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The non-linear analysis of cybernetic models. Guidelines for model formulation

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Abstract

A set of guidelines are formulated, using tools from bifurcation theory, that describe the qualitative characteristics of the different forms of competition for key cellular resources present within the cybernetic framework. These guidelines establish the basis of a modular approach for the construction of abstracted cybernetic models of microbial processes. This methodology, employed in the subsequent papers of this series, affords the construction of entire classes of cybernetic models that are guaranteed to possess desired dynamic features, thus reducing the model formulation burden and yielding insight into the necessary level of metabolic pathway abstraction.

Keywords: Cybernetic models; Non-linear analysis; Mathematical models

1. Introduction

The central theme of mathematical modeling is the abstraction of physical phenomena into a suitably simplified mathematical formalism. There exist a number of perspectives that guide the level of abstraction and hence the method of formulation of acceptable mathematical models. One school of thought, termed first principles or white box modeling, relies upon developing an abstracted view of the process in which unnecessary physical detail has been neglected. If the intuition based abstraction of the physical system is correct, then the appropriate phenomena is predicted by the mathematical model. If, however, the conception of the system is erroneous then the model formulation fails. Clearly, a deep and fundamental understanding of the phenomena is required for a consistent level of first principles modeling success. In the context of biological systems, this intuitive approach requires the presumption of which metabolic intermediates and pathways are crucial to system behavior and the specific regulatory role they play. Given that metabolic path-

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ways can consist of large numbers of coordinated reactions, this approach may require a number of iterations to be successful in the general case.

In the area of process control, another methodology for the development of mathematical models, termed black box modeling, takes a fundamentally different tact. This methodology relies upon the fitting of basis functions to experimental data. Thus, instead of translating physical intuition into mathematical structure, black box approaches force mathematical structure to obey experimental observations. Black box approaches, such as Volterra series methods, or the use of Neural networks have enjoyed a fair degree of success in a number of different instances where first principles modeling has proven to be difficult (Doyle et al., 1995; Pearson et al., 1996). The attractiveness of black box approaches stems from the strong advantage that they are guaranteed to describe the system behavior, at least locally. In complicated systems, this negates the need to develop a deep intuitive understanding of the process, potentially yielding a large time and effort savings.

The cybernetic framework developed by Ramkrishna and co-workers has long sought to abstract biological systems into highly idealized mathematical structures that are capable of predicting complex nutrient uptake and growth profiles (Kompala et al., 1986; Baloo and Ramkrishna, 1991; Straight and Ramkrishna, 1991). However, these past endeavors have suffered from the pitfalls of a purely first principles perspective. As such the development and application of the framework has been limited in scope to a few example systems. Therefore, the objective of this work is to approach the problem of cybernetic model formulation from a different perspective. Our vision for the development of cybernetic models of microbial systems falls somewhere between the two extreme schools of thought. We seek to formulate a methodology that produces a degree of biological insight higher than black box modeling while simultaneously guaranteeing the description of macroscopic biological phenomena, such as growth and nutrient assimilation profiles. Moreover, this methodology does not rely solely upon physical intuition as is true of white box approaches. Rather, it draws inspiration from a set of operating postulates that govern the dynamic features displayed by particular topological pathway structures. Thus, upon inspection of the topological structure of a model framework, the qualitative dynamic characteristics are easily identified. This is somewhat akin, by analogy, to assembling a jigsaw puzzle. Imagine that we possess a library of elementary pieces that can be used to assemble the puzzle. Each piece has a topological structure associated with it. Furthermore, as a consequence of the structure, each elementary piece has a particular function. For example, some pieces may be corner pieces, whereas others may only fit in the center of the puzzle. What ever the case may be, clearly, the shape of the piece reflects its purpose or function in the puzzle.

We propose something very similar, in spirit, for the formulation of abstracted cybernetic models. We postulate that a global metabolic map can be decomposed into abstracted elementary components, similar to pieces in a puzzle. The converse of this postulate implies that given an appropriate library of elementary components, we can assemble model structures from abstracted elementary pieces. Bear in mind that each elementary component has a distinct mathematical configuration, thus, this represents the structure side of the argument, i.e. what is the mathematical structure needed to describe a microbial process. At issue, however, is the ability to a priori construct model formulations that are guaranteed to possess desired dynamic features. The investigation of this issue is the particular focus of the work at hand.

Our library of elementary components consists of the four elementary pathways derived by Straight and Ramkrishna (1994) (see Fig. 1). These pathways can be subdivided on the basis of topology, as well as, the type of competitive structure for key cellular resources required for expression of the enzymatic machinery. It is the latter issue, i.e. the competitive structure, that dictates the dynamics and moreover the function of the elementary piece. Thus, by understanding the dynamics associated with each type of competitive structure, we gain insight into the dynamics dis-

played by each elementary member of the model construction library. The next step in the development must be an understanding of the manner in which the competitive structure of individual elementary pathways is influenced upon assembly. This is a key issue given the modular theme of the methodology. These two pieces of information can then be employed to formulate a set of postulates that guide the construction of mathematical models so that specific desired features are obtained.

1.1. Scope of investigation

The objective of this work is the analysis, using tools from bifurcation theory, of the characteristics of substitutable and complementary competition as defined by Straight and Ramkrishna (1994). The linear and convergent elementary pathways experience substitutable competition, whereas, the divergent and cyclic units possess a complementary competitive structure. The distinction between competitive type rest upon the choice of the cybernetic objective function and resource constraint rather than the topology of the pathway. The hallmark of substitutable competition is the objective of maximization of the level of an end product subject to a constraint upon available resources. Thus, the objective of a linear and convergent pathway is the maximization of the end product or the focus of the pathway. Complementary competition, however, does not strive towards this type of objective. Rather, the objective of complementary structures is the maximization of the mathematical product of the pathway end products or ring metabolites (in the case of a cyclic unit.)

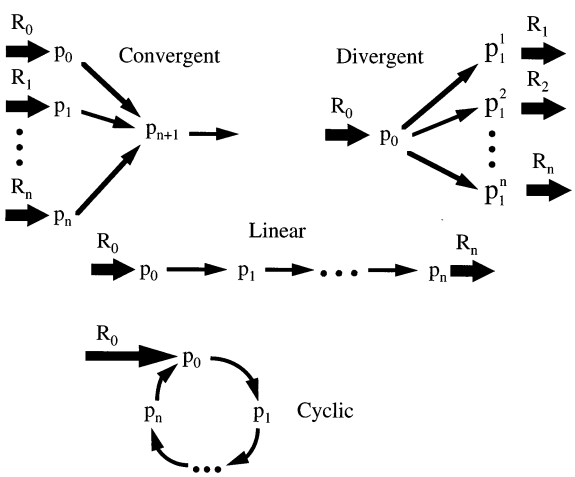

Fig. 1. Four elementary pathways derived by Straight and Ramkrishna.

In what follows, we analyze the elementary convergent and divergent pathways to determine the qualitative characteristics of each type of competition as a function of system parameters. In the larger picture, we are examining the qualitative characteristics of the elementary pathways that compose our formulation library. As per our modular theme, after completing the analysis of individual elementary pathways, we combine a convergent and a divergent pathway to determine the qualitative effects upon system behavior of the interaction of substitutable and complementary competition. However, before we introduce and analyze the model formulations, we begin by discussing some mathematical preliminaries. In particular, we present an overview of Lyapunov–Schmidt reduction and the objectives of bifurcation theory. These pieces of mathematical machinery are used, subsequently, to analyze the qualitative aspects of cybernetic model behavior. Those readers who seek an understanding of the development solely on an application level may move directly to Section 1.4 where the analysis objectives are summarized. The detail of the bifurcation analysis is presented as an appendix to this development thereby limiting unnecessary confusion stemming from involved mathematical manipulation.

1.2. Mathematical preliminaries: Lyapunov–Schmidt reduction

Lyapunov–Schmidt reduction is an elegant operator technique that has its origins in Singularity theory (Golubitsky and Schaeffer, 1985). This technique allows for the isolation of the aspect of a dynamic system that is responsible for qualitative changes in system behavior. Once this portion has been isolated, tools from bifurcation theory can be applied to examine the exact nature of the

qualitative change. Often, using a reduction technique such as Lyapunov–Schmidt allows the study of the qualitative nature of an n-dimensional system to be reduced to a q-dimensional subsystem, where q is equal to the number of zero eigenvalues of the system Jacobian evaluated around an equilibrium point, thereby greatly simplifying the analysis problem. In a more exact mathematical sense, Lyapunov–Schmidt reduction is centered upon the use of appropriately defined projection operators to isolate the regions of system space that are responsible for bifurcation phenomena. In the subsequent development, we leave much of the detail to the reader in the interest of brevity, however, in what follows we present a general treatment of the material so to acquaint the reader with the arguments of the mathematical development.

Let the time evolution of an arbitrary dynamic system in the state variable $\mathbf{x}$ be governed by the differential equation set

$$\frac{d\mathbf{x}}{d\tau} = \Phi = \mathbf{F}(\mathbf{x}, \kappa), \quad \mathbf{x} \in \mathbf{R^n} \quad \mathbf{F}: \mathbf{R^n} \times \mathbf{R^k} \rightarrow \mathbf{R^n} \tag{1.1}$$

where κ is the k-dimensional vector of system parameters. Furthermore, denote a steady state solution of Eq. (1.1) as

$$\mathbf{F}(\mathbf{x}^*, \kappa^*) = 0 \tag{1.2}$$

where * denotes the steady state value. Assuming that $\mathbf{F}(\mathbf{x}, \kappa)$ is smooth around $(\mathbf{x}^*, \kappa^*)$, if the Jacobian of equation set (1.1) evaluated at $(\mathbf{x}^*, \kappa^*)$, denoted as $d\mathbf{F}|_{\mathbf{x}^*, \kappa^*}$ has a non-zero determinant in an arbitrary neighborhood of the equilibrium point, then equation set (1.1) contains no bifurcation behavior by the implicit function theorem. If however, there exist a point in parameter space, denoted by $\boldsymbol{k}^\circ$ along solution $\mathbf{x}^*$ at which $det\ (d\mathbf{F}|_{\mathbf{x}^*, \kappa^\circ}) = 0$, equation set (1.1) has the possibility of multiple equilibrium solutions. Formally the point $(\mathbf{x}^*, \kappa^\circ)$ is then termed a critical point. Without loss of generality we assume that $(\mathbf{x}^*, \kappa^\circ)$ is translated to the origin and the critical Jacobian in the translated system is given by

$$\mathbf{L} \equiv \mathbf{dF}|_{\mathbf{0,\,0}} \tag{1.3}$$

The eigenvalues of the critical operator $\mathbf{L}$ form the basis for the decomposition of system space, and eventually, for the isolation of the aspect of the dynamic system that is responsible for the bifurcation behavior. We assume for the sake of ease (although the technique is applicable in any case) that $\mathbf{L}$ is diagonalizable. Note this is almost always possible given matrix operators because the original system basis can be rotated to be equivalent to the eigenvector directions, hence, producing a diagonal Jacobian. The only requirement of the rotation is a non-defective Jacobian for all values of the bifurcation parameter, i.e. the algebraic multiplicity is equal to geometric multiplicity. Assuming this is true, the system space can easily be decomposed into the components

$$\mathbf{X} = \mathbf{KerL} \oplus \mathbf{RngL} \tag{1.4}$$

where the null space of the critical Jacobian is spanned by the eigenvector set

$$\mathbf{z^N} = \{\{\mathbf{z}_j\} : \mathbf{L}\mathbf{z}_\mathbf{j} = \mathbf{0}, \forall\, j\} \tag{1.5}$$

and the range space is spanned by

$$\mathbf{z^R} = \{\{\mathbf{z_k}\} : \mathbf{L}\mathbf{z_k} = \sigma_\mathbf{k}\mathbf{z_k}, \forall\, k\} \tag{1.6}$$

Note that because we have assumed the critical Jacobian is diagonal the eigenvector set $\mathbf{z^N}$, $\mathbf{z^R}$ form the standard basis on R^n. The projection operator $\mathbf{E}$ which projects elements of the system space onto $\mathbf{RngL}$ can then be defined as

$$\mathbf{E}(\cdot) \equiv \sum_{k=1}^{dim(\mathbf{z^R})} \langle \cdot, \mathbf{z_k^R} \rangle \mathbf{z_k^R} \tag{1.7}$$

This operator is crucial to the decomposition of the dynamical system into regions where bifurcation is present and absent. More precisely, bifurcation is limited to the aspect of the system that lies in the $\mathbf{KerL}$. Using the projection operator $\mathbf{E}$ we isolate the range space variables in terms of the null space variables, which can be substituted into the null space system aspect. The latter set of equations contain the bifurcation problem and are termed the reduced set or equation.

1.3. Mathematical preliminaries: bifurcation theory

Bifurcation theory is the mathematical study of the qualitative change in system behavior as a function of parameters. Thus, given the reduced equation, i.e. the aspect of the system that is responsible for qualitative changes in behavior, bifurcation theory could be employed to study where and how system behavior changes. In what follows we present a very simplified overview of the salient aspects of the theory so that the reader is familiar with the operations that follow in the analysis section of the development. For a complete discussion of bifurcation theory the reader is referred to Iooss and Joseph (1990).

Suppose the reduced equation is given by

$$\frac{dz}{d\tau} = G(z, \kappa), \quad z \in R^1 \tag{1.8}$$

with the equilibrium solution

$$G(0, \kappa) = 0 \tag{1.9}$$

where we have assumed the reduced equation to be 1-dimensional, i.e. the original n-dimensional system has only a single zero eigenvalue. We need not make this assumption, although for most bifurcation problems encountered such a simplification is not restrictive. The study of higher dimensional bifurcation problems (greater than 2-dimensional) is not a well-defined endeavor above a 2-dimensional null space (Mcleod and Sattinger, 1973), accordingly, such situations are beyond the scope of this introduction in any event. It can be shown that the equilibrium solutions of Eq. (1.8) correspond to equilibrium solutions in the original system. Thus, by studying Eq. (1.8) we are analyzing how the original system changes as a function of the system parameters. Bifurcation theory concerns itself with examining the system behavior in regions where the conditions of the implicit function theorem fail. More exactly, if the 1-dimensional eigenvalue given by the partial

$$g_z \equiv \left.\frac{\partial G}{\partial z}\right|_{0,\,\kappa} \tag{1.10}$$

is non-zero for all arbitrary neighborhoods of the equilibrium point $(0, \kappa)$ then Eq. (1.8) displays no bifurcation behavior, i.e. the implicit function theorem is satisfied for all values of κ. If however, g_z vanishes at some point, denoted as (z_0, κ_0), this point is a possible bifurcation point. The objective of bifurcation theory is then to determine the qualitative system properties in the neighborhood of κ_0.

For bifurcation to occur, two conditions must be satisfied at the bifurcation point. Firstly, alternative equilibrium solutions must exist in a neighborhood around the bifurcation point with the additional property that at least two solution branches intersect at the bifurcation point. To determine the existence of alternative solutions we simply solve Eq. (1.9) for $z_j = z_j(\kappa)$, $j = 1, 2, \ldots n$, i.e. the equilibrium solution branches parameterized by the bifurcation parameter κ. Note when the closed form solution of Eq. (1.9) is not practical, approximations of the equilibrium behavior can be determined using the tools outlined in Iooss (Iooss and Joseph, 1990). These n branches represent the various modes of system behavior that are possible given κ. Note the number of solution branches, as well as, the physical significance of each branch may change as κ is varied, thus not all n branches may be accessible at any given κ. The second component of bifurcation is an exchange of stability at the bifurcation point between a base solution and a bifurcating, alternative solution. This aspect of bifurcation is described by the system eigenvalues. In particular, points of possible bifurcation are marked by zero eigenvalues. The significance of these points, in a mathematical sense, was discussed earlier in that they mark points where the conditions of the implicit function theorem are violated. The points where both properties are present mark the location where the system of equations may assume different qualitative long term properties.

1.4. Analysis objectives

We now, in a formal sense, outline the objectives of our analysis and discussion

1. To determine the qualitative properties of key enzymes that compete in substitutable and complementary environments

Table 1
Model parameter set(s) where $j = 0, 1, 2$ and $k = a, b$

Parameter set: elementary pathways			
K_j	0.01[a]	K_{e_j}	0.01[a]
$K_{e_0^k}$	0.01[a]	α_j	0.001[b]
α_0^k	0.001[b]	$\mu_g^{\max}$	0.75[c]
R_j	0.1[b]	β	0.05[c]

[a] Units: g gDw^{-1}.
[b] Units: g gDw^{-1} h^{-1}.
[c] Units: h^{-1}.

2. To determine how the qualitative properties of purely complementary/purely substitutable elementary pathways are influenced by assembly
3. To hypothesize a resource allocation basis for the qualitative properties of the various cybernetic competitive environments
4. To formulate a set of guidelines that can be employed to rationally construct abstracted cybernetic models of microbial processes.

2. Materials and methods

All model systems discussed in this development were constructed and simulated within the Simulink environment of Matlab. The model equations were evaluated using the ODE23s routine within Matlab. Unless otherwise noted all model systems were simulated using the parameter described by Table 1.

3. Model formulation

In this section we formulate and analyze, using the tools of bifurcation theory, the cybernetic models of the elementary convergent and divergent metabolic pathways shown in Fig. 1. In a larger sense we are analyzing the behavior of substitutable and complementary competition. This allows the results of the analysis to be extended to the linear and cyclic elementary pathways because these share a common cybernetic structure. In each case we begin by discussing the process equations and kinetics that constitute the model, and then make the formulation complete by deriving the appropriate forms of the cybernetic variables that modify the rates of reaction and enzyme synthesis. Once the model systems have been formulated we turn to the analysis portion of the development and analyze each elementary competitive structure in turn.

3.1. Elementary convergent pathway

The framework of the elementary convergent pathway is shown in Fig. 1. The specific material flux into the elementary pathway is denoted by

$$R_k, \quad k = 0, 1, \ldots, n \tag{3.1}$$

and is considered a constant. This is consistent with the notion of investigating pathway features under balanced growth conditions. The metabolites $p_j, j = 0,1,\ldots, n$ are degraded by key enzymes e_j at the specific rate

$$r_j = \mu_j^{\max}\left(\frac{e_j}{e_i^{\max}}\right)\frac{p_j}{K_j + p_j}, \quad j = 0, 1, \ldots, n \tag{3.2}$$

where $\mu_j^{\max}$, K_j denotes the rate and saturation constants governing the formation of p_{n+1} and $e_j^{\max}$ denotes the maximum specific level of key enzyme e_j. The key enzyme e_j is assumed to be induced by the intermediate p_j and is expressed at the specific rate

$$r_{e_j} = \alpha_j\frac{p_j}{K_{e_j} + p_j} \quad j = 0, 1, \ldots, n \tag{3.3}$$

where α_j, K_{e_j} denote the rate and saturation constants governing the expression of e_j. The intermediate p_{n+1} is assumed to be consumed at the specific rate

$$r_{n+1} = \mu_j^{\max}\frac{p_{n+1}}{K_{n+1} + p_{n+1}} \tag{3.4}$$

where $\mu_{n+1}^{\max}$, K_{n+1} denote the rate and saturation constants governing the consumption of p_{n+1}. We assume $e_{n+1} \sim e_{n+1}^{\max}$.

The formulation of the cybernetic regulation for the elementary convergent pathway was presented by Straight and Ramkrishna (1994) and is presented here for the sake of completeness. The objective of an elementary convergent pathway is assumed to be the maximization of the level of

intermediate p_{n+1}. This objective is subject to a constraint upon the resources available for enzyme expression. These statements formally become the constrained optimization problem

$$\max \sum_{j=0}^{n} p_{n+1}^{j}(R_j) \quad \text{subject to}$$
$$g = R_0 + R_1 + \cdots + R_n \equiv R \tag{3.5}$$

where p_{n+1}^{j} denotes the level of p_{n+1} produced by the j^{th} route and R_j denotes the amount of resource allocated to the j^{th} source of p_{n+1}. The optimality condition given by

$$\frac{dp_{n+1}^{0}}{dR_0} = \frac{dp_{n+1}^{1}}{dR_1} = \cdots = \frac{dp_{n+1}^{n}}{dR_n} \tag{3.6}$$

can be rearranged to yield the matching condition

$$\frac{dp_{n+1}^{j}}{dp_{n+1}^{j} + \sum_{q=0,j}^{n} dp_{n+1}^{q}} = \frac{dR_j}{dR_j + \sum_{q=0,j}^{n} dR_q}, \quad j = 0, 1, \ldots, n \tag{3.7}$$

where the lower summation limit indices denote the exclusion of the j^{th} element from the set. Eq. (3.7) states the optimum operation of the convergent pathway occurs when the fractional return on investment is equal to the fractional allocation of critical resource. If we assume the allocation policy described by Eq. (3.7) is implemented at every instant in time and that allocation takes place in time dt, the return on resource investment can be measured as the reaction rate. Accordingly, the cybernetic variable that governs the allocation of critical resource earmarked for convergent pathway operation to the synthesis of key enzyme e_j takes the form

$$u_j^s = \frac{r_j}{r_j + \sum_{q=0,j}^{n} r_q}, \quad j = 0, 1, \ldots, n \tag{3.8}$$

where superscript s denotes substitutable. The derivation of the cybernetic variable that governs the activity of a convergent key enzyme, denoted as v_j^s, follows from the cybernetic proportional law. This simply states that enzyme activity is proportional to the reaction rate that it catalyzes. Functionally, this implies the relationship

$$v_j^s \sim \lambda r_j, \quad j = 0, 1, \ldots, n \tag{3.9}$$

The cybernetic variable $v_j^s, \forall j$ is constrained to obey

$$0 \le v_j^s \le 1, \quad \forall j \tag{3.10}$$

which implies the proportionality constant λ is governed by

$$0 \le \lambda \le \frac{1}{r_j}, \quad \forall j \tag{3.11}$$

Eq. (3.11) must be valid $\forall j$ which implies λ is of the form

$$\lambda = \frac{1}{\max\{r_0, r_1, \ldots r_n\}} \tag{3.12}$$

Accordingly, the cybernetic variable that governs the activity of convergent pathway key enzymes is given by

$$v_j^s = \frac{r_j}{\max(r_0, r_1, \ldots, r_n)}, \quad j = 0, 1, \ldots, n \tag{3.13}$$

We now make the model formulation complete by modifying the rates of reaction and enzyme synthesis with the appropriate cybernetic variables. The intermediate p_{n+1} is synthesized via key enzyme e_j, $j = 0, 1, \ldots, n$ at the modified specific rate

$$r_j v_j^s, \quad j = 0, 1, \ldots, n \tag{3.14}$$

where r_j denotes the specific rate of p_{n+1} formation and v_j^s denotes the cybernetic variable that governs the activity of $e_j, j = 0, 1, \ldots, n$. The key enzyme e_j is expressed at the modified specific rate

$$r_{e_j} u_j^s, \quad j = 0, 1, \ldots, n \tag{3.15}$$

where r_{e_j} denotes the specific rate of e_j expression and u_j^s denotes the cybernetic variable that governs the expression of e_j. Consistent with the assumption of maximal e_{n+1} level, we assume the cybernetic variable that governs e_{n+1} activity takes the value $v_{n+1}^s \sim 1$.

The complete set of mass balances governing the evolution of the convergent pathway model that reflects the input of metabolic regulation is given by the set

$$\frac{dp_j}{dt} = R_j - r_j v_j^s - r_g p_j, \quad j = 0, 1, \ldots, n$$

$$\frac{dp_{n+1}}{dt} = \sum_{j=0}^{n} r_j v_j^s - r_{n+1} v_{n+1}^s - r_g p_{n+1},$$
$$\frac{de_j}{dt} = r_{e_j} u_j^s - (r_g + \beta)e_j + r_{e_j}^*, \quad j = 0, 1, \ldots, n \tag{3.16}$$

where r_g denotes the specific growth rate and $r_{e_j}^*$ denotes the rate of constitutive enzyme synthesis of e_j. The parameter β denotes the rate constant governing the first order decay of e_j.

3.2. Elementary divergent pathway

The framework for the elementary divergent pathway is shown in Fig. 1. Again, consistent with the development of Straight and Ramkrishna (1994), we assume the elementary divergent pathway is an isolated local element of a larger global metabolic map whose only interaction with the rest of the pathway is through material flux connections. The metabolic flux into the branch point is denoted by

$$R_0 \tag{3.17}$$

and is assumed to be constant. This is consistent with exploring the system dynamics under conditions of balanced growth. The formation of $p_1^j, j = 1, 2, \ldots, n$ from p_0 is catalyzed by the key enzyme $e_0^j, j = 1, 2, \ldots, n$ at the specific rate

$$r_0^j = \mu_0^{j,\max}\left(\frac{e_0^j}{e_0^{j,\max}}\right)\frac{p_0}{K_0^j + p_0}, \quad j = 1, 2, \ldots, n \tag{3.18}$$

where $\mu_0^{j,\max}$, K_0^j denote the rate constant and saturation constants that govern the formation of p_1^j and $e_0^{j,\max}$ denotes the maximum level of key enzyme e_0^j. The key enzyme(s) e_0^j are assumed to be induced by p_0 and expressed at the specific rate

$$r_{e_0^j} = \alpha_0^j \frac{p_0}{K_{e_0^j} + p_0}, \quad j = 1, 2, \ldots, n \tag{3.19}$$

where α_0^j, $K_{e_0^j}$ denote the rate and saturation constants governing the expression of e_0^j. The intermediates p_1^j are assumed to be precursors for downstream metabolic activity and consumed at the specific rate

$$r_1^j = \mu_1^{j,\max}\frac{p_1^j}{K_1^j + p_1^j}, \quad j = 1, 2, \ldots, n \tag{3.20}$$

where $\mu_1^{j,\max}$, K_1^j denote the rate and saturation constants governing the utilization of p_1^j for downstream metabolic activity. For the sake of simplicity we assume $e_1^j \sim e_0^{j,\max}$, $j = 1, 2, \ldots, n$. This assumption is consistent with the notion of restricting our analysis to solely the divergent pathway element.

Straight and Ramkrishna (1994) have postulated the objective of a divergent branch point is the maximization of the mathematical product of the branch point metabolites. This objective function is subject to a constraint upon the resources available for the expression of branch point key enzymes. These statements formally become the constrained optimization problem

$$\max\left(\prod_{j=1}^{n} p_1^j(R_0^j)\right) \quad \text{subject to} \quad g = \sum_{j=1}^{n} R_0^j \equiv R \tag{3.21}$$

where $R_0^j, j = 1, 2, \ldots, n$ denotes the amount of resources allocated to the expression of $e_0^j, j = 1, 2, \ldots, n$. The optimality condition given by

$$p_1^2 p_1^3 \ldots p_1^n \frac{dp_1^1}{dR_0^1} = p_1^1 p_1^3 \ldots p_1^n \frac{dp_1^2}{dR_0^2} = \ldots$$
$$= p_1^1 p_1^2 \ldots p_1^{n-1}\frac{dp_1^n}{dR_0^n} \tag{3.22}$$

can be rearranged to yield the matching condition

$$\frac{dp_1^j/p_1^j}{dp_1^j/p_1^j + \sum_{q=1,j}^{n} dp_1^q/p_1^q} = \frac{dR_0^j}{dR_0^j + \sum_{q=1,j}^{n} dR_0^q} \tag{3.23}$$

Eq. (3.23) states that optimum divergent pathway operation occurs when the fractional return on investment is equal to the fractional allocation of resources. If we assume Eq. (3.23) is implemented at every instant in time and allocation takes place on the time scale of dt, the cybernetic variable that governs the allocation of critical resources for the expression of $e_0^j, j = 1, 2, \ldots, n$ is given by

$$u_{0j}^c = \frac{r_0^j/p_1^j}{r_0^j/p_1^j + \sum_{q=1,j}^{n} r_0^q/p_1^q}, \quad j = 1, 2, \ldots, n \tag{3.24}$$

where superscript c denotes complementary process. The cybernetic variable that governs the

activity of $e_0^j = 1, 2,\ldots, n$ follows from a restatement of the proportional law. In the case of a complementary pathway the proportional law is given by

$$v_{0j}^c \sim \lambda \frac{r_0^j}{p_1^j}, \quad j = 1, 2,\ldots, n \tag{3.25}$$

Note that the rate of reaction is scaled by its product, this is a feature distinct to complementary processes and is an artifact of the objective function. The cybernetic variable v_{0j}^c is constrained to obey

$$0 \leq v_{0j}^c \leq 1, \quad j = 1, 2,\ldots, n \tag{3.26}$$

which implies the proportionality constant is bounded by

$$0 \leq \lambda \leq \frac{1}{r_0^j/p_1^j}, \quad j = 1, 2,\ldots, n \tag{3.27}$$

Eq. (3.27) must hold $\forall\, j$, thus, it follows v_{0j}^c is of the form

$$v_{0j}^c = \frac{r_0^j/p_1^j}{\max(r_0^1/p_1^1, r_0^2/p_1^2,\ldots, r_0^n/p_0^n)}, \quad j = 1, 2,\ldots, n \tag{3.28}$$

We make the model system complete by modifying the rates of reaction and enzyme expression by the appropriate cybernetic variables. We then present the complete set of mass balances that constitute the elementary divergent pathway. The branch metabolite precursor p_0 is consumed to form the branch metabolites p_1^j via the key enzyme e_0^j at the modified specific rate

$$r_0^j\, v_{0j}^c, \quad j = 1, 2,\ldots, n \tag{3.29}$$

where r_0^j denotes the specific rate of p_1^j formation and v_{0j}^c denotes the cybernetic variable that regulates the activity of e_0^j. The key enzyme e_0^j is induced by p_0 and expressed at the modified specific rate

$$r_{e_0^j}\, u_{0j}^c, \quad j = 1, 2,\ldots, n \tag{3.30}$$

where $r_{e_0^j}$ denotes the specific rate of e_0^j expression and u_{0j}^c denotes the cybernetic variable that regulates the expression of e_0^j. The complete set of mass balances governing the time evolution of the elementary divergent pathway which include the cybernetic variables are given by the set:

$$\begin{aligned}
\frac{dp_0}{dt} &= R_0 - \sum_{j=1}^{n} r_0^j\, v_{0j}^c - r_g\, p_0,\\
\frac{dp_1^j}{dt} &= r_0^j\, v_{0j}^c - r_1^j - r_g\, p_1^j, \quad j = 1, 2,\ldots, n\\
\frac{de_0^j}{dt} &= r_{e_0^j}\, u_{0j}^c - (r_g + \beta) e_0^j + r_{e_0^j}^*, \quad j = 1, 2,\ldots, n
\end{aligned} \tag{3.31}$$

where r_g denotes the specific growth rate and $r_{e_0^j}^*$ denotes the specific rate of constitutive enzyme synthesis of the j^{th} key enzyme. The parameter β denotes the rate constant for the first order decay of enzyme e_0^j.

4. Analysis and discussion

4.1. Substitutable competition

To begin the analysis of the $2n + 2$-dimensional elementary convergent pathway model we rescale with respect to the following set of dimensionless variables and parameters

$$\sigma_j \equiv \frac{p_j}{K_j} \qquad \epsilon_j \equiv \frac{e_j\, \delta}{\alpha_j} \qquad \kappa_j \equiv \frac{\mu_j^{\max}}{\delta} \tag{4.1}$$

$$\tau \equiv \delta t \qquad n_j \equiv \frac{r_{e_j}^*}{\alpha_j} \qquad \bar{K}_{i,j} \equiv \frac{K_{e_i}}{K_j} \tag{4.2}$$

where $\delta \equiv (r_g + \beta)$ and $j = 0, 1,\ldots, n$. By assumption we are investigating the dynamics of the elementary convergent pathway during balanced growth conditions. It follows that $r_g \sim \mu_g^{\max}$ under this assumption. Furthermore, we assume $\delta \sim \mu_g^{\max}$, i.e. the rate constant governing the first order decay of enzyme, denoted as β, is small compared with the maximum specific growth rate. Substituting the resealed variables and parameters into the convergent pathway model under the above assumptions yields the dimensionless system in vector form

$$\frac{d\mathbf{x}}{d\tau} = \mathbf{F}(\mathbf{x}, \kappa, \eta), \quad F{:}\mathbf{R^{2n+2}}\mathbf{x}\mathbf{R^{n+1}}\mathbf{x}\mathbf{R^{n+1}} \rightarrow \mathbf{R^{2n+2}} \tag{4.3}$$

where the state vector $\mathbf{x}$ and the rate constant vector κ are given by

$$\mathbf{x} \equiv \{\sigma_0, \sigma_1,\ldots \sigma_n, \epsilon_0,\ \epsilon_1,\ldots, \epsilon_n\}^T \tag{4.4}$$

$$\kappa \equiv \{\kappa_0, \kappa_1, \ldots, \kappa_n\}^T, \tag{4.5}$$

$$\eta \equiv \{\eta_0, \eta_1, \ldots, \eta_n\}^T \tag{4.6}$$

The goal of our analysis is to characterize the qualitative features of the convergent pathway as a function of the parameter vector κ. To achieve such a goal we call upon tools from nonlinear analysis, specifically bifurcation and singularity theory, to determine the location and nature of possible bifurcation points. We then determine the manner in which the system behavior is altered as the parameter values are varied through a critical value. To assure a manageable problem that still provides a hint of the nature of the general n-dimensional problem, we consider the case of $n=2$. The mathematical details of the analysis are presented in the appendix, we choose only to summarize and discuss the results in a qualitative light here.

Let us assume that the fastest rate of p_3 formation is r_0. The mathematical purpose of such an assumption is detailed in Appendix A, in short, it allows the cybernetic v variable to be decomposed into a form that is more amenable to analysis while still maintaining the qualitative features of the cybernetic regulatory description. As is true of all cybernetic developments, the qualitative portrait of the system dynamics follows directly from resource allocation policy implemented by the microorganism. In the case of the convergent pathway, the key enzymes that catalyze the parallel routes to p_3 formation compete for cellular resources from a single resource pool. Thus, by assuming $r_0 \gg r_1, r_2$ the key enzyme e_0 is seen as a more attractive resource investment from the perspective of the microorganism because it satisfies the local objective better than the other key enzymes, namely, the production of p_3. It follows that e_0 receives the lion's share of the critical resources and the synthesis of the remaining elementary pathway enzymes is repressed. This is the base solution from which we explore the qualitative features of the system behavior. When choosing different values for the constant vector κ we are in effect altering how the microorganism views the key enzymes in terms of their attractiveness as resource investments.

It is shown in the appendix that the elementary convergent pathway in which the fastest rate of p_3 formation is r_0, i.e. e_0 is allocated all the critical resources while e_1 and e_2 are repressed undergoes bifurcation (pitchfork) when the condition

$$K_1\left(\frac{\kappa_1 \psi_1}{(1+\psi_1)r_0^*} - 1\right)K_2\left(\frac{\kappa_2 \psi_2}{(1+\psi_2)r_0^*} - 1\right) = 0 \tag{4.7}$$

is satisfied where r_0^* denotes the steady state rate of p_3 formation catalyzed by e_0 and $\psi_j \equiv R_j/\delta$. More precisely, condition (4.7) marks the location in parameter space in which the microbe views the other enzymes that catalyze p_3 formation as attractive resource investments. Notice there exists three distinct parameter conditions that allow condition (4.7) to be satisfied, i.e.

$$\frac{\kappa_j}{\kappa_0} = \left(\frac{1+\psi_j}{\psi_j}\right)\theta \quad j = 1, 2 \tag{4.8}$$

or both $j=1, 2$ where θ is given by

$$\theta \equiv \frac{\epsilon_0^* \sigma_0^*}{1+\sigma_0^*} \tag{4.9}$$

and is approximately independent of the system parameters in a small neighborhood of the bifurcation point. In terms of the qualitative features of the dynamics, these bifurcation points mark where $e_j, j=1, 2$ assumes non-zero equilibrium solutions. Interestingly, the right hand side of the condition (4.8) is approximately unity, thus, it follows that as the maximum rate of two competing routes of p_3 formation approaches similar values the microbe views each as an attractive investment and allocates critical resources to the synthesis of the key enzymes that catalyze each parallel route. Condition (4.7) implies three distinct regions of system behavior all of which spring forth from the base solution. These regions are shown by simulation of the original model system in Fig. 2. The first region is the base solution from which we examine the system bifurcation behavior, i.e. e_0 assumes a non-zero steady state and the other system enzymes are forced to zero, because of a lack of resource allocation. This follows from $\kappa_0 \gg \kappa_1$ and $\kappa_0 \gg \kappa_2$, i.e. the maximum rate of p_3 formation catalyzed by e_0 is much greater than all other rates making it the microbes preferred resource investment. The sec-

ond region marks a situation in which e_0 and e_1 are considered good resource investments while e_2 is forced to zero due to a lack of allocation. This region is parametrically characterized by $\kappa_0 \simeq \kappa_1$ and $\kappa_0 \gg \kappa_2$. By symmetry region four follows from $\kappa_0 \simeq \kappa_2$ and $\kappa_0 \gg \kappa_1$. Region three marks the unique situation in which all three key enzymes are viewed, from the perspective of the microorganism as good resource investments. Thus, it follows that all three key enzymes are synthesized in this region. Parametrically, this allocation pattern follows from $\kappa_0 \simeq \kappa_1$ and $\kappa_0 \gg \kappa_2$.

From this analysis we see one of the features of substitutable competition is the presence of an enzymatic zero solution. This follows from a resource allocation argument that stipulates that enzyme repression is a consequence of a lack of resource allocation. The question at hand is how this feature is effected by the competitive structure as well as other factors such as constitutive enzyme synthesis. We reserve comment on the influence of constitutive enzyme synthesis until the discussion in Section 4.3 and consider the competitive structure issue presently by examining the qualitative features complementary competition.

4.2. Complementary competition

To ease the algebraic complexity of the analysis and guide the understanding of the qualitative interaction present within the equation set, we propose the following set of dimensionless variables and parameters

$$\rho_0 \equiv \frac{p_0}{K_0^1}, \qquad \rho_j \equiv \frac{p_1^j}{K_1^j}, \qquad \epsilon_j \equiv \frac{\delta}{\alpha_j} e_0^j,$$

$$\kappa_j \equiv \frac{\mu_0^{j,\max}}{\delta}, \qquad \bar{\kappa}_j \equiv \frac{\bar{\mu}_1^{j,\max}}{\delta}, \qquad \Gamma_{q,j} \equiv \frac{K_0^q}{K_0^j}$$

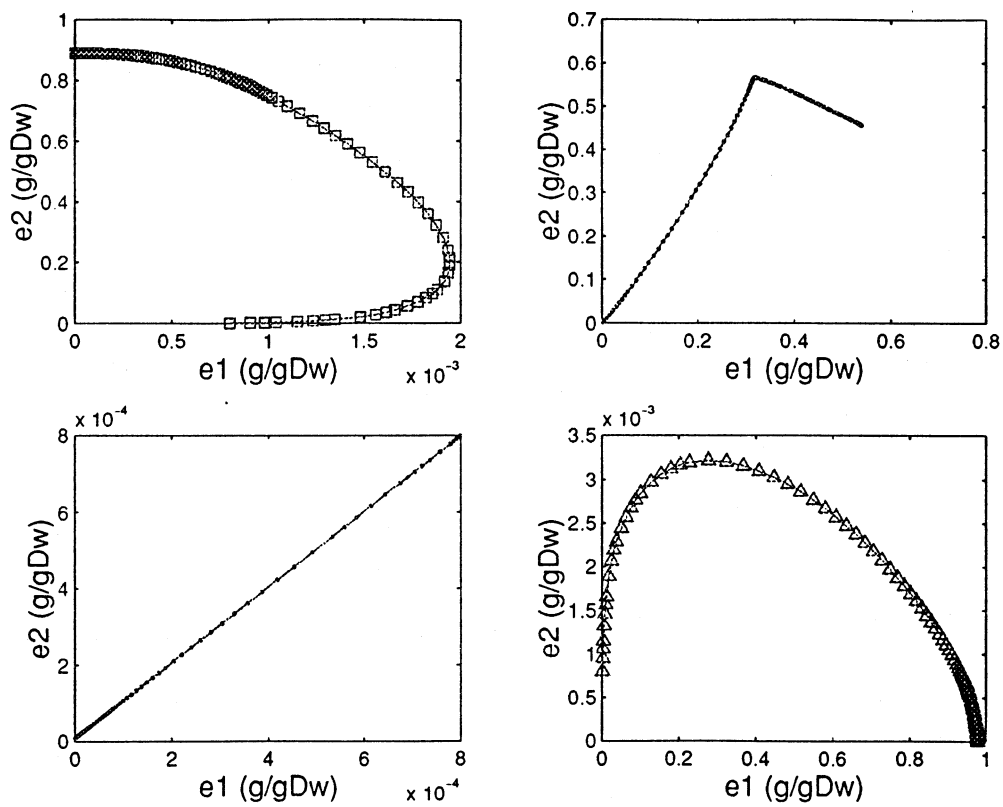

Fig. 2. Simulation results for the elementary convergent pathway in the four regions of system behavior.

$$\bar{K}_{q,j} \equiv \frac{K_{e_0^q}}{K_0^j}, \qquad \psi_0 \equiv \frac{R_0}{\delta}, \qquad \eta_k \equiv \frac{r^*_{e_k}}{\delta}$$

where $\delta \equiv (r_g + \beta)$, $\tau \equiv \delta t$ and $j = 1, 2, \ldots, n$. Assuming balanced growth and slow enzymatic decay, substitution of the resealed variables and parameters into the elementary divergent system model yields the resealed system

$$K_0^1 \frac{d\rho_0}{d\tau} = \psi_0 - \sum_{j=1}^{n} r_j v_j^c - K_0^1 \rho_0$$

$$K_1^q \frac{d\rho_q}{d\tau} = r_q v_q^c - \bar{r}_q - K_1^q \rho_q, \quad q = 1, 2, \ldots, n$$

$$\frac{d\epsilon_k}{d\tau} = r_{\epsilon_k} u_k^c - \epsilon_k + \eta_k, \quad k = 1, 2, \ldots, n \tag{4.10}$$

where the resealed rates take the form(s)

$$r_q = \kappa_q \, \epsilon_q \left(\frac{\rho_0}{\Gamma_{q,1} + \rho_0} \right), \quad q = 1, \ldots, n$$

$$\bar{r}_k = \bar{\kappa}_k \left(\frac{\rho_k}{1 + \rho_k} \right), \quad k = 1,2, \ldots, n \tag{4.11}$$

and

$$r_{\epsilon_j} = \frac{\rho_0}{\bar{K}_{j,1} + \rho_0}, \quad j = 1, \ldots, n \tag{4.12}$$

The parameter ψ_0 denotes the resealed specific rate of material influx into p_0, and by assumption is considered as a constant.

It is shown in Appendix A by analysis of the resealed divergent pathway model that key enzymes experiencing complementary competition assume non-zero equilibrium levels for all values of the system parameters. From a resource allocation perspective, this implies that no key enzyme can dominate over the others under this type of competitive environment. This interesting feature follows as a consequence of the class of objective functions that characterize complementary competition. The class of objective functions that defines substitutable competition consist of maximizing the level of a particular intermediate or end product of an isolated pathway, whereas, the objective of complementary competition is the maximization of the mathematical product of end products. This implies, in the case of complementary objectives, that all routes for the production of end products, for example p_1^1, p_1^2,…, p_1^n, must be operating at all times to ensure a non-zero objective. Thus, although the level of any one key enzyme is influenced by the others through resource sharing, it can not be driven to zero as was true of substitutable competition.

4.3. Combination of elementary pathways

Using a modular approach, we seek to construct lumped abstracted model frameworks of microbial processes. However, the problem that has plagued modelers since the beginning of time is at work in this instance as well. Namely, what level of abstraction is sufficient to suitably describe physical reality? This is an especially arduous question in the modeling of microbial processes because of the complexity of biological systems. The approach that we have proposed relies upon the construction of model frameworks from elementary components in such a way as to guarantee the description of desired qualitative behavioral features. The requirement of the approach is a fundamental understanding of the dynamics displayed by the elementary components in isolation coupled with awareness of the impact upon system dynamics of the interaction stemming from assembly. In other words, we must understand the characteristics of each of the elementary pieces, and moreover, how these characteristics interact when the elementary components are assembled to form the model. The purpose of the previous section was the exploration of the former, accordingly, we now focus our attention upon the ramifications to system behavior of assembling elementary units.

The model structure that we consider is shown in Fig. 3. This is an overlapping combination of an elementary convergent and divergent pathway. Notice that key enzyme e_1^a is a member of both elementary pathways, i.e. is a point of overlap between the two elementary components. As such we expect, intuitively, the allocation to e_1^a to be influenced by e_0 as well as e_1^b competition. However, the competitive structure experienced by e_1^a, as well as, the other system enzymes is neither complementary nor substitutable. Rather it is some hybrid combination of the two. We explore the deeper significance of these statements subse-

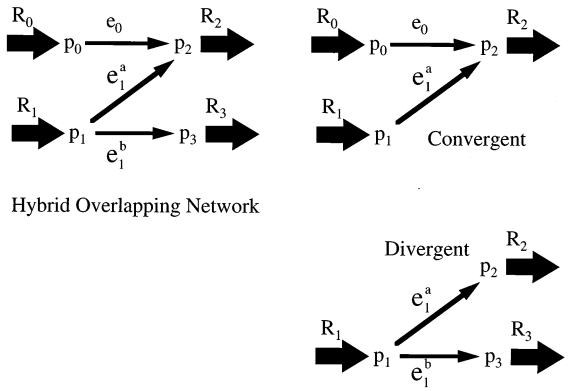

Fig. 3. Hybrid network composed of an overlapping convergent and divergent pathway.

quently, however, in the meantime we formulate the model equations that constitute the framework.

The material flux into intermediates p_0 and p_1 is assumed to be constant and denoted as

$$R_j, \quad j = 0, 1 \tag{4.13}$$

The intermediate p_0 is consumed via the key enzyme e_0 to produce the intermediate p_2 at the specific rate

$$r_0 = \mu_0^{\max}\left(\frac{e_0}{e_0^{\max}}\right)\frac{p_0}{K_0 + p_0} \tag{4.14}$$

where $\mu_0^{\max}$, K_0 denote the rate and saturation constants that govern p_2 formation and $e_0^{\max}$ denotes the maximum level of key enzyme e_0. The key enzyme e_0 is assumed to be induced by the presence of p_0 and is expressed at the specific rate

$$r_{e_0} = \alpha_0 \frac{p_0}{K_{e_0} + p_0} \tag{4.15}$$

where α_0, K_{e_0} denote the rate and saturation constants that govern the expression of e_0. The intermediate p_1 can be consumed by two routes. Firstly, the consumption of p_2 via key enzyme e_1^a to produce the intermediate p_2 occurs at the specific rate

$$r_1^a = \mu_1^{a,\max}\left(\frac{e_1^a}{e_1^{a,\max}}\right)\frac{p_1}{K_1^a + p_1} \tag{4.16}$$

where $\mu_1^{a,\max}$, K_1^a denote the rate and saturation constants that govern the formation of p_2 from p_1 and $e_1^{a,\max}$ denotes the maximum specific level of key enzyme e_1^a. The key enzyme e_1^a is assumed to be induced by the presence of p_1 and is expressed at the specific rate

$$r_{e_1^a} = \alpha_1^a \frac{p_1}{K_{e_1^a} + p_1} \tag{4.17}$$

where α_1^a, $K_{e_1^a}$ denote the rate and saturation constants that govern the expression of e_1^a. The intermediate p_1 can also be consumed via key enzyme e_1^b to produce the intermediate p_3 at the specific rate

$$r_1^b = \mu_1^{b,\max}\left(\frac{e_1^b}{e_1^{b,\max}}\right)\frac{p_1}{K_1^b + p_1} \tag{4.18}$$

where $\mu_1^{b,\max}$, K_1^b denote the rate and saturation constants that govern the formation of p_3 and $e_1^{b,\max}$ denotes the maximum specific level of key enzyme e_1^b. The key enzyme e_1^b is assumed to be induced by the presence of p_1 and is expressed at the specific rate

$$r_{e_1^b} = \alpha_1^b \frac{p_1}{K_{e_1}^b + p_1} \tag{4.19}$$

where α_1^a, $K_{e_1}^b$ denote the rate and saturation constants that govern the expression of e_1^b. The intermediates p_2 and p_3 are assumed to be precursors for downstream metabolites and are consumed at the specific rate

$$r_j = \mu_j^{\max}\frac{p_j}{K_j + p_j}, \quad j = 2, 3 \tag{4.20}$$

We assume for the sake of simplicity that the key enzymes which mediate the consumption of p_2 and p_3 are near their maximum levels, i.e. $e_j \sim e_j^{\max}, j = 2, 3$ and can be considered as constant.

To make the model formulation complete we must derive the appropriate forms of the cybernetic variables that regulate the expression and activity of key enzymes within the network. The derivation of the elementary cybernetic variables that regulate the convergent and divergent elementary pathways was shown in the previous section(s) and by Straight and Ramkrishna (1994), so we neglect its reintroduction. We construct the cybernetic regulation for the modular network using the tenets of the modular approach described by Varner and Ramkrishna (1998). To

review, this formulation assumes that metabolic regulation can be decomposed into a regulatory hierarchy consisting of three interacting layers, namely, elementary, local and global. This type of formulation was developed to provide a systematic description of cybernetic regulation for models systems formulated from elementary components. The elementary regulatory layer controls the allocation of critical resources from the resource pools associated with the elementary pathways to the synthesis of key enzymes competing for resources within a particular elementary pathway. These cybernetic variables are formulated following standard cybernetic doctrine, i.e. we postulate an objective function that is subject to a resource constraint and solve the resulting constrained optimization problem to yield the forms of the cybernetic variables. The local regulatory layer describes the interaction of the elementary regulatory components, i.e. overlapping key enzymes such as e_1^a. Because e_1^a is a member of two elementary pathways, it can receive critical resources from each elementary resource pool. Thus, the local layer of regulatory action describes how the multiple elementary allocation sources interact. Lastly, the highest postulated level of metabolic control is termed global control. This regulatory layer controls local metabolic activity. In a larger sense, this layer of regulatory action imparts the higher understanding of the microorganism to local metabolic activity. For example, we postulate the regulatory signals that control nutritional state dependent metabolic activity such as maintenance function or storage product synthesis are global signals. These signals translate the regulatory significance of the nutritional state into control action. The cybernetic variable that reflects all three regulatory levels is termed the complete cybernetic variable. We dispense with further introduction of the approach because this is not the main focus of the present development, however, interested readers are strongly encouraged to consult the above reference.

Within the present context, we assume no key enzyme is subject to global regulation, which implies the global cybernetic regulatory component in this case is unity. The derivation of the local cybernetic variables follows from the pathway topology and the type of intersection of elementary pathways.

The key enzyme e_0 is a member of only a single elementary pathway (convergent). Thus, it competes for key cellular resources only from the pool earmarked for convergent pathway operation. It follows that the local regulatory component consists solely of the elementary substitutable regulation, i.e.

$$u_0^l = u_0^s = \frac{r_0}{r_0 + r_1^a} \tag{4.21}$$

where the superscript l denotes local control. Because e_0 is not sensitive to any global regulatory signals, the complete cybernetic variable that regulates the expression of e_0 is given functionally as

$$u_0 = u_0^l \tag{4.22}$$

The complete cybernetic variable that governs the activity of e_0 follows by analogy and is given functionally as

$$v_0 = v_0^l = \frac{r_0}{\max(r_0, r_1^a)} \tag{4.23}$$

The key enzyme e_1^a is a member of both the elementary convergent, as well as, divergent pathway(s). As such, its synthesis machinery competes for key cellular resources from both elementary resource pools. The elementary cybernetic variable that governs the allocation of critical resources from the pool earmarked for convergent pathway operation, denoted as u_{1a}^s, was derived previously and is given functionally as

$$u_{1a}^s = \frac{r_1^a}{r_1^a + r_0} \tag{4.24}$$

where superscript s denotes substitutable. The key enzyme e_1^a is also a member of the elementary divergent pathway. The elementary cybernetic variable that governs the allocation of critical resources from the resource pool earmarked for operation of the elementary divergent pathway, denoted as u_{1a}^c, was derived previously and is given functionally as

$$u_{1a}^c = \frac{r_1^a/p_2}{r_1^a/p_2 + r_1^b/p_3} \tag{4.25}$$

where superscript c denotes complementary. The local cybernetic variable that governs the expression of e_1^a, denoted by u_{1a}^1 must reflect both possible allocation sources because e_1^a is sensitive to the objectives of both elementary pathways. Therefore, we postulate the local cybernetic variable consists of the product of the elementary regulatory components, i.e.

$$u_{1a}^1 = u_{1a}^c\, u_{1a}^s \tag{4.26}$$

where the multiplication follows as a consequence of the multiple allocation sources. Because e_1^a is not subject to any global regulatory signals, the complete cybernetic variable that governs e_1^a synthesis consists solely of the local component and takes the form

$$u_{1a} = u_{1a}^1 \tag{4.27}$$

The functional form of the complete cybernetic variable that governs the activity of e_1^a follows by analogy and is given functionally as

$$v_{1a} = v_{1a}^1 = v_{1a}^c\, v_{1a}^s \tag{4.28}$$

where the elementary cybernetic variables take the form

$$v_{1a}^c = \frac{r_1^a/p_2}{\max(r_1^a/p_2,\, r_1^b/p_3)} \qquad v_{1a}^s = \frac{r_1^a}{\max(r_1^a,\, r_0)} \tag{4.29}$$

Lastly, the key enzyme e_1^b is only a member of the elementary divergent pathway which implies that it can only receive resources from the pool earmarked for divergent pathway operation. The cybernetic variable that governs the allocation of critical resources for the expression of e_1^b, denoted as u_{1b}^c, was derived previously and takes the form

$$u_{1b}^c = \frac{r_1^b/p_3}{r_1^a/p_2 + r_1^b/p_3} \tag{4.30}$$

Because the key enzyme e_1^b is a member of only a single elementary pathway, the local cybernetic regulatory component that regulates its expression is given solely by the elementary component, i.e.

$$u_{1b}^1 = u_{1b}^c \tag{4.31}$$

Moreover, e_1^b expression is not sensitive to global regulatory concerns. Accordingly, the complete cybernetic variable that governs the synthesis of e_1^b is given by

$$u_{1b} = u_{1b}^1 \tag{4.32}$$

The functional form of the cybernetic variable that regulates the activity of e_1^b follows by analogy and is given by

$$v_{1b} = v_{1b}^1 = \frac{r_1^b/p_3}{\max(r_1^a/p_2,\, r_1^b/p_3)} \tag{4.33}$$

We now make the model formulation complete by modifying the rates of reaction and enzyme synthesis by the appropriate cybernetic variables.

The production of p_2 from p_0 is catalyzed by the key enzyme e_0 at the modified specific rate

$$r_0\, v_0 \tag{4.34}$$

where r_0 denotes the specific rate of p_2 formation from p_0 and v_0 denotes the complete cybernetic variable that governs the activity of e_0. The key enzyme e_0 is induced in the presence of p_0 and is expressed at the modified specific rate

$$r_{e_0}\, u_0 \tag{4.35}$$

where r_{e_0} denotes the specific rate of expression of e_0 and u_0 denotes the complete cybernetic variable that governs the synthesis of e_0.

The intermediate p_1 is consumed to produce p_2 via key enzyme e_1^a at the modified specific rate

$$r_1^a\, v_{1a} \tag{4.36}$$

where r_1^a denotes the specific rate of p_2 formation from p_1 and v_{1a} denotes the complete cybernetic variable that governs the activity of e_1^a. The key enzyme e_1^a is assumed to be induced in the presence of p_1 and is expressed at the modified specific rate

$$r_{e_1^a}\, u_{1a} \tag{4.37}$$

where $r_{e_1^a}$ denotes the specific rate of e_1^a expression and u_{1a} denotes the complete cybernetic variable governing the synthesis of e_1^a.

The intermediate p_1 can also be consumed to produce p_3 via key enzyme e_1^b at the modified specific rate

$$r_1^b\, v_{1b} \tag{4.38}$$

where r_1^b denotes the specific rate of p_3 formation and v_{1b} denotes the complete cybernetic variable that governs the activity of e_1^b. The key enzyme e_1^b

is assumed to be expressed at the modified specific rate

$$r_{e_1^b}\, u_{1b} \tag{4.39}$$

where $r_{e_1^b}$ denotes the specific rate of e_1^b expression and u_{1b} denotes the complete cybernetic variable that governs the synthesis of e_1^b.

The model framework, including the influence of metabolic regulation, is given by the set of differential equations

$$\frac{dp_0}{dt} = R_0 - r_0\, v_0 - r_g\, p_0$$

$$\frac{dp_1}{dt} = R_1 - r_1^a\, v_{1a} - r_1^b\, v_{1b} - r_g\, p_1$$

$$\frac{dp_2}{dt} = r_0\, v_0 + r_1^a\, v_{1a} - r_2 - r_g\, p_2$$

$$\frac{dp_3}{dt} = r_1^b\, v_{1b} - r_3 - r_g\, p_3$$

$$\frac{de_0}{dt} = r_{e_0}\, u_0 - (r_g + \beta) e_0 + r_{e_0}^*$$

$$\frac{de_1^j}{dt} = r_{e_1^j}\, u_{1j} - (r_g + \beta) e_1^j + r_{e_1^j}^*, \quad j = a, b \tag{4.40}$$

where r_g denotes the specific growth rate and β denotes the rate constant governing the first order decay of the k^{th} key enzyme.

As alluded to earlier, the hypothetical framework shown in Fig. 3 is a combination of a convergent and a divergent elementary pathway. More exactly, the key enzyme e_1^a is a member of both the convergent and divergent elementary components. From a cybernetic perspective, this implies that e_1^a competes for key cellular resources in both a substitutable, as well as, a complementary environment. In the analysis section we showed that substitutable and complementary competition are marked by qualitatively different types of dynamic behavior. Specifically, in a substitutable environment, it is possible for key enzymes to assume zero steady states, depending upon the system parameters, whereas, a complementary environment is characterized by a lack of bifurcation behavior. Bear in mind, that these behavioral traits are inherent to the isolated elementary pathways. Intuitively, we expect e_0 to behave much like a purely substitutable enzyme, and e_1^b as a complementary enzyme, however, what behavior should we expect from e_1^a? In other words, does e_1^a behave like a substitutable enzyme, i.e. it has a zero solution or does it behave in a complementary fashion by only assuming non-zero steady states? The answer to this question can be obtained by understanding the resource allocation structure and the properties of elementary pathways. To this end we postulate the following:

Definition 4.1. A key enzyme that is a member of both a substitutable and a complementary elementary pathway competes for key cellular resources in both competitive environments. Such an enzyme is termed partially substitutable/partially complementary.

Postulate 4.1. A partially substitutable/partially complementary key enzyme, because of the resource allocation structure, has the possibility of a zero solution whose stability is a function of system parameters.

Postulate 4.1 is proven in Appendix A so we dispense with a formal discussion here. Rather we focus upon the qualitative aspects of the system behavior and moreover the manner in which this behavior follows straightaway from the resource allocation structure. We have seen in the preceding sections that substitutable competition affords the possibility of enzymatic zero solutions, whereas, complementary competition yields no such behavior. From the modular formulation of the cybernetic variables described above and formulated in Varner and Ramkrishna (1998) the local level of regulation stems directly from solution of the isolated constrained optimization problems. In particular, key enzyme e_1^a competes for cellular resources in two distinct competitive environments, i.e. it experiences partially complementary/partially substitutable competition for key cellular resources. The cybernetic variable that governs the synthesis of e_1^a because of the possibility of joint allocation is given by

$$u_{1a} = u_{1a}^s\, u_{1a}^c \tag{4.41}$$

where the functional forms of the elementary cybernetic variables $u_{1a}^j, j = s, c$ follow from the solution of the isolated constrained optimization

problem. We postulate the presence of the substitutable regulatory component affords the possibility of a zero solution for e_1^a.

The equilibrium behavior of e_1^a (characterized in a rigorous sense in Appendix A) can be borne out through qualitative resource allocation arguments. The substitutable aspect of the allocation of critical resources to e_1^a competes with e_0 to produce the intermediate p_2, whereas, the complementary aspect of the cybernetic regulation competes to maximize the mathematical product of p_2 and p_3. Given this, one could imagine having already characterized the qualitative nature of substitutable competition that if $r_0 \gg r_1^a$ the intermediate p_2 is produced solely by e_0, i.e. e_0 is a better resource investment from the microorganism viewpoint for the synthesis of p_2 and consequently e_1^a levels are driven to zero. The simulation results for this case are shown in Fig. 4. Some remarks are in order before we proceed.

Remark 4.1. Note the complementary objective is satisfied under this condition as well as the substitutable.

Remark 4.2. Bear in mind e_1^b always assumes a non-zero equilibrium solution because it competes in only a complementary environment.

Remark 4.3. Note that investigation of the case $r_0 \gg r_1^a$ yields no bifurcation behavior. Thus, in this allocation regime e_1^a is always viewed as a bad resource investment for the production of p_2 relative to e_0.

However, e_1^a does not compete solely in a complementary environment. Rather it feels the influence of both types of competitive structures. In contrast to the case above, imagine the situation in which $r_0 \ll r_1^a$. This case is marked by e_1^a assum-

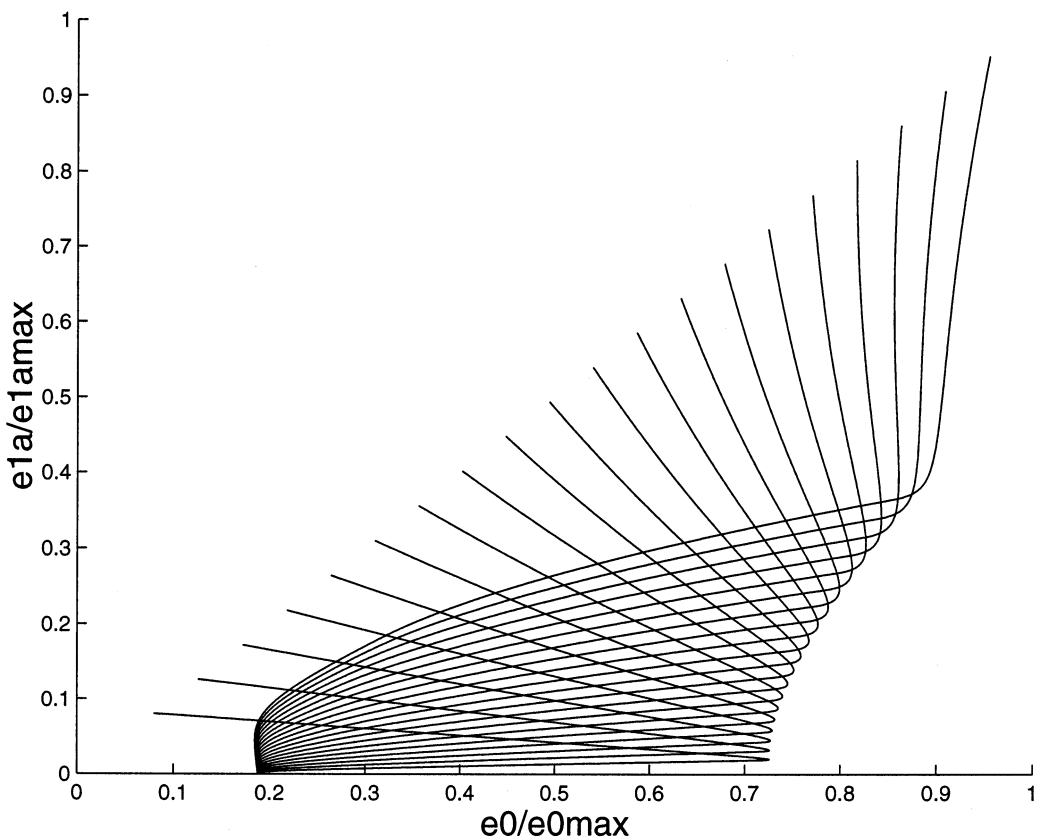

Fig. 4. Parallel network evolution when $r_1^a \ll r_0, \forall t$. The key enzyme e_1^a evolves toward a zero steady state.

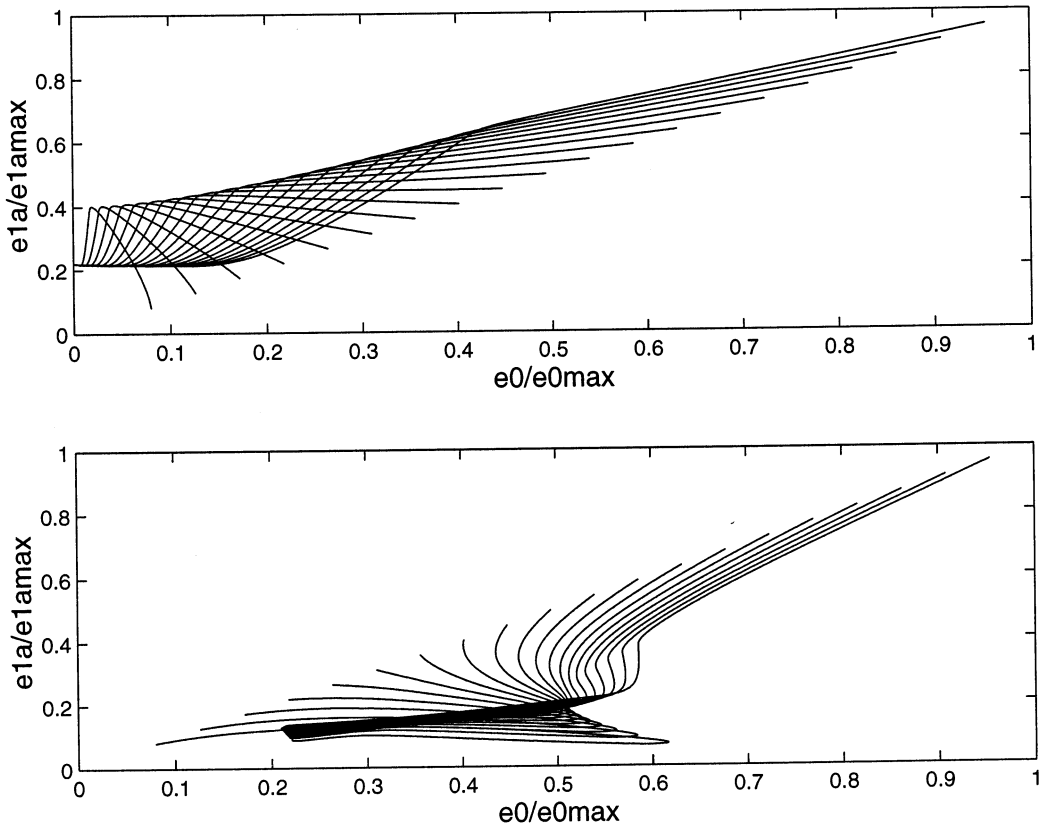

Fig. 5. Simulation of hybrid network for $r_1^a \gg r_0$. Note that e_0 can assume both a zero and non-zero steady state. Steady state values; Top plate: $(e_0/e_0^{max}, e_1^a/e^{a,\,max}) = (0.2167, 0.1194)$. Bottom plate: $(e_0/e_0^{max}, e_1^a/e_1^{a,\,max}) = (0, 0.2205)$.

ing a non-zero equilibrium solution and e_0 levels being driven to zero. Following the same logic as the substitutable analysis, as e_0 becomes a more attractive resource investment from the microorganisms viewpoint it assumes a non-zero equilibrium solution. In fact it is shown in Appendix A that the base zero solution ($e_0 = 0$, $e_1^a \neq 0$) bifurcates to a non-zero level of e_0 when the condition

$$\frac{\kappa_0}{\kappa_1^a} = \Theta \tag{4.42}$$

is satisfied where Θ is the compound parameter

$$\Theta \equiv \frac{\sqrt{\kappa_2\,\kappa_3}}{\kappa_3} \tag{4.43}$$

Notice that condition (4.42) is very similar in structure to the bifurcation condition determined in the pure substitutable case with the exception of Θ. The compound parameter Θ can be shown to be the influence stemming from the complementary aspect of the system regulation. Clearly, this is the case because Θ reflects the regulatory influence stemming from the metabolite feedback loops that are a characteristic of complementary competition. In particular, the complementary regulatory aspect influences e_0 expression (via e_1^a synthesis) depending upon the rate of removal of the intermediates p_2 and p_3. If Θ is small, the maximum rate of formation of the intermediate p_2 via key enzyme e_1^a must be large when compared with κ_0 for e_0 to be an attractive resource investment. The converse being true if Θ is large. This case is simulated using the original model system and the results are shown in Fig. 5.

Thus, a partially substitutable/partially complementary key enzyme can assume both a non-zero and a zero equilibrium solution depending upon the system parameters. In particular, if $r_0 \gg r_1^a$ then e_0 is seen as an attractive resource investment and e_1^a levels are driven to zero. In this case the intermediate p_2 is produced in a substitutable manner. However, if $r_1^a \gg r_0$ using the same arguments, e_0 levels can be driven to zero because e_1^a represents a better resource investment from the perspective of the microorganism. It follows in this case p_2 is produced in a complementary fashion. Lastly, as $\kappa_0 > \Theta\kappa_1 e_0$ and e_1 are both synthesized and p_2 is produced via both routes. This behavioral switch follows as a consequence of the substitutable aspect of the system regulation.

4.4. Effect of constitutive enzyme synthesis

The presence of constitutive enzyme synthesis does nothing to qualitatively alter the complementary aspect of the system regulation, however, as alluded to previously it can significantly alter the characteristics of substitutable environments. More precisely, it destroys the possibility of substitutable zero solutions. Intuitively, one would expect such a phenomena because those enzymes that are seen as poor resource investments can not be driven to zero because some expression is taking place, i.e. constitutive expression, independently of the inducible portion. From a mathematical perspective, the presence of constitutive enzyme synthesis is formally treated as a system imperfection or breaking problem as outlined by Iooss and Joseph (1990). The details of the analysis are presented in Appendix A. Within the present discussion, the parametric location and the physical significance of the bifurcation are altered somewhat by the presence of constitutive enzyme synthesis. More precisely, bifurcation in this context is between two non-zero equilibrium solutions rather than between a zero and non-zero equilibrium behavior. Note this statement assumes all enzymes possess a constitutive synthesis element.

5. Topological abstraction guidelines

The analysis of the model framework(s) shown above shed light upon the type of behavior that can be expected from particular competitive structures. The objective of this section is to translate this understanding into a set of guidelines that can be used to formulate cybernetic model systems. In reality, these guidelines are nothing more than a restatement of the previous results in condensed form. We choose to state the guidelines in the form of postulates and illustrate the application of these postulates in subsequent papers.

Postulate 5.1. The qualitative dynamics of a cybernetic model can be determined by inspection of the topological structure. Specifically, the dynamics follow from the competitive structure that a key enzyme is experiencing. The competitive structure is a non-unique function of the topology. Convergent and linear fragments are substitutable, whereas, divergent and cyclic units are complementary.

Remark 5.1. This assumes that the abstracted model framework is constructed from the elementary pieces as derived by Straight and Ramkrishna. In a more general sense, the behavior stems from the structure of the competition for cellular resources and in particular the form of the objective function and resource constraint.

Postulate 5.2. Key enzymes that compete for cellular resources in a substitutable manner have the possibility of both non-zero, as well as, zero equilibrium solutions depending upon the system parameters. As a rule of thumb, in the absence of constitutive enzyme synthesis, the fastest process in a substitutable competitive environment dominates the allocation process, thus, driving the level of key enzyme(s) that catalyze the slower processes to zero.

Postulate 5.3. Key enzymes that compete for cellular resources in a complementary manner evolve only toward non-zero steady states for all values of the system parameters.

Postulate 5.4. Overlapping enzymes that lie in multiple allocation regimes can receive key cellular resources from each of the elementary resource pools. Thus, because the optimization for each elementary allocation problem is performed in

isolation, overlapping key enzymes possess the characteristics of the totality of competitive structures in which it competes.

Postulate 5.5. Key enzymes that compete in partially complementary/partially substitutable environments assume the qualitative properties of the substitutable environment, i.e. have the possibility of assuming a zero solution in the face of severe competition for key cellular resources.

Remark 5.2. In the case of partially substitutable/partially complementary competition, the complementary competitive aspect alters the location of bifurcation of the substitutable competition. The communication between elementary pathways is a consequence of having a single key enzyme compete for resources from both elementary pools.

Postulate 5.6. Constitutive enzyme synthesis destroys the possibility of zero solutions in a substitutable competitive environment. It has no qualitative effect upon the dynamics of complementary structures.

These postulates represent a set of rules that can be used to guide the construction of abstracted cybernetic models. In the general case, given an arbitrary model structure, they can be employed to determine the qualitative dynamics that a particular model system displays. We illustrate this approach in subsequent papers by considering the construction and analysis of model systems capable of describing complex uptake patterns.

6. Conclusions

In this work we have investigated the qualitative nature of cybernetic models using tools from bifurcation theory. Specifically, we have determined the qualitative properties of substitutable and complementary competition for key cellular resources as a function of the system parameters. The hallmark of substitutable competition is the possibility of an enzymatic zero solution. In cybernetic terms this implies the key enzyme which catalyzes the fastest rate dominates the allocation of critical resources, leaving little resource for the remaining enzymes. Moreover, as a function of system parameters substitutable competition was shown to undergo bifurcation between states of equitable sharing of resources and complete dominance by specific key enzymes. Complementary competition, on the other hand, is marked by the absence of an enzymatic zero solution. In fact, no bifurcation behavior was observed for all values of the system parameters. The combination of substitutable and complementary competition resulted in a regulatory structure whose dynamic features bear a striking resemblance to purely substitutable competition. The partially substitutable partially complementary structure shares the qualitative features of elementary substitutable competition, namely, the existence of an enzymatic zero solution. However, the parametric location of bifurcation is influenced by the complementary aspects of the system.

In a larger sense this work serves as an introduction to a conceptual methodology that can be employed to guide the abstraction of metabolic pathways. In particular, we have solved the cybernetic structure function relationship. In other words, we now have an understanding of the qualitative features that can be expected from substitutable and complementary competition. This is the function side of the equation. The structural aspect, i.e. the abstraction of metabolic pathways, stems from a modular construction concept. This methodology follows from the postulate that an arbitrary metabolic network can be decomposed and abstracted into elementary components, similar to pieces in a jigsaw puzzle. By understanding the function associated with a particular piece, and moreover, how this function is altered by interaction with other elementary components we gain insight into the qualitative nature of the dynamics of the abstracted pathway model. This affords the ability to approach the problem of formulating a cybernetic model with a desired set of dynamical features from a grey box modeling perspective. In other words, using this approach, we can produce entire classes of model systems with desired behavioral traits, simply by assembling the elementary pieces in a particular fashion.

This paper is the first of a series to be published elsewhere in the literature. The objective of the

remaining papers is to illustrate these concepts by considering a number of example systems. Firstly, we consider the mineralization of Aniline in the presence/absence of secondary carbon and nitrogen sources. For this system we present two degenerate cybernetic model formulations, both of which are capable of describing experimental nutritional uptake patterns. The last two examples are purely illustrative in scope and are presented in a more general spirit. The first formulation is a cybernetic model that is capable of describing an arbitrary nutritional uptake pattern given three substrates. This model is a generalization of the model framework recently published by Ramakrishna et al. (1997). We then having analyzed this structure, generalize it to the n-dimensional mixture problem and present a model framework that is capable of predicting uptake patterns for n carbon sources.

Appendix A. Bifurcation analysis of competitive environments

A.1. Bifurcation analysis: substitutable competition

To begin the analysis of the $2n+2$-dimensional elementary convergent pathway model we rescale with respect to the following set of dimensionless variables and parameters

$$\sigma_j \equiv \frac{p_j}{K_j} \qquad \epsilon_j \equiv \frac{e_j\,\delta}{\alpha_j} \qquad \kappa_j \equiv \frac{\mu_j^{\max}}{\delta} \tag{A.1}$$

$$\tau \equiv \delta t \qquad \eta_j \equiv \frac{r_{e_j}^*}{\alpha_j} \qquad \bar{K}_{i,j} \equiv \frac{K_{e_i}}{K_j} \tag{A.2}$$

where $\delta \equiv (r_g + \beta)$ and $j = 0, 1, \ldots, n$. By assumption we are investigating the dynamics of the elementary convergent pathway during balanced growth conditions. It follows that $r_g \sim \mu_g^{\max}$ under this assumption. Furthermore, we assume $\delta \sim \mu_g^{\max}$, i.e. the rate constant governing the first order decay of enzyme, denoted as β, is small compared with the maximum specific growth rate. Substituting the recealed variables and parameters into the convergent pathway model under the above assumptions yields the dimensionless system in vector form

$$\frac{d\mathbf{x}}{d\tau} = \mathbf{F}(\mathbf{x}, \kappa, \eta), \quad F{:}\mathbf{R^{2n+2}} \times \mathbf{R^{n+1}} \rightarrow \mathbf{R^{2n+2}} \tag{A.3}$$

where the state vector $\mathbf{x}$ and the rate constant vector κ are given by

$$\mathbf{x} \equiv \{\sigma_0, \sigma_1, \ldots, \sigma_n, \epsilon_0, \epsilon_1, \ldots, \epsilon_n\}^T \tag{A.4}$$

$$\kappa \equiv \{\kappa_0, \kappa_1, \ldots, \kappa_n\}^T, \tag{A.5}$$

$$\eta \equiv \{\eta_0, \eta_1, \ldots, \eta_n\}^T \tag{A.6}$$

The goal of our analysis is to characterize the qualitative features of the convergent pathway as a function of the parameter vector κ. To achieve such a goal we call upon tools from non-linear analysis, specifically bifurcation and singularity theory, to determine the location and nature of possible bifurcation points. We then determine the manner in which the system behavior is altered as the parameter values are varied through a critical value. To assure a manageable problem that still provides a hint of the nature of the general n-dimensional problem, we consider the case of $n = 2$. Before we can begin the bifurcation analysis, however, we address a technical issue related to the form of the cybernetic v variable.

For the j^{th} substitutable process, the elementary cybernetic variable that governs the activity of e_j, denoted as v_j^s, takes the form

$$v_j^s = \frac{r_j}{\max(r_1, r_2, \ldots, r_j, \ldots, r_n)}, \qquad \forall\, j \tag{A.7}$$

The max function in the denominator of v_j^s makes the system very difficult to deal with analytically. Accordingly, we employ a methodology that has been developed to combat this concern, yet still maintains the essential information that the v variable imparts to the system. Let us suppose that the i^{th} rate of p_{n+1} formation is the fastest rate. Dividing the numerator and the denominator of (A.7) by r_i yields

$$v_j^s = \frac{\gamma_{j,i}}{\max(\gamma_{1,i}, \gamma_{2,i}, \ldots, \gamma_{j,i}, \ldots, \gamma_{n,i})}, \qquad \forall\, j,$$

$$\gamma_{j,i} \equiv \frac{r_j}{r_i} \tag{A.8}$$

Following the same logic the elementary substitutable cybernetic variable that governs the expression of e_j, denoted as u_j^s, given by

$$u_j^s = \frac{r_j}{r_j + \sum_{k=1,j}^{n} r_k}, \qquad \forall\, j \tag{A.9}$$

can be rewritten as

$$u_j^s = \frac{\gamma_{j,i}}{\gamma_{j,i} + \sum_{q=1,j}^{n} \gamma_{q,i}}, \qquad \forall\, j \tag{A.10}$$

where we divided the numerator and denominator by the fastest rate of p_{n+1} formation. We arbitrarily assign $r_i \equiv r_0$. This does not have to be so, however, it eases the algebraic complexity while not restricting the generality of analysis. Accordingly, we rewrite Eqs. (A.8) and (A.10) with $n = 2$ to yield

$$v_j^s = \frac{\gamma_{j,0}}{\max_q(\gamma_{q,0})},$$

$$u_j^s = \frac{\gamma_{j,0}}{\gamma_{j,0} + \sum_{q=0,j}^{2} \gamma_{q,0}}, \quad j = 0, 1, 2 \tag{A.11}$$

When $j = 0$ we arrive at the control variable set

$$v_0^s = \frac{1}{\max(1, \gamma_{1,0}, \gamma_{2,0})}, \qquad u_1^s = \frac{1}{1 + \sum_{q=1}^{2} \gamma_{q,0}} \tag{A.12}$$

Because we have assumed the r_0 is the fastest rate, for some period of time $t^* \in [t_1, t_2]$ $v_0^s = 1$. During t^* the other v^s variables, i.e. v_1^s, v_2^s are given by

$$v_1^s = \gamma_{1,0}, \qquad v_2^s = \gamma_{2,0} \tag{A.13}$$

Following the same logic, the cybernetic variable u_0^s can be rewritten as

$$u_0^s = \frac{1}{1 + \sum_{q=1}^{2} \gamma_{q,0}} \tag{A.14}$$

If $1 \gg (\gamma_{1,0} = \gamma_{2,0})$ while $t^* \in [t_1, t_2]$, then $u_0^s \simeq 1$ and

$$u_1^s \simeq \gamma_{1,0}, \qquad u_2^s \simeq \gamma_{2,0}, \qquad \forall\, t^* \tag{A.15}$$

This breakdown has the effect of reducing our original system model to two distinct sets of equations which we term the unregulated and the regulated set, respectively. The unregulated set is composed of the mass balances for the quantities that take part in the fastest process. Because we have assumed r_0 to be the fastest, the unregulated set is made up of the σ_0 and ϵ_0 balances

$$K_0 \frac{d\sigma_0}{d\tau} \simeq \psi_0 - \kappa_0\, \epsilon_0\, \bar{\sigma}_0 - K_0\, \sigma_0, \quad \bar{\sigma}_0 \equiv \frac{\sigma_0}{1 + \sigma_0} \tag{A.16}$$

$$\frac{d\epsilon_0}{d\tau} \simeq r_{e_0} - \epsilon_0 + \eta_0 \tag{A.17}$$

where $v_0^s = 1$, $u_0^s \simeq 1$ and $\psi_j \equiv R_j/\delta$ denotes the rescaled material input flux towards p_0. By contrast, the regulated set consists of the remainder of the system mass balances, i.e.

$$K_j \frac{d\sigma_j}{d\tau} \simeq \psi_j - \kappa_j\, \epsilon_j\, \bar{\sigma}_j\, \gamma_{j,0} - K_j\, \sigma_j, \quad j = 1, 2 \tag{A.18}$$

$$\frac{d\epsilon_i}{d\tau} \simeq \frac{\sigma_i}{\bar{K}_{i,j} + \sigma_j}\, \gamma_{i,0} - \epsilon_i + \eta_i, \quad i = 1, 2 \tag{A.19}$$

These two sets of equations (regulated and unregulated) define what is termed the region set and can be recast in vector form as

$$\frac{d\mathbf{x_{RS}}}{d\tau} \simeq \mathbf{H}(\mathbf{x_{RS}}, \kappa, \eta),$$

$$\mathbf{x_{RS}} \equiv \{\mathbf{x_{UR}}, \mathbf{x_R}\}^T \tag{A.20}$$

where $\mathbf{x_{UR}}$, $\mathbf{x_R}$ are the state variable vectors of the unregulated and regulated set, respectively and $\mathbf{H}(\mathbf{x_{RS}}, \kappa, \eta)$ is defined as

$$\mathbf{H}(\mathbf{x_{RS}}, \kappa, \eta) \equiv \{\mathbf{f}(\mathbf{x_{UR}}, \kappa, \eta), \mathbf{g}(\mathbf{x_{RS}}, \kappa, \eta)\}^T \tag{A.21}$$

where $\mathbf{f}(\mathbf{x_{UR}}, \kappa, \eta)$, $\mathbf{g}(\mathbf{x_{RS}}, \kappa, \eta)$ are the vectors corresponding to the right hand sides of the unregulated and regulated sets, respectively. The equation set (A.20) defines a region in the solution state space in which the cybernetic regulatory machinery can be broken down as shown. Clearly, this decomposition hinges upon the rate r_0. If r_0 remains the fastest rate for $\forall\, t$ then this region becomes invariant with respect to time. However, if r_0 remains the fastest rate for only some finite period of time, i.e. there exist non-infinite boundaries for the interval $[t_1, t_2]$ then the regulatory system can be broken down as shown only as long as $t \in t^*$. This distinction has a profound

effect upon system behavior. If the regulatory region is invariant, then the long time behavior of the original system is the stable equilibrium behavior of equation set (A.20). Conversely, if we enter and/or leave the regulatory region in some finite period of time, the behavior of equation set (A.20) will appear only as a transient in the overall long time behavior of the full system model. Moreover, it can be shown that there will always exist, if the cybernetic system possesses a stable steady state, an invariant regulatory region. Thus, those regions which appear as transients simply represent the manner in which the regulatory picture changes with respect to time as we move towards a stable steady state. In other words, we move through the transient regions on our way towards the invariant region as time progresses.

The dynamics displayed by the unregulated set are approximately independent of the rest of the system, thus, as $t \to \infty$ $\mathbf{x_{UR}}$ can be approximated by the long time equilibrium behavior of equation set (A.16). To determine this behavior we must solve the system

$$\mathbf{f}(\mathbf{x_{UR}}, \kappa, \eta) = \mathbf{0} \tag{A.22}$$

i.e. determine the equilibrium behavior of the unregulated system. If we assume $\bar{K}_{0,0} \ll \sigma_0, \ \forall\, t$ (this assumption implies enzyme expression is saturated with respect to inducer metabolite) then (A.22) has the solution set $(\epsilon_0^*, \sigma_0^*)^T$

$$\epsilon_0^* = 1 + \eta_0,$$
$$K_0(\sigma_0^*)^2 + (K_0 + \kappa_0(1+\eta_0) - \psi_0)\sigma_0^* - \psi_0 = 0 \tag{A.23}$$

which can be shown to be a stable equilibrium point for all values of the system parameters. The regulated set given by

$$\frac{d\mathbf{x_R}}{d\tau} \simeq \mathbf{g}(\mathbf{x_R}, \kappa, \mathbf{0}) \tag{A.24}$$

has an equilibrium solution set $\mathbf{x_R^*} = (\sigma_1^*, \epsilon_1^*, \sigma_2^*, \epsilon_2^*)^T$

$$\mathbf{x_R^*} = \{\psi_1, 0, \psi_2, 0\}^T \tag{A.25}$$

Notice that we have defined our equilibrium set as the solutions of the system $\mathbf{g}(\mathbf{x_R}, \kappa, \mathbf{0}),\ \eta = \mathbf{0}$. This is done so as to consider η (which denotes the scaled specific rate of constitutive enzyme synthesis) as an imperfection parameter. Solution set (A.25) denotes the system zero solution for enzymes $e_j, j = 1, 2$. Thus, even at this early stage, we see that substitutable competition has an allocation structure that allows for some enzyme levels to be driven to zero. From a resource allocation viewpoint, this implies that one key enzyme, if competitive enough, can dominate over the others. Introducing the new variable $\mathbf{y} \in \mathbf{R}^4$ given by

$$\mathbf{x_R} = \mathbf{x_R^*} + \mathbf{y} \tag{A.26}$$

translates equilibrium set (A.25) to the origin. This solution set forms the base set from which we investigate any possible bifurcation that may arise in the system. A point of possible bifurcation is marked by a zero eigenvalue, i.e. the Jacobian matrix becomes singular at this point. In a more precise sense we seek the parameter value κ_0 such that $Det\{J(\kappa_0)\} = 0$. Physically, by examining bifurcation behavior with respect to the system parameters, specifically the process rate constants, we are exploring the ramifications of different levels of competitive vigor amongst the enzyme expression systems. The translated system given by

$$\frac{d\mathbf{y}}{d\tau} \simeq \mathbf{g}(\mathbf{y}, \kappa, \mathbf{0}) \tag{A.27}$$

has the *Det* (**J**) at the origin, where $j_{i,k} \equiv \dfrac{\partial g_i}{\partial y_k}$, given by

$$K_1\left(\frac{\kappa_1\,\psi_1}{(1+\psi_1)r_0^*} - 1\right) K_2\left(\frac{\kappa_2\,\psi_2}{(1+\psi_2)r_0^*} - 1\right) \tag{A.28}$$

where r_0^* is the rate of the fastest process evaluated at the equilibrium point of the unregulated system. If we define

$$r_0^* \equiv \kappa_0\,\theta, \quad \theta \equiv \frac{\epsilon_0^*\,\sigma_0^*}{1+\sigma_0^*} \tag{A.29}$$

then the critical value of the system parameters i.e. points of possible bifurcation are given by

$$\beta_j = \frac{\kappa_j}{\kappa_0}\left(\frac{\psi_j}{(1+\psi_j)\theta}\right) - 1, \quad j = 1, 2 \tag{A.30}$$

Examination of Eq. (A.30) clearly indicates three distinct bifurcation possibilities exist for the regulated system. Firstly, β_2 could independently cross through a critical value i.e. $\beta_2 = 0$, $\beta_3 \neq 0$ which implies

$$\frac{\kappa_1}{\kappa_0} = \left(\frac{1+\psi_1}{\psi_1}\right)\theta \tag{A.31}$$

Similarly β_3 could cross through zero independently ($\beta_2 \neq 0$, $\beta_3 = 0$) which implies

$$\frac{\kappa_2}{\kappa_0} = \left(\frac{1+\psi_2}{\psi_2}\right)\theta \tag{A.32}$$

Both of the previous cases mark simple crossings with a 1-dimensional Jacobian null-space. The last case, however, involves $\beta_2 = \beta_3 = 0$ simultaneously which implies a 2-dimensional null-space and is a slightly more involved problem.

The physical significance of the parametric relationship amongst the critical condition(s) has an interesting interpretation in terms of resource allocation. If the material flux into the elementary convergent pathway is large, i.e. $\psi_j \gg 1$ then the right hand side of the critical condition is approximately unity. This implies that bifurcation occurs when the ratio of the rate constants is unity. From a resource allocation viewpoint, this condition marks the point where the investment attractiveness of competing processes is approximately equal.

We begin the bifurcation analysis with the first two cases, then comment upon the 2-dimensional null-space problem.

A.1.1. Case a, b: 1-dimensional null-space

The Jacobian matrix for the regulated balances when evaluated on the base solution can easily be shown to be of the form

$$\mathbf{J} = \begin{pmatrix} \mathbf{J_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{J_2} \end{pmatrix} \tag{A.33}$$

where $\mathbf{0}$ denotes a 2×2 null block and the blocks $\mathbf{J_i}$ are given by

$$\mathbf{J_i} = \begin{pmatrix} -K_i & 0 \\ 0 & \beta_i \end{pmatrix}, \quad i = 1, 2 \tag{A.34}$$

Two observations are useful at this point. Firstly note that the Jacobian of the regulated system is diagonal. This will be of great use subsequently when we employ a reduction technique to isolate the bifurcation problem. Secondly, note that the balances for the second and third process are not coupled and are identical. The latter feature follows as a consequence of the regulatory region decomposition. We take advantage of these properties because they imply we have two degenerate bifurcation problems. We need only to study one to gain information about both systems.

As is standard practice, we utilize a reduction technique to isolate the portion of the system that is responsible for the bifurcation. In our case, because the Jacobian is diagonal and hence possesses a complete orthonormal family of eigenvectors we utilize Lyapunov–Schmidt reduction (Golubitsky and Schaeffer, 1985) to obtain the reduced system or equation. Furthermore, because our bifurcation problem is 1-dimensional, the reduced system will consist of a single equation whose behavior will control the qualitative aspects of the system dynamics. In this development we skip much of the technical detail related to the reduction procedure and present only the reduced equation. However, firstly, to make the algebra more tractable, we approximate the system by a Taylor series around the origin retaining up to cubic terms. Note, because of the degeneracy of the system we need only expand and study one set of balances. Accordingly, to keep the analysis general, we expand and study the balances around the i^{th} process i.e. r_i where $i = 1, 2$. More precisely if we define $\mathbf{z}^k \equiv \{y_k, y_{k+1}\}^T$ and further define $\overline{\mathbf{g}^{\mathbf{k}}} \equiv \{g_k, g_{k+1}\}^{\mathrm{T}}$ where $k = 1$ or 3 then the degenerate problem can be caste in vector form

$$\frac{d\mathbf{z}^k}{d\tau} = \overline{\mathbf{g}^k}(\mathbf{z}, \kappa, \eta), \quad \mathbf{z} \in \mathbf{R}^2 \tag{A.35}$$

(see Eq. (A.27)). Expanding Eq. (A.35) in a Taylor series about the origin yields

$$\frac{d\mathbf{z}^k}{d\tau} = \mathbf{J_k}\begin{cases} k=1 & \mathbf{J_1}(\beta_1) \\ k=3 & \mathbf{J_2}(\beta_2) \end{cases}\mathbf{z^k} + \mathbf{G_2^k}(\mathbf{z^k}, \beta, \eta) + \mathcal{O}(3), \quad \mathbf{k} = 1 \text{ or } 3$$

where $\mathbf{G_2^k}(\mathbf{z^k}, \beta, \eta)$ denotes the vector of second order terms. In a more practical sense, the symmetry of **J** implies that for a 1-dimensional crossing one bifurcation parameter is held constant while the other is varied. Accordingly, we assume β_1 is held constant while we vary β_2, i.e. we vary the parametric nature of e_2 consumption. Applying Lyapunov–Schmidt reduction to equation set (A.36) and relaxing the requirement that $\eta_j = 0$ yields the reduced equation

$$\frac{d\zeta}{d\tau} = \beta_2\,\zeta - a(\beta_2)\zeta^3 + \eta_2,$$

$$a(\beta_2) = \left(\frac{\theta(1+\beta_2)^3\kappa_1\,K_2}{\psi_2(K_2+\psi_2)}\right) \tag{A.37}$$

where ζ is the reduced variable. Note in the absence of constitutive enzyme synthesis Eq. (A.37) is the pitchfork bifurcation normal form. Thus, as β_2 is varied through the critical value we expect ζ to bifurcate from the zero solution to a non-zero equilibrium solution. As a matter of convenience, we choose to eliminate the parameter dependence of the coefficient of ζ^3. Accordingly, we introduce the scaled reduced variable

$$\zeta \equiv \frac{u}{f(\beta_2)} \tag{A.38}$$

where $f(\beta_2) \neq 0,\ \forall\ \beta_2$. Substituting this rescaling into Eq. (A.37) yields

$$\frac{du}{d\tau} = \beta_2\,u - u^3 + \bar{\eta}_2(\beta_2) \tag{A.39}$$

$$f(\beta_2) \equiv \sqrt{a(\beta_2)} \tag{A.40}$$

$$\bar{\eta}_2(\beta_2) \equiv \eta_2(\sqrt{a(\beta_2)}) \tag{A.41}$$

The solutions of the reduced Eq. (A.39) control the qualitative nature of the dynamics of the full system. More exactly, these solutions correspond, qualitatively, with the equilibrium behavior of e_2. Thus, by examining Eq. (A.39) we gain insight as to how the dynamics change as a function of system parameters. In our case we are using the compound parameter β_2 as the bifurcation parameter. Physically, this parameter reflects the influence of the rescaled reaction rate constants as well as the rescaled specific input flux and saturation constants. In the absence of constitutive enzyme synthesis, Eq. (A.39) has the equilibrium solutions

$$u^1 = 0 \qquad u^{2\pm} = \pm\sqrt{\beta} \tag{A.42}$$

As expected in the neighborhood of the bifurcation point two (physically realistic) solutions are possible for the reduced variable ξ. In biological terms, this implies that two solutions are possible for the key enzyme e_2 as a function of the system parameters. The solution u^1 corresponds directly with the zero solution of (A.25). However, $u^{2\pm}$ corresponds to a non-zero level of e_2. Which solution is observed is a function of the stability properties. The stability of the solution set (A.42) to small disturbances is governed by the sign of the eigenvalue associated with the j^{th} solution branch. The eigenvalue in 1-dimension is really nothing more than the linear coefficient of the taylor series expansion of the reduced equation with respect to the reduced variable u around the bifurcation point, denoted as (β_0, u_0^j), as a function of β. More precisely, the 1-dimensional eigenvalue of the general reduced equation $h(\beta, u)$ is given by

$$\lambda_j = \left.\frac{\partial h}{\partial u}\right|_{\beta,\,u^j} = h_u \tag{A.43}$$

where λ_j denotes the eigenvalue along the j^{th} solution branch. It is easily shown that h_u vanishes at the bifurcation point for double point bifurcation, yielding no stability information. Accordingly, we must appeal to the stability information contained in the higher order terms of the eigenvalue expansion. Expanding h_u around the bifurcation point with respect to the parameter β and retaining the linear terms yields

$$\lambda_{\beta,j} = \simeq \left(\frac{\partial h_u}{\partial \beta} + \frac{\partial h_u}{du}d\beta\right)\delta\beta + \mathcal{O}(|\delta\beta|^2) \tag{A.44}$$

where $\delta\beta \equiv \beta - \beta_0$ and where $\lambda_{\beta,j}$ denotes the eigenvalue expansion along the j^{th} solution branch. Given the form of Eq. (A.39), $\lambda_{\beta,j}$ reduces to the set

$$\lambda_{\beta,1} = \delta\beta \quad \lambda_{\beta,2\pm} = -2\delta\beta \tag{A.45}$$

This indicates that solution 1, i.e. the zero solution is stable when $\beta > \beta_0$ and unstable when $\beta < \beta_0$, the converse being true for solution 2. In the present case, $\beta_0 = 0$ implies the zero solution strictly exchanges stability (pitchfork bifurcation) with the bifurcating solution. The stability analysis results are summarized in Table 2 where system behavior is summarized as a function of (β_1, β_2). The analysis prediction are simulated and the results presented in Fig. 2.

Let us return to the discussion of bifurcation behavior as a consequence of the system resource allocation structure. We have shown that bifurcation (in the absence of constitutive enzyme synthesis) occurs when $\beta_j = 0$. This condition physically, as alluded to previously, marks a location in which the resource investment attractiveness of one process versus another is approximately equal. In particular we have shown that as β_j moves through zero from the left the enzymatic zero solution exchanges stability with a non-zero enzyme level. Recast in terms of resource allocation, $\beta_j = 0$ marks the boundary at which e_j can be driven to zero. In other words, as β_j is varied through the critical value, the attractiveness of the j^{th} process as a resource investment increases and finally becomes significant enough to warrant investment by the microorganism.

Table 2
System behavior as a function of (β_1, β_2)[a]

Parameter region	e_0	e_1	e_2
$\beta_1>0, \beta_2>0$	+	+	+
$\beta_1>0, \beta_2<0$	+	+	0
$\beta_1<0, \beta_2<0$	+	0	0
$\beta_1<0, \beta_2>0$	+	0	+

[a] The + symbols indicate non-zero enzymatic steady states whereas 0 indicates stable zero solutions. Note results obtained under the assumption of no constitutive enzyme synthesis.

A.2. Two-dimensional null space

Investigation of the double degenerate case is actually a more general development than the 1-dimensional null space analysis and yields similar information with one notable exception. In the previous section we, in effect, analyzed the crossing on the β_j axis at any point other than through the origin. Analysis of the double degenerate system affords this possibility. However, given the previous results, it is easily seen from Fig. 2 that a crossing through the origin of the (β_1, β_2) plane results in the symmetric allocation reversal between e_1 and e_2. In other words, in quadrant 2 of the (β_1, β_2) plane e_1 is synthesized and e_2 assumes a zero solution. As we move through the origin into quadrant 4, this steady state profile is symmetrically reversed, i.e. e_2 becomes a more attractive resource investment than e_1 and the allocation decision to express e_1 over e_2 is reversed. Thus, from a qualitative perspective no new information is gained by pursuing the double degenerate analysis. Accordingly, we neglect the consideration of this case with the understanding that such an endeavor is readily available given the tools presented by Iooss (Iooss and Joseph, 1990) and Mcleod (Mcleod and Sattinger, 1973).

A.3. Constitutive enzyme synthesis

In the previous analysis we have neglected constitutive enzyme synthesis, however, from the appearance of η_j in Eq. (A.39) its presence may qualitative alter the dynamics of the system. In fact, upon closer examination of Eq. (A.39) an important feature can be borne out, namely, the destruction of the system zero solution. Presently, we neglect the consideration of this case and choose to revisit the issue subsequently in Section A.5.

A.4. Bifurcation analysis: elementary divergent pathway

To ease the algebraic complexity of the analysis and guide the understanding of the qualitative interaction present within the equation set, we propose the following set of dimensionless variables and parameters

$$\rho_0 \equiv \frac{p_0}{K_0^1}, \qquad \rho_j \equiv \frac{p_1^j}{K_1^j}, \qquad \epsilon_j \equiv \frac{\delta}{\alpha_j} e_0^j,$$

$$\kappa_j \equiv \frac{\mu_0^{j,\max}}{\delta}, \qquad \bar{\kappa}_j \equiv \frac{\bar{\mu}_1^{j,\max}}{\delta}, \qquad q,\ j \equiv \frac{K_0^q}{K_0^j}$$

$$\bar{K}_{q,j} \equiv \frac{K_{e_0^q}}{K_0^j} \qquad \psi_0 \equiv \frac{R_0}{\delta} \qquad \eta_k \equiv \frac{r^*_{e_k}}{\delta}$$

where $\delta \equiv (r_g + \beta)$, $\tau \equiv \delta t$ and $j = 1, 2, \ldots, n$. Assuming balanced growth and slow enzymatic decay, substitution of the rescaled variables and parameters into the elementary divergent system model yields the rescaled system

$$K_0^1 \frac{d\rho_0}{d\tau} = \psi_0 - \sum_{j=1}^{n} r_j v_j^c - K_0^1 \rho_0$$

$$K_1^q \frac{d\rho_q}{d\tau} = r_q v_q^c - \bar{r}_q - K_1^q \rho_q, \quad q = 1, 2, \ldots, n$$

$$\frac{d\epsilon_k}{d\tau} = r_{\epsilon_k} u_k^c - \epsilon_k + \eta_k, \quad k = 1, 2, \ldots, n \tag{A.46}$$

where the rescaled rates take the form(s)

$$r_q = \kappa_q \epsilon_q \left(\frac{\rho_0}{\Gamma_{q,1} + \rho_0} \right), \quad q = 1, \ldots, n$$

$$\bar{r}_k = \bar{\kappa}_k \left(\frac{\rho_k}{1 + \rho_k} \right), \quad k = 1, 2, \ldots, n \tag{A.47}$$

and

$$r_{e_j} = \frac{\rho_0}{\bar{K}_{j,1} + \rho_0}, \quad j = 1, \ldots, n \tag{A.48}$$

As was true in the convergent pathway, we must employ the regulatory region concept to simplify the structure of the cybernetic regulation. For a general elementary complementary process, the cybernetic variable that governs the activity of the j^{th} key enzyme, denoted as v_j^c, is given functionally as

$$v_j^c = \frac{r_0^j / p_1^j}{\max(r_0^1/p_1^1, r_0^2/p_1^2, \ldots, r_1^n/p_1^n)}, \quad j = 1, 2, \ldots, n \tag{A.49}$$

Dividing the numerator and denominator by the largest scaled rate

$$\frac{r_0^k}{p_1^k} \tag{A.50}$$

yields the rescaled v variable

$$v_j^c = \frac{\dfrac{r_0^j}{p_1^j}\left(\dfrac{p_1^k}{r_0^k}\right)}{\max\left(\left(\dfrac{r_0^1}{r_0^k}\right)\left(\dfrac{p_1^k}{p_1^1}\right), \ldots, \left(\dfrac{r_0^j}{r_0^k}\right)\left(\dfrac{p_1^k}{p_1^j}\right), \ldots, \left(\dfrac{r_0^n}{r_0^k}\right)\left(\dfrac{p_1^k}{p_1^n}\right)\right)}$$

$$j = 1, 2, \ldots, n \tag{A.51}$$

If we define the quantity $\bar{\zeta}_{h,j}$ as

$$\bar{\zeta}_{h,j} \equiv \left(\frac{r_0^h}{r_0^j}\right)\left(\frac{p_1^j}{p_1^h}\right) \tag{A.52}$$

Eq. (A.51) can be recast in the form

$$v_j^c = \frac{\bar{\zeta}_{j,k}}{\max(\{\bar{\zeta}_{q,k}\}_{q=1}^n)}, \quad j = 1, 2, \ldots, n \tag{A.53}$$

The cybernetic variable that regulates the expression of the j^{th} enzyme belonging to an elementary complementary pathway, denoted as u_j^c, is given functionally as

$$u_j^c = \frac{r_0^j / p_1^j}{\sum_{q=1}^{n} r_0^q / p_1^q}, \quad j = 1, 2, \ldots, n \tag{A.54}$$

Division of the numerator and denominator by Eq. (A.50) and substituting Eq. (A.52) yields the control variable

$$u_j^c = \frac{\bar{\zeta}_{j,k}}{\sum_{q=1}^{n} \bar{\zeta}_{q,k}}, \quad j = 1, 2, \ldots, n, \qquad \forall\, k \tag{A.55}$$

Our intention is to invoke the regulatory region concept developed in the analysis of the elementary convergent process to decompose the regulation in this case. However, because of the structure of the system of equations, we cannot decompose the model into unregulated and regulated portions while still maintaining the inherent mathematical structure of the cybernetic regulation. Accordingly, we turn to time scale arguments and the properties of the cybernetic variables to decompose the system regulation. To ease the analysis we consider the $n = 2$ case.

Because we are analyzing an elementary process, i.e. one in which all enzymes present can only receive resources from the pool earmarked for the operation of the k^{th} elementary pathway the cybernetic u variables have the property

$$\sum_{j=1}^{n} u_j^c = 1 \tag{A.56}$$

Using this property we are able to rewrite u_q^c as

$$u_q^c = 1 - \sum_{j=1,\, q}^{n} u_j^c \tag{A.57}$$

If we assume, for the sake of argument, that $r_0^1/p_1^1 \gg r_0^j/p_1^j$, $\forall j$ for some time t^* on the interval $t^* \in [t_0, t_1]$, $t_1 \geq t_0$ then Eq. (A.55) can be recast as the set

$$u_1^c \sim 1, \qquad u_2^c \sim \zeta_{2,1} \tag{A.58}$$

Using Eqs. (A.58) and (A.57) the elementary divergent enzyme balances can be rewritten as

$$\frac{d\epsilon_1}{d\tau} \simeq r_{\epsilon_1}(1 - \zeta_{2,1}) - \epsilon_1 + \eta_1, \tag{A.59}$$

$$\frac{d\epsilon_2}{d\tau} \simeq r_{\epsilon_2}\zeta_{2,1} - \epsilon_2 + \eta_2 \tag{A.60}$$

Following the same logic, the cybernetic v variables can be decomposed as

$$v_1^c \sim 1, \qquad v_2^c \sim \zeta_{2,1} \tag{A.61}$$

which implies the balances on the dimensionless metabolites $\rho_j, j = 1, 2, \ldots, n$ take the form

$$K_0^1 \frac{d\rho_0}{d\tau} \simeq \psi_0 - r_0^1 - r_0^2\,\zeta_{2,1} - K_0^1\,\rho_0$$

$$K_1^1 \frac{d\rho_1}{d\tau} \simeq r_0^1 - r_1^1 - K_1^2\,\rho_1$$

$$K_1^2 \frac{d\rho_2}{d\tau} \simeq r_0^2\,\zeta_{2,1} - r_1^2 - K_1^2\,\rho_1 \tag{A.62}$$

Before we proceed, we make some observations about the decomposed enzyme and metabolite balances. Notice, as an artifact of the original rescaling, the j^{th} time derivative term of the metabolite balances is multiplied by the $(j)^{\text{th}}$ saturation constant. If we define the relationship

$$K_0^1 = \gamma_{1,j}\,K_0^j, \quad j = 1, 2, \ldots, n \tag{A.63}$$

($\gamma_{1,1} = 1$) then the metabolite balances can be redefined in vector form as

$$K_0^1 \frac{d\rho}{d\tau} \simeq \Gamma\mathbf{F}(\rho, \epsilon, \mathbf{K}) \tag{A.64}$$

where $\rho \equiv (\rho_0, \rho_1, \rho_2)^T$, $\{\gamma_{j,k} = 0,\ \gamma_{j,j} \neq 0\} \in \Gamma$ and $\mathbf{F}(\rho, \epsilon, \mathbf{K})$ denotes the vector of the right hand sides of the decomposed metabolite balances. In a similar way, we rewrite the enzyme balances in vector form

$$\frac{d\epsilon}{d\tau} \simeq \mathbf{G}(\rho, \epsilon, \mathbf{K_0}) \tag{A.65}$$

where $\epsilon \equiv (\epsilon_0, \epsilon_1)$ and $\mathbf{G}(\rho, \epsilon, \mathbf{K_0})$ denotes the vector of right hand sides of the decomposed enzyme balances. The system

$$K_0^1 \frac{d\rho}{d\tau} \simeq \Gamma\mathbf{F}(\rho, \epsilon, \mathbf{K})$$

$$\frac{d\epsilon}{d\tau} \simeq \mathbf{G}(\rho, \epsilon, \mathbf{K}_0) \tag{A.66}$$

clearly operates on multiple time scales, i.e. the parameter K_0^1 can act as a singular perturbation parameter that can be employed to separate the time evolution of the system into slow and fast moving components. More exactly, if we consider the limiting case of $\|K_0^1\| \ll 1$ the metabolite balances are approximately constant and the only dynamic movement is with respect to enzyme level, i.e.

$$\Gamma\mathbf{F}(\rho, \epsilon, \mathbf{0}) \simeq 0 \tag{A.67}$$

$$\frac{d\epsilon}{d\tau} \simeq \mathbf{G}(\rho, \epsilon, \mathbf{0}) \tag{A.68}$$

However, if we define a new time scale λ given by

$$\lambda \equiv \frac{\tau}{K_0^1} \tag{A.69}$$

Eq. (A.66) can be recast as

$$\frac{d\rho}{d\lambda} \simeq \Gamma\mathbf{F}(\rho, \epsilon, \mathbf{K}) \tag{A.70}$$

$$\frac{d\epsilon}{d\lambda} \simeq K_0^1\,\mathbf{G}(\rho, \epsilon, \mathbf{K_0^1}) \tag{A.71}$$

In the limiting case of $K_0^1 \to 0$ the λ time scale is infinite and the system displays no dynamic movement with respect to enzyme level, however, the metabolite balances are free to evolve, i.e. the system evolution as $K_0^1 \to 0$ is governed by

$$\frac{d\rho}{d\lambda} \simeq \Gamma\mathbf{F}(\rho, \epsilon, \mathbf{K})$$

$$\frac{d\epsilon}{d\lambda} \simeq 0 \tag{A.72}$$

These two time scales represent the limiting behavior of the system. In physical terms, the τ time scale is the short time scale in which enzyme synthesis is occurring. On this scale, the level of metabolites is approximately constant. The dynamic evolution of metabolite level on the λ time scale is a much longer time scale and reflects the physical argument that enzymatic catalyst is required to effect changes in the metabolite level. Of course, $\|K_0^1\| \ll 1$ need not be true in which case some mixture of the behavior inherent to the individual time scales prevails. Presently, the time scale of enzyme synthesis represents the aspect of system in which bifurcation can occur, i.e. the qualitative nature of enzyme level drives the qualitative dynamics of metabolite level because of the requirement for enzymatic catalyst. Accordingly, to determine the qualitative dynamics of the system with respect to changes in parameters we need only to investigate the dynamics of the enzyme balances. This implies in a formal sense, we need only investigate the qualitative dynamics of the system

$$\Gamma\mathbf{F}(\rho, \epsilon, \mathbf{0}) \simeq 0 \tag{A.73}$$

$$\frac{d\epsilon}{d\tau} \simeq \mathbf{G}(\rho, \epsilon, \mathbf{0}) \tag{A.74}$$

with respect to changes in the system parameters. To determine a closed form solution of the algebraic equation $\mathbf{F}(\rho, \epsilon, \mathbf{0}) = 0$ certain assumptions must be made to simplify the structure of the system. Firstly, we assume that all metabolic function is saturated with respect to the branch point precursor p_0 (consistent with the assumption of $\|K_0^1\| \ll 1$), this includes both catalytic, as well as, enzyme expression function. This allows the balances for $\rho_j, j = 1, 2$ to be decoupled from the ρ_0 balance. Secondly, we assume that $\rho_j \ll 1, j = 1, 2$, i.e. the consumption of the branch metabolites $\rho_j, j = 1, 2$ is subsaturated with respect to metabolite level. Under these assumptions, the algebraic equation $\mathbf{F}(\rho, \epsilon, \mathbf{0}) = 0$ has a solution set of the form

$$\rho_q \sim \left(\frac{\kappa_q}{\sqrt{\bar{k}_q\,\bar{k}_1}}\right)\epsilon_q, \quad q = 1, 2, \ldots, n \tag{A.75}$$

Substituting Eq. (A.75) into the decomposed system regulation i.e. $\zeta_{2,1}$ yields

$$\zeta_{2,1} = \frac{\sqrt{\bar{\kappa}_1\,\bar{\kappa}_2}}{\bar{\kappa}_1} \tag{A.76}$$

which implies the decomposed enzyme balances take the functional form

$$\frac{d\epsilon_1}{d\tau} \simeq \left(1 - \frac{\sqrt{\bar{\kappa}_1\,\bar{\kappa}_2}}{\bar{\kappa}_1}\right) - e_1 + \eta_1 + \mathcal{O}(\zeta_{2,1})$$

$$\frac{d\epsilon_2}{d\tau} \simeq \frac{\sqrt{\bar{\kappa}_1\,\bar{\kappa}_2}}{\bar{\kappa}_1} - \epsilon_2 + \eta_2 + \mathcal{O}(\zeta_{2,1}) \tag{A.77}$$

Equation set (A.77) controls the qualitative picture of enzyme expression in the limit of small K_0^1. There are some interesting observations that come forth upon examination of this set. Firstly, the enzyme balances are coupled only through the kinetic nature of branch metabolite consumption, i.e. the parameters $\bar{\kappa}_j, j = 1, 2$. This is a consequence of the feedback repression/inhibition of the branch leg key enzyme by its product as described by the cybernetic variables. Secondly, even in the absence of constitutive enzyme synthesis, no zero solution is possible for the enzyme balances. This is in stark contrast to the general convergent elementary pathway in which both zero and non-zero steady state enzyme levels are possible.

Even at this level of approximation, the qualitative features of the elementary divergent pathway are evident. In particular, there exists a rather complex interplay between the repression of the j^{th} branch point key enzyme by its corresponding product and the activation of the opposite leg by the respective branch metabolites. This regulatory feature is evident from the sensitivity of steady state enzyme level to the parameter(s) $\bar{\kappa}_j, j = 1, 2$. The steady state level of key enzyme ϵ_1 is positively sensitive to the kinetic parameter $\bar{\kappa}_1$ and negatively sensitive to $\bar{\kappa}_2$. From Eq. (A.75), as $\bar{\kappa}_1$ increases, the level of branch metabolite p_1 decreases, i.e. the branch metabolite is being consumed for downstream metabolite formation at a greater rate resulting in a lower steady state level. In turn, the lowered level of p_1 eases the repressive effect felt by the

ϵ_1 expression machinery and ϵ_1 synthesis proceeds at a faster rate resulting in a higher steady state enzyme level. As $\bar{\kappa}_2$ is increased, the promotion of ϵ_1 expression (as described by $1-\zeta_{2,1}$) decreases. Physically, this implies that as downstream ρ_2 consumption is increased, the steady state level of ϵ_1 falls and vice-versa. Accordingly, the expression of ϵ_0 is positively sensitive to ρ_2 levels. The same type of arguments can be developed to explain the parameter dependence of the steady state level of ϵ_2. Note, however, that ϵ_2 level is positively sensitive to $\bar{\kappa}_2$ and negatively sensitive to the parameter $\bar{\kappa}_1$. This is an artifact of the assumption of $r_0^1/\rho_1 \gg r_0^2/\rho_2$ and can be shown to be a symmetric effect. In a deeper sense this effect is the result of the cybernetic objective function. The objective of maximizing the mathematical product of the branch metabolite level implies the optimum solution occurs when the branch metabolite levels are equal. The assumption of $r_0^1/\rho_1 \gg r_0^2/\rho_2$ (assuming $\kappa_1 \sim \kappa_2$) indicates a disparity in the relative level of branch point metabolite. Specifically, this assumption imparts the condition $\rho_2 \gg \rho_1$. It follows that ϵ_2 expression in this instance is severely repressed because of the high levels of branch metabolite. If the downstream consumption of the metabolite ρ_2 is increased, the repression force upon the ϵ_2 expression machinery is eased and ϵ_2 synthesis can proceed at an increased rate. However, because the level of ρ_2 drops the expression of ϵ_1 suffers and ρ_1 decreases in turn reducing ϵ_2 expression and so forth. Thus, the joint positive effector relationship between the opposite branch metabolites and the opposite branch enzymatic machinery coupled with the repressive effects of product inhibition/repression act to lock the branch intermediate level.

The qualitative significance of the findings for complementary competition are best understood when contrasted with the substitutable counterpart. In particular, we have shown that no bifurcation behavior is possible, and moreover, no enzymatic zero solution for an elementary structure experiencing complementary competition. Thus, the possibility of zero solutions is reserved for substitutable environments only.

A.5. Bifurcation analysis: partially substitutable/partially complementary environments

We choose to prove postulate 4.1 by construction using the same non-linear analysis techniques employed in the previous section. Specifically, for postulate 4.1 to hold, e_1^a must have a zero solution whose stability is a function of system parameters. Accordingly, we must firstly show that $e_1^a = 0$ is a possible equilibrium solution, and secondly, that $e_1^a = 0$ can undergo bifurcation as a function of system parameters.

To simplify the algebraic aspects of the analysis and bring forth clearly the qualitative interactions of the system equations we rescale with respect to the following set of dimensionless variables and parameters:

$$\rho_0 \equiv \frac{p_0}{K_0}, \qquad \rho_1 \equiv \frac{p_1}{K_1^a}, \qquad \rho_2 \equiv \frac{p_2}{K_2}, \qquad \rho_3 \equiv \frac{p_3}{K_3}$$

$$\epsilon_0 \equiv \left(\frac{\delta}{\alpha_0}\right) e_0, \qquad \epsilon_1^a \equiv \left(\frac{\delta}{\alpha_1^a}\right) e_1^a, \qquad \epsilon_1^b \equiv \left(\frac{\delta}{\alpha_1^b}\right) e_1^b,$$

$$\psi_j \equiv \frac{R_j}{\delta}, \quad j = 0, 1$$

$$\kappa_j \equiv \frac{\mu_j^{\max}}{\delta}, \quad j = 0, 2, 3 \qquad \kappa_1^j \equiv \frac{\mu_1^{j,\max}}{\delta}, \quad j = a, b$$

$$\Gamma_{q,j} \equiv \frac{K_1^q}{K_1^j}, \qquad K_{q,j} \equiv \frac{K_{e_q}}{K_{e_j}}$$

$$\bar{K}_{q,j} \equiv \frac{K_{e^q}}{K_j}, \qquad \tau \equiv \delta t, \qquad \eta_0 \equiv \frac{r_{e_0}^*}{\delta},$$

$$\eta_1^j \equiv \frac{r_{e_1^j}^*}{\delta}, \quad j = a, b$$

where $\delta \equiv (r_g + \beta)$. We assume, consistent with the assumption of investigation of network behavior during balanced growth, that $r_g \sim \mu_g^{\max}$ and enzyme decay is slow. If we substitute the dimensionless variables and parameters into the model equations we arrive at the rescaled set

$$K_0 \frac{d\rho_0}{d\tau} = \psi_0 - \kappa_0\, \epsilon_0\, \bar{\rho}_0\, v_0 - K_0\, \rho_0$$

$$K_1^a \frac{d\rho_1}{d\tau} = \psi_1 - \kappa_1^a\, \epsilon_1^a\, \bar{\rho}_1^a\, v_{1a} - \kappa_1^b\, \epsilon_1^b\, \bar{\rho}_1^b\, v_{1b} - K_1^a\, \rho_1^a$$

$$K_2 \frac{d\rho_2}{d\tau} = \kappa_0\, \epsilon_0\, \bar{\rho}_0 + \kappa_1^a\, \epsilon_1^a \bar{\rho}_1^a\, v_{1a} - r_2 - K_2\, \rho_2$$

$$K_3 \frac{d\rho_3}{d\tau} = \kappa_1^b\, \epsilon_1^b\, \bar{\rho}_1^b\, v_{1b} - r_3 - K_3\, \rho_3$$

$$\frac{d\epsilon_0}{d\tau} = u_0 - \epsilon_0 + \eta_0$$

$$\frac{d\epsilon_1^j}{d\tau} = u_{1j} - e_1^j + \eta_1^j \quad j = a, b \tag{A.78}$$

where

$$\bar{\rho}_0 \equiv \frac{\rho_0}{1+\rho_0} \qquad \bar{\rho}_1^a \equiv \frac{\rho_1}{1+\rho_1} \qquad \bar{\rho}_1^b \equiv \frac{\rho_1}{\Gamma_{b,a}+\rho_1} \tag{A.79}$$

and the rescaled rates r_2 and r_3 take the form

$$r_2 = \kappa_2 \frac{\rho_2}{1+\rho_2} \qquad r_3 = \kappa_3 \frac{\rho_3}{1+\rho_3} \tag{A.80}$$

We assume for the sake of simplicity that all enzyme expression is saturated with respect to inducer metabolite. The rescaled equation set, as was true previously, clearly operates on multiple time scales. Enzyme expression operates on the short time scale, and the metabolite balances are approximately constant in the limit of $\|K_0\| << 1$ where we have assumed the relationship

$$K_0 = \gamma_{0,j}\, K_j, \quad j = 0, 2, 3$$
$$K_0 = \gamma_{0,1a}\, K_1^a \quad \gamma_{0,j} \sim \forall\, j \tag{A.81}$$

Using arguments similar to the elementary divergent pathway analysis, the metabolite balances written with respect to the time scale of enzyme expression are given by the set of algebraic equations

$$\psi_0 - \kappa_0\, \epsilon_0\, v_0 \sim 0$$
$$\psi_1 - \kappa_1^a\, \epsilon_1^a\, v_{1a} - \kappa_1^b\, \epsilon_1^b\, v_{1b} \sim 0$$
$$\kappa_0\, \epsilon_0\, v_0 + \kappa_1^a\, \epsilon_1^a\, v_{1a} - \kappa_2\, \rho_2 \sim 0$$
$$\kappa_1^b\, \epsilon_1^b\, v_{1b} - \kappa_3\, \rho_3 \sim 0 \tag{A.82}$$

where we have assumed saturation with respect to $\rho_j, j = 0, 1$ and subsaturation with respect to $\rho_j, j = 2, 3$, i.e.

$$\rho_j >> 1, \quad j = 0, 1 \qquad \rho_j << 1, \quad j = 2, 3 \tag{A.83}$$

The hallmark of substitutable competition is the existence of enzymatic zero solutions (assuming no constitutive enzyme expression.) More exactly, we have shown that the fastest rate in substitutable set dominates the allocation of critical resources. Using this as a guide, we decompose the cybernetic regulation into two regions. Firstly, we assume the production of p_2 from p_0 catalyzed by key enzyme e_0 is much faster that any other route. In this region we expect e_0 to receive the lions share of substitutable resources. The second region we consider is the converse in which the production of p_2 via p_0 is much slower than any other route. In each case we assume $r_1^a/\rho_2 >> r_1^b/\rho_3$ and $K_2 \sim K_3$.

A.5.1. $r_0 >> r_1^a$

In this region the cybernetic variables that regulate enzyme expression can be decomposed into the set

$$u_0 \sim 1 - \frac{r_1^a}{r_0} \qquad u_{1a} \sim \left(\frac{r_1^a}{r_0}\right)\left(\frac{r_1^a\, \rho_3}{r_1^b\, \rho_2}\right)$$
$$u_{1b} \sim 1 - \frac{r_1^a\, \rho_3}{r_1^b\, \rho_2}$$

where we make use of the k^{th} elementary pathway property

$$\sum_j u_j^k = 1, \qquad \forall\, k \tag{A.84}$$

The rates of reaction, because of the assumption of saturation with respect to p_0 and p_1 take the form

$$r_0 = \kappa_0\, \epsilon_0 \qquad r_1^j = \kappa_1^j\, \epsilon_1^j, \quad j = a, b \tag{A.85}$$

The cybernetic variables that regulate enzyme activity can be decomposed as

$$v_0 \sim 1 \qquad v_{1a} \sim \left(\frac{r_1^a}{r_0}\right)\left(\frac{r_1^a\, \rho_3}{r_1^b\, \rho_2}\right) \qquad v_{1b} \sim 1 \tag{A.86}$$

where the rates of reaction are given above. The metabolite balances, written with respect to the time scale of enzyme synthesis, are approximately constant. However, the balances can not be discarded straightaway because of the explicit metabolite dependence stemming from the complementary regulatory component. Accordingly,

we substitute the regulatory decomposition shown above into the metabolite balances and explicitly solve for the level of ρ_2 and ρ_3 as a function of the enzyme level and the system parameters. These functions can then be substituted into the enzyme balances so as to decouple them from the remaining portion of state space. Solving the metabolite balances for ρ_2 and ρ_3 yields the solutions

$$(\kappa_2\,\kappa_0\,\epsilon_0)\rho_2^2-(\kappa_0\,\epsilon_0)^2\rho_2-\frac{(\kappa_1^a\,\epsilon_1^a)^3}{\kappa_3}\simeq 0 \tag{A.87}$$

$$\rho_3\simeq\frac{\kappa_1^b\,\epsilon_1^b}{\kappa_3} \tag{A.88}$$

Note the quadratic equation in ρ_2 is sensitive to both modes of ρ_2 synthesis. As per our original assumption regarding the relative magnitudes of reaction rates given the substitutable nature of e_1^a expression, we assume that $e_1^a\rightarrow 0$ as time evolves. This implies that ρ_2 during enzyme expression is on the order

$$\rho_2\sim\frac{\kappa_0\,\epsilon_0}{\kappa_2} \tag{A.89}$$

Substituting ρ_2 and ρ_3 into the enzyme balances yields the dynamic set

$$\frac{d\epsilon_0}{d\tau}\simeq\left(1-\frac{\kappa_1^a\,\epsilon_1^a}{\kappa_0\,\epsilon_0}\right)-\epsilon_0+\eta_0 \tag{A.90}$$

$$\frac{d\epsilon_1^a}{d\tau}\simeq\left(\frac{\kappa_1^a\,\epsilon_1^a}{\kappa_0\,\epsilon_0}\right)(\Delta)-\epsilon_1^a+\eta_1^a \tag{A.91}$$

$$\frac{d\epsilon_1^b}{d\tau}\simeq\left(1-\frac{\kappa_1^a\,\epsilon_1^a}{\kappa_0\,\epsilon_0}\right)-\epsilon_1^b+\eta_1^b \tag{A.92}$$

where the compound parameter Δ is given by the ratio

$$\Delta\equiv\frac{\kappa_2}{\kappa_3} \tag{A.93}$$

Notice that ϵ_1^b is a coupled to the expression of the remaining enzymes as a slave variable. In other words, it is driven by the movement of e_1^a, however, exerts no influence upon the remaining system variables. Accordingly, as time evolves e_1^b moves towards

$$\epsilon_1^b\simeq\left(1-\frac{\kappa_1^a\,\epsilon_1^a}{\kappa_0\,\epsilon_0}\right)+\eta_1^b \tag{A.94}$$

irrespective of the dynamics of e_0 and e_1^a. This implies the qualitative nature of enzyme expression (in the limit of small ϵ_1^a) is controlled solely by the ϵ_1^a and ϵ_0 balances which can be recast in the vector form

$$\frac{d\epsilon}{d\tau}\simeq\mathbf{G}(\epsilon,\,\kappa,\,\eta) \tag{A.95}$$

where the state vector $\epsilon\equiv\{\epsilon_0,\,\epsilon_1^a\}^T$ and $\kappa,\,\eta$ denote the parameter and rescaled constitutive enzyme synthesis vector(s), respectively. The equilibrium behavior of equation set (A.95) is determined from the solutions of the algebraic system

$$\mathbf{G}(\epsilon^{\mathbf{0}},\,\kappa^{\mathbf{0}},\,\mathbf{0})=\mathbf{0} \tag{A.96}$$

where $\epsilon^{\mathbf{0}}$, $\kappa^{\mathbf{0}}$ denote the steady state rescaled enzyme and parameter vector(s), respectively. Also note that we have assumed $\eta=\mathbf{0}$ at equilibrium. This assumption allows for the investigation of the effects of constitutive enzyme synthesis as a breaking parameter, or an imperfection. This is similar to the arguments presented during the analysis of the elementary convergent pathway, and will be touched on subsequently. A possible solution of Eq. (A.113) is given by the set

$$\epsilon_0=1\qquad\epsilon_1^a=0 \tag{A.97}$$

Notice solution (A.97), when coupled with the equilibrium solution for ϵ_1^b, is indicative of completely parallel operation. In other words, the metabolites ρ_2 and ρ_3 are produced only from ρ_0 and ρ_1, respectively. We use solution (A.97) as a base from which we explore the parameter dependence of the equilibrium behavior. More exactly, we 'ride' along solution (A.97) as a function of the system parameters and search for possible points of bifurcation which are marked by zero eigenvalues of the system Jacobian. To ease the algebraic burden, we propose the translation

$$\epsilon=\epsilon^{\mathbf{0}}+\mathbf{x}\quad\mathbf{x}\in\mathbf{R^2} \tag{A.98}$$

which simply slides the equilibrium point to the origin of the new state space variable $\mathbf{x}$. We approximate vector Eq. (A.95) in the new state variable $\mathbf{x}$ around the origin as the Taylor series retaining up to cubic terms

$$\frac{d\mathbf{x}}{d\tau} = \mathbf{J}\mathbf{x} + \mathbf{F_2}(\mathbf{x}, \kappa) + \mathcal{O}(\mathbf{x^3}) \tag{A.99}$$

where $\mathbf{F_2}(\mathbf{x}, \kappa)$ denotes the vector of second order terms and $\mathbf{J}$ denotes the Jacobian matrix. The Jacobian matrix for the translated enzyme system evaluated at the origin is given functionally as

$$\mathbf{J} = \begin{pmatrix} -1 & -\dfrac{\kappa_1^a}{\kappa_0} \\ 0 & -1 \end{pmatrix} \tag{A.100}$$

The eigenvalues of the Jacobian are $-1, -1$ which is indicative of a globally stable solution, i.e. no possibility of bifurcation. Thus, in the present regulatory region, the parallel operation of the system is the only type of behavior that is possible. If the regulatory decomposition stemming from the assumption $r_1^a \ll r_0$ is invariant, the system trajectories once entering the region remain for all time. It follows that, under these conditions, the long time behavior of the original set of equations evolved towards parallel network operation. The simulation of this case is shown in Fig. 4.

With respect to the behavior postulate, we have shown that ϵ_1^a does possess a zero solution in accordance with the substitutable component of the local regulation. Let us now consider a second case in which we assume $r_1^a \gg r_0$.

A.5.2. $r_1^a \gg r_0$

In this region the cybernetic variables that regulate enzyme expression decompose into the set

$$u_0 \sim \frac{r_0}{r_1^a} \qquad u_{1a} \sim \left(1 - \frac{r_0}{r_1^a}\right)\left(\frac{r_1^a \rho_3}{r_1^b \rho_2}\right)$$
$$u_{1b} \sim \left(1 - \frac{r_1^a \rho_3}{r_1^b \rho_2}\right) \tag{A.101}$$

where, again, we make use of the elementary pathway property

$$\sum_q u_q^k = 1, \qquad \forall\, k \tag{A.102}$$

The cybernetic variables that govern the enzyme activity decomposes into the set

$$v_0 \sim \frac{r_0}{r_1^a} \qquad v_{1a} \sim \left(\frac{r_1^a \rho_3}{r_1^b \rho_2}\right) \qquad v_{1b} \sim 1 \tag{A.103}$$

Because the metabolite balances operate on the slow time scale the level of the various metabolite appears to be approximately constant on the time scale of enzyme synthesis. Therefore, we can solve equation set (A.82) for the metabolite level as a function of enzyme concentration and system parameters. Notice, because of the assumption of saturation with respect to ρ_0, the regulatory decomposition is not coupled to the ρ_0 balance. However, the complementary regulatory component does contain ρ_2 and ρ_3, thus, we solve the ρ_2 and ρ_3 balances written with respect to the time scale of enzyme synthesis to yield the equations

$$\kappa_2(\kappa_1^a \epsilon_1^a)\rho_2^2 - (\kappa_0 \epsilon_0)^2 \rho_2 - \frac{(\kappa_1^a \epsilon_1^a)^3}{\kappa_3} \sim 0 \tag{A.104}$$

$$\rho_3 \sim \frac{\kappa_1^b \epsilon_1^b}{\kappa_3} \tag{A.105}$$

Interestingly, the quadratic in ρ_2 indicates the two possible modes of formation of ρ_2. Given the assumption of $r_1^a \gg r_0$, we assume $\epsilon_0 \to 0$ as time evolves which implies that the ρ_2 is on the order of

$$\rho_2 \sim \left(\frac{\kappa_1^a}{\sqrt{\kappa_2\, \kappa_3}}\right)\epsilon_1^a \tag{A.106}$$

Note Eq. (A.106) is identical in form to the branch metabolite level shown in the elementary divergent pathway analysis. This is to be expected because with $\epsilon_0 \sim 0$, the production of ρ_2 is purely complementary. Substituting ρ_2 and ρ_3 into the enzyme balances yields the enzyme expression set

$$\frac{d\epsilon_0}{d\tau} \simeq \left(\frac{\kappa_0 \epsilon_0}{\kappa_1^a \epsilon_1^a}\right) - \epsilon_0 + \eta_0$$
$$\frac{d\epsilon_1^a}{d\tau} \simeq \left(1 - \frac{\kappa_0 \epsilon_0}{\kappa_1^a \epsilon_1^a}\right)\Theta - \epsilon_1^a + \eta_1^a \tag{A.107}$$
$$\frac{d\epsilon_1^b}{d\tau} \simeq (1 - \Theta) - \epsilon_1^b + \eta_1^b \tag{A.108}$$

where Θ is given as

$$\Theta \equiv \frac{\sqrt{\kappa_2\, \kappa_3}}{\kappa_3} \tag{A.109}$$

Equation set (A.107) controls the qualitative properties of the system in the limit of small ϵ_0. Notice the similarities of this equation set with those derived during the analysis of the individual elementary pathways. The influence of each of the individual types of elementary competition is clearly visible from the ϵ_1^a balance. In particular, the regulatory decomposition is composed of the two distinct elementary components. The term

$$\left(1-\frac{\kappa_0\,\epsilon_0}{\kappa_1^a\,\epsilon_1^a}\right) \tag{A.110}$$

corresponds to the substitutable influence of the ϵ_1^a regulation. In particular, note the dependence of the substitutable regulatory input upon ϵ_0. As the ϵ_0 level increases, the share of resources allocated to e_1^a expression must decrease because of the limited amount of critical resources. This reflects the give and take relationship inherent to substitutable competition. The term Θ is the regulatory influence stemming from the elementary complementary pathway. As was true in the purely complementary case, this compound parameter reflects the complex interaction between metabolite inhibition/repression and the opposite branch positive effector relationship. Thus, the promotion of e_1^a expression is sensitive to both types of local resource competition.

The ϵ_1^b balance is not dynamically coupled to the remaining set, however, parametrically ϵ_1^a and ϵ_1^b are linked. This implies that as time evolves, the key enzyme ϵ_1^b assumes the steady state

$$\epsilon_1^b \simeq (1-\Theta)+\eta_1^b \tag{A.111}$$

independently of the dynamics of ϵ_1^a and ϵ_0. Accordingly, the qualitative dynamics of the hybrid network are controlled solely by the ϵ_0 and ϵ_1^a balances recast in vector form as

$$\frac{d\epsilon}{d\tau} \simeq \mathbf{G}(\epsilon,\kappa,\eta) \tag{A.112}$$

where the enzymatic state vector ϵ is given by $\epsilon=(\epsilon_0,\epsilon_1^a)^T$ and $\mathbf{G}(\epsilon,\kappa)$ denotes the vector of right hand sides of the ϵ_0 and ϵ_1^a balances. The equilibrium behavior of system (A.112) can be determined by solving the set of algebraic equations given by

$$\mathbf{G}(\epsilon^{\mathbf{0}},\kappa^{\mathbf{0}},\mathbf{0})=\mathbf{0} \tag{A.113}$$

where ϵ^0, κ^0 denote the equilibrium rescaled enzyme level and parameter vector, respectively. A possible solution of equation set (A.113) is given by

$$\epsilon_0^0=0 \qquad \epsilon_1^{a,0}=\Theta \tag{A.114}$$

Note the above solution corresponds to purely complementary operation of the network. Equilibrium solution (A.114) serves as the base from which we explore the qualitative nature of the system. Accordingly, we propose the translation (translates the equilibrium point to the origin)

$$\epsilon=\epsilon^{\mathbf{0}}+\mathbf{x}, \qquad \mathbf{x}\in\mathbf{R^2} \tag{A.115}$$

where $\mathbf{x}$ denotes the translated state vector. For simplicity, we approximate system (A.112) as a Taylor series (retaining up to cubic terms) around the origin

$$\frac{d\epsilon}{d\tau}=\mathbf{J}\mathbf{x}+\mathbf{F_2}(\mathbf{x},\kappa)+\ \mathcal{O}(\mathbf{x}^3) \tag{A.116}$$

where $\mathbf{F_2}(\mathbf{x},\kappa)$ denotes the vector of second order terms and $\mathbf{J}$ denotes the Jacobian matrix. Our intention is to utilize Lyapunov–Schmidt reduction to abstract the portion of the system that is responsible for the qualitative changes in system dynamics. This task is greatly simplified if the Jacobian is self-adjoint with respect to the usual inner product on $\mathbf{R}^n$, i.e. the Jacobian is symmetric. Accordingly, we propose the coordinate transformation

$$\mathbf{y}=\mathbf{M}\mathbf{x}, \quad \mathbf{y}\in\mathbf{R^2} \tag{A.117}$$

which simply rotates the coordinate axis such that the principle directions are equivalent to the eigenvector directions. The 2×2 modal matrix $\mathbf{M}$ consists of the system eigenvectors and is given functionally as

$$\mathbf{M}=\begin{pmatrix} 0 & -\dfrac{1}{\Theta} \\ 1 & 1 \end{pmatrix} \tag{A.118}$$

Note the modal matrix $\mathbf{M}$ exists for all values of the system parameters as long as ρ_2 and ρ_3 are

precursors for downstream metabolic activity. Substituting transformation (A.117) into equation set (A.116) yields the rotated system

$$\frac{d\mathbf{y}}{d\tau} = \mathbf{M}^{-1}\mathbf{G}(\mathbf{y}, \kappa) = \mathbf{B}\mathbf{y} + \mathbf{H_2}(\mathbf{y}, \kappa) + \mathcal{O}(3) \tag{A.119}$$

where

$$\mathbf{B} \equiv \mathbf{M}^{-1}\mathbf{J}\mathbf{M} \qquad \mathbf{H_2}(\mathbf{y}, \kappa) \equiv \mathbf{M}^{-1}\mathbf{F_2}(\mathbf{y}, \kappa) \tag{A.120}$$

The rotated Jacobian, denoted by **B**, is a 2×2 diagonal matrix given functionally as

$$\mathbf{B} = \begin{pmatrix} -1 & 0 \\ 0 & \dfrac{\kappa_0}{\kappa_1^a \Theta} - 1 \end{pmatrix} \tag{A.121}$$

Clearly, there exists a possibility of bifurcation, i.e. a zero eigenvalue when the system parameters obey the relationship

$$\frac{\kappa_0}{\kappa_1^{\mathrm{a}} \Theta} = 1 \tag{A.122}$$

Note that the critical condition is very similar to the purely substitutable case, with the exception of Θ. More exactly, it was shown, that purely substitutable structures bifurcate when the ratio of the rate constants of competing processes are in the neighborhood of unity. This structure is clearly visible in the form of the critical condition shown above, with the addition of the complementary regulatory input described by the compound parameter Θ. The complementary regulatory input alters the parametric location of the bifurcation of the substitutable system, however, does not destroy the existence of bifurcation. Let us examine this in more detail.

For the sake of algebraic simplicity, the critical condition is translated to the origin of parameter space by the transformation

$$\lambda = -1 + \frac{\kappa_0}{\kappa_1 \Theta} \tag{A.123}$$

which implies $\lambda = 0$ is the new point of possible bifurcation. Application of Lyapunov–Schmidt reduction to the rotated system, relaxing the conditions upon $\eta = \mathbf{0}$, yields the reduced system

$$\frac{d\chi}{d\tau} = g = \lambda\,\chi - \frac{1+\lambda}{\Theta}\,\chi(\Theta\,\eta_0 + \eta_1^a + \chi) - \Theta\,\eta_0 \tag{A.124}$$

where χ denotes projected null space state variable. Eq. (A.124) controls the qualitative aspects of enzyme evolution. Specifically, the equilibrium behavior of Eq. (A.124) corresponds to equilibrium solutions e_0.

The analysis of the reduced equation is centered upon two broad cases, namely, the presence and absence of constitutive enzyme synthesis. We begin by considering the latter case. The reduced equation in this instance becomes

$$\frac{d\chi}{d\tau} = g = \lambda\,\chi - \frac{(1+\lambda)}{\Theta}\,\chi^2 \tag{A.125}$$

where λ denotes the bifurcation parameter. To eliminate the parameter dependence of the quadratic term coefficient, we propose the rescaling

$$\zeta \equiv \frac{\chi}{f(\lambda)}, \qquad f(0) \neq 0 \tag{A.126}$$

which after substitution yields the rescaled reduced system

$$\frac{d\chi}{d\tau} = g = \lambda\,\zeta - \zeta^2 \tag{A.127}$$

Eq. (A.127) is the transcritical bifurcation normal form. The rescaled reduced equation possess a zero solution (which can be shown to be equivalent to (A.114)) whose stability is governed by the sign of the bifurcation parameter λ. Additionally, a second solution $\zeta = \lambda$ is also possible. This solution can be shown to correspond to the case in which both e_1^a, as well as, e_0 are present (we expand this discussion subsequently.) The stability of the system, i.e. which equilibrium solution is observed, is dependent upon the sign of the parameter λ. In the general case, the bifurcation parameter can be parameterized as

$$\lambda = \lambda(\zeta) \tag{A.128}$$

along the equilibrium trajectory, i.e.

$$g(\lambda(\zeta), \zeta) = 0 \tag{A.129}$$

which implies

$$\frac{dg}{d\zeta} = \frac{\partial g}{\partial \lambda}\frac{d\lambda}{d\zeta} + \frac{\partial g}{\partial \zeta} \tag{A.130}$$

The sign of $\frac{dg}{d\zeta}$ evaluated along the j^{th} solution branch determines the stability. If $\frac{dg}{d\zeta} < 0$ when evaluated along the j^{th} equilibrium solution, then this solution is stable, the converse being true for unstable solutions. For our system, $\frac{dg}{d\zeta}$ takes the form

$$\frac{dg}{d\zeta} = \lambda - 2\zeta \tag{A.131}$$

It follows straightaway that $\zeta = 0$ is stable when $\lambda < 0$, otherwise, $\zeta = \lambda$ is the stable system behavior. At the point of bifurcation, i.e. $\lambda = 0$ Eq. (A.131) vanishes yielding no stability information as to the exchange of stability. Expanding g_ζ with respect to λ around the bifurcation point and retaining up to linear terms yields

$$g_{\zeta,\lambda} = \left(\frac{\partial g_\zeta}{\partial \lambda} + \frac{\partial g_\zeta}{\partial \zeta}\left(\frac{d\zeta}{d\lambda}\right)\right)\delta\lambda + \mathcal{O}\lambda^2 \tag{A.132}$$

where $\delta\lambda \equiv \lambda - \lambda_0$. Given Eq. (A.131), the eigenvalue expansion reduces to

$$g_{\zeta,\lambda} = \left\{1 - 2\left(\frac{d\zeta}{d\lambda}\bigg|_j\right)\right\}\delta\lambda + \mathcal{O}\lambda^2 \tag{A.133}$$

The zero solution is stable when $\lambda < 0$, whereas, the bifurcating solution assumes stability as λ is varied through the origin. The system undergoes transcritical bifurcation at $\lambda = 0$.

In terms of the original system, the solution(s) $\zeta = 0$ and $\zeta = \lambda$ correspond to

$$\epsilon_0 = 0 \qquad \epsilon_0 = 1 - \frac{\kappa_0}{\kappa_1^a\,\Theta} \tag{A.134}$$

$$\epsilon_1^a = \Theta \qquad \epsilon_1^a = \frac{\kappa_0}{\kappa_1^a}, \tag{A.135}$$

respectively. Accordingly, the original model system assumes a solution in the neighborhood of the zero solution when the system parameters obey the inequality

$$\kappa_0 > \kappa_1^a\,\Theta \tag{A.136}$$

otherwise the bifurcating solution is the stable solution and the system evolves towards

$$\epsilon_0 = 1 - \frac{\kappa_0}{\kappa_1^a\,\Theta} \qquad \epsilon_1^a = \frac{\kappa_0}{\kappa_1^a} \tag{A.137}$$

These results are illustrated by simulation of the original system model in Fig. 6.

The inclusion of constitutive enzyme synthesis breaks the symmetric nature of bifurcation and results in a qualitatively different dynamics. If we relax the assumption $\eta = \mathbf{0}$ the original reduced equation in χ becomes

$$g = \lambda\,\chi - \frac{1+\lambda}{\beta}(\chi(\beta\,\eta_0 + \eta_1^a + \chi)) - \beta\,\eta_0 \tag{A.138}$$

Proceeding in much the same manner as the previous case, let us rescale the reduced variable by some function of the bifurcation parameter λ, i.e.

$$\zeta \equiv \frac{\chi}{f(\lambda)}, \qquad f(0) \neq 0 \tag{A.139}$$

which after substitution in Eq. (A.138) yields

$$g = \left(\lambda - \frac{1+\lambda}{\beta}\eta^*\right)\zeta - \zeta^2 - \eta_0(1+\lambda) \tag{A.140}$$

where η^* and $f(\lambda)$ take the form

$$\eta^* \equiv (\beta\,\eta_0 + \eta_1^a) \qquad f(\lambda) \equiv \frac{\beta}{1+\lambda} \tag{A.141}$$

Eq. (A.140) has several features that distinguish it from the previous case. Firstly, note that no mathematical zero solution is possible in the $\eta_j \neq 0, \forall\, j$ case. Thus, the presence of constitutive enzyme synthesis, as discussed during the substitutable analysis section, acts to disrupt the competition between competing enzyme systems and affords a non-zero minimum level. In practical terms this implies no matter how fierce the competition to produce p_2, e_0 and e_1^a are present at non-zero levels for all values of the system parameters. The exact level, however, can bifurcate between two possible equilibrium solutions which exchange stability at

$$\left(\lambda - \frac{1+\lambda}{\beta}\eta^*\right) = 0 \tag{A.142}$$

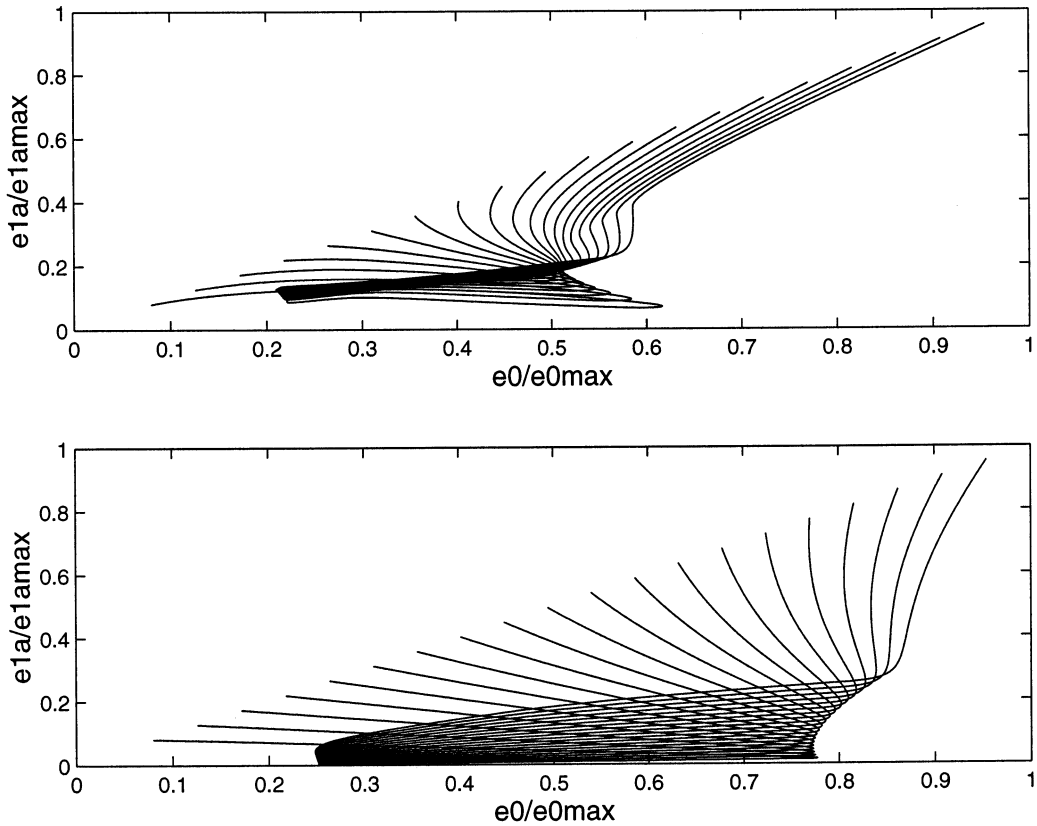

Fig. 6. Simulation of hybrid overlapping network in the absence of constitutive enzyme synthesis. Notice e_1^a can assume both a zero and a non-zero steady state solution depending upon system parameters. Steady state values; Top plate: $(e_0/e_0^{max}, e_1^a/e_1^{a,max}) = (0.2167, 0.1194)$. Bottom plate: $(e_0/e_0^{max}, e_1^a/e_1^{a,max}) = (0.2526, 0)$.

Because our goal is only to determine the qualitative aspects of this regulatory region, the conclusion that constitutive enzyme synthesis no longer allows the existence of a zero solution is sufficient. Thus, we dispense with a formal analysis of the bifurcation behavior of reduced Eq. (A.140) with the understanding that the bifurcation that Eq. (A.140) undergoes is between two non-zero levels of enzyme e_1^a. In fact, guidelines have been developed by Iooss and Joseph that govern how the bifurcation profile of an unperturbed system behaves under perturbation, we neglect such considerations leaving the interested reader to further pursue the subject.

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