

The Dynamic Systems Approach to Control and Regulation of IntraCellular Networks

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Abstract

Systems theory and cell biology have enjoyed a long relationship that has received renewed interest in recent years in the context of *systems biology*. The term ‘systems’ in systems biology comes from *systems theory* or *dynamic systems theory*: Systems biology is defined through the application of systems- and signal-oriented approaches for an understanding of inter- and intra-cellular dynamic processes. The aim of the present text is to review the systems and control perspective of dynamic systems. The biologist’s conceptual framework for representing the variables of a biochemical reaction network, and for describing their relationships, are pathway maps. A principal goal of systems biology is to turn these static maps into dynamic models which can provide insight into the temporal evolution of biochemical reaction networks. Towards this end we review the case for differential equation models as a ‘natural’ representation of causal entailment in pathways. Block-diagrams, commonly used in the engineering sciences, are introduced and compared to pathway maps. The stimulus-response representation of a molecular system is a necessary condition for an understanding of dynamic interactions among the components that make up a pathway. Using simple examples, we show how biochemical reactions are modelled in the dynamic systems framework and visualized using block-diagrams.

Keywords: pathways, biochemical reaction networks, dynamics, feedback, block diagrams.

Abbreviations: ODE - Ordinary Differential Equations, PDE - Partial Differential Equations.

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*A cell is built up of molecules, as a house is with stones.
But a soup of molecules is no more a cell
than a heap of stones is a house.*

1 Pathways as Dynamic Systems

In an amusing article, Yuri Lazebnik [1] argues that with the ever increasing flood of information about the components that are involved in any of the cell functions, like apoptosis, we are failing to improve our understanding of cell functions. Put specifically, by simply collecting and cataloguing the components and their molecular properties, we lose sight of how the components interact functionally. By comparing an engineering approach to systems with that of biology, he argues that a more systematic and formal approach is necessary to move from molecular characterization towards an understanding of cell function through the interactions of the components involved. The functions of the cell do not reside in the molecules themselves but in their interactions, just as life is an emergent, rather than an inherent, property of matter. Although life, or the functions of the cell, arise from the material world, they cannot be reduced to a plain description of the component parts. A central dogma of systems biology is that it is the dynamic interactions of molecules and cells that give rise to biological function. The understanding of inter- and intra-cellular networks defines the agenda of the (re-)emerging area of systems biology. The principle aim is to understand intra- and inter-cellular processes [2]:

1. How do the components within a cell interact, so as to bring about its structure and function?
2. How do cells interact to develop higher levels of organization, including, cell clusters, tissue and organs?

To support an understanding of the functioning and function of cells, in our view systems biology ought to focus on mathematical modelling and simulation of the dynamics associated with biochemical reaction networks (pathways).

With the many reviews and special issues available on the subject, the present article instead focusses on the differential equation or dynamic systems approach that underlies the majority of noteworthy publications that may be attributed to this area. One notices a trend in the current literature that the term ‘mathematical’ is frequently avoided and replaced by ‘computational’ to make mathematical modelling and simulation more acceptable to experimentalists. Systems biology is trying to establish a close link between experimental data and mathematical models in molecular and cell biology. Central to any computational approach or simulation is a *model*, a mathematical model. Variables in a dynamic system change with time and for these changes to be ordered and meaningful they must be organized in a formal model. Control systems analysis provides a way of formalizing such organization by using structured mathematical descriptions of dynamical systems, and graphical representations of system component interactions. The purpose of this article is to provide a review of system- and signal-oriented methods for biologists. On the way, we learn about various ways to represent and visualize our understanding, including the biologist’s pathway diagrams, the biochemists reaction equations, pathway maps, the mathematician’s differential equation models, and the control engineer’s block diagrams. These tools serve as an interface between the practical experiment and a theoretical model of system dynamics.

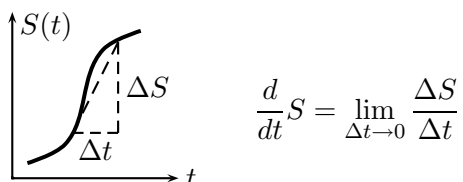
2 Differential Equations as a Language for Observed Changes

There are a range of formalisms available for modelling and/or simulation of biochemical networks, including formal languages, stochastic models and differential equations. The choice of a suitable framework is not only guided by which formalism would provide the most realistic representation. The large number of variables and nonlinear relationships force us to make assumptions. The experimental difficulties in quantifying intra-cellular concentrations renders most models in molecular and cell biology *phenomenological*. We are thus not in the business of building *in silico* replica models of actual physical interactions of molecules. Instead, we ought to choose a conceptual framework that is best suited to support the biologist’s reasoning in making sense of observations.

Cell functions, including cell growth, cell differentiation, proliferation, stress response etc. are dynamic processes. We observe through temporal changes in concentrations, counts or copy numbers. A natural approach to describe dynamic processes in terms of rates of change are differential equations. Differential equations come in two flavors: *ordinary* differential equations (ODEs), describing changes over time and *partial* differential equations (PDEs), describing changes in space and time. While the latter seem intuitively more appropriate for modelling intra- and inter-cellular processes, they require mathematical tools and experimental data that in most practical cases are not available. We hereafter focus therefore on ODEs as the most commonly adopted, although not only approach for modelling intra-cellular dynamic processes. To motivate differential equation modelling we use a very simple model of proteolysis. Let us consider a protease E , which cleaves a specific peptide bond in a substrate protein S , and thereby activating it to yield the modified cleaved form P . In the first step we would assume or hypothesize the principle:

“The rate of proteolysis is inversely proportional to the amount of substrate.”

Assuming the rate of cleavage (proteolysis) is inversely proportional to the amount of inactive substrate S , we can translate this into a mathematical model by first considering a notation for the rate of change of substrate S : dS/dt . The change over time is related to the slope of the concentration profile:



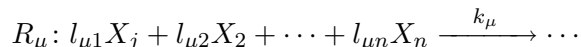
The mathematical model for changes in the substrate concentration is subsequently obtained as

$$\frac{d}{dt}S = -k_p S(t)$$

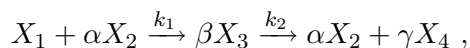
\swarrow variable (changes)
 \searrow parameter (fixed)

The parameter k_p defines the rate coefficient and includes assumptions about (constant) temperatures and volume. The concentration profile of the cleaved form, $P(t)$ is readily obtained from $P(t) = S_0 - S(t)$, where S_0 denotes the initial substrate concentration. The *state* of the system is therefore completely determined by $S(t)$. For simple differential equation models we find an analytical or formal *solution*. This is another equation which describes the curve $S(t) = S_0 \cdot e^{-k_p t}$. For more complex systems we will often not be able to find such solutions in analytical form but instead obtain solutions through numerical integration of the differential equations.

With the chosen framework of ODEs we now look at more complex networks. Pathways are the concept by which knowledge of interactions of proteins in cell functions is organized. A *pathway map* exhibits the names of the molecular components, whose interactions govern the basic cell functions. This leads us to a definition of pathways as biochemical *networks*. A large number of pathway maps are collected in biological databases (e.g. <http://www.kegg.org>). Whether we are aiming for stochastic models or a differential equation model, one possible approach to bring these static diagrams to life through modelling and simulation, is to decompose large reaction networks into a set of unidirectional *reaction channels* R_μ



where X denotes a chemical species participating in a reaction, the ‘+’ signs represent a combination, the arrow represents a transformation proceeding with rate k_μ and $l_{\mu j} \geq 0$ defines the number of molecules of X_j involved in the reaction [3]. For instance, consider the following example



which can be split into two reaction channels



When a reaction occurs, the changes to molecule populations can be summarized in form of vectors

$$\nu_1 = (-1, -\alpha, \beta, 0) , \quad \nu_2 = (0, \alpha, -\beta, \gamma) .$$

That is, if the first reaction channel is active, the population of X_1 molecules decreases by one, the population of X_2 by α molecules and so forth. Applying the *law of mass action*, a differential equation model is easily derived. Denoting with x_1, \dots, x_4 the dynamic variables corresponding to chemical species X_1, \dots, X_4 we have

$$\begin{aligned} \frac{d}{dt}x_1 &= -k_1x_1(t)x_2^\alpha(t) \\ \frac{d}{dt}x_2 &= -\alpha k_1x_1(t)x_2^\alpha(t) + \alpha k_2x_3^\beta(t) \\ \frac{d}{dt}x_3 &= \beta k_1x_1(t)x_2^\alpha(t) - \beta k_2x_3^\beta(t) \\ \frac{d}{dt}x_4 &= \gamma k_2x_3^\beta(t) . \end{aligned}$$

Looking at the structure of these equations, we recognize the generalized representation

$$\frac{d}{dt}x_i(t) = \sum_{\mu=1}^M \nu_{\mu i} k_\mu \prod_{j=1}^N x_j^{l_{\mu j}}(t) \quad i = 1, 2, \dots, N \quad (1)$$

where the units of the concentrations x are mol per liter, $M=\text{mol/L}$. For simplicity, the commonly used square brackets $[]$ to denote concentrations are omitted. Equation (1) is already a quite general representation, suitable for a large class of pathways it allows for further assumptions leading to Michaelis-Menten type kinetics. The beauty of mathematical analysis is that it identifies particular cases as examples of a more general framework. We can thus further generalize this representation, ignoring the specific structure on the right-hand side. The most commonly employed framework to model nonlinear dynamic systems is the *state-space model* [4, 5, 6, 7]

$$\begin{aligned} \dot{x} &= f(x) + \sum_{i=1}^m g_i(x)u_i , \\ y_j &= h_j(x) \quad 1 \leq j \leq p . \end{aligned} \quad (2)$$

where \dot{x} is shorthand for the *rate of change* $dx(t)/dt$ of the n variables summarized in the vector x . At any time t , x , represented through the variables $x_1(t), \dots, x_n(t)$ defines the state of the system. This system has m inputs and p outputs, the dependence on t is omitted to simplify notation. A fundamental assumption in using (2) is that together with some initial condition $x_0 = x(t=0)$ the state completely defines the future behavior of the system.

Control variable u represents some independent stimulus. In cell signalling $u(t)$ would typically model ligands binding to receptors. In many situations we will not be able to observe all state-variables directly. Through the response variable y and the mapping h we can capture this situation. For example, we may only be able to measure a total concentration phosphorylation assay. The f, g and h in (2) are *mathematical morphisms* or mappings, relating the variables on the right-hand side of the equation to rates on the left-hand side. Note that while x, u and y are functions of time, f, g, h do not explicitly depend on time. This means that we hereafter consider only *time-invariant systems*, i.e., dynamic systems where the variables evolve in time but where the system properties remain unchanged.

The state-space model (2) is applicable to a wide range of systems. Although spatial aspects are not represented explicitly, this would require PDEs, for many practical cases it is either possible to assume rapid diffusion and thus ignore it, or different regions of the cell may also be modelled by introducing additional variables to the model. The same protein would thus have two variables in the model, representing different

compartments such as, for example, the nucleus and cytosol of the eucaryotic cell. In our view, the greatest weakness of (2), and any other conventional differential equation model, are the consequences of protein translocation, e.g. in and out of the cell nucleus. The nucleocytoplasmic export of molecules that play a role nearer the membrane and receptors, introduces time-delays [8]. As is well known from dynamic systems theory, transport delays have a significant influence on the behavior of a dynamic system. *Delay differential equations* are a natural framework to account for such phenomena explicitly. However, as with partial differential equations we trade a more realistic model with an increase in the complexity of a formal analysis. On the other hand, including delays in numerical simulations does not pose any difficulties but requires detailed knowledge of parameter values.

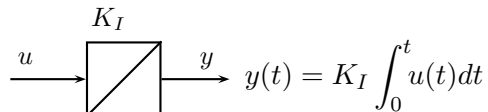
Implicit in the definition of a dynamic system (2) is the assumption that we have an idea of what are the independent and dependent variables of a system – corresponding to stimulus u and response y respectively. For many intracellular processes it is far from clear which proteins can be considered “drivers” and which “followers”. For signal transduction pathways ligands binding to cell surface receptors may be considered the input to the system and gene expression as the output or response to the stimulus $u(t)$. The area of cell signalling [9] is therefore most susceptible to the control perspective on intra-cellular dynamics.

Rather than continuing with more abstract representations, as beautiful they may be to the theoretician, we now develop concrete models from basic examples. Before this we are however introducing an unambiguous graphical representation of differential equation models.

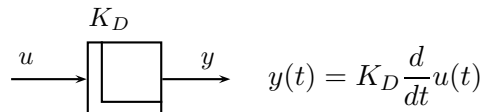
3 Pathway Block Diagrams

Pathway maps used are for most cases a graphical representation that lacks a standard and for which it is not clear which mathematical model should/could be used to simulate the system. We here introduce a *block diagram* representation of nonlinear dynamic systems, which is an unambiguous translation of the mathematical model (2). Admittedly it is therefore only suitable for differential equations. The biologist’s conception of a pathway map is similar to block diagrams that are widely used in the physical- and engineering sciences. Arbitrary complex systems can be built up from four basic building blocks:

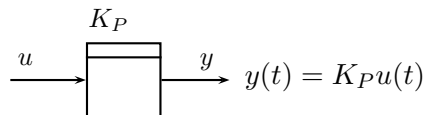
Integrator:



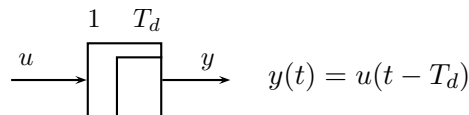
Differentiator:



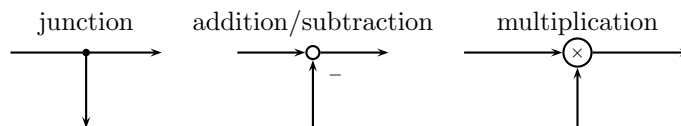
Gain:



Transport Delay:



The most important block we are going to focus on is that of an *integrator*, which describes an accumulation or growth process. The differentiator is simply the reverse operation to the integrator. As alluded to above, the *transport delay* block is of particular importance in simulating the effect of protein translocation, nucleocytoplasmic export and related spatial effects. Block diagrams differ to pathway maps in that they show the processing of *signals*. Block-diagrams are thus a *signal-oriented* approach, an arrow in these diagrams is associated with a variable that is changing over time. The arrows are thus not simply defining ‘associations’, plus/minus signs indicating amplification/inhibition but instead they are numbers that are added or subtracted. Towards this end, blocks or subsystems are connected through signals via the following nodes:



For the addition/subtraction node, if there is no sign, a “+” is assumed. These basic building blocks form a de facto standard for graphical modelling of control systems circuits. While the value and use of diagrammatic representations of pathway models and tools to visualize them are discussed, for example⁵, in [10, 11, 1], there are no established standards for pathway maps. Given that we are discussing the value of control concepts in pathway modelling, we hereafter consider a couple of well studied examples of biochemical systems and investigate (a) how control block diagram representations might be used and (b) how a control analyst might incorporate feedback loops in pathway models. A discussion of how the more conventional pathway maps can serve as information organizers and simulation guides is discussed in [12].

4 The Role of Feedback

Differential equations models, such as the control system (2) are particularly suited to study the role of feedback loops. One of the first biologists who recognized the importance of biological feedback was René Thomas [13]. For any process that is to maintain, optimize or adapt a condition or value, information about the ‘is-state’ has to be fed back into the decision on any change that should occur. In other words, *feedback loops* are the basis for any form of regulation and/or control.

Control engineers distinguish between two principal kinds of control systems with different purposes: a) *reference tracking*, and b) *disturbance rejection*. We hereafter refer to the first case, where the system is *sensitive* to inputs, as the ability to make changes as required, e.g., to track or follow a reference signal, as *control*. On the other hand, we refer to *regulation* as the maintenance of a regular or desirable state, making the system *robust* against perturbations. Regulation that maintains the level of a variable is also referred to as *homeostasis*. Here we should distinguish two forms of robustness in a control system. The first is robustness against external disturbances (disturbance regulation). In a biochemical pathway, a disturbance might be caused by unwanted cross-talk from a neighboring signalling pathway. The second form of robustness, is one which tolerates parameter changes in a system, without significantly changing the system performance. Both forms of robustness are important properties in understanding pathways.

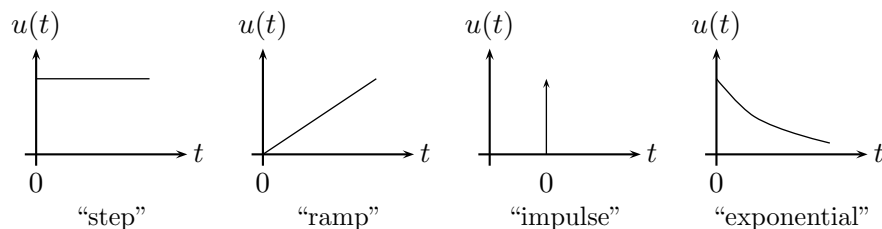


Figure 1: Test signals that can be used to investigate the dynamic behavior of pathways.

A central objective of systems biology is to devise methods that allow the detection and description of feedback loops in pathways [14, 15]. An important result from systems theory is that this is only possible through perturbation studies, where the the system is stimulated with a well defined signal. Unfortunately, experiments in molecular and cell biology are difficult to set up in a way that suits systems-theoretic approaches. A major hurdle for the success of systems biology arises therefore from the need to conduct expensive, time consuming, complex perturbation experiments.

A superficial view of feedback would say that positive feedback is bad (destabilizing) and negative feedback is good (stabilizing). Indeed, the description of the role of feedback often implies that in the absence of negative feedback, a system is unbounded, unstable and not resistant to perturbations. In fact

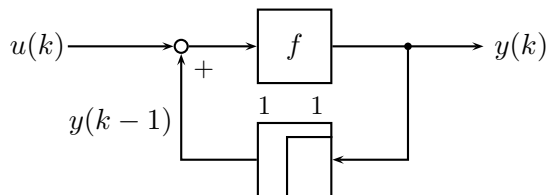
⁵See also http://discover.nci.nih.gov/kohnk/interaction_maps.html

this is not the case, most dynamical systems exist in a stable manner without the need for feedback. A better way in which to describe the role of feedback is as a modifier of the dynamical behavior of a system. Depending upon the nature of the feedback, it can either stabilize, destabilize, sensitize or de-sensitize the behavior of a process. While positive feedback is conventionally associated with destabilization the truth is more complex, and in many circumstances negative feedback can have unwelcome effects. However, in the context of the special dynamical model forms found in pathway modelling, there are certain special dynamical features induced by feedback that are important to understand. The following simple models of accumulation or growth processes will illustrate some of these features as they manifest themselves within cells.

As an initial demonstration of the features associated with feedback, consider the simple model of growth (e.g. of a cell or of a population of molecules in the cell). Let $u(k)$ denote the stimulus of the system at time k and y the response. Let us take the view that the present depends not only on the current state but also on the past, leading to a discrete version of a differential equation, called *difference equation*:

$$y(k) = f(y(k-1), u(k)) . \quad (3)$$

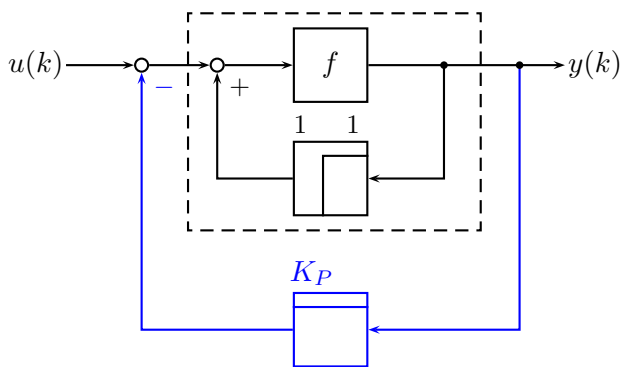
where f describes the detailed functional relationship between the stimulus, the past of y and the current response $y(k)$. One way to illustrate this is by the following block-diagram:



In the diagram the two numbers above the transport delay block denote an amplification of the signal, respectively the unit sampling time delay. For instance, let us look at a linear system, where f is realized by the following law

$$y(k) = u(k) + y(k-1) .$$

For initial conditions $y_0 = 0$, $u_0 = 0$ if we stimulate the system with a step input, $u(k) = 1$ for $k \geq 1$, a simulation reveals a linearly increasing, unbounded signal (Figure 2, left). Whatever the initial conditions, $y_0 \geq 0$, the system is unstable and an unrealistic model for most purposes. Let us therefore see what happens if we add a *negative* feedback loop to the system:



The temporal evolution of the response signal is modelled by the following equation

$$y(k) = (u(k) - K_P \cdot y(k)) + y(k-1) .$$

A simulation reveals a bounded signal (Figure 2, right).

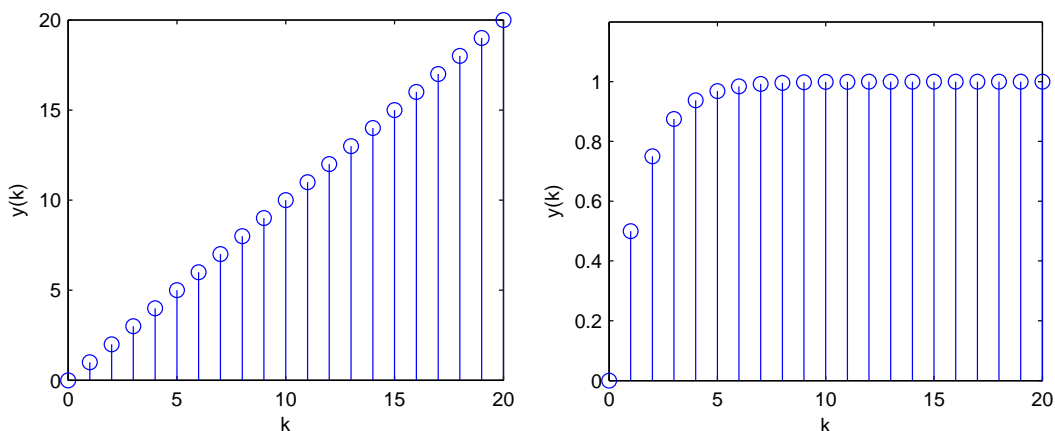


Figure 2: Illustration of the stabilizing effect of a negative feedback loop in a discrete-time system. Left: Unstable system with a positive feedback loop. Right: Negative feedback loop with $K_P = 1$. In both cases the response to unit step input signal is shown.

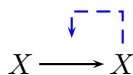
5 Tutorial Examples

In the following we present very simple examples of biochemical reactions, which are subsequently translated into a set of mathematical (differential) equations. These in turn maybe related to a standard positive/negative feedback representation drawn from control engineering. In general, we say a component or variable of a system is subject to *negative feedback* when it inhibits its own level of activity. For example, a gene product that acts as a repressor for its own gene is applying negative feedback. Likewise, a component of a system is subject to *positive feedback* when it increases its own level of activity. Through these examples we are going to review the concepts of the biochemist’s reaction equation, pathway maps, differential equations and block diagrams.

Returning to our proteolysis example from the introductory section, we generalize it in the context of the framework outlined above. Consider a simple monomolecular reaction where chemical species X is transformed. The change in concentration of X at time t depends on the concentration of X at time t in that the rate by which the reaction proceeds is proportional to the concentration at each time instant,

$$\frac{dx(t)}{dt} \propto x(t)$$

with a certain positive *rate constant* k . A diagrammatic representation of this biochemical process illustrates the fact that chemical species X “feeds back” on itself:



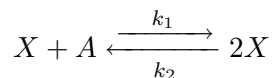
A linear mathematical ODE model of the process is given by

$$\frac{d}{dt}x(t) = k \cdot x(t) .$$

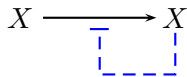
Here X acts as a *substrate* being converted and the *product*. There is *positive feedback* in that the larger the product X , the greater the rate of change by which substrate X is transformed. A simulation of this system reveals the expected unbounded growth in the concentration of X ,

$$x(t) = x_0 \cdot e^{kt} ,$$

where $x_0 = x(t = 0)$ denotes the initial condition. With increasing x , the growth rate dx/dt also increases in this system, leading to an unbounded growth. Next we look at the *autocatalytic* reaction



where for a given X molecule, A facilitates the doubling. A pathway map of this process would be



In pathway maps we use a bar at the end of the arrow to denote an inhibition or negative feedback loop. If A is considered to have a constant concentration, generalizing the law of mass action, we arrive at the following differential equation model:

$$\begin{aligned} \frac{d}{dt}x(t) &= k_1ax(t) - k_2x^2(t) \\ &= k_1ax(t) \left(1 - \frac{k_2}{ak_1}x(t)\right) . \end{aligned}$$

Why we rewrote the equation in the form given in the second line will be clarified below. In this autocatalytic reaction the ‘product’ has a strong inhibitory effect on the rate at which X is transformed. In order to indicate the internal feedback mechanisms at work in this system, we will label the right-hand bracketed term $(1 - k_2x(t)/(ak_1))$ as a control input variable $u(t)$

$$\frac{d}{dt}x(t) = k_1au(t)x(t) .$$

Because of the product term on the right-hand side this equation is also referred to as a model of a *bilinear system*. If we consider variable x to represent the state of the system, and we write $dx(t)/dt = \dot{x}$ for short, this system becomes an example of (2), in particular:

$$\begin{aligned} \dot{x} &= f(x) + g(x)u , & x(t_0) &= x_0 , \\ y &= h(x) . \end{aligned}$$

We can alternatively write:

$$u(x) = \alpha - \beta x ,$$

where the constant α is called the *intrinsic growth rate* of the population and α/β corresponds to the maximum attainable population. The model we thus obtain is specified by the equation

$$\begin{aligned} \frac{dx}{dt} &= \alpha x \left(\frac{\alpha/\beta - x}{\alpha/\beta}\right) \\ &= \alpha x(t) \left(1 - \frac{\beta}{\alpha}x(t)\right) . \end{aligned} \tag{4}$$

This model form is called the *logistic growth model* and is equivalent to the autocatalytic reaction introduced above. The model describes the real growth rate as a proportion of the intrinsic growth rate. This proportion however decreases with an increase in the population, leading to a more realistic scenario of a system that remains within bounds (Figure 3). Both previous examples, echo the observations made in the discrete-time example of a simple growth process with added negative feedback.

For two molecular species we can generalize the control of the system into

$$\begin{aligned} \dot{x}_1 &= u_1(x_1, x_2)x_1 , \\ \dot{x}_2 &= u_2(x_1, x_2)x_2 . \end{aligned}$$

If we specify for u_1 and u_2 ,

$$\begin{aligned} u_1(x_1, x_2) &= k_1a - k_2x_2 , \\ u_2(x_1, x_2) &= k_2x_1 - k_3 , \end{aligned}$$

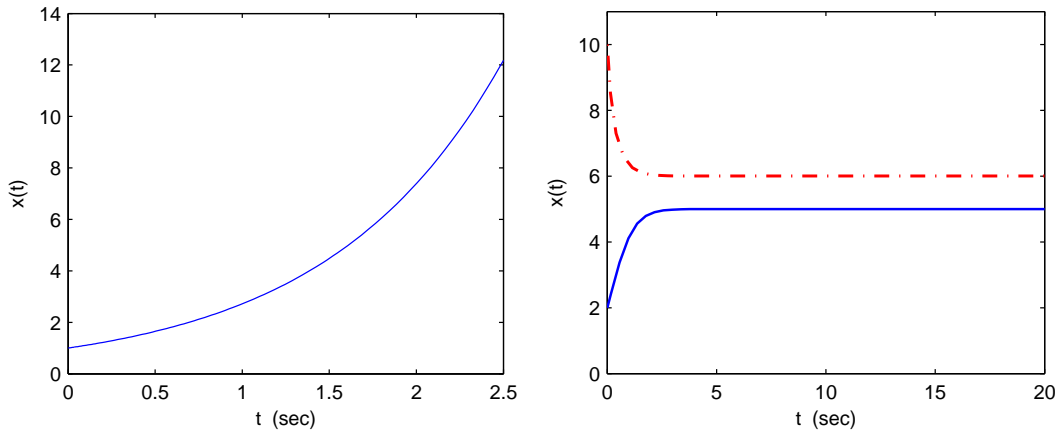
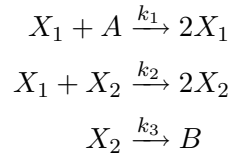
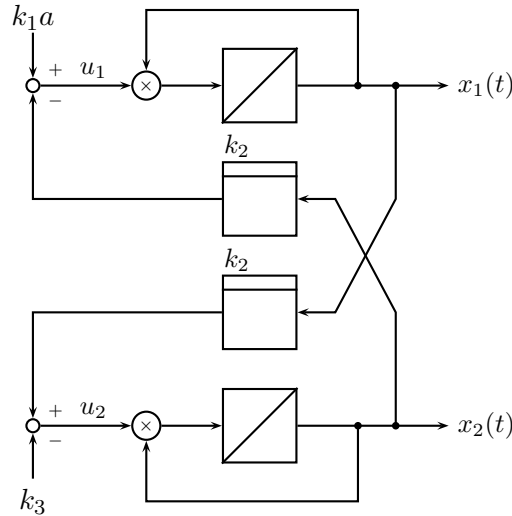


Figure 3: Unbounded and limited growth. Left: Simulation of the monomolecular reaction with positive feedback. Right: Simulation of an autocatalytic reaction (logistic equation) with negative feedback. For the solid line $x_0 = 2$, $a = 2$, $b = 1/2.5$ and for the dashed line $x_0 = 10$, $a = 2$, $b = 1/3$.

we obtain the well known *Lotka-Volterra model* of two competing populations. If variables x_1 and x_2 correspond to the chemical species X_1 and X_2 , the biochemical representation of this system is

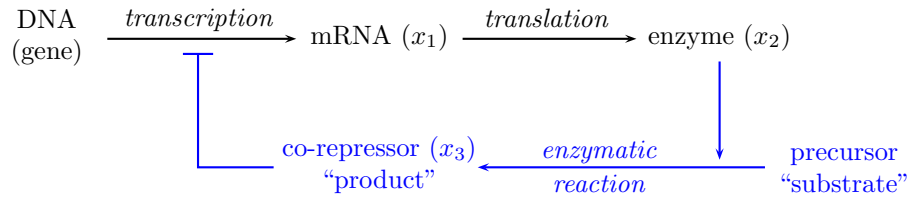


where A is maintained at a constant concentration and B corresponds to the degradation of X_2 . The first two reactions are autocatalytic. Compared to the limited growth model from above, this system is capable of showing oscillatory behavior. The block diagram for the Lotka-Volterra model can be drawn directly from those equations:



The Lotka-Volterra model of competing species gives an opportunity to discuss the purpose of mathematical models as a mechanism for illuminating basic principles, while not necessarily describing the details of a particular case. Specifically, the Lotka-Volterra model would nowadays be considered an *unrealistic* model for modelling animal population dynamics. However as an abstraction it has proven very *useful*, helping scientists to establish a conceptual approach and ask the right questions [16]. It is in this spirit that models of intracellular dynamics are, or should be, developed in systems biology. The systems considered here are frequently used for an introduction to differential equations. The prototypical biological example of

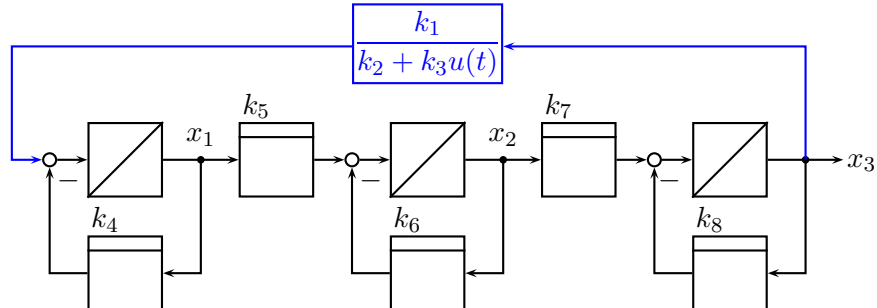
a regulatory system is the protein synthesis model of Jacob and Monod [17]. The conceptual model explains how the production of mRNA (x_1), is feedback controlled by a repressor (x_3). A simplified pathway map of this process is shown in the following diagram:



A differential equation model of this regulatory mechanism of protein synthesis is:

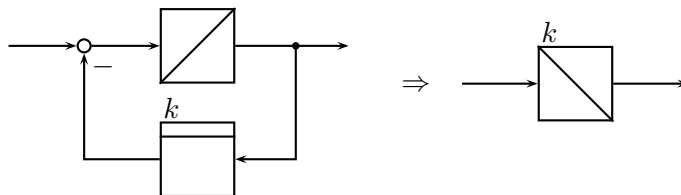
$$\begin{aligned} \frac{d}{dt}x_1 &= \frac{k_1}{k_2 + k_3x_3(t)} - k_4x_1(t) \\ \frac{d}{dt}x_2 &= k_5x_1(t) - k_6x_2(t) \\ \frac{d}{dt}x_3 &= k_7x_2(t) - k_8x_3(t) . \end{aligned}$$

For each of these equations, the last term describes degradation of the molecules. k_5 is the rate of synthesis for the protein that facilitates the production of the co-repressor. Note that there is no minus sign to indicate the negative feedback as in previous examples. The greater x_3 in the numerator of the first term of the rate equation for x_1 , the smaller its contribution towards the rate of change of x_1 . In contrast to the previous example where the feedback was linear, i.e., a simple additive or negative term, in this example the feedback is nonlinear. To illustrate the use of block-diagrams more clearly, let us consider the block-diagram for the Jacob-Monod model of protein synthesis.

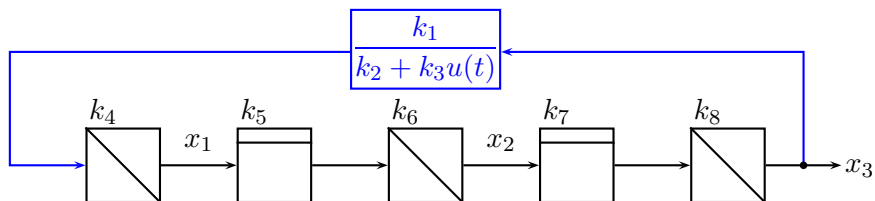


We are now alerted to the fact that *negative feedback* does not necessarily coincide with an explicit form of *negative feedback loop*. Specifically, we have in the block diagram arbitrarily chosen to arrange the figure such that $x_3(t)$ appears as the term fed-back to $x_1(t)$ and that because of the nonlinear form of the feedback it will in fact for small perturbations be negative. The arbitrary nature of the feedback variable is because there is no explicit control input. In such autonomous systems, it is the physical/biological structure that will determine what we (the analyst) chose to call the feedback signal. When the differential equation for x_1 is linearized by Taylor series expansion the $x_3(t)$ appears as a negative feedback term. Whether or not linearization is feasible depends on the system considered. For a more comprehensive discussion of this system and further references we refer to [16].

In the block diagram above we have also noticed that degradation is represented by an integrator with a negative feedback loop around it. This motif we can summarize into a single block:



Note that this is not just an arbitrary graphical simplification, the inner structure of the block remains unambiguously defined. That is, we do not lose information or accuracy in presentation by scaling the block diagram in this way. Finally, the protein synthesis model can be simplified to



Although the systems we have considered here are fairly simplistic, the consequences of feedback loops we have observed remain akin for more complex processes. For a related instructive discussion of the dynamic systems approach in biochemical pathway modelling we recommend [18, 19, 20].

6 Discussion and Conclusions

Although a pathway or pathway map describes molecules, their physical state and interactions, it is an *abstraction*, with no physical embodiment. A pathway map is thus a model; which proteins and what physical states of the molecules should be considered for experiments and the model is what we call the *art* of modelling.

Feedback loops are the essence of control and regulation, for only if information about the consequences of some output is fed back, the system can adjust itself or respond in an appropriate way. Using ordinary differential equations to model biochemical networks, we have shown that feedback loops can stabilize and destabilize a system, keep its variables and signals bounded, they can make the system robust against perturbations, they allow the system to adapt to changes, or track an input stimulus.

Another relevant feature of control systems is that they have specific intent, and control systems analysts have theories for understanding intent and methods for achieving a required intent or purpose [21]. In a modelling framework, the causal structure of a control system provides a framework for the dynamical manipulation of information with a purposeful objective. This is topical and relevant in the light of recent discussion of the value of systems biology compared with mathematical biology [22]. In this same spirit, feedback loops lie at the heart of the causal/purposeful mechanisms of control and regulation in dynamic systems. Specifically, it is only if information about the consequences or some output is fed back, can the system automatically adjust itself or respond in an appropriate way. Feedback is not always beneficial, for feedback loops can stabilize or destabilize a system. Feedback can keep a system's variables and signals bounded, or it can induce oscillations or unbounded growth. Likewise, feedback loops can make a system robust against perturbations, but at the same time they allow the system to adapt to changes, or track an input stimulus.

Apart from the role of feedback loops, the text has tried to survey alternative and complementary representations and visualizations, including the biochemist's reaction equations, the mathematician's differential equation models, the control engineer's block diagrams and the biologist's pathway maps. Block diagrams are well established in the engineering sciences as a means of describing dynamic systems in general. Through the integrators used, these diagrams are inherently linked to differential equation models and are therefore less general than those molecular interaction maps [11], commonly used to visualize relationships in pathways. On the other hand, block diagrams are a direct and unambiguous visualization of the mathematical

model. These diagrams also do not explicitly represent spatial aspects. While the transport of a protein from the nucleus to the cytosol can be modelled, compartments are realized by introducing more than one variable in the model for the same molecular species in different regions of the cell. For the analysis of the nonlinear differential equations models we only used time plots. Visualization is no less important to theoreticians than it is to biologists and so there are a range of tools available we have not mentioned here, including stimulus-response curves, phase-plane and bifurcation analysis (e.g. [23, 24, 16, 20]). For an application of these mathematical tools applied to a model of the yeast cell cycle, we refer to the expositions of Novak and Tyson [25, 26, 20]. The building block approach to an understanding of systems, when associated with purpose, is very similar to the causality principles that are embedded in the dynamical system modelling methods of control engineering. One question we investigated here was whether the control engineer's proficiency with block diagram models and modular representations can contribute to systems biology by facilitating the translation of biological concepts into mathematical representations.

The cell is made up of molecules, like a car is made up from plastic and metal. But a soup of molecules is no more a cell than a heap of plastic and metal is a car. To understand the *functioning* and *function* of a cell we need to know the *relations* and *interactions* of the components that constitute it. If the central dogma of systems biology is that *it is dynamics that determines biological function*, we would refine this statement and argue that *the dynamical manifestation of feedback determines the development and maintenance of biological processes*.

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