed* letter by letter into mRNA. *Translation* is the next step. The information in the mRNA is read, and *proteins* are assembled out of amino acids.

Proteins are the primary components of living things, and the main players shown in Fig.1. They form receptors that endow the cell with sensing capabilities, actuators that make muscles move (myosin, actin), detectors for the immune response, enzymes that catalyze chemical reactions, switches that turn genes on or off, provide structural support, help in the transport of smaller molecules, and help direct the breakdown and reassembly of other cellular elements such as lipids and sugars. Ultimately, one might say that cell life is about proteins and how and when they are produced.

**Cells as Dynamical Systems.** The term *genotype* refers to the genetic blueprint encoded in the DNA of a given individual, while *phenotype* refers to the actual observable physical manifestations of that information. Different species may be close in genotype. Even in a given species, a

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mutation in a single letter in an individual's DNA may have a catastrophic phenotypical consequence, as with cystic fibrosis in humans. There are surely many factors contributing to the "discontinuity" in going from genotype to phenotype, but one explanation that developmental biologists and others have proposed is that cells behave as *nonlinear dynamical systems*, and so bifurcation phenomena play a major role. Proteins interact, often through feedback loops, directly through enzymatic action or binding and indirectly via control of gene expression. Feedback is a dynamic phenomenon, where quantities (concentrations of proteins, RNA, metabolites, etc.) are seen as functions of time.

Bifurcations, i.e. transitions between behaviors such as mono- and multiple-stability, or the onset of oscillations, are phenomena which frequently arise when parameters are modified. In molecular biology modeling, a parameter may represent a concentration of an external ligand, a voltage applied to a voltage-gated channel, the concentration of a signaling molecule (as an input to a cellular subsystem), an enzyme concentration affecting a reaction, or the degree of effective cooperativity (Hill coefficient) of a reaction.

An important theme in current molecular biology thought is the understanding cell behavior in terms of interconnections of elementary "modules." Cells are composed of subsystems involved in various processes such as cell growth and maintenance, division, and death. The hope is that one should be able to decompose into simpler subsystems and then study the emergent properties of interconnections. The control and systems-theory paradigm of input/output systems, built out of simpler components interconnected according to certain rules, is natural in this context, as it may permit the recursive verification of important properties through the use of standard analysis tools such as passivity, small-gain, or input to state stability. Even if the entire system were autonomous, in order to be able to define such interconnections, one would be forced to consider subsystems that process time-dependent input signals into output signals. But, in fact, cells are not autonomous systems. They process external information, provided by physical (UV or other radiation, mechanical, temperature) or chemical (drugs, growth factors, hormones, nutrients) inputs. They also produce signals which we may view as outputs, such as chemical signals sent to other cells, commands to motors that move flagella or pseudopods, or the internal activation of transcription factors which may be monitored by measurement technologies. Thus, the control-theory formalism is natural. Once viewed in control-theory terms, one can pose synthesis questions, dealing with the *control* of cellular systems through drugs or genetic modifications.

Control and systems researchers are, indeed, addressing many of these questions for biomolecular systems. Nevertheless, the author has argued, in [25], [26], that in spite of its immense success in engineering, "off the shelf" application of known control theory is not always appropriate. This is because detailed models are hard to come by: it is virtually impossible to experimentally validate the forms of nonlinearities in reaction terms or to accurately estimate coefficients (parameters). In addition, issues such as robustness, multi-scale modeling, continuous/discrete interfaces, and seamless integration of hybrid stochastic/deterministic systems, although treated to various degrees in the control field, cannot often be handled with the tools available, which were developed for engineering applications. Even though many problems in systems biology resemble standard problems in control theory, on closer inspection they often turn out to differ in fundamental ways, and these differences are challenging and worth exploring. See more discussion, and examples, in [25], [26]. The remaining part of this article will focus on one particular topic.

**The "Data-Rich/Data-Poor" Paradox.** Although an impressive amount of *qualitative* network (schematic modeling) knowledge of the type shown in Fig.1 is available, little of this knowledge is *quantitative* at the level of precision demanded by most control and system theoretic analysis tools. The problem of exploiting this qualitative knowledge, and effectively integrating relatively sparse quantitative data, is among the most challenging issues confronting systems biology. New tools must to be developed in order to bridge this "data-rich/data-poor" dichotomy.

In systems biology, one often sets up a model based on biological knowledge, estimates parameter ranges, and explores the spaces of parameters and initial conditions through simulations, bifurcation analysis, and model reduction. There are several shortcomings to this approach, however. The *form* of nonlinearities often cannot be well-justified, and parameters such as reaction rates are based on rough guesses or on data from different and perhaps inconsistent sources, and usually are obtained from *in vitro* experiments as opposed to *in vivo* measurements. In addition, parameter and state spaces are of high dimension, which makes convergence of numerical techniques questionable and at best local. Some of these problems are intrinsic, and cannot be solved by better technology or algorithms; for example, parameters such as enzyme concentrations vary from cell to cell, even within cells of the same type. In addition, a purely numerical approach does not provide fundamental understanding. This argues for the desirability of approaches which, while taking advantage of the huge, and growing, amount of qualitative network "schematic" knowledge such as shown in Fig.1, take into account the uncertainties inherent in biological measurements and effectively integrate relatively sparse quantitative data. We describe next one such approach, based upon the systems theory paradigm of I/O systems and combining information on network structure with steady-state step response data on subsystems.

## II. Consistency and Monotonicity

Our main themes may be summarized as follows:

- Network structure (qualitative knowledge) constrains behavior (e.g. periodic behavior may not be possible).
- The forms of reactions and parameters matter (bifurcation phenomena), but such information is often unavailable.
- The interplay of structure and reaction forms can sometimes be fruitfully studied by breaking up systems into well-

behaved building-blocks and using only a *restricted amount* of input/output quantitative data (such as step responses) for these subsystems in order to characterize global behavior.

A particularly appealing class of candidates for "well behaved" subsystems are *monotone systems*, introduced by Moe Hirsch in the 1980s. They are a class of dynamical systems for which many behaviors (including "chaos") are ruled out. Even though they may have arbitrarily large dimensionality, monotone systems behave in many ways like one-dimensional systems. Bounded trajectories generically converge to steady states, and there are no stable oscillatory behaviors. Actually, see [1], one must extend the notion of monotone system so as to incorporate input and output channels.

An interconnection of monotone subsystems may or may not be monotone: positive feedback (in a sense that can be made precise) preserves monotonicity, while "negative feedback" destroys it. Positive feedback is central to regulation, metabolism, and development, but 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. [3]). A rich theory is beginning to arise, characterizing the behavior of monotone and non-monotone interconnections.

One way to introduce monotonicity is through a *sign-consistency* property for the graph which describes how each state variable influences each other variable in a given system. We consider graphs whose edges are labeled by "+" or "−" signs. Sometimes we use as in Fig.1, respectively activating "→" or inhibiting "⊣" arrows; see Fig.2. Such

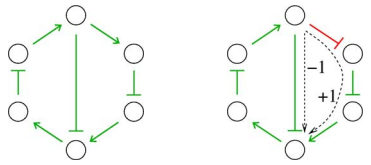


Fig. 2. A consistent and an inconsistent graph

a graph is said to be *sign-consistent* (or "coherent") if all paths between any two nodes have the same net sign, or equivalently, all closed loops have positive parity, i.e. an even number, possibly zero, of negative edges. (For technical reasons, one ignores the direction of arrows, looking only at undirected graphs. Also, self-edges are ignored.) Thus, the first graph in Fig.2 is consistent, but the second one, which differs in just one edge from the first one, is not (two paths with different parity are shown).

Now consider a system of ordinary differential equations $\dot{x}{=}f(x)$, with no inputs nor outputs for the time being. We assume that the system is sign-definite: for each two components $x_i$ and $x_j$, either $x_i$ always inhibits $x_j$ or $x_i$ always activates $x_j$, as in Fig.1. In molecular biological applications, this is usually —though not always– a very reasonable restriction (ambiguous effects can often be explained by an additional variable operating at a different time scale). Mathematically, sign-definiteness means that $\frac{\partial f_i}{\partial x_j}(x)$ does not change sign as a function of $x$, for each pair of distinct indices $i$ and $j$, where $f_i$ denotes the $i$th component of $f$ (ignoring diagonal terms $\frac{\partial f_i}{\partial x_i}(x)$). To any sign-definite system in $\mathbb{R}^n$ one associates an incidence graph $G$ on the nodes $\{1,\ldots,n\}$, drawing an edge from node $j$ to node $i$ if $\frac{\partial f_i}{\partial x_j}(x){\not\equiv}0$, and assigning a $+$ sign to this edge if $\frac{\partial f_i}{\partial x_j}(x){>}0$ for some $x$, and $-$ sign otherwise.

Systems whose incidence graphs are consistent are examples of monotone systems. A *monotone* system is one for which there is some partial order in the state space so that the evolution operator preserves the order. Denoting the order by "$\leq$" this means that $x(0){\leq}y(0)$ implies $\varphi(t,x(0)){\leq}\varphi(t,y(0))$ for all $t{\geq}0$, where we are denoting by $\varphi(t,\xi)$ the solution at time $t$ of the initial value problem $\dot{x} = f(x)$, $x(0) = \xi$, and we assume for simplicity that solutions are unique and defined for all $t \geq 0$. An example of a partial order in $\mathbb{R}^2$ is the "Northeast" order, in which we declare that $(x,y) \leq (x',y')$ provided that both $x \leq x'$ and $y \leq y'$, and more generally for every $n$, $x \leq y$ provided that $x_i \leq y_i$ for each $i = 1,\ldots,n$. More generally, one can define partial orders associated to any possible orthant in $\mathbb{R}^n$, for example in $\mathbb{R}^2$ the "Northwest" order: $(x,y) \leq (x',y')$ provided that both $x \geq x'$ and $y \leq y'$, i.e., $(x',y') - (x,y)$ belongs to the second quadrant $K = \{(a,b) \mid a < 0, b > 0\}$. A system with a consistent incidence graph is monotone with respect to some such order: in each connected component of the graph, just pick one node $N$, label it $+$, and assign to any other node $M$ in the same connected component the sign of a path from $N$ to $M$. In this way, an assignment of signs to nodes is obtained, and the system can be easily shown to be monotone with respect to the order associated to the corresponding orthant.

Under an additional hypothesis of *irreducibility* (basically, strong connectedness of the incidence graph), one obtains what are called *strongly* monotone systems: $x(0){\leq}y(0)$ but $x(0){\neq}y(0)$ implies that $x(t){=}\varphi(t,x(0)){<}\varphi(t,y(0)){=}y(t)$ for $t{>}0$ in a strict sense which we not define here for general orders, but which, for systems that are monotone with respect to orthants, amounts to: $x_i(t){<}y_i(t)$ for every coordinate $i = 1,\ldots,n$. Strongly monotone systems are very well-behaved in a dynamical sense. According to a beautiful result of Hirsch (cf. [22]), almost every bounded solution of such a system converges to the set of equilibria. By "almost any" one means every solution except for a measure-zero set of initial conditions, or, in a different version of the theorem, every solution except for those starting from a thin set in the Baire category sense. In particular, no chaotic or other "strange" dynamics can occur; in fact, not even limit cycles can arise in strongly monotone systems.

*Often in applications, a system that is not monotone as originally modeled turns out to be so under some simplifications.* An elementary illustration of this phenomenon is as follows. Suppose that an enzyme $B$ catalyzes conversion of $C$ to $A$, as in the left panel of Fig.3. (The reverse reaction is not required for the point to be made.) Thus, $B$ negatively affects the concentration of $C$, and positively affects that of $A$. Let lower case variables $a, b, c$ denote concentrations of the three species; under simplifying assumptions, a set

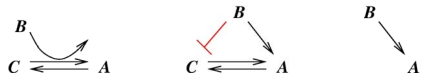

Fig. 3. Consistency after elimination

of ideal mass-action equations for this reaction is $da/dt = k_1bc - k_2a$, $db/dt = 0$, and $dc/dt = -k_1bc + k_2a$. The incidence graph for this set of differential equations will look as the middle panel of Fig.3, and thus be inconsistent. It would then appear that monotone theory cannot be applied to this example. However, since there is a conservation law $a(t) + c(t) \equiv c_0$ constant, one may eliminate $c$ (or $a$) from the system of differential equations, leading to $da/dt = k_1b(c_0 - a) - k_2a$, $db/dt = 0$, and this reduced system is now consistent, since there are no loops (remember self-loops are ignored), cf. the right panel in Fig.3. To analyze solutions of the differential equation, we may first restrict to an appropriate hyperplane, which depends on the initial conditions, and monotone theory can therefore be applied. This observation is key in applications to signaling cascades, where $A$ and $C$ might correspond to un-phosphorylated and phosphorylated forms of the same protein, for example. Very often, much less obvious eliminations and coordinate changes are reqired to as to apply monotone theory; the search for such transformations is an active area of research, see e.g. [11], [13], [1], [2], [4], [12], [6].

*Time-scale separation may also lead to monotonicity.* A non-monotone system might be a singular perturbation of a monotone system. (The fast system a "quasi-steady state" approximation.) A trivial linear example is $\dot{x} = -x - y$, $\varepsilon\dot{y} = -y + x$, with $\varepsilon > 0$. This is not monotone with respect to any orthant. But, for $\varepsilon \ll 1$, the fast variable $y$ tracks $x$, so the slow dynamics is well-approximated by $\dot{x} = -2x$ (monotone, since every scalar system is). More generally, one may consider $\dot{x} = f(x, y)$, $\varepsilon\dot{y} = g(x, y)$ such that the fast system $\dot{y} = g(x, y)$ has a unique globally asymptotically stable steady state $y = h(x)$ for each $x$ (and possibly a mild ISS-like requirement), and the slow system $\dot{x} = f(x, h(x))$ is (strongly) monotone. Then (see [27]) the original system inherits global convergence properties for $\varepsilon$ small enough. This can be established in two ways: using the theory of asymptotically autonomous systems (viewing $y - h(x)$ as an input to the slow system), or through geometric invariant manifold theory. In the second approach, one uses the existence of a manifold $M_\varepsilon$ invariant for the dynamics, which attracts all near-enough solutions, with an asymptotic phase property. The system restricted to the invariant manifold $M_\varepsilon$ is a regular perturbation of the fast ($\varepsilon = 0$) system, and hence inherits strong monotonicity properties. So, solutions in the manifold will be generally well-behaved, and asymptotic phase implies that solutions track solutions in $M_\varepsilon$, and hence also converge to equilibria if solutions on $M_\varepsilon$ do.

**I/O Monotone Systems.** A system $\dot{x} = f(x, u)$, $y = h(x)$ is *monotone* if there are nontrivial orders in the state, input, and output spaces, such that $\xi_1 \leq \xi_2$ and $u_1 \leq u_2$ imply $x(t, \xi_1, u_1) \leq x(t, \xi_2, u_2)$ for all $t \geq 0$, with respect to the state and input orders, and the output map $h$ preserves the order as well. Here, $x(t, \xi, u)$ is the solution at time $t$ for initial state $\xi$ at $t = 0$ and input $u(\cdot)$; and $u_1 \leq u_2$ for controls means that $u_1(t) \leq u_2(t)$ for all $t$. When there are no inputs nor outputs, this reduces to the earlier definition of monotone systems. (Discrete-time systems may be studied similarly, as can delay-differential systems, reaction-diffusion PDE's, and more abstract flows in metric spaces, cf. [15].) The generalization to I/O systems is from [1], [2], and it was motivated by the types of problems that we are discussing here. Orders are typically defined by positivity cones $K$, by defining $\xi_1 \leq \xi_2$ to mean $\xi_2 - \xi_1 \in K$, and similarly for input and for output values. For cones, monotonicity can be checked in infinitesimal terms, not requiring solution of differential equations. A very special but most important case is that of monotonicity with respect to cones that happen to be orthants in Euclidean space. Suppose that a system is sign-definite, meaning that we can draw unambiguous sign-graphs for the Jacobians of $f$ and $h$, analogously to what we did for systems with no inputs nor outputs. More precisely, $(\partial f_i/\partial x_j)(x, u)$ has a constant sign $\varepsilon_{ij} \in \{0, +, -\}$ for all $(x, u)$ and all $i \neq j$ (we may ignore self-loops), and, for all $i, j$ and $(x, u)$, $(\partial f_i/\partial u_j)(x, u)$ has a constant sign $\alpha_{ij} \in \{0, +, -\}$ and $(\partial h_i/\partial x_j)(x)$ has a constant sign $\beta_{ij} \in \{0, +, -\}$. A system is monotone with respect to *some* orthant if and only if its incidence graph does not contain any negative cycles (once again, ignoring direction of edges).

**Properties of Monotone Systems.** For the monotone system $\dot{x} = f(x, u)$, $y = h(x)$ consider step, i.e. constant, inputs $u(t) \equiv u$. One can prove, under weak boundedness assumptions, that for each $u$, there is at least one steady state: $f(x, u) = 0$, and that for each periodic input $u(t+T) = u(t)$, there is a corresponding periodic solution. Now assume that for each such constant input it holds that all solutions are bounded (this is frequently the case in biological systems, due to conservation laws), and that there is a *unique* steady state $x_u$ corresponding to this value of the input. Under weak additional hypotheses ([12]), one can the prove that $x_u$ must be a global attractor, i.e. all solutions of $\dot{x} = f(x, u)$ converge to $x_u$ as $t \to \infty$. We say in this case that the system has a *monostable steady-state step response* and define (composing with the output map) the *characteristic* or *steady state step response* of the system as the map $u \mapsto k(u) := h(x_u)$.

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. Moreover, there are asymptotic gain estimates: the omega-limit sets satisfy $k(\liminf u(t)) \leq \Omega^+[x(t, u)] \leq k(\limsup u(t))$ for any $u(\cdot)$, and in particular if $u(t) \to \bar{u}$, then the output satisfies $y(t) \to k(\bar{u})$.

The most important fact in the present discussion is that characteristics *can often be measured experimentally*. They are often called dose-response curves, or signal vs input concentration, receptor activity, or steady-state phosphorylation plots, and are usually interpolated from a large number of measurements, (see [25], [26] for more discussion of this fact). This is in contrast to actual system parameters (or functional forms), which are typically hard to estimate. *In*

*our approach, we blend qualitative information about the system, specifically monotonicity, with quantitative information, specifically characteristics*. We view this as one way to bridge the "data-rich data-poor" gap, but we are also confident that other approaches, most probably totally unrelated to monotonicity and characteristics, will be developed in the future to similarly combine qualitative and quantitative data.

**Some Theorems.** Some of the main results for monotone I/O systems characterize the location and stability of equilibria of closed-loop systems. The results represent nontrivial generalizations of elementary properties of one- and two-dimensional systems.

For simplicity, we assume from now on that inputs and outputs are scalar, i.e. $m{=}p{=}1$, and that the order in the input and output value spaces is the usual one in $\mathbb{R}$. The main positive-feedback theorem is from [2], and is as follows.

Suppose given two monotone systems with well-defined characteristics $k, g$ and interconnected in feedback as shown in Fig.4 (left), Interconnections of such systems form the

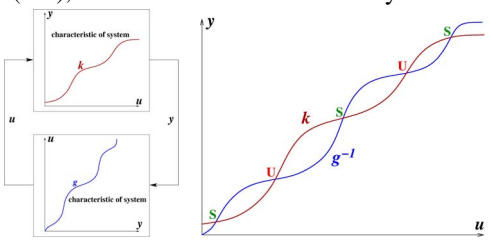


Fig. 4. Monotone systems with characteristics in feedback

basis of switches and other multi-stable systems that enable memory and binary decisions in cells. Plot $k$ together with the inverse of $g$, and label each intersection between the two graphs by an "S" or an "U" depending on whether the slope of the graph of $k$ is smaller ($k'(u){<}(g^{-1})'(u)$), or larger respectively, than that of $g^{-1}$, Fig.4 (right). The conclusion is then that steady-states of the closed-loop system, which are in a one-to-one correspondence with the intersections, are so that almost every bounded trajectory (with the possible exception of a set of measure zero of initial conditions such as those in the stable manifolds of saddle points associated to values U) converges to a steady state associated to one of the stable states associated to a value S.

The above statements assume that the graphs intersect transversally, and a nondegeneracy assumption of strong monotonicity for the closed-loop. See [18] for weakenings of these assumptions, as well as allowing for *set characteristics*, where the characteristics might be multi-valued. Generalizations to $m, p > 1$ are given in [16].

Moreover ([6]), often a complete bifurcation diagram can be derived immediately, with no further computation, if the graphs are parameterized, and hysteresis behavior can be understood in this way, for systems for which the closed-loop exhibits multiple stable steady states. In turn, a theory of *relaxation oscillations* can be built in this fashion ([19]) if there is a slow feedback adaptation of one of the parameters.

We also remark (see [25] for more discussion) that *the result remains true even if arbitrary delays are allowed in the feedback loop* (infinite phase margin) *as well as if diffusion is added*. By the latter statement we mean that for the reaction-diffusion equation $\frac{\partial x}{\partial t} = D\Delta x + f(x,u)$ for $x = x(t,q)$ with $q$ belonging to a convex domain and with no-flux (Neumann) boundary conditions, diagonal diffusion matrix $D$, and assuming a discrete set of equilibria, almost all solutions converge to one of the uniform states predicted if the corresponding ODE is analyzed. In other words, *no Turing-like pattern formation due to diffusive instability can occur.* These results follow from the corresponding facts for monotone dynamical systems surveyed in [22].

A feedback involving two monotone systems is still monotone, so this result is one about monotone systems. The main point is that *conclusions about the closed loop system, which may have arbitrarily large dimension, are derived from looking at a simple one-dimensional picture.*

Non-monotone systems arise when "negative feedback" is involved. Let us discuss next a negative-feedback result, for simplicity again restricting ourselves to the case $m{=}p{=}1$. The theorem, from [1], is as follows. Let us call a system *anti-monotone* if the internal dynamics are monotone but the output map $h$ inverts the order. Examples of such output maps, if outputs are scalar, would be $h(x) = -x$ (hence the name negative feedback) or, in a biochemical context where quantities cannot be negative, an "inhibition" function $h(x) = 1/(1+x)$. Negative feedback underlies homeostasis (regulation) as well as the construction of oscillators. Suppose given two systems, one monotone and one anti-monotone, with characteristics $k$ and $g$ respectively. We once more plot $k$ and $g^{-1}$, see Fig.5. The intersection between the

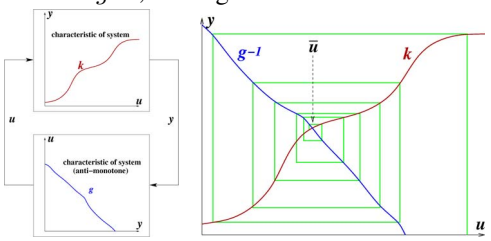


Fig. 5. Monotone/anti-monotone systems with characteristics in feedback

plots, if it exists, is necessarily unique. We consider the scalar discrete time ("spider-web") iteration $u_{i+1} = (g{\circ}k)(u_i)$. The result is that, if this iteration has a globally attractive fixed point $\bar{u}$ (cf. Fig.5), then the closed-loop system, provided that trajectories are bounded, has a globally attracting steady state (corresponding to the i/o signal value $\bar{u}$). *Once again, a one-dimensional picture completely characterizes global behavior, even for systems of arbitrary dimension.*

As with the positive-feedback case, this *result remains true if arbitrary delays are inserted in the feedback loop, and if diffusion is allowed.* Extensions to $m, p{>}1$, and in fact to a large class of systems evolving on Banach spaces, including delay-differential equations, can be found in [15]. The extension to reaction-diffusion pde's, saying roughly that the same result holds when the diffusion terms are ignored, is in [14]. Applications of the negative feedback monotone system result to species competition problems are given in [13], to circadian rhythm models in *Drosophila* in [3], and to a model of testosterone dynamics in [17]. See [26]

for an application to a model of Mitogen-Activated Protein Kinase cascades, a common "signaling module" involved in proliferation, differentiation, and development,

**Almost-Monotonicity.** Any sign-definite system may be decomposed into an interconnection of monotone subsystems, through the "pulling out" of "inconsistent" connections. The original system is thus viewed as a "negative feedback" loop around an otherwise consistent system. (In fact, even sign-definiteness can sometimes be dispensed with, if an indefinite term is be written as a difference of two increasing ones, and one of the two terms is "pulled out" as feedback.) When attempting to apply the theory reviewed above (with, generally, $m, p > 1$), it tends to be the case that *the fewer the number of interconnections among components, i.e. the number of variables being fed-back when viewing the decomposition as a negative feedback, the easier it is to obtain useful conclusions.* Let us call the smallest number of edges that must be removed in order to obtain a consistent graph the *consistency deficit (CD)* of the graph. For example, for the particular graph shown in Fig.6, one edge (the diagonal positive one)

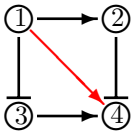


Fig. 6. Dropping the diagonal edge gives consistency

suffices. The paper [10] studies the computational complexity of the question of computing CD; it provides a relaxation-based polynomial-time approximation algorithm guaranteed to solve the problem to about 87.9% of the optimum solution, based on semidefinite programming relaxation and proves that it is not possible to have a polynomial-time algorithm with performance too close to the optimal. The algorithm is applied to a Drosophila segmentation network and to an Epidermal Growth Factor Receptor pathway model.

In addition, and independently from the theory mentioned above, one might speculate that nature tends to favor systems that are decomposable into small monotone interconnections, since "negative" feedback loops, although required for homeostasis and for periodic behavior, have potentially destabilizing effects, especially if there are signal propagation delays. Informally, let us say that a graph is *almost-consistent* —or an associated dynamical system *almost monotone*— if the CD is small compared to the original number of edges in the graph. The work [21] examines an *E. coli* transcriptional regulation map and estimates a much smaller CD than for a randomized version of the same network. These preliminary results provide a strong indication that almost-consistency is ubiquitous in biological networks. In the same large-network statistical analysis spirit, one may ask if smaller CD is correlated with more ordered (less "chaotic") behavior. It is hard to perform this type of analysis on differential equations, but for Boolean networks, the paper [23], shows, using a mean-field calculation of sensitivity, that networks of Boolean functions behave in a sense in a more and more "orderly" fashion the closer that their components are to being monotone.

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