Some new directions in control theory inspired by systems biology

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Abstract: This paper, addressed primarily to engineers and mathematicians with an interest in control theory, argues that entirely new theoretical problems arise naturally when addressing questions in the field of systems biology. Examples from the author's recent work are used to illustrate this point.

1 Introduction

We are in the midst of revolutionary developments in the biological sciences. Literally each day brings new discoveries, and proposals for novel organising principles, which hold the promise of altering the understanding of life itself. The potential impact of these developments, ranging from the extension of life spans to the cure of diseases, is impossible to overstate.

Concomitant with these advances, leading biologists have recognised that new systems-level knowledge is urgently required. Position papers emphasising this need regularly appear in influential publications such as *Nature* and *Science*. The loosely defined field of *systems biology* has arisen, having as its goal the unravelling and conceptualisation of the basic dynamic processes, feedback control loops, and signal processing mechanisms underlying life. As evidence of the seriousness of this effort, consider the fact that entire new academic departments have been recently created, such as the Department of Systems Biology at Harvard University Medical School, and new educational programs have been established, such as MIT's Computational and Systems Biology Ph.D. program. Even the Institute for Advanced Studies, home to Albert Einstein and John von Neumann, now has a Centre for Systems Biology.

The field offers control theorists and engineers an abundance of opportunities and challenges. These opportunities and challenges may be broadly classified into several categories, the first three of which I will not discuss further in this article:

(1) The role of control and signal processing techniques in the design of instrumentation for high-precision biological measurements and manipulation. This rich subject is hugely varied in scope, and well covered in bioengineering journals, such as for instance the *IEEE Transactions on Biomedical Engineering*, on *Medical Imaging*, on *Information Technology in Biomedicine*, and on *Systems, Man and Cybernetics*.

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(2) The use of existing techniques from identification, gain quantification, sensitivity analysis, optimal control, and other well-developed areas of control theory in the analysis and solution of problems of interest to biologists. These applications are also well-addressed in the literature: the reader is referred to control journals such as *IEE Proceedings on Control Theory & Applications*. *Automatica*, *IEEE Transactions on Automatic Control*, or *IEEE Transactions on Control Technology*, as well as journals focused on mathematical biology or systems biology, such as *Systems Biology*, *IEEE Transactions on Computational Biology and Bioinformatics*, *Mathematical Biosciences and Engineering*, *Mathematical Biosciences*, *Bulletin of Mathematical Biology*, *Journal of Mathematical Biology*, *Mathematical Medicine and Biology*, and *Biosystems*. In addition, the related fields of metabolic control theory [1, 2] and biochemical systems theory [3] provide complementary insights to control issues in biology.

(3) The abstraction, from biological research, of new ideas for control and sensor engineering. Evolution has resulted in systems that are highly fault-tolerant, non-linear, feedback-rich, and truly hybrid (in the sense that the digital information encoded in DNA controls chemical concentrations in cells). Recent advances in genomic research are continually adding detailed knowledge of such systems' architecture and operation, and one may reasonably argue that they will constitute a rich source of inspiration for innovative solutions to problems of control and communication engineering, as well as sensor and actuator design and integration.

(4) The formulation of entirely new theoretical control and systems theory problems, motivated by systems biology research. I will focus this article on this last point.

2 Cells as I/O systems

One may view cell life as a collection of 'wireless networks' of interactions among proteins, RNA, DNA, and smaller molecules involved in signalling and energy transfer. These networks 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.

Research in molecular biology, genomics, and proteomics has provided, and will continue to produce, a wealth of data describing the elementary components of such networks, as well the mapping of intra- and inter-cellular signalling networks. The genome encodes, through a particular ordering of the four possible (A,T,C,G) bases in

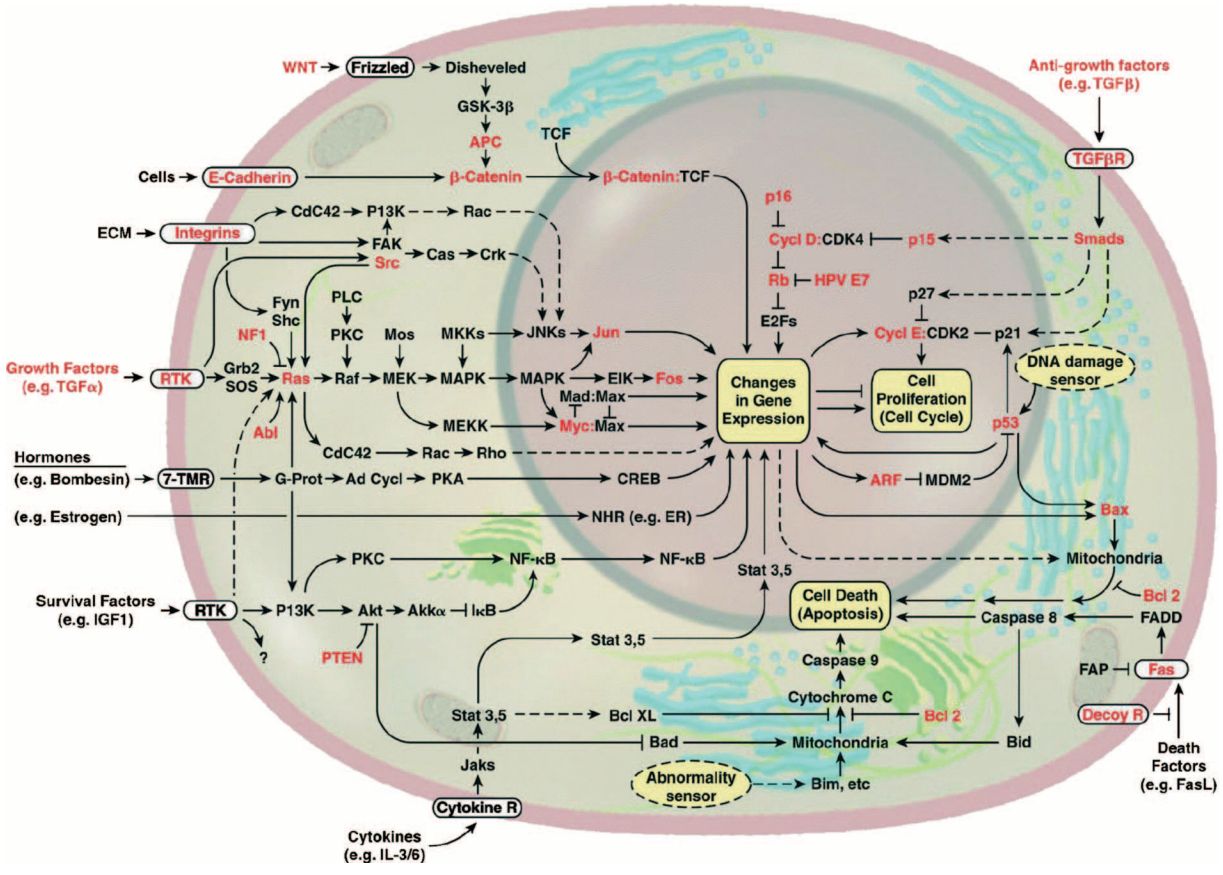

Fig. 1 *Signalling circuitry of the mammalian cell, from [6]. The illustration shows signalling pathways for growth, differentiation, and apoptosis commands, which instruct the cell to die. Highlighted in red are some of the genes known to be functionally altered in cancer cells. The analogy with electronic integrated circuits is striking. Reproduced from [6] with permission from Elsevier*

its DNA sequence, a *parts list* for the proteins that are potentially present in every cell of a given organism. Genomics research has as its objective the complete decoding of this information, both the parts common for a species as a whole as well as the cataloging of differences among individual members. The shape of proteins is what largely determines their function, and thus the elucidation of their three-dimensional *structure* is a goal of proteomics research. Proteins, which interact with each other through lego-like fitting of parts in lock and key fashion, are the primary components of living things. Among other roles, they form receptors that endow the cell with sensing capabilities, actuators that make muscles move (myosin, actin), detectors for the immune response, enzymes that catalyse chemical reactions, and switches that turn genes on or off. They also provide structural support, and help in the transport of smaller molecules, as well as in directing the breakdown and reassembly of other cellular elements such as lipids and sugars. (An intermediate link between genetic information and the proteins that DNA encodes for is RNA. Until recently, RNA was not believed to be a direct player in cell control mechanisms, but research into microRNA conducted within the past two years is forcing a complete rethinking of their role.) Massive amounts of data are being generated by genomics and proteomics projects, facilitated by sophisticated genetic engineering tools (gene knock-outs and insertions, PCR), and measurement technologies (green fluorescent protein, microarrays, FRET), and there is a widely recognised need to organise and interpret these data.

The control and systems-theory paradigm of input/output systems, built out of simpler components that are interconnected according to certain rules, is a most natural one in this context. Cells receive external information through inputs that may be physical (UV or other radiation, mechanical, or temperature) as well as chemical (drugs, growth factors, hormones, nutrients), and their measurable outputs include chemical signals to other cells, the movement of flagella or pseudopods, the activation of transcription factors, and so forth. Each cell can be thought of, in turn, as composed of a large number of subsystems, involved in processes such as cell growth and maintenance, division, and death. Indeed, an important theme in the current molecular biology literature [4, 5] is the attempt to understand cell behaviour in terms of cascades and feedback interconnection of elementary 'modules'.

As a simple illustration, consider the diagram shown in Fig. 1, extracted from the paper on cancer research [6], which describes the wiring diagram of the growth signalling circuitry of the mammalian cell. Of course, such a diagram leaves out a lot of information, some known but omitted for simplicity, and some unknown: much of the system has not been identified yet, and the numerical values of most parameters as well as the functional forms of interactions are only very approximately known. However, data is being collected at an amazing rate and better and better models are being constantly obtained. Many of the natural systems-theoretic questions that one would normally pose for such a system are precisely those that leading biologists are asking, if sometimes in a different language: What is special about the information-processing capabilities, or input/output behaviours, of such networks, and how does one characterise these behaviours? How do the different signal transduction pathways interact? How does one find the forms of reactions, and values of parameters (identification, reverse

engineering)? Once these forms of reactions are known, how does one estimate time-varying internal states, such as the concentrations of proteins and other chemical substances, from input/output experiments (observer problem)? What subsystems appear repeatedly? Where lie the main sensitivities affecting robustness of the system? What is the reason that there are cascades and feedback loops? More generally, what can one say, if anything, about stability, oscillations, and other dynamical properties of such complex systems? In addition to analysis questions there are, of course, also synthesis ones, dealing with the *control* of cellular systems through drugs or genetic modifications.

Many publications address the above types of problems for cell signalling systems, but the field is still in its infancy, and a major and long-term research effort will continue toward their solution. For the rest of this article, however, I would like to focus not on what *existing* control theory and tools can do for systems biology but rather on a sort of converse, namely: *do new questions in control theory arise from looking at problems in systems biology?* (Not surprisingly, my answer will be a resounding 'yes!'.) This is of interest for several reasons. First of all, control theory draws one of its main strengths from its origins in concrete applications. Abstraction is necessary and useful, but should be grounded on concepts whose motivation lies outside of pure theory. Second, it is by being open-minded and receptive to the possibility of modifying our paradigmatic problems that the control theory (and more generally the mathematical) community may have an even greater impact upon this new field.

One of the main messages that I wish to convey it is that, often, problems in systems biology 'sound like' standard problems in control theory, but that on closer inspection, they differ in fundamental ways, and that these differences are challenging and worth exploring. As I hope to convince the reader through the brief discussion of selected examples, new and interesting theoretical questions do arise. These examples are unabashedly picked from my own research, and, obviously, they constitute but a small sample of the new problems that suggest themselves. For example, no mention is made of multi-scale aspects in time and space, of the need for effective hybrid techniques to handle the interface of discrete and continuous components, or of the study of stochastic aspects, which are particularly important in systems involving small numbers of molecules.

3 A sample of questions

In the rest of this paper, I will briefly describe a few new research directions in control theory that were motivated by the study of particular systems biology problems. The first one deals with the analysis of the dynamical behaviour of interconnections of monotone systems with well-defined characteristics, and is discussed in more detail than the rest.

3.1 A mix of qualitative and quantitative modelling

The analysis of signalling networks constitutes one of the central questions in systems biology. There is a pressing need for powerful mathematical tools to help understand, quantify, and conceptualise their information processing and dynamic properties.

The most widespread approach to the modelling of such complex systems involves the use of biological knowledge in the design of large-scale simulation models. Extensive simulations, often combined with bifurcation analysis and dimensionality-reducing techniques such as time-scale decompositions, are used in order to explore the space of parameters and initial conditions. A shortcoming of this approach is that it is virtually impossible to experimentally validate the form of the non-linearities used in reaction terms. Moreover, even when such forms are known, the accurate estimation of coefficients (parameters) *in vivo* is extremely hard — if not impossible — even in principle, since enzyme and other chemical concentrations may greatly vary from cell to cell. Coupled with the need for a huge number of simulations in order to explore state spaces of large dimension, and the fact that numerical algorithms cannot be guaranteed to produce accurate results, due to problems of local vs global convergence, numerical error, and so forth, this argues for the necessity of more effective theoretical tools. In addition, theory has the virtue of providing fundamental understanding, by focusing on the essential principles underlying given behaviours.

Of course, it would be naïve to search for a general theory that would encompass all interesting phenomena exhibited by cell networks. The range of potential non-linear dynamical behaviours is too wide for that. But there is hope, substantiated by the results to be described next, that large classes of signalling systems may be profitably studied by first decomposing them into several subsystems, each of which is endowed with certain *qualitative* mathematical properties which — in conjunction with a relatively small amount of *quantitative* data — allow the behaviour of the entire, reconstituted system, to be easily deduced from the behaviour of its parts. This paradigm of decomposition and reconnection has always been one of the basic principles in systems theory and control engineering. Recently, a certain class of subsystems that appear to be very suitable for the analysis of enzymatic cascades and feedback loops was identified in [7–9], in work done with David Angeli (see also [10]). This novel approach emerged originally from our study of possible multi-stability and oscillations in feedback loops involving MAPK cascades.

Mitogen-activated protein kinase (MAPK) cascades represent a biological module, or subcircuit, which is ubiquitous in eukaryotic cell signal transduction processes [11–13] and is a critical component of pathways involved in cell proliferation, differentiation, movement, and death. They are activated by diverse stimuli (cytokines, growth factors, neurotransmitters, hormones, cellular stress, cell adherence), and their outputs drive different cellular responses, including DNA transcription. The MAPK cascade motif appears in different forms in distinct organisms from yeast to humans, and even in any given cell, and different MAPK cascades involve different chemicals, but their basic architecture is conserved. A MAPK pathway is a three-component module, consisting of three kinases that establish a sequential activation pathway comprising a MAPK kinase kinase (MKKK), a MAPK kinase (MKK), and the final MAPK itself [13]. (A kinase is an enzyme that catalyses the transfer of phosphate groups from a high-energy phosphate-containing molecule, such as ATP or ADP, to a substrate.) The diagram in Fig. 2 illustrates one standard biological model for this process. The output of the system is represented by the 'activated' form of MAPK, denoted as $MAPK^{**}$ in the diagram. The precise meaning of this 'activation' need not concern us here, but we remark that MAPK must be phosphorylated on both a threonine and tyrosine residue for its activation, a dual phosphorylation catalysed by activated-MKK. The concentration $MAPK^{**}(t)$ of this activated form is controlled by the amount $MKK^{**}(t)$ of

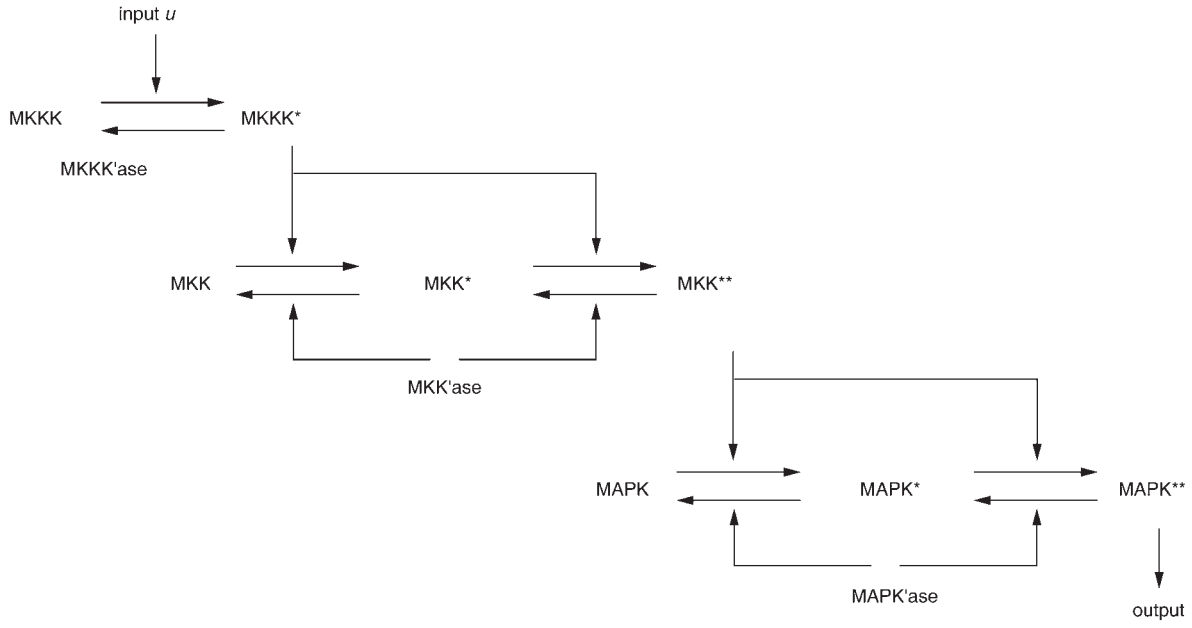

Fig. 2 *Schematic of a MAPK signaling module. The output of the system is represented by the concentration of the 'activated' form of MAPK, denoted as MAPK**. Vertical arrows indicate that this concentration is in turn driven by the time-varying concentration of MKK**, which in turn depends on the previous level of the cascade*

activated MKK present at any given time. (The intermediate MAPK$^*(t)$ corresponds to a partially-activated form of MAPK, where only one phosphorylation has taken place.) A reverse reaction of dephosphorylation takes place as well, not controlled by activated MKK, but controlled by a phosphatase (an enzyme that removes phosphate groups), whose concentration is assumed to be constant in the time-scale being studied. Similarly, the concentrations of partially and completely activated MKK's are controlled by the concentration of activated MKKK. The input u to the complete subsystem represents the concentration of a different kinase or of a small GTP-binding protein, that phosphorylates, and hence activates, MKKK.

Differential equation models of MAPK signalling modules take as states the concentrations of MAPK(t), MAPK$^*(t)$, and other variables; thus, corresponding to the diagram in Fig. 2 there would be a set of eight differential equations. (Variations in the literature include those in which the first subsystem is simplified to be one-dimensional, or conversely, in which one includes additional states corresponding to elementary intermediate chemical reactions.) For example (see the references cited earlier), the formula for $d\text{MAPK}/dt$ would include a term '$+f(\text{MAPK}^*(t))$' to represent the effect of dephosphorylation of MAPK*, where f is some non-decreasing function with $f(0)=0$, and a term '$-g(\text{MKK}^{**}(t), \text{MAPK}(t))$' to represent the effect of phosphorylation by MKK**, where g is non-decreasing function on each argument and $g(0,\cdot)=g(\cdot,0)=0$. There are, in addition, *conservation laws*. For instance, $\text{MAPK}(t)+\text{MAPK}^*(t)+\text{MAPK}^{**}(t)$ must be constant, since these variables represent modified forms of the same enzyme, the amount of which remains constant unless one takes into account the slower time-scale processes of transcription and translation of the protein and its degradation.

The initial motivation for the work reported in [7–9] had to do with the possible onset of oscillations, due to a Hopf bifurcation, when inhibitory ('negative') feedback is introduced from the output to the input of a MAPK module, cf [14, 15]. Such oscillations are not typically observed in cells in their natural states, even though several inhibitory feedbacks are known to exist, so it was natural to ask what conditions on the feedback strength would guarantee that no oscillations result. Small-gain theorems provide a routine control theory approach to such problems, so a first reaction when confronted with this problem might be to attempt to apply one such result (linearly for local analysis, or H^∞ gain, or an ISS-small gain theorem, for example). However, a technical problem arises: in this application — and typically in biological applications — the *location of equilibria are changed* by feedback. While in engineering we are used to feedback laws of the form '$u=k(x)$' where k preserves the equilibrium of interest (so, for instance, for the open-loop system $\dot{x}=-x+u$, the equilibrium $x=0$ is preserved under $u=-kx$), in biological applications the equilibrium location may depend on the numerical values of feedback gains. For example, consider the system $\dot{x}=-x+u$, but suppose that the inhibitory feedback has the form $u=\frac{a}{b+x}$. Then the open-loop equilibrium at $x=0$ will change under feedback; for instance it becomes $x=1$ for $\dot{x}=-x+\frac{4}{3+x}$. This shift of steady-state locations is especially hard to handle with classical methods when we wish to study global, as opposed to simply local, asymptotic stability. Even more challenging, one should remember that the exact forms of the non-linearities in the original system, and certainly parameter values, are very hard to estimate; in general, only rough forms are known. For instance, in the diagram shown in Fig. 1, the only information given about interactions is whether they are activating (arrows '$\rightarrow$') or inhibitory ('$\dashv$' symbols). Thus, a problem that resembles a standard one in control theory turns out, on further inspection, to be rather different in technical details.

These considerations led us to the introduction of a different type of gains — 'Cauchy gains' — for systems [16] and more generally to the theory of *monotone I/O systems with characteristics* in [7]. Using these concepts, we could provide new forms of 'small-gain theorems' that are very well suited for MAPK cascades, and as it turned out, a large number of other applications in biology (see for instance [17–19]).

Moreover, once we developed the basic results for monotone systems with characteristics, we realised that

a wealth of other interesting questions, ranging from the detection of bistability and hysteresis in systems biology models to the study of delay-induced oscillations, could be handled in the same fashion (see for instance [8, 9, 20, 21]). The key idea is to decompose a network into subsystems each of which has the following two properties: *input/output (I/O) monotonicity* and *existence of I/O characteristics*.

I/O monotonicity is defined mathematically through an order-preservation property for flows (see below for more details). The concept represents a generalisation of the notion of monotone autonomous system studied by Hirsch, Smale, Smith, and others [22–25]. It can often be verified by simply studying the 'sign structure' of an incidence graph associated to the Jacobian of the dynamics; in other words, by simply inspecting which connections in a diagram such as the one shown in Fig. 1 are activating ('→') and which are inhibitory ('⊣'). Interconnections of monotone systems remain monotone, and monotone systems — to be precise, a slightly stronger strict version, in which an appropriate margin is required — have very appealing global convergence properties. Almost all bounded trajectories converge to equilibria, and therefore no limit cycles, chaotic behavior, and so forth, can occur. In particular, all one-dimensional systems are automatically monotone. An analogy to passivity was suggested, to us by Rodolphe Sepulchre: just as statements about interconnections of integrators often generalise to statements about the same interconnections of passive systems of arbitrary dimensions, statements about interconnections of scalar systems often generalise to statements about monotone systems in arbitrary dimensions. One might even speculate that I/O monotone systems may end up playing a role in biological modelling not unlike that of passive systems in mechanical and electrical systems.

Existence of characteristics means that there is a monostable steady-state response to any constant external input. This is, of course, a very strong constraint, but we should remember that this constraint is only imposed on the elementary components of our interconnections — indeed, the whole point of the theory is to predict what more interesting dynamical behaviour emerges when several such components are interconnected. In any event, we have devoted considerable efforts to the characterisation of subsystems as appear in systems biology problems for which this property is always satisfied, including receptor-ligand models [26], certain general types of chemical reactions [27], and very general processive modifications such as phosphorylation as with the special case of MAPK modules, but not necessarily restricted to two steps, see [9, 28].

When coupled, these two properties, monotonicity and existence of characteristics, allow the characterisation of limit sets of arbitrary trajectories, under all possible external forcing signals. When several systems with these two properties are interconnected, possibly in feedback, and possibly destroying monotonicity of the overall system — when negative feedback is used, the resulting system is not monotone anymore — this characterisation allows the reduction of global stability questions to simple graphical tests involving external signals. These graphical tests do not require knowledge of the dynamics, but merely of the steady-state response to constant inputs. Steady-state responses—called sometimes dose response curves, or activity assays for receptor binding — are routinely provided by biologists; they constitute the *quantitative* information that our analysis requires. Monotonicity, on the other hand, is a *qualitative* property which is characterised by the topology of the network (and the signs of interactions).

3.1.1 *Monotone systems:*

A continuous-time finite-dimensional system, in the standard sense of control theory:

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

(one also studies delay-differential systems, reaction-diffusion PDE's, and more abstract flows in metric spaces) is monotone if there are non-trivial orders in the state, input, and output spaces, such that

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

with respect to the state and input orders, and the output map h preserves the order as well. As usual, $x(t,\xi,u)$ means the solution at time t if the initial state is ξ at $t=0$ and the input is $u(\cdot)$; and $u_1 \leq u_2$ for controls means that $u_1(t) \leq u_2(t)$ for all t (see [7, 8] for more technical details). Orders are typically defined by positivity cones, and one may check monotonicity in infinitesimal terms, not requiring solution of differential equations (see the references). 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. 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. Thus, for instance, the following system (evolving on non-negative states and with non-negative inputs) is monotone:

$$\begin{aligned} \dot{x}_1 &= -x_1 + \frac{1}{1+u} \\ \dot{x}_2 &= -x_1 x_2 + x_3 u \\ \dot{x}_3 &= x_2 - x_1 x_3 \\ y &= x_3 - x_1 \end{aligned}$$

since there are no negative-parity cycles (including cycles when directions of arrows are ignored) in its incidence graph shown in Fig. 3. It is easy to show, using this graphical characterization, that many systems of interest in systems biology are indeed monotone (see [7–9, 28]).

3.1.2 *Steady-state responses:*

The only 'quantitative' component needed for the theory is the steady-state response to step inputs. For our elementary subsystems, we

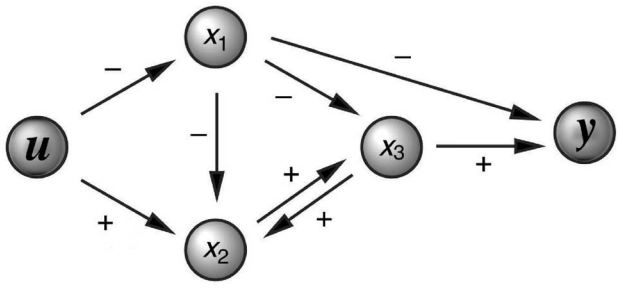

Fig. 3 *Incidence graph for system* $\dot{x}_1 = -x_1 + \frac{1}{1+u}$, $\dot{x}_2 = -x_1 x_2 + x_3 u$, $\dot{x}_3 = x_2 - x_1 x_3$ *with output* $y = x_3 - x_1$. *For example, the negative arrow from u to* x_1 *represents the fact that* $\frac{dx_1}{du} < 0$

require not just monotonicity but also that, for each constant input $u(t) \equiv v$ there be a globally asymptotically stable state x_v of $\dot{x} = f(x, v)$. As we discussed earlier, this is in principle a very strong constraint, but we have shown that many 'modules' in systems biology have this property. For further analysis, all that is required is the knowledge of the graph of the steady-state output $y = h(x_v)$ as a function of the constant input value being applied. We call this map the 'I/O characteristic' of the system.

3.1.3 Applications: In [7], we provided a small-gain theorem for global stability of negative feedback loops involving monotone systems with well-defined steady-state responses. Leaving out some technical details, we may summarise the results as follows. Suppose that $\dot{x} = f(x, u)$, $y = h(x)$ is monotone and has characteristic $y = k(u)$, and we wish to study the effect of a feedback $\ell: y \mapsto u$ that is monotone decreasing. We assume for simplicity here that the inputs and outputs are scalar. The result was that the closed-loop has a globally attractive equilibrium provided that the *scalar* discrete time iteration

$$u_{i+1} = F(u_i) \quad (F := \ell \circ k : u \mapsto u)$$

has a globally attractive equilibrium $\bar{u}$ (see Fig. 4). As with classical small-gain theorems, phase is irrelevant, and arbitrary delays are allowed in the feedback loop with no change in the result. This was applied to the analysis of the examples given in the literature of oscillations in MAPK cascades.

Another early application of this new approach was to the analysis of *bistability* (and more generally multi-stability). A bistable system is one that admits two discrete, alternative stable steady states. Early biological examples of bistable systems included the lambda phage lysis-lysogeny switch and the hysteretic *lac* repressor system [29, 30]. More recent examples have included several MAPK cascades in animal cells [31, 32], as well as cell cycle regulatory circuits in *Xenopus* and *S. cerevisiae* [33, 34]. Bistable systems are thought to be involved in the generation of switch-like biochemical responses [31, 35, 36], the establishment of cell cycle oscillations and mutually-exclusive cell cycle phases [34, 35], the production of self-sustaining biochemical memories of transient stimuli [37, 38], and the rapid lateral propagation of receptor tyrosine kinase activation [39]. Our papers [8–10] showed how often — and in particular, in the case of MAPK signaling cascades — bistability and hysteretic behaviour can be analysed in terms of decompositions into modules each of which is I/O monotone and admits an I/O characteristic. The method led us to establish properties of MAPK cascades at a level of generality largely independent of functional forms and parameter values, leading to conclusions regarding the global stability of behaviour.

One way in which multi-stability arises is through positive-feedback loops involving monotone systems with well-defined steady-state responses. We assume once again that the inputs and outputs are scalar (the multivariate case is treated in [40]). The main result in [8] (again omitting some technical details) was as follows. Suppose that $\dot{x} = f(x, u)$, $y = h(x)$ is monotone and has characteristic $y = k(u)$, and we wish to study the effect of a feedback $\ell: y \mapsto u$ which is strictly increasing. We plot the characteristic k together with the graph of the inverse of the mapping ℓ, see Fig. 5 (where we use $\ell(y) = y$ for simplicity, that is, we only illustrate unity feedback). Next, we label the intersection points between these two plots with a symbol 'S' or 'U' depending on whether the derivative k' is less than that of the inverse mapping ℓ^{-1} or not. The theorem guarantees then that there are as many steady states in the complete closed loop system $\dot{x} = f(x, \ell(y))$ as there are intersections (this part is quite trivial to show), and that among these states, those corresponding to the intersections marked S are global attractors, in the sense that all trajectories, except for those originating in a set of measure zero in the state space, converge to them. (A technical condition, not hard to check, of strong monotonicity is needed as well, see [8].) Note that this conclusion is only based on a qualitative piece of data, the monotonicity of the open-loop system — which one may typically check using information from incidence graphs — and one quantitative piece of data, the plot of the characteristic — which is often readily available from experiments. In [10], we illustrated this technique through the analysis of bistability of a positive-feedback loop involving progesterone-induced Xenopus oocyte maturation. These results represent a broad generalisation of classical work on feedbacks on cascades of

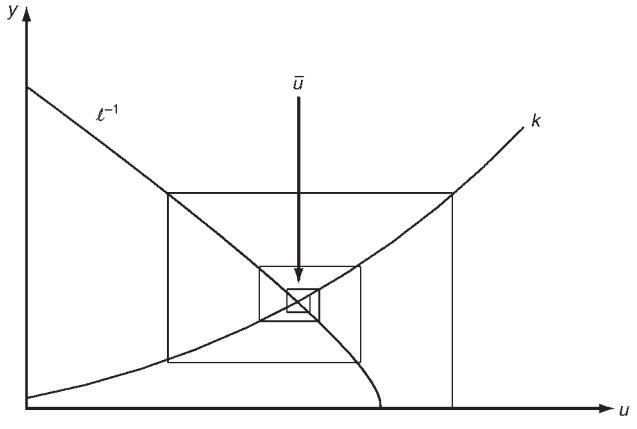

Fig. 4 *Small-gain theorem for monotone system with I/O characteristic k, under feedback ℓ. The spiderweb diagram, shows convergence of the discrete one-dimensional iteration to an input/output value $\bar{u}$; this is enough to guarantee that the closed-loop system, which may have arbitrary dimensions, is globally asymptotically stable*

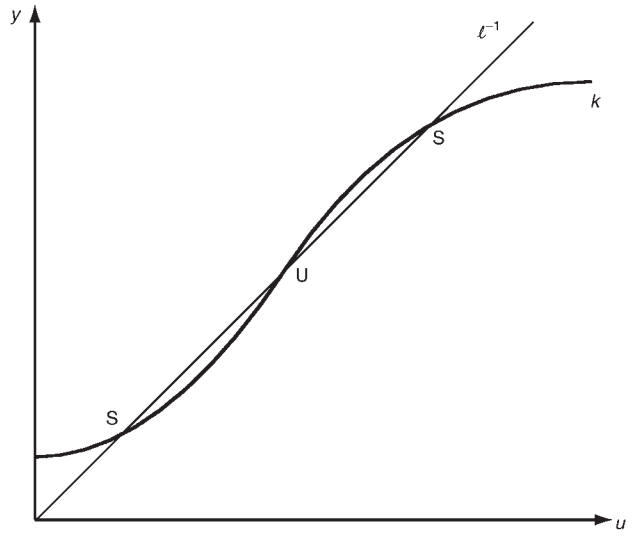

Fig. 5 *Multi-stability theorem for monotone system with I/O characteristic k, under feedback ℓ (here taken to be the identity). The intersection points between the plots of k and ℓ^{-1} are in a one-to-one correspondence with internal steady states; those marked 'S' correspond to those states (here, two) which attract almost all trajectories*

one-dimensional systems such as described in [41] and references there.

Ordinary differential equation models such as those considered above implicitly assume that reactions proceed in a well-mixed environment. While this is a reasonable assumption when diffusion is fast compared to the time scales of reactions (see for instance [42], Ex. 11.5), it is other times important to explicitly incorporate spatial inhomogeneity. This leads to reaction-diffusion partial differential equations: instead of a dynamics $\dot{x} = f(x)$ (omitting now for simplicity the inputs u), one must consider equations such as $\frac{\partial x}{\partial t} = D\Delta x + f(x)$, where now $x = x(t, q)$ depends on both time t and space variables q, Δx is the Laplacian of the vector x (that is, its ith coordinate is $\sum_{j=1}^{n} \partial^2 x_i / \partial q_j^2$) with respect to the space variables, and D is a diagonal matrix of positive diffusion constants.

It is a surprising and non-trivial fact that many stability conclusions derived for the ordinary differential equation $\dot{x} = f(x)$ remain valid, with little or no change, for the PDE $\dot{x} = D\Delta x + f(x)$, subject to no-flux (Neumann) boundary conditions, provided that the system $\dot{x} = f(x)$ is (strongly) monotone. For example, stable steady states x_0 of $\dot{x} = f(x)$ remain stable as homogeneous states $(x(q) \equiv x_0)$ of the PDE, see [25], Remark 7.6.1. Thus, diffusive instability, which is the basis of Turing's mechanism for pattern formation, cannot arise in monotone systems. Unstable states of $\dot{x} = f(x)$ are also unstable when viewed as homogeneous steady states of the associated diffusion equation, since they are unstable already with respect to homogeneous perturbations. On the other hand, in principle there could be inhomogeneous (not constant on space variables q) steady states of the diffusion system. However, if the shape of the space in which the variables q evolve is convex (for example, if a cell is modelled by a circle, sphere, or ellipsoid), then there are no stable inhomogeneous states, i.e. the stable points of the ODE and PDE systems coincide, see e.g. [43–46]. These facts, applied to the closed-loop system, provide an extension to the reaction-diffusion case of the discussed results regarding multi-stability and global behaviour of trajectories under positive feedback.

3.2 Some other problems

To close, let me quickly mention a few other new theoretical questions in control theory that arose from looking at systems biology problems.

3.2.1 Disturbance rejection with signal detection: One strength of control theory is that it predicts that certain structures *must* be present in systems, in order for regulation objectives to be met. Such insight may help guide biological research, as it suggests a search for the corresponding structures. Conversely, the absence of a critical subsystem might be an indication that the biological entity being studied does not regulate its behaviour in some hypothesised sense.

The authors of [47] brought this point of view to a wide biological audience. The setup was that of *E. coli* chemotaxis: these bacteria move in response to the temporal sensing of a chemical concentration *gradient*; for constant concentrations, there is no net movement. (Actually the story is more complicated, since the bacteria really perform a kind of stochastic line search, with random tumbles to reorient themselves when no gradient has been detected, but this simplified version suffices for the point that we wish to make.) It is experimentally known that the response, as measured by the activity of motors driving flagella, has a zero DC gain, and a zero at the origin suggests a feedback structure containing an integrator. One may view this behaviour as disturbance rejection of step signals, and thus motivated, the paper [47] proposed that the internal model principle (IMP) due to Francis and Wonham predicts the existence of an integrator in the *E. coli* chemotatic subsystem; a possible mechanism — see also [48] — was described. Recall that the classical IMP tells us that if a controller regulates a system against external disturbances in some family — such as steps — and if this regulation is *structurally stable* in a precise mathematical sense, then the controller must necessarily contain a subsystem — such as an integrator — that can itself generate all such disturbances, and which is driven by a suitable error signal.

But there is a potential drawback when attempting to use this theorem in biological applications. The structural stability criterion may be impossible to check: it would imply, for instance, regulation even in the presence of direct connections from inputs, such as cell receptors, to outputs, such as flagellar motors. Furthermore, the distinction between 'controller' and 'plant' in a cell is not at all obvious.

In addition, there is another characteristic that distinguishes the more classical engineering disturbance rejection setup from the problem of interest in cell biology: *signal detection* is of great importance. The cell *should* detect the signal before regulating — in fact, a large overshoot is often desirable, in order to 'start up' a downstream subsystem. (On the other hand, adaptation after the signal is processed is also often important: the reaction might be metabolically too expensive to be kept 'on' for very long). The analogy would be the design of an automobile active suspension system in which a requirement is that passengers hit the car roof as hard as possible, when a bump in the road is encountered, before the control system takes over and stabilises the oscillation.

Thus, once again, we are faced with a problem that resembles a standard one in control theory, yet which mathematically differs in a subtle fashion. Motivated by this, in [49], we provided an internal model theorem that did not require the assumption of structural stability, nor an *a priori* requirement for the system to be partitioned into separate plant and controller components. In lieu of structural stable regulation, a signal detection criterion, expressed through zero-dynamics and relative degree for non-linear systems, was imposed in order to force the existence of an internal model. Much work remains to be done on this subject; the paper [49] barely scratched the surface.

3.2.2 Systems identification and reverse engineering: One of the most active current research areas in systems biology is that of reverse engineering of gene and protein networks. From measurements of how certain measured variables — concentrations of proteins, amount of RNA being transcribed, etc. — change in response to probing inputs, one wishes to determine the internal structure of the system. At this abstract level of description, this appears to be a perfect candidate for the use of systems identification and realisation theory techniques. And indeed, there is substantial work being carried out, using control theoretic techniques to approach such reverse engineering problems. In the spirit of this paper, however, let me point out some twists which often distinguish this type of problem from more standard formulations of systems identification.

One of the main features of biological problems is that it is often extremely expensive, if not impossible, to apply

arbitary test signals to the system. More often than not, biologists will only be able to consider step inputs, or perhaps a small combination of such inputs and ramps. Although sufficient for local identification of linearised models, this is certainly not enough information to characterise the behaviour of a non-linear system. Thus, an interesting question has to do with the amount of experimentation needed in order to identify a system if only a finitely-parameterised family of inputs is available; the paper [50] looked into this problem and established that the best possible answer, assuming exact measurements, is $2r + 1$ experiments, where r is the number of parameters. Moreover, in a precise mathematical sense, a generic set of such experiments suffices. This resembles, but is technically different in a fundamental way, from similar results for embeddings of chaotic systems or for generic observability.

An even more important set of questions has to do with the fact that often only *steady-state* measurements are available. In mathematical terms, there is an unknown system $\dot{x} = f(x, p)$, where the vector of state variables evolves in some manifold M and p is a vector of parameters, and for each particular value of p in some neighbourhood of a default value p_0, one may have measurements of a steady state $\xi(p)$ satisfying $f(\xi(p), p) = 0$. In cellular networks, for instance, the state vector might represent the concentrations of certain proteins, mRNA, or other substances at different time instants, the parameters might represent the concentration levels of certain enzymes that are maintained at a constant value during a particular experiment, and the experiment measures concentrations after the system has relaxed to a steady state. As discussed earlier, in cell biology applications it is often the case that the equations defining the system — that is, the functions f_i describing the vector field — are unknown, even in general form, but one wishes nonetheless to determine the interaction graph of the system, that is to say, to know which variables directly influence which variables, as well as the relative strengths of these interactions. Suppose the steady-state measurements induce a smooth map ξ from parameters to states, at least locally, so that perturbation experiments, corresponding to the possible vector fields Y in parameter space induce, by push-forward, vector fields $X = \xi_*(Y)$ on the state space, which in turn determine a distribution $\mathcal{D} := \text{span}\{X\} \subseteq TM$. Under suitable genericity conditions, $\dim \mathcal{D} \equiv n - 1$, and with the main technical assumption described in [51], one can obtain that $df_i \in \mathcal{D}^{\perp}(\langle df_i, \mathcal{D}\rangle = 0)$ for appropriate coordinates of the motion, which determines each df_i projectively. That is, the interaction graph is completely characterised, and strengths are identified up to a constant common multiple. See [51] for details, as well as [52] for further results and [53] for new problems in combinatories and computational complexity also suggested by this problem.

3.2.3 *Adaptive control of bifurcation parameters:*

Some biological systems are believed to operate at a critical point between stability and instability, which brings up the issue of how bifurcation parameters may be automatically tuned to this critical value. The papers [54–56] were motivated by two such instances from the literature: neural integration in the nervous system, and hair cell oscillations in the auditory system. In both examples, the question arises as to how the required fine-tuning may be achieved and maintained in a robust and reliable way. As with the other questions illustrated here, this led to a new type of problem in control theory, related but in fact different from other work on bifurcation control in the literature. We formulated this question in the language of adaptive control, and presented solutions in some simple instances.

3.2.4 *Robust stability from structure:*

Designers strive to engineer as much robustness as possible into control systems. However, few systems perform acceptably under truly large variations in parameters. In biology, in contrast, there is often a very large variability in intracellular concentrations of chemicals, due to, for instance, unequal division among daughter cells during mitosis, gene duplications, or mutations. If functions critical to the survival of the organism are not affected, this means that evolution must have selected for appropriately robust structures. Their study is of great theoretical interest, and may also potentially suggest ideas for novel robust designs in engineering applications.

One such type of problem arose during a fascinating series of talks [57] given by a mathematical immunologist, Carla Wofsy. Among other tasks, the immune system is charged with the destruction and elimination of invading organisms and of the toxic products that they produce, as well as the destruction of virus-infected or mutated cells. One of the most challenging problems in the study of the immune system is to understand how it manages to distinguish among self and other while still being able to react fast. One approach to this question was proposed by McKeithan [58], who suggested that a chain of modifications of the T-cell — a type of immune cell — receptor complex, via tyrosine phosphorylation and other reactions, may give rise to both increased sensitivity and selectivity of response. This process, which he called kinetic proofreading because of its analogy to an older model proposed by Hopfield for DNA error correction, was modelled in [58] for T-cell receptor (TCR) and peptide-major histocompatibility complex (MHC) interactions by means of a certain set of non-linear ordinary differential equations. The steady states that result are interpreted as a signal of antigen recognition. Thus, it was natural to ask if the steady states of this system are unique, if they are asymptotically stable, and so forth.

No standard technique from control theory seemed appropriate for handling this example, but it soon became apparent that the beautiful theory of chemical network stability studied by Feinberg, Horn, and Jackson in the 1970s [59, 60] provides a rich approach to establishing uniqueness and asymptotic stability of equilibria for the system in [58] as well as, it turns out, many other types of problems in receptor-ligand dynamics [26]. The theorems that result are valid essentially for all parameters appearing in the system description, as long as the interconnection structure of the system satisfies certain rules. In [27], we extended the results in [59, 60] to handle global stability as well as to provide estimates of robustness to unmodelled dynamics, and also gave solutions to a stabilisation problem that one may formulate for such systems; an observer theory was subsequently developed in work with Madalena Chaves [61, 62]. In this case, a systems biology problem did not suggest an entirely new theoretical development, but instead led to bringing into control theory, tools from an area of mathematical chemistry that had not attracted so much previous attention in the control theory community.

4 Conclusions

I have argued, through a selection of examples, that new problems in control theory arise most naturally when looking at systems biology problems. In my experience, it is necessary to listen carefully to the questions that our biology colleagues are asking. Many of these questions may

sound like routine questions in control theory, but they often turn out to lead to challenging and interesting, and ultimately highly rewarding, new directions of theoretical research.

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