A Frequency Domain Approach to Sensitivity Analysis of Biochemical Networks

Brian P. Ingalls*

Department of Applied Mathematics, University of Waterloo, Waterloo, Ontario N2L 3G1 Canada

Received: August 28, 2003; In Final Form: October 30, 2003

Steady state sensitivity analysis, as exemplified by work in metabolic control analysis and biochemical systems theory, has proved a potent tool in the analysis of biochemical networks. A limitation of this method is that it treats the response of a system only under step perturbations. Extension of these results to arbitrary disturbances, as addressed in this paper, can be carried out directly using the frequency domain tools of signal processing and control theory. This analysis, while it remains linear (and hence local), allows improved theoretical understanding as well as new ways of describing the behavior of systems of interest. The theory can be interpreted as a natural extension of metabolic control analysis and as such provides generalizations of the summation and connectivity theorems. The results are illustrated by application to some simple biochemical feedback networks.

1. Introduction

Much work has been devoted to determining the responses of biochemical networks to changes in their environment or their internal components. These studies have been motivated both by direct application to metabolic engineering and pharmaceutics as well as by the desire to improve our understanding of the behavior of these systems.

This sensitivity analysis has focused primarily on the steady state (i.e., asymptotic) response of a system to constant (i.e., step) changes in parameters. This is in part due to the relative difficulty of obtaining experimental data on time-varying behavior as opposed to measurements of steady states. Moreover, in many cases steady-state analysis is of primary interest; mechanisms that are under homeostatic control tend to exhibit uniform behavior, with only small and often insignificant transients. In such cases, steady-state analysis provides a perfectly adequate description of system behavior.

However, there are cases in which a dynamic analysis of system response is crucial. This is clearly the case for mechanisms whose nominal behavior is time-varying, e.g., the cell cycle. Furthermore, even systems for which steady-state behavior is the norm exhibit dynamic responses to time-varying perturbations, and such nonconstant disturbances are ubiquitous in the cellular environment. Investigation of the transient behavior invoked in signal transduction networks or the role of Ca^{2+} oscillations as a second messenger demands a dynamic analysis. This paper represents a step toward extending the classical steady-state sensitivity analysis to this more general case.

As mentioned, the lack of understanding of dynamic responses is in large part due to experimental difficulties in dealing with time-varying systems. This situation is improving, as new technologies are making measurements of time course data easier to obtain. In addition, now that the molecular mechanisms underlying these networks are being revealed in sufficient detail, faithful computational models are being developed that can probe a network's dynamic behavior.

Analytic tools for the study of the sensitivity of biochemical systems have been developed within the fields of metabolic control analysis (MCA)[1−3] and biochemical systems theory (BST)[4,5]. The analysis is carried out in a linear (or log-linear) regime in which only small perturbations are addressed. This restriction is necessary since it is only after linearization that the analysis becomes tractable. In particular, the main results of MCA − the summation and connectivity theorems − are valid only in the domain of this local analysis.

The same approach is taken in this paper: the linearized response of a biochemical system is considered. The sensitivity analysis is extended by considering the response not just to constant parameter changes but also to time varying perturbations. This is achieved through a frequency domain analysis which describes the response of the system to a canonical set of inputs. The response to arbitrary perturbations can be reconstructed from this analysis by use of the Fourier transform.

This analysis can be interpreted as an extension of MCA by defining control and response coefficients as functions of the frequency of the oscillatory perturbation. The stoichiometric nature of the network imposes constraints on the system behavior which can be expressed by generalizations of the theorems of MCA.

Other generalizations of MCA to time-varying behavior have appeared in the literature. Analysis of time-varying sensitivity has been carried out.[6−10] The work presented in refs 11 and 12 is closely related to this paper, as it makes use of Fourier analysis to treat oscillatory behavior. In those papers the authors investigate the sensitivity of a forced oscillating system to step changes in its parameter values, which is an orthogonal approach to that taken here. In the current work, the system is assumed to be at steady state in the absence of disturbances, and the response to time varying perturbations is examined. A similar analysis has been carried out in ref 13.

This paper is organized as follows. The linearized model of a stoichiometric network is introduced in section 2. This model is interpreted as an input-output system in section 3. In section 4 the frequency response of the system is addressed. Generalizations of the theorems of MCA are reported in section 5. Section 6 describes applications of the analysis to some simple models. Finally, a brief discussion of the results and relations to other work is presented in section 7.

* E-mail: bingalls@math.uwaterloo.ca

10.1021/jp036567u CCC: $27.50
Published on Web 12/19/2003

2. Linear Model of a Stoichiometric Network

A key feature that differentiates the study of biochemical networks from general dynamical systems is their stoichiometric structure. In the results that follow, the terminology and examples will be drawn from biochemical applications, but the analysis applies to stoichiometric systems in general.

Consider a stoichiometric network

$$\dot{\mathbf{s}}(t) = \mathbf{N}\mathbf{v}(\mathbf{s}(t), \mathbf{p}(t)) \quad \text{for all } t \geq 0 \tag{1}$$

where $\mathbf{s}$ is an n-vector of states, $\mathbf{p}$ is an m-vector of parameters and $\mathbf{v}(\cdot,\cdot)$ is an r-vector valued function. These describe the species concentrations, internal and external parameters, and reaction rates, respectively. The $n \times r$ matrix $\mathbf{N}$ describes the stoichiometry of the system. We assume that for each initial condition and for each piece-wise continuous parameter history $\mathbf{p}(\cdot)$ the unique solution of eq 1 is defined for all $t \geq 0$.

Biochemical systems regularly exhibit structural conservations (e.g., conserved moieties) which make some of the species concentrations dependent on others. In such cases it is prudent to eliminate such dependent species before proceeding with any analysis. Following ref 14 (see also ref 15), we note that each structural conservation manifests itself as a row of $\mathbf{N}$ which is linearly dependent upon the others. Removing such rows from $\mathbf{N}$ leads to a reduced stoichiometry matrix $\mathbf{N}_R$, from which $\mathbf{N}$ can be recovered through the link matrix $\mathbf{L}$:

$$\mathbf{N} = \mathbf{L}\mathbf{N}_R$$

The states that correspond to the rows of $\mathbf{N}_R$ form the vector of independent species $\mathbf{s}_i$. The full set of species concentrations can be recovered from $\mathbf{s}_i$ through the relation

$$\mathbf{s}(t) = \mathbf{L}\mathbf{s}_i(t) + \mathbf{T} \tag{2}$$

with an appropriate choice of the constant vector $\mathbf{T}$. The reduced system is then given by

$$\mathbf{s}_i(t) = \mathbf{N}_\mathbf{R}\mathbf{v}(\mathbf{L}\mathbf{s}_i(t) + \mathbf{T}, \mathbf{p}(t)) \tag{3}$$

All analyses can be restricted to this simplified system since the extension to the original network follows immediately through eq 2.

Local analysis of system (3) will be carried out in the neighborhood of a steady state $(\mathbf{s}_i^0, \mathbf{p}^0)$ of interest. This point is brought to the origin by a change of variables in the states: $\mathbf{x}(t) = \mathbf{s}_i(t) - \mathbf{s}_i^0$, and in the parameters: $\mathbf{u}(t) = \mathbf{p}(t) - \mathbf{p}^0$. The n-vector $\mathbf{x}$ and the m-vector $\mathbf{u}$ indicate the deviation from the nominal state and parameter values of eq 3, respectively. The linearized system then takes the form

$$\dot{\mathbf{x}}(t) = \left[\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right] \mathbf{x}(t) + \left[\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{p}}\right] \mathbf{u}(t) \tag{4}$$

where the derivatives are taken at $(\mathbf{s}^0, \mathbf{p}^0) = (\mathbf{L}\mathbf{s}_i^0 + \mathbf{T}, \mathbf{p}^0)$. By construction, this linearized system has steady state $(\mathbf{x},\mathbf{u}) = (0,0)$.

The behavior of the original system (eq 3) is approximated by the behavior of the linearized system eq 4 near the nominal operating point. In particular, the linearized model faithfully represents the response of the original system to small changes in the parameters (i.e., functions $\mathbf{u}(\cdot)$ which remain near zero). Standard sensitivity analysis involves gauging the response of system (4) to constant (i.e., step) changes in the parameter levels. In extending this analysis to nonconstant perturbations, it is useful to introduce the terminology used in systems and control theory for analyzing such systems.

3. Input-Output Systems

The standard model of a linear time-invariant input-output system has the form

$$\dot{\mathbf{x}}(t) = \mathbf{A}\mathbf{x}(t) + \mathbf{B}\mathbf{u}(t) \quad \text{for all } t \geq 0 \tag{5}$$

where $\mathbf{x}$ is an n-vector, $\mathbf{u}$ is an m-vector, and $\mathbf{A}$ and $\mathbf{B}$ are matrices of dimension $n \times n$ and $n \times m$, respectively. The linearized model (eq 4) takes this form with

$$\mathbf{A} = \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\bigg|_{\mathbf{s}=\mathbf{s}^0,\mathbf{p}=\mathbf{p}^0}$$

and

$$\mathbf{B} = \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{p}}\bigg|_{\mathbf{s}=\mathbf{s}^0,\mathbf{p}=\mathbf{p}^0}$$

The components of the input vector $\mathbf{u}$ can play a number of roles in the system. In control engineering, three of the most common are: reference input, control input, and disturbance.

A reference input provides an external signal which the system is expected to track. This reference could be either constant (e.g., the set-point of a thermostat) or time-varying (e.g., the prescribed trajectory of a missile). The input to information-processing systems often plays such a role. For example, ligand binding is a reference input for many signal transduction networks − the associated cellular activity should track the ligand level in an appropriate manner.

A control is an input that is manipulable in some way. System design typically leads to feedback laws acting through control channels, whereby the control input is chosen as a function of the state: $\mathbf{u}(t) = \boldsymbol{k}(\mathbf{x}(t))$. In biochemical systems, experimentally manipulable parameters can be considered as control inputs. A more subtle analogy comes from the conceptual division of systems into subnetworks acting on one another. For example, thinking of the influence of genetic networks on metabolism as the action of a controller on a system, one can ask what measure of authority the controlling system possesses and what sort of feedback has been implemented.

A disturbance input can be included in the model as an attempt to incorporate the effect of external perturbations on the system. Engineers typically design systems with these effects in mind; control laws are chosen based on their ability to attenuate the effect of harmful disturbances. The ability of biochemical networks to continue their function in the presence of perturbations is a central feature of life. This homeostatic regulation is achieved through the appropriate use of feedback control.

In system analysis and design, it is often the case that certain functions of the state and input are of specific interest. These are defined as the system outputs. A common example is an output consisting of a single component of the state vector, e.g., the concentration of a particular molecular species. In the linear system framework, an output vector $\mathbf{y}$ is included by appending the definition

$$\mathbf{y}(t) = \mathbf{C}\mathbf{x}(t) + \mathbf{D}\mathbf{u}(t) \tag{6}$$

to eq 5, where $\mathbf{C}$ and $\mathbf{D}$ are matrices of appropriate dimensions.

In addressing biochemical systems, there are several outputs that may be of interest, including species concentrations, reaction rates, pathway fluxes, transient times, and rates of entropy

production (cf. section 5.8.1 of ref 16). In what follows, two output vectors of primary interest will be addressed.

The first is the vector of independent species concentrations, or more precisely, the deviations of these concentrations from the nominal level. In the linearized model (eq 4), these deviations are described by the state **x**. This choice of output is thus characterized by

$$\mathbf{y}(t) = \mathbf{x}(t)$$

which is eq 6 with $\mathbf{C} = \mathbf{I}$ (the $n \times n$ identity matrix) and $\mathbf{D} = \mathbf{0}$.

The second output of interest is the vector of reaction rates. Again, it is the deviation from the nominal rates that is the natural choice for **y**. This is approximated by the linearization of the reaction rate function $\mathbf{v}(\cdot,\cdot)$ at the nominal point as follows:

$$\mathbf{y}(t) = \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\mathbf{x}(t) + \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \mathbf{u}(t)$$

where the derivatives are evaluated at $(\mathbf{s}^0, \mathbf{p}^0)$. This output takes the form of eq 6 with $\mathbf{C} = \partial\mathbf{v}/\partial\mathbf{s}\ \mathbf{L}$ and $\mathbf{D} = \partial\mathbf{v}/\partial\mathbf{p}$.

4. Frequency Response

Sensitivity analysis is concerned with determining the steady-state response of a system to constant (i.e., step) disturbances, e.g., an instantaneous change in the activity of an enzyme from one constant level to another. Extending that analysis to the determination of the asymptotic response to arbitrary time-varying perturbations may seem a daunting task. Indeed, this is an intractable problem in general. However, when restricting to linear systems, a satisfactory result can be achieved.

There are two features of linear systems that allow this analysis. The first is simply the linear nature of their input-output behavior, which implies an additive property; provided the system starts with initial condition $\boldsymbol{x}(0) = \mathbf{0}$ (which corresponds to the nominal steady state of the biochemical network), the output produced by the sum of two inputs is the sum of the outputs produced independently by the two inputs. That is, if input $\boldsymbol{u}_1(\cdot)$ elicits output $\boldsymbol{y}_1(\cdot)$ and input $\boldsymbol{u}_2(\cdot)$ yields output $\boldsymbol{y}_2(\cdot)$, then input $\boldsymbol{u}_1(\cdot) + \boldsymbol{u}_2(\cdot)$ leads to output $\boldsymbol{y}_1(\cdot) + \boldsymbol{y}_2(\cdot)$.

The additive property allows a reductionist approach to the analysis of system response: if a complicated input can be written as a sum of simpler signals, the response to each of these simpler inputs can be addressed separately and the original response can be found through a straightforward summation. This leads to a satisfactory procedure provided one is able to find a family of "simple" functions with the following two properties: (1) the family has to be "complete" in the sense that an arbitrary signal can be decomposed into a sum of functions chosen from the family; and (2) the asymptotic response of a linear system to an input chosen from the family must be easily characterized. The family of sinusoids (sines and cosines) satisfies both of these conditions.

That the set of sinusoids is sufficiently rich to allow any function to be written as a combination of these functions was recognized by Fourier in the early 19th century and is a cornerstone of the theory of signal processing.[17,18] In general, if a function $f(t)$ is periodic with period 2π, then one can write

$$f(t) = a_0 + a_1 \cos(t) + b_1 \sin(t) + a_2 \cos(2t) + b_2 \sin(2t) + \cdots$$

where

$$a_k = \frac{1}{\pi} \int_{-\pi}^{\pi} f(t) \cos(kt)\, dt$$

and

$$b_k = \frac{1}{\pi} \int_{-\pi}^{\pi} f(t) \sin(kt)\, dt$$

That these coefficients can be found so readily is a consequence of the orthogonality of the family of sinusoids. A more concise decomposition can be reached by expressing the sinusoids in terms of exponentials through

$$\cos(t) = \frac{1}{2}(e^{it} + e^{-it}) \qquad \text{and} \qquad \sin(t) = \frac{-i}{2}(e^{it} - e^{-it})$$

where $i = \sqrt{-1}$. Rewriting in this form leads to the decomposition

$$f(t) = \sum_{k=-\infty}^{\infty} c_k e^{ikt} \tag{7}$$

where

$$c_k = \frac{1}{2\pi} \int_{-\pi}^{\pi} f(t) e^{-ikt}\, dt$$

Such a decomposition allows an alternative characterization of the function $f(\cdot)$ in terms of the list of Fourier coefficients ..., c_{-2}, c_{-1}, c_0, c_1, c_2, ... These coefficients describe the frequency content (or spectrum) of the function, recording the "density" of the corresponding sinusoid within the signal $f(\cdot)$.

Functions that are not periodic demand an extended analysis since they cannot be expressed as a sum of sines and cosines at discrete frequencies. Such functions can be expressed as a combination of sinusoids only if components are allowed at all frequencies. The discrete list of frequency content is thus extended to a function $F(\omega)$ that describes the content at each frequency ω. This function is known as the Fourier integral or Fourier transform. The continuum of components expressed in $F(\omega)$ can be "summed" by integration to yield the original function $f(t)$ as follows,

$$f(t) = \int_{-\infty}^{\infty} F(\omega)\, e^{i\omega t}\, d\omega \tag{8}$$

where

$$F(\omega) = \frac{1}{2\pi} \int_{-\infty}^{\infty} f(t)\, e^{-i\omega t}\, dt$$

The analogy between eq 7 and eq 8 is immediate.

In both cases the record of the frequency content of the function (i.e., the transform) is an alternative characterization of the original function. While complete recovery of a signal from its transform involves computation of the integral in eq 8, important aspects of the nature of the signal can be gleaned directly from the graph of the transform. In particular, one can determine what sort of variations dominate the signal (e.g., low frequency or high frequency). As an example, consider the functions $f(t) = 1/(1+t^2)$ and $g(t) = \cos(4t)\ 1/(1+t^2)$ shown in Figure 1. These functions share the same basic "profile". However, $f(t)$ follows a slowly varying path, while $g(t)$ varies much more rapidly. This difference in their nature is evident in comparison of their Fourier transforms, shown in Figure 2. The

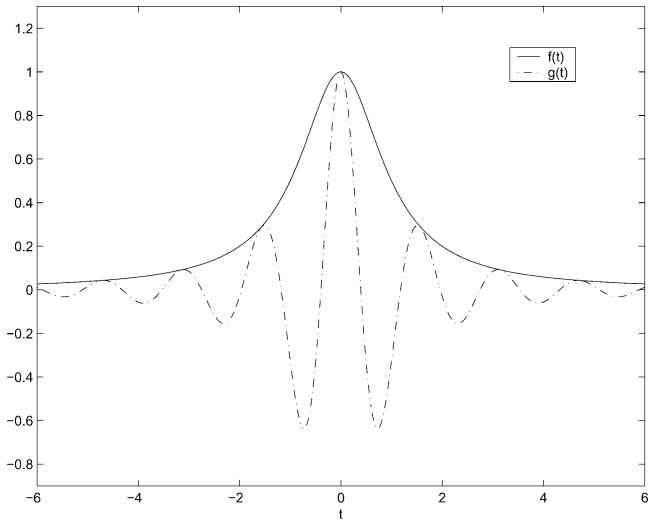

Figure 1. Functions $f(t) = 1/(1+t^2)$ and $g(t) = \cos(4t)\ 1/(1+t^2)$.

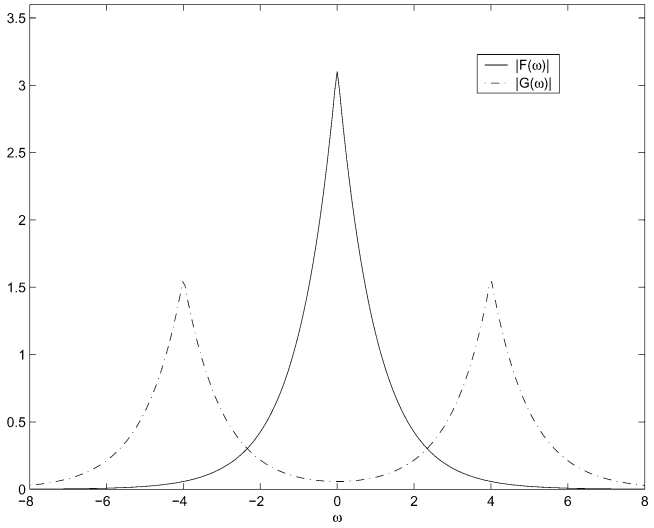

Figure 2. Fourier transforms of $f(t)$ and $g(t)$.

transform of $f(\cdot)$ is dominated by content at near-zero frequencies (corresponding to slowly varying sinusoids), while the content of $g(\cdot)$ is concentrated at $\omega = 4$, which is the frequency of its quasi-periodic behavior. (The introduction of "negative frequencies" is a consequence of the exponential description of the sinusoids. The symmetry of the transform allows attention to be restricted to positive values of ω in what follows.)

Now that it has been shown that any function can be decomposed into sinusoids, and that linearity can be used to exploit such a decomposition, one question still remains. How is it that the response of a system to these "simple" sinusoidal inputs can be recorded in a useful way? After all, the procedure outlined above demands that one "sum" over a continuum of responses to arrive at the response to a given signal. This will be a useful strategy only if the "simple" responses are easily characterized. The second crucial property of linear systems which will be used is that, as mentioned above, their response to sinusoidal inputs can be very easily described. (Indeed, it is this property of sines and cosines which makes Fourier analysis a useful tool for analyzing linear time-invariant systems. There are many other complete and orthogonal families of functions into which one can decompose arbitrary signals as described above, but only the sinusoids produce appropriately convenient outputs from linear systems. The widespread use of Fourier analysis by electrical engineers is not due to the ubiquity of alternating (i.e. oscillatory) electrical signals but rather to mathematical necessity.)

Consider the case of a system for which the input and output are scalars, referred to as a single-input-single-output (SISO) system. For such systems, an oscillatory input elicits an asymptotic response that can be characterized by just two numbers, as follows. The output of a linear SISO system whose input is a sinusoid of frequency ω tends asymptotically to a sinusoid of frequency ω. More precisely, an oscillatory input of the form $u(t) = e^{i\omega t}$ produces an output $y(t)$ which converges asymptotically to $Ae^{i(\omega t + \phi)}$. This asymptotic response can be described by two numbers: A, the amplitude of the oscillatory output, known as the system gain; and ϕ, the phase of the oscillatory output, known as the phase shift. For systems that are not SISO, there is one such pair of numbers that characterizes the response of each output "channel" (i.e., component) to each input channel. In the current context, this extension is simply a matter of bookkeeping.

The conclusion of this discussion is that the behavior of the system can be completely described by the values of the gain A and phase shift ϕ at each frequency ω. These two numbers can be conveniently described in terms of a single complex number $Ae^{i\phi}$ with modulus A and argument ϕ. The system, then, can be characterized by a complex-valued function defined over all frequencies. This function, called the frequency response of the system, can be derived through an algebraic calculation involving the Laplace transform of the system, cf., e.g., ref 12. (The Laplace transform is an extension of the Fourier transform, see e.g., ref 18.) The frequency response for the system described by eq 5 with output given by eq 6 is

$$\mathbf{H}(i\omega) = \mathbf{C}(i\omega\mathbf{I} - \mathbf{A})^{-1}\mathbf{B} + \mathbf{D}$$

for all real ω. This function will in general be matrix-valued but is scalar-valued in the SISO case. The frequency response is usually derived as the restriction of a function of a complex variable z to the imaginary axis. The complete function $\mathbf{H}(z)$ is known as the transfer function of the system.

Considering the two outputs of interest indicated above, the corresponding frequency response functions are found as follows. For the independent species concentration output, we have $\mathbf{C} = \mathbf{I}$ and $\mathbf{D} = \mathbf{0}$, and so the frequency response takes the form

$$\mathbf{H}_{s_i}(i\omega) = \left(i\omega\mathbf{I} - \mathbf{N}_R\frac{\partial \mathbf{v}}{\partial \mathbf{s}}\right)^{-1}\mathbf{N}_R\frac{\partial \mathbf{v}}{\partial \mathbf{p}} \tag{9}$$

For the reaction rate output, $\mathbf{C} = \partial\mathbf{v}/\partial\mathbf{s}\ \mathbf{L}$ and $\mathbf{D} = \partial\mathbf{v}/\partial\mathbf{p}$ give

$$\mathbf{H}_{v}(i\omega) = \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\left(i\omega\mathbf{I} - \mathbf{N}_R\frac{\partial \mathbf{v}}{\partial \mathbf{s}}\mathbf{L}\right)^{-1}\mathbf{N}_R\frac{\partial \mathbf{v}}{\partial \mathbf{p}} + \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \tag{10}$$

These expressions define matrix-valued frequency responses of systems that have multiple output and input channels. Each element of such a matrix-valued function is a scalar-valued function that describes the response of one output channel to one input channel. For each such input/output channel pair, the complex-valued function that describes the system behavior can be plotted in a number of ways. Perhaps the most useful of these visualizations is the Bode plot, in which the magnitude and argument of the frequency response are plotted separately. The magnitude of the function value (the system gain) is plotted on a log−log scale, where the gain is measured in decibels (dB) (defined by x dB $= 20\log x$). The argument of the function value (the phase shift) appears on a semilog plot, with log

frequency plotted against phase in degrees. Examples of Bode plots are shown in section 6.

The response of a system to a constant input (which can be thought of as a sinusoid with frequency zero) is characterized by the frequency response at $\omega = 0$. Making this substitution into eqs 9 and 10, the response of the system is found as

$$\mathbf{H}_{s_i}(0) = -\left(\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{p}}$$

and

$$\mathbf{H}_{v}(0) = -\frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L} \left(\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \mathbf{L}\right)^{-1} \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{p}} + \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \qquad (11)$$

These expressions can be derived from a standard sensitivity analysis of eq 3. A framework for such an analysis is provided by MCA, which is addressed in the next section.

5. Metabolic Control Analysis

The expressions of system sensitivity in eq 11 are called "response coefficients" in MCA. This definition can be generalized to address the entire frequency response as follows. The (frequency dependent) unscaled concentration response coefficients of the system described by eq 3 are the elements of the matrix function

$$\mathbf{R}_{s_i}(\omega) := \mathbf{H}_s(i\omega) = \left(i\omega \mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \qquad (12)$$

The (frequency dependent) unscaled rate response coefficients of the system described by eq 3 are the elements of

$$\mathbf{R}_{v}(\omega) := \mathbf{H}_{v}(i\omega) = \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L} \left(i\omega\, \mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} \frac{\partial \mathbf{v}}{\partial \mathbf{p}} + \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \qquad (13)$$

Further, define the (frequency dependent) unscaled concentration control coefficients and rate control coefficients as the elements of

$$\mathbf{C}_{s_i}(\omega) = \left(i\omega \mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1}$$

and

$$\mathbf{C}_{v}(\omega) = \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L} \left(i\omega \mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} \mathbf{N}_R + \mathbf{I} \qquad (14)$$

These response coefficients exhibit the frequency response of the system. For nonzero frequency ω, these coefficients will in general be complex-valued, describing both the gain and the phase shift of the system response. The control coefficients (which can be thought of as a special case of the response coefficients in which $\partial \mathbf{v}/\partial \mathbf{p} = \mathbf{I}$) describe the response of the system to oscillatory perturbations in the individual reaction rates (since $\partial \mathbf{v}/\partial \mathbf{p} = \mathbf{I}$ implies that there are r parameters, each of which has a direct effect on a single reaction). While the response coefficients are useful in investigating the behavior of the system under external influences, the control coefficients are often more relevant in addressing system design and function.

The definitions given above describe unscaled sensitivities, as opposed to the more commonly used scaled sensitivities of MCA. This "scaling" refers to the treatment of relative, rather than absolute, sensitivities. These relative sensitivities can be recovered from the definitions above through multiplication by a scaling factor. To that end, define the diagonal matrices

$$\mathbf{D}^{s_i} = \mathrm{diag}\,[\mathbf{s}_i^0] \qquad \mathbf{D}^{v} = \mathrm{diag}\,[\mathbf{v}(\mathbf{s}^0, \mathbf{p}^0)] \qquad \mathbf{D}^{p} = \mathrm{diag}\,[\mathbf{p}^0]$$

as in ref 15. The scaled response coefficients describing the relative sensitivities are then given by

$$\tilde{\mathbf{R}}_{s_i}(\omega) = [\mathbf{D}^{s_i}]^{-1} \mathbf{R}_{s_i}(\omega)\, \mathbf{D}^{p} \qquad \tilde{\mathbf{R}}_{v}(\omega) = [\mathbf{D}^{v}]^{-1} \mathbf{R}_{v}(\omega)\, \mathbf{D}^{p}$$

where the tilde ($\sim$) denotes the scaled coefficient. Scaled control coefficients are defined similarly.

5.1. MCA Theorems. The stoichiometric nature of a biochemical system enforces certain relations on the sensitivities of the system. For step perturbations, such relations are described by the summation and connectivity theorems of MCA. These relations can be extended beyond the $\omega = 0$ case to the frequency response defined above. These generalized theorems are stated below as algebraic conditions involving the control coefficients, following statements found in refs 16 and 17. The statements can be translated into results involving scaled coefficients by the appropriate multiplications.

Theorem 1: Summation Theorem. If a vector $\mathbf{k}$ lies in the nullspace of $\mathbf{N}_R$ (and hence of $\mathbf{N}$), then

$$\mathbf{C}_{s_i}(\omega)\, \mathbf{k} = \mathbf{0} \qquad \text{and} \qquad \mathbf{C}_{v}(\omega)\, \mathbf{k} = \mathbf{k}$$

for all $\omega \geq 0$.

The statement follows directly from the definition of the control coefficients (eq 14). This result can be interpreted in terms of the system response as follows.

Interpretation: If the vector of parameters $\mathbf{p}$ is chosen so that the columns of $\partial \mathbf{v}/\partial \mathbf{p}$ lie in the nullspace of $\mathbf{N}_R$ then the responses are given by

$$\mathbf{R}_{s_i}(\omega) = 0 \qquad \text{and} \qquad \mathbf{R}_{v}(\omega) = \frac{\partial \mathbf{v}}{\partial \mathbf{p}}$$

for all $\omega \geq 0$. This result is immediate from the form of the linearized system (eq 4), since such a parameter $\mathbf{p}$ leads to $\mathbf{B} = \mathbf{N}_R\, \partial \mathbf{v}/\partial \mathbf{p} = \mathbf{0}$. In this case the species concentrations are completely decoupled from changes in $\mathbf{p}$, leading to the output responses indicated.

Theorem 2: Connectivity Theorem. For the control coefficients as described above,

$$\mathbf{C}_{s_i}(\omega) \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L} = -\mathbf{I} + i\omega \left(i\omega \mathbf{I} - \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\right)^{-1}$$

and

$$\mathbf{C}_{v}(\omega) \frac{\partial \mathbf{v}}{\partial \mathbf{s}} = i\omega \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L} \left(i\omega \mathbf{I} - \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1}$$

Proof: We note that since

$$\left(i\omega \mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} \left(i\omega \mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right) = \mathbf{I}$$

it follows that

$$\left(i\omega \mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} i\omega \mathbf{I} - \left(i\omega \mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L} = \mathbf{I}$$

so

$$\left(i\omega\mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L} = -\mathbf{I} + \left(i\omega\mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} i\omega\mathbf{I}$$

which gives the first statement. The second follows directly.

Interpretation: Note that if the parameter vector **p** is chosen so that the columns of $\partial\mathbf{v}/\partial\mathbf{p}$ lie in the span of the columns of $\partial\mathbf{v}/\partial\mathbf{s}$ **L**, then there exists a matrix **M** so that $\partial\mathbf{v}/\partial\mathbf{p} = \partial\mathbf{v}/\partial\mathbf{s}$ **LM**. In this case the system response is described by

$$\mathbf{R}_{s_i}(\omega) = \left[-\mathbf{I} + i\omega\left(i\omega\mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\right)^{-1} \mathbf{L}\right] \mathbf{M}$$

and

$$\mathbf{R}_{v}(\omega) = i\omega \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L} \left(i\omega\,\mathbf{I} - \mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right)^{-1} \mathbf{M}$$

This result is not as easy to interpret as that provided by the summation theorem. It is insightful, at the least, in the limiting case of low-frequency disturbances. For the case $\omega = 0$ the responses are given by

$$\mathbf{R}_{s_i}(0) = -\mathbf{M} \qquad \text{and} \qquad \mathbf{R}_{v}(0) = 0$$

which agrees with the standard statement of the connectivity theorem.[12] Thus, for step disturbances (i.e., $\omega = 0$), the connectivity theorem provides a result orthogonal to the summation theorem in that it indicates which perturbations will affect the species concentrations while leaving the reaction rates unchanged. This conclusion can be extended by observing that for parameters which satisfy the condition above, slowly varying inputs (i.e., consisting of frequencies ω small compared to the eigenvalues of $\mathbf{N}_R\,\partial\mathbf{v}/\partial\mathbf{s}$ **L**) will have only a small effect on the reaction rates.

Considering the form of system described by eq 4 provides an immediate derivation of the standard connectivity theorem (i.e., the $\omega = 0$ case). Under the condition that $\partial\mathbf{v}/\partial\mathbf{p} = \partial\mathbf{v}/\partial\mathbf{s}$ **LM** the system reduces to

$$\begin{aligned}\dot{\mathbf{x}} &= \left[\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right] \mathbf{x} + \left[\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right] \mathbf{M}\mathbf{u} \\ &= \left[\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}} \mathbf{L}\right] (\mathbf{x} + \mathbf{M}\mathbf{u})\end{aligned}$$

If $\mathbf{N}_R\,\partial\mathbf{v}/\partial\mathbf{s}$ **L** is assumed nonsingular, a constant input **u** yields a unique steady state of $\mathbf{x} = -\mathbf{M}\mathbf{u}$, corresponding to the form of the species response $\mathbf{R}_{s_i}(0)$. Moreover, in this case the reaction rate output takes the form

$$\mathbf{y} = \frac{\partial \mathbf{v}}{\partial \mathbf{s}} (\mathbf{x} + \mathbf{M}\mathbf{u})$$

and so is necessarily zero at steady state.

It can be noted that the limiting case of high-frequency oscillations has an interpretation for general perturbations. Considering the definitions of the response coefficients in eqs 12 and 13, we see that

$$\lim_{\omega \to \infty} \mathbf{R}_{s_i}(\omega) = 0$$

and

$$\lim_{\omega \to \infty} \mathbf{R}_{v}(\omega) = \frac{\partial \mathbf{v}}{\partial \mathbf{p}}$$

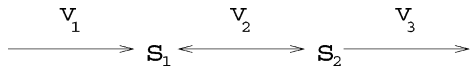

Figure 3. Simple pathway.

These are precisely the responses produced under the summation theorem condition that $\mathbf{N}_R\,\partial\mathbf{v}/\partial\mathbf{p} = 0$, i.e., no effect on the species concentrations and direct effect on the reaction rates. This response to high-frequency oscillation is characterized by the system's bandwidth − the frequency above which the states of the system are insensitive to oscillatory inputs. Perturbations with frequencies above the bandwidth act more quickly than the time scale of the system. Essentially, the state cannot "keep up" with the perturbations, and so reacts only to their average− in this case the zero input.

6. Illustrations of the Frequency Response

The frequency response will now be illustrated by application of the analysis described above to some simple biochemical networks. The first example will be a toy model of an unbranched metabolic chain. This trivial system will serve to demonstrate the theorems of MCA. Next, simple models of two biological processes (tryptophan biosynthesis and regulation of bacterial chemotaxis) will be addressed. These networks employ distinct feedback mechanisms. The effect of this feedback structure on the system's behavior will be considered.

6.1. Illustration of Theorems: Unbranched Metabolic Pathway. Consider the simple network shown in Figure 3 with linear kinetics given by

$$v = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix} = \begin{bmatrix} k_1 \\ k_2 s_1 - k_{-2} s_2 \\ k_3 s_2 \end{bmatrix}$$

Thus

$$\frac{\partial \mathbf{v}}{\partial \mathbf{s}} = \begin{bmatrix} 0 & 0 \\ k_2 & -k_{-2} \\ 0 & k_3 \end{bmatrix}$$

The connectivity theorem can be illustrated by considering changes in the parameter k_2, as $\mathbf{p} = k_2$ yields

$$\frac{\partial \mathbf{v}}{\partial \mathbf{p}} = \begin{bmatrix} 0 \\ s_1 \\ 0 \end{bmatrix}$$

which is a scalar multiple of the first row of $\partial\mathbf{v}/\partial\mathbf{s}$. (Note **L** is the identity matrix in this case.)

With nominal parameter values of $k_1 = 6$, $k_2 = 2$, $k_{-2} = 1$ and $k_3 = 3$, the steady-state concentrations are $(s_1^{ss}, s_2^{ss}) = (4, 2)$. Setting $\mathbf{x} = (x_1, x_2) = (s_1 - 4, s_2 - 2)$, and $\mathbf{u} = k_2 - 2$, the system can be written in the form of eq 4 as

$$\begin{aligned}\dot{\mathbf{x}}(t) &= \left[\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{s}}\right] \mathbf{x}(t) + \left[\mathbf{N}_R \frac{\partial \mathbf{v}}{\partial \mathbf{p}}\right] \mathbf{u}(t) \\ &= \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} 0 & 0 \\ 0 & -1 \\ 0 & 3 \end{bmatrix} \mathbf{x}(t) + \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} -1 \\ 4 \\ -1 \end{bmatrix} \mathbf{u}(t) \\ &= \begin{bmatrix} -2 & 1 \\ 2 & -4 \end{bmatrix} \mathbf{x}(t) + \begin{bmatrix} -4 \\ 4 \end{bmatrix} \mathbf{u}(t)\end{aligned}$$

The asymptotic response of the system to the input $\mathbf{u}(\cdot)$ is described by the connectivity theorem. In particular, for a step

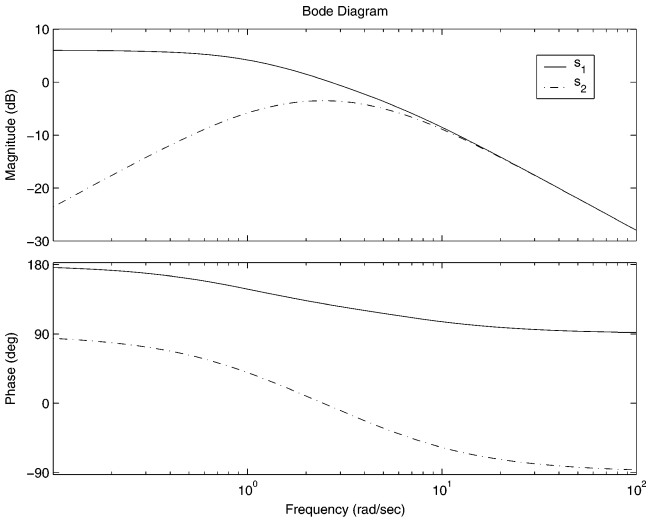

Figure 4. Bode plot of species concentration response.

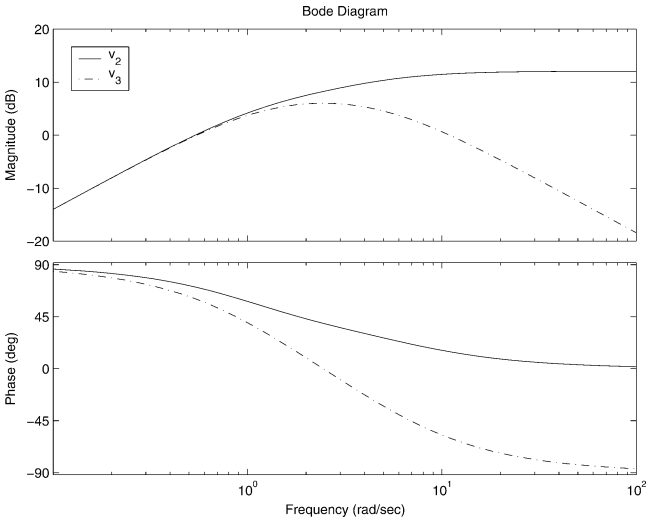

Figure 5. Bode plot of reaction rate response.

increase (i.e., $\omega = 0$) there will be a steady state decrease in s_1 while s_2 and all reaction rates will tend to their nominal steady-state values. This result is evident in the Bode plots for the outputs.

Frequency responses for s_1 and s_2 are shown in Figure 4 (constructed by choosing $\mathbf{C} = [1\ 0]$, $\mathbf{D} = 0$ and $\mathbf{C} = [0\ 1]$, $\mathbf{D} = 0$, respectively). These plots verify the expected behavior of the system. For low-frequency inputs, the concentration of s_1 responds asymptotically with a 180 degree phase shift and a gain of 6.02 dB. This corresponds to the output being -2 times the input (as -2 has argument 180 degrees in the complex plane, and $20\log(2) = 6.02$). As described by the connectivity theorem, the asymptotic response of s_2 to this input tends to zero at low frequencies (and indeed is zero at $\omega = 0$ though this cannot appear on the log scale). Both species concentrations show a reduced response to high-frequency inputs, characterizing the system's bandwidth.

The asymptotic response of the reaction rates is shown in Figure 5 (found by taking $\mathbf{C} = [2\ -1]$, $\mathbf{D} = 4$ for v_2 as output and $\mathbf{C} = [0\ 3]$, $\mathbf{D} = 0$ for v_3). As the theorem indicates, the asymptotic response of both reaction rates vanishes as the frequency tends to zero. At high frequencies, the nature of the response is dominated by the "feedthrough" term $\mathbf{D}$, which is 4 ($= 12.04$ dB) for v_2 and 0 for v_3.

In addition, the theorems describe the response of the system to two other sets of inputs. The connectivity theorem indicates

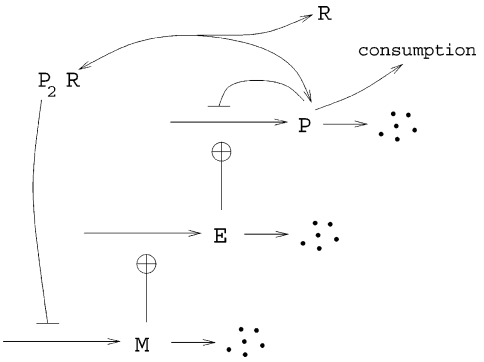

Figure 6. Model of tryptophan biosynthesis.

that if k_{-2} and k_3 are perturbed appropriately, the asymptotic response to low-frequency inputs will be zero for s_1, v_2, and v_3. This can be easily verified, yielding Bode plots that are similar to those presented above. The coordinated input prescribed by the theorem can be achieved by replacing k_{-2} with $k_{-2} + \mathbf{u}$ and k_3 with $k_3 + 3\mathbf{u}$ (so any change in k_{-2} is accompanied by a 3-fold greater change in k_3).

Finally, the effect of the summation theorem can be reached by replacing k_1 with $k_1 + 4\mathbf{u}$, k_2 with $k_2 + \mathbf{u}$, and k_3 with $k_3 + 2\mathbf{u}$. In this case the dynamics are independent of the input, as $\mathbf{B} = \mathbf{0}$. The frequency responses are then constant across all frequencies (0 for species concentrations, 4, 1, and 2 for reaction rates v_1, v_2, and v_3, respectively) as described by the theorem.

The input−output behavior of feedback structures will be considered in the next subsections. In each case, it is the gain of the system which is of primary interest, so attention will be restricted to the magnitude part of the Bode plot. (The phase shift of the system is of major importance in addressing the interconnections of systems, a topic which will not be addressed here.) In the interest of brevity, a single input/output pair will be addressed for each system. A more complete analysis would address the response of several components of the system (outputs) to various inputs and disturbances, the choice of which must be tailored to the particular features of the network that are under investigation.

6.2. Negative Feedback: Regulation of Tryptophan Production. The effect of negative feedback on a system will be illustrated by an analysis of the *trp* operon of bacteria, which is responsible for tryptophan production. A number of models of bacterial tryptophan biosynthesis have appeared in the literature, originating with the work of Goodwin.[19] The model of Xiu et al.[20] will be considered here. (A more complete model, including explicit time delays, has recently appeared.[21])

The model of Xiu et al. involves three state variables: the concentration of tryptophan P; the concentration of mRNA transcribed from the *trp* operon M, and the amount of expressed enzyme E. (It is an abstraction of the model that tryptophan synthesis is catalyzed by a single enzyme.) The dynamics of the model describe production of mRNA, enzyme, and tryptophan, as well as the degradation and dilution (due to cell growth) of each of these species. Cellular consumption of tryptophan is also included. In addition, two negative feedbacks are incorporated. The first is the inhibition of enzyme E by tryptophan. The second is the repression of transcription of mRNA, also tryptophan dependent. This genetic regulation is achieved through the activity of a repressor molecule R which, when bound to two units of tryptophan, interacts with an operator region of the operon, thus blocking transcription. The interactions are shown in Figure 6.

The dynamics are as follows:

$$\frac{dM}{dt} = K_m D \frac{O_t (P + K_d)}{P + K_d + \frac{R_t}{K_o} P} - (K_1 + \mu) M$$

$$\frac{dE}{dt} = K_e M - (K_2 + \mu) E$$

$$\frac{dP}{dt} = K_p E \frac{K_I^2}{K_I^2 + P^2} - (K_3 + \mu) P - 2 k_2 \frac{R_t P}{P + K_d} - (P_{\text{pro}}^m + \beta\mu) \mu C \frac{P}{P + K_s}$$

The parameters in the model are the gene concentration D, the total operator concentration O_t, the total repressor concentration R_t, the growth rate of the cells μ, the maximum protein concentration P_{pro}^m, the influence of growth rate on cellular protein concentration β, the molar percentage of tryptophan in cellular protein C, dissociation constants K_d, K_o, and K_I, a saturation constant K_s, and rate constants K_m, K_e, K_p, K_1, K_2, K_3, and k_2.

Nondimensionalizing and taking appropriate parameter values (see ref 20) leads to the equations

$$\frac{dx(t)}{dt} = \frac{z(t) + 1}{1 + (1 + r)\, z(t)} - 0.909\, x(t)$$

$$\frac{dy(t)}{dt} = x(t) - 0.0293\, y(t)$$

$$\frac{dz(t)}{dt} = y(t) \frac{5210000}{5210000 + z(t)^2} - 0.00936\, z(t) - 0.024 \frac{z(t)}{z(t) + 1} - \alpha_5 \frac{0.00870\, z(t)}{z(t) + 0.00500}$$

where x, y, and z are dimensionless concentrations of mRNA, enzyme, and tryptophan, respectively. The behavior of the system under changes in the value of α_5 will be addressed, with a nominal value of $\alpha_5 = 430$. The effect of the enzyme inhibition on this response will be illustrated by considering two values of the parameter r: strong feedback is exhibited with $r = 10$, while weaker feedback will be addressed by taking $r = 5$. The concentration of tryptophan (x) is taken as the output of the system.

The (magnitude) frequency responses to changes in α_5 are shown in Figure 7. As discussed in ref 20, α_5 describes the effect of cellular demand for tryptophan. The behavior shown in the figure is typical of a negative feedback system. With weak feedback ($r = 5$), the effect of the input on asymptotic tryptophan levels decreases monotonically as the frequency grows larger. Strengthening the feedback (to $r = 10$) has two effects. The first is that the low-frequency response is improved: as a standard sensitivity analysis would show, increasing the feedback reduces the effect of perturbations on the output. The other feature of stronger negative feedback is an increase in sensitivity at higher frequencies—to the point that the feedback actually makes the system *more* sensitive to disturbances over a certain frequency range.

The knowledge that negative feedback can introduce such resonance effects is crucial to the design of feedback systems. The unavoidable tradeoff between improved response at low frequencies and increased sensitivity at higher frequencies can be made explicit (for certain linear systems) by a constraint known as Bode's integral formula.[22] System designers work around this "performance constraint" by implementing feedback which introduces increased sensitivity only at frequency ranges over which the system is unlikely to be excited. One could postulate that the same is true of feedback mechanisms within the cell: they have been crafted by natural selection in such a way that a tradeoff is made between improved response to common low-frequency inputs and amplification of rare disturbances at higher frequencies. (Such an analysis has recently been applied to a model of glycolysis.[23])

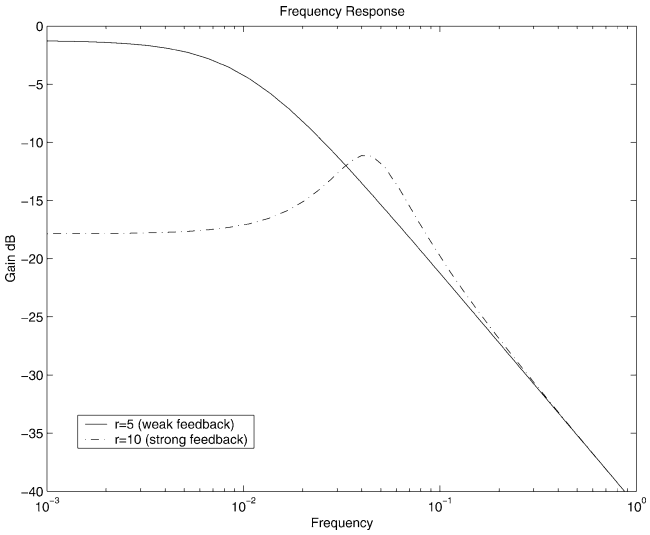

Figure 7. Frequency response for *trp* operon model.

6.3. Integral Control: Regulation of Bacterial Chemotaxis. Last, a system employing integral feedback will be addressed. It was argued in ref 24 that the signal transduction network responsible for regulating bacterial chemotaxis achieves perfect adaptation through negative feedback involving an integral of the regulated variable. This powerful design is a regular feature in engineered feedback systems and no doubt is a common scheme in biochemical regulation. The analysis below is based on a model presented in ref 25. A simplification of the model, which retains the feedback structure, has been derived[26] as shown in Figure 8. The model describes four states for the receptor complex R, which can be bound or unbound by ligand L, and methylated or unmethylated. The reaction rates are given as

$$v_1 = k_1 [\text{R}] [\text{L}] - k_{1m} [\text{RL}]$$

$$v_2 = k_r([\text{R}] + [\text{RL}]) [\text{R}] - k_r a_1^u [\text{Rm}]$$

$$v_3 = k_r([\text{R}] + [\text{RL}]) [\text{RL}] - k_r a_1^o [\text{RmL}]$$

$$v_4 = k_1 [\text{Rm}] [\text{L}] - k_{1m} [\text{RmL}]$$

where $k_r(\cdot)$ is a function which will be defined below. Nominal parameter values are chosen as

$$k_1 = 1000, \quad k_{1m} = 1000, \quad k_b = 1$$

$$a_1^u = 1, \quad a_1^o = 0.25$$

Initial conditions are chosen so that the total receptor complex concentration (which is invariant) is unity. The response to changes in the ligand concentrations will be considered, with a

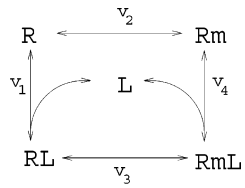

Figure 8. Model of regulation of bacterial chemotaxis.

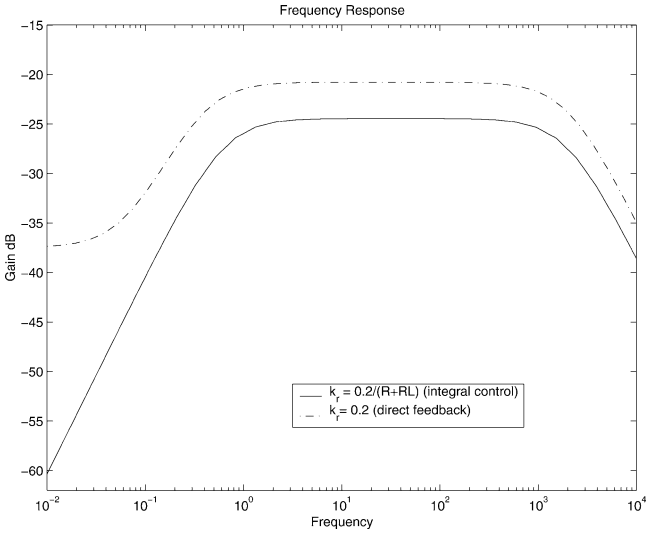

Figure 9. Frequency response of chemotaxis model.

nominal value of [L] = 1. The output of interest is the system activity level, which is taken as

$$A = a_1^u\,[\mathrm{Rm}] + a_1^o\,[\mathrm{RmL}]$$

Two choices for the function $k_r(\cdot)$ will be addressed. As described in ref 25 and ref 24, the system exhibits integral control action provided that the methylation reaction proceeds at saturation. This can be achieved by taking $k_r([R] + [RL]) = 0.2/[R] + [RL]$. In this case a simple calculation shows that

$$\frac{\mathrm{d}}{\mathrm{d}t}([\mathrm{Rm}] + [\mathrm{RmL}]) = -A + 0.2$$

so that the steady-state value of A is clearly independent of [L]. The magnitude Bode plot showing the response in A to changes in [L] is shown in Figure 9. As expected, the response tends to zero at low frequencies and is zero at $\omega = 0$. The response reaches a maximum over a range of frequencies before dropping again at the system's bandwidth.

Alternatively, the methylation reaction could be chosen as nonsaturated. This can be implemented by choosing linear dynamics with $k_r(\cdot) = 0.2$. This choice leads to the second frequency response shown in Figure 9. The behavior at higher frequencies is very similar to that described above. However, at low frequency a nonzero response is exhibited, indicative of negative feedback which does not employ integral action.

Other dynamics can be considered by alternative choices for the function $k_r(\cdot)$. By choosing saturable nonlinear dynamics (e.g., Michaelis−Menten type) one can explore the range between the frequency responses shown here. Such an analysis might capture the "true" nature of the chemotaxis control system. Although the system appears to exhibit an integral controller, there can be no doubt that the implementation is through a "leaky" integrator, and so the behavior will be somewhere between the two extremes considered above.

7. Discussion

As mentioned in the Introduction, the results in this paper join a growing list of generalizations of steady-state sensitivity analysis to time-varying behavior. In the standard analysis, both the perturbation and the response are treated as constant (i.e., the asymptotic response to step inputs is addressed). This treatment can be generalized to include a description of time-varying behavior in either of two ways.

First, the time-varying response of the system to a constant disturbance can be addressed. The result of such an analysis will be a description of the sensitivity of the system as a function of time (e.g., time-varying response coefficients ($\mathbf{R}_s(t)$). To be consistent with the classical analysis, these time-varying responses must converge to the steady-state responses as time gets large. This approach to the study of time-varying behavior has been taken in several papers.[6,11,7−10,27]

Alternatively, one can restrict attention to the asymptotic behavior of the system, but allow time-varying disturbance inputs, as was done in this paper. This is standard practice in the study of linear input−output systems, but has seen only limited use in addressing biochemical networks. One example of a study employing frequency domain techniques is described in ref 13 in which the authors address the filtering properties of biochemical systems in an attempt to elucidate their function. For such analyses to be consistent, the asymptotic response to an input with frequency zero (i.e., $\omega = 0$) must coincide with the steady-state response of classical sensitivity analysis.

The results in this paper provide a means to describe the (linearized) asymptotic response of a biochemical system to arbitrary perturbations. However, in addressing inputs with no regular structure, the analysis must be interpreted carefully, as it may not be straightforward to even *define* the asymptotic response in such cases. Fortunately, there are several classes of disturbances for which the technique provides direct results.

The first is the class of constant (i.e., step) inputs, in which case the frequency domain technique simply recovers the results of steady-state sensitivity analysis at $\omega = 0$. A second natural set of inputs are periodic or near-periodic signals. For example, a perturbation that is nearly sinusoidal has the bulk of its frequency content at a single frequency, say ω_0, and so the response of the system can be described by a single (complex) number (e.g., $\mathbf{R}(\omega_0)$). Periodic behavior is common at both the cellular and multicellular levels (e.g., mitotic, circadian, and Ca^{2+} oscillations[28] and periodic action potentials[29]). The behavior of cellular networks that respond to (or are perturbed by) the resulting periodic signals can be elegantly described by frequency domain analysis.

A more general approach treats classes of inputs that exhibit some regularity in their frequency content. For example, disturbances that are slowly varying have most of their content at low frequencies. Such signals are approximately bandwidth limited. The corresponding system behavior can be discovered by considering the frequency response at low frequencies only. Disturbances that have their content mostly at high frequency or in other frequency bands can be treated similarly. Many biochemical systems lend themselves well to this kind of catagorization due to the often widely different time scales on which dynamics occur. Signals produced by systems evolving on "slow" time scales will be dominated by low-frequency content, while disturbances produced by "fast" systems may have most of their content in a relatively higher frequency band. As an example, variations in enzyme concentrations (produced at the genetic level) would in most cases be low-frequency inputs to metabolic systems. Conversely, variation in the level of

metabolic products might appear as a high-frequency disturbance to a genetic network.

8. Conclusion

Local dynamics of biochemical networks can be faithfully modeled by linear differential equations. Identifying relevant input and output channels leads to a linear control system model. The behavior of this system under general time-varying disturbances can be characterized by the frequency response—the system's response to a family of canonical (oscillatory) disturbances. This theory has been presented as a natural extension of the sensitivity theory of biochemical networks, and as such provides generalizations of the summation and connectivity theorems of metabolic control analysis. The results were used to illustrate the role of feedback in simple biochemical systems.

Acknowledgment. The author would like to thank Tau-Mu Yi for stimulating interest in this work and also Herbert Sauro for his insightful comments. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

References and Notes

(1) Fell, D. A. *Biochem. J.* **1992**, *286*, 313−330.
(2) Heinrich, R.; Rapaport, T. A. *Eur. J. Biochem.* **1974**, *42*, 89−95.
(3) Kascer, H.; Burns, J. A. *Symp. Soc. Exp. Biol.* **1973**, *27*, 65−104.
(4) Savageau, M. A. *Biochemical Systems Analysis. A Study of Function and Design in Molecular Biology*; Addison-Wesley: Reading, MA, 1976.
(5) Voit, E. O. *Computational Analysis of Biochemical Systems*; Cambridge University Press: Cambridge, 2000.
(6) Acerenza, L.; Sauro, H. M.; Kacser, H. *J. Theor. Biol.* **1989**, *151*, 423−444.
(7) Heinrich, R.; Reder, C. *J. Theor. Biol.* **1991**, *151*, 343−350.
(8) Ingalls, B. P.; Sauro, H. M. *J. Theor. Biol.* **2003**, *222*, 23−36.
(9) Kholodenko, B. N.; Demin, O. V.; Westerhoff, H. V. *J. Phys. Chem. B* **1997**, *101*, 2070−2081.
(10) Kohn, M. C.; Whitley, L. M.; Garfinkel, D. *J. Theor. Biol.* **1979**, *76*, 437−452.
(11) Demin, O. V.; Westerhoff, H. V.; Kholodenko, B. N. *J. Phys. Chem. B* **1999**, *103*, 10695−10710.
(12) Morris, K. *Introduction to Feedback Control*; Harcourt Academic Press: London, 2001.
(13) Samoilov, M.; Arkin, A.; Ross, J. *J. Phys. Chem. A* **2002**, *106*, 10205−10221.
(14) Reder, C. *J. Theor. Biol.* **1988**, *135*, 175−201.
(15) Hofmeyr, J.-H. S. In *Proceedings of the International Conference on Systems Biology*, Pasadena, California, 2000; p. 291−300.
(16) Heinrich, R.; Schuster, S. *The Regulation of Cellular Systems*; Chapman & Hall: New York, 1996.
(17) Lynn, P. A. *An Introduction to the Analysis and Processing of Signals*, 2nd ed,; MacMillan Press: London, 1982.
(18) Strang, G. *Introduction to Applied Mathematics*; Wellesley-Cambridge Press: Wellseley, MA, 1986.
(19) Goodwin, B. *Adv. Enzyme Regulation* **1965**, *3*, 425−438.
(20) Xiu, Z.-L.; Zeng, A.-P.; Deckwer, W.-D. *J. Biotechnol.* **1997**, *58*, 125−140.
(21) Santillán, M.; Mackey, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1364−1369.
(22) Bode, H. W. *Network Analysis and Feedback Amplifier Design*; Van Nostrand: New York, 1945.
(23) Doyle, J., personal communication.
(24) Yi, T.-M.; Huang, Y.; Simon, M. I.; Doyle, J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 4649−4653.
(25) Barkai, N.; Leibler, S. *Nature* **1997**, *387*, 913−917.
(26) Iglesias, P. A.; Levchenko, A. In *Proceedings of the 40th IEEE Conference on Decision and Control*, Orlando, FL, **2001**; p. 843−848.
(27) Reijenga, K. A.; Westerhoff, H. V.; Kholodenko, B. N.; Snoep, J. L. *Biophys. J.* **2002**, *82*, 99−108.
(28) Goldbeter, A. *Biochemical Oscillations and Cellular Rhythms*; Cambridge University Press: Cambridge, 1996.
(29) Keener, J.; Sneyd, J. *Mathematical Physiology*; Springer-Verlag: New York, 1998.