Title: Mathematical Modeling of Cell Metabolism

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Mathematical Modeling of Cell Metabolism

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Definition

Suppose that a system has m different chemicals, $A_1, \ldots, A_m$, and define a *complex* to be an m-vector of non-negative integers. A metabolic network is a directed graph, not necessarily connected, whose vertices are complexes. There is an edge from complex C to complex D if there exists a chemical reaction in which the chemicals in C with non-zero components are changed into the chemicals in D with non-zero components. The non-zero integer components represent how many molecules of each chemical are used or produced in the reaction. Metabolic networks are also called biochemical networks.

Description.

Chemicals inside of cells are normally called *substrates* and the quantity of interest is the concentration of the substrate that could be measured as mass per unit volume or, more typically, number of molecules per unit volume. In Figure 3, below, the substrates are indicated by rectangular boxes that contain their acronyms. A chemical reaction changes one or more substrates into other substrates and the function that describes how the rate of this process depends on substrate concentrations and other variables is said to give the *kinetics* of the reaction. The simplest kind of kinetics is *mass action kinetics* in which a unimolecular reaction (one substrate), $A \xrightarrow{k} B$, proceeds at a rate proportional to the concentration of the substrate, i.e. $k[A]$, and a bimolecular reaction, $A+B \xrightarrow{k} C$ proceeds at a rate proportional to the product of the concentrations of the substrates, $k[A][B]$, and so forth. Given a chemical reaction diagram, such as Figure 1, the differential equations for the concentrations of the substrates simply state that the

rate of change of each substrate concentration is equal to the sum of the rates of the reactions that make it minus the rates of the reactions that use it. A simple reaction diagram and corresponding differential equations are shown in Figure 1.

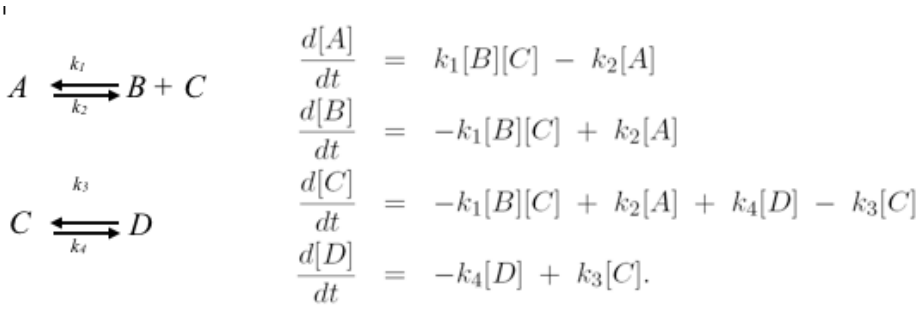

Figure 1. On the right are the differential equations corresponding to the reaction diagram if one assumes mass-action kinetics.

Figure 2 shows the simplest reaction diagram for an enzymatic reaction in which an enzyme, E, binds to a substrate, S, to form a complex, C. The complex then dissociates into the product, P, and the enzyme that can be used again. One can write down the four differential equations for the variables S, E, C, P but they can not be solved in closed form. It is very useful to have closed form formula for the overall rate of the reaction $S \longrightarrow P$ because that formula can be compared to experiments and the constants can be determined. Such an approximate formula was derived by Leonor Michaelis and Maud Menten:

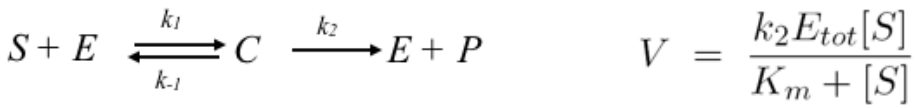

Figure 2. A simple enzymatic reaction and the Michaelis-Menten formula.

Here E_{tot} is the total enzyme concentration, k_2 is indicated in Figure 2, and K_m is the so-called Michaelis-Menten constant. The quantity $k_2 E_{tot}$ is called the V_{max} of the reaction because that is the maximum rate obtained as $[S] \to \infty$. There is a substantial

mathematical literature about when this approximation is a good one Segal [33]. For further discussion of kinetics and references, see Keener and Sneyd [24].

The biological goal is to understand how large biochemical systems that accomplish particular tasks work, that is, how the behavior of the whole system depends on the components and on small and large changes in inputs. So, for example, the folate cycle in Figure 3 is central to cell division since it is involved in the production of purines and pyrimidines necessary for copying DNA. Methotrexate, a chemotherapeutic agent binds to the enzyme DHFR and slows down cell division. Why? And how much methotrexate do you need to cut the rate of cell division in half? The enzyme DNMT catalyzes the methylation of DNA. How does the rate of the DNMT reaction depend on the folate status of the individual, that is, the total concentration of the six folate substrates?

Difficulties.

It would seem from the description so far that the task of an applied mathematician studying metabolism should be quite straightforward. A biologist sets the questions to be answered. The mathematician writes down the differential equations for the appropriate chemical reaction network. Using data bases or original literature, the constants for each reaction, like K_m and V_{max}, are determined. Then the equations are solved by machine computation and one has the answer. For many different reasons the actual situation is much more difficult and much more interesting.

What's the network? The metabolism of cells is an exceptionally large biochemical network and it is not so easy to decide on the "correct" relatively small network that corresponds to some particular cellular task. Typically the substrates in any small network will also be produced and used up by other small networks and thus the behavior in those other networks affects the one under study. How should one draw the

boundaries of a relatively small network so that everything that is important for the effect one is studying is included?

Enzyme properties. The rates of reactions depend on the properties of the enzymes that catalyze them. Biochemists often purify these enzymes and study their properties when they are combined with substrates in a test tube. These experiments are typically highly reproducible. However, enzymes may behave very differently in the context of real cells. They are affected by ph and by the presence or absence of many other molecules that activate them or inhibit them. Thus their K_m and V_{max} may depend on the context in which they are put. Many metabolic pathways are very ancient, for example the folate cycle occurs in bacteria, and many different species have the "same" enzymes. But, in reality, the enzymes may have different properties because of differences in the genes that code for them.

Gene expression levels. Enzymes are proteins that are coded for by genes. The V_{max} is roughly proportional to the total enzyme concentration, which is itself dependent on gene expression level and the rate of degradation of the enzyme. The expression level of the gene that codes for the enzyme will depend on the cell type (liver cell or epithelial cell) and on the context in which the cell finds itself. This expression level will vary between different cells in the same individual, between individuals of the same species, and between different species that have the same gene. Furthermore, the expression level may depend on what other genes are turned on or the time of day. Even more daunting is the fact that identical cells (same DNA) in exactly the same environment often show a 30% variation in gene expression levels Sigal et al [36]. Thus, it is not surprising that the K_m and V_{max} values (that we thought the biochemists would determine for us) vary sometimes by 2 or 3 orders of magnitude in public enzyme databases.

Is the mean field approximation valid? When we write down the differential equations for the concentrations of substrates using mass-action, Michaelis-Menten, or other kinetics, we are assuming that the cell can be treated as a well-mixed bag of chemicals. There are two natural circumstances where this is not true. First the number of molecules of a given substrate may be very small; this is particularly true in biochemical networks related to gene expression. In this case stochastic fluctuations play an important role. Stochastic methods are discussed below. Secondly, some biochemical reactions occur only in special locations, for example the cell membrane or the endoplasmic reticulum. In this case there will clearly be gradients, the well-mixed assumption is not valid, and partial differential equations will be required.

Are these systems at steady state? It is difficult to choose the right network and determine enzyme constants. However, once that is done surely the traditional approach in applied mathematics to large non-linear systems of ODEs should work. First one determines the steady-states and then one linearizes around the steady-states to determine which ones are asymptotically stable. Unfortunately, many cellular systems are not at or even near steady state. For example, amino acids concentrations in the blood for the hours shortly after meals increase by a factor of 2 to 6. This means that cells are subject to enormous fluctuations in the inputs of amino acids. The traditional approach has value, of course, but new tools, both technical and conceptual, are needed for studying these systems of ODEs.

Long-range interactions. Many biochemical reaction diagrams do not include the fact that some substrates influence distant enzymes in the network. These are called long-range interactions and several are indicated by red arrows in Figure 3. The substrate SAM activates the enzyme CBS and inhibits the enzymes MTHFR and BHMT. The substrate 5mTHF inhibits the enzyme GNMT. We note that "long-range" does

not indicate distance in the cell; we are assuming the cell is well-mixed. "Long-range" refers to distance in the network. It used to be thought that it was easy to understand the behavior of chemical networks by walking through the diagrams step by step. But if there are long-range interactions this is no longer possible; one must do serious mathematics and/or extensive machine experimentation to determine the system properties of the network.

But what do these long-range interactions do? In the cases indicated in Figure 3 this is understood. After meals the methionine input goes way up and the SAM concentration rises dramatically. This activates CBS and inhibits BHMT, which means that more mass is sent away from the methionine cycle via the CBS reaction and less mass is recycled within the cycle via the BHMT reaction. So these two long-range interaction roughly conserve mass in the methionine cycle. The other two long range interactions keep the DNMT reaction running at almost a constant rate despite large fluctuations in methionine input. Here's a verbal description of how this works. If SAM starts to go up, the enzyme MTHFR is more inhibited so there will be less of the substrate 5mTHF. Since there's less 5mTHF, the inhibition of GNMT is partly relieved and the extra SAMs that are being produced are taken down the GNMT pathway, leaving the rate of the DNMT reaction about constant Nijhout et al [28]. We see that in both cases the long-range interactions have specific regulatory roles and probably evolved for just those reasons. The existence of such long-range interactions makes the study of chemical reaction networks much more difficult.

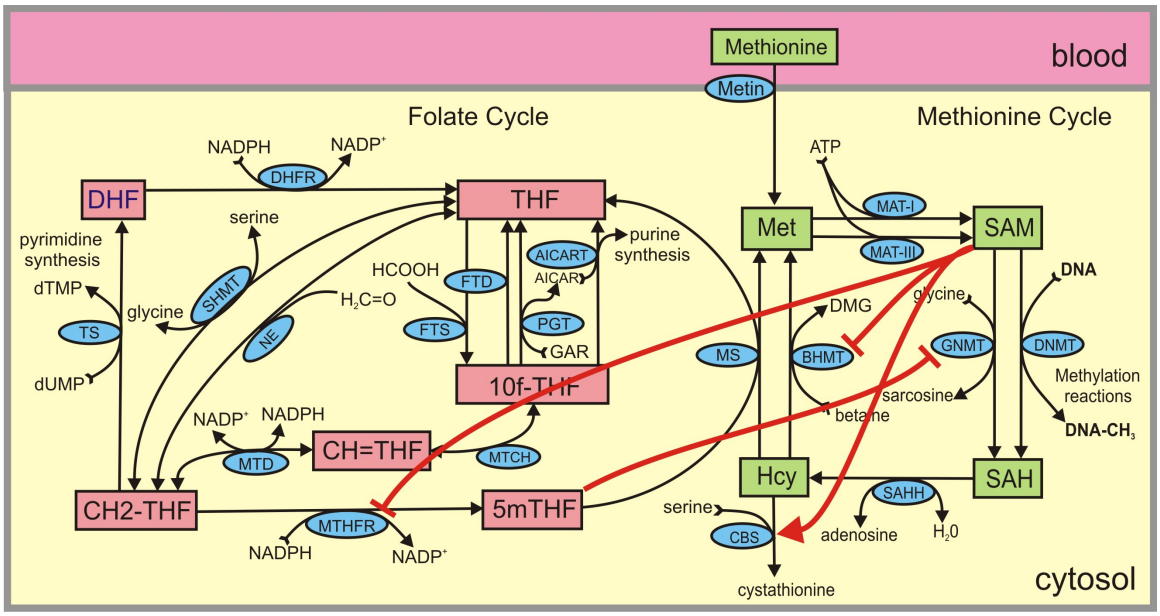

Figure 3. Folate and methionine metabolism. The rectangular boxes represent substrates whose acronyms are in the boxes. All the pink boxes are different forms of folate. Each arrow represents a biochemical reaction. The acronyms for the enzymes that catalyze the reactions are in the blue ellipses. The TS and AICART reactions are important steps in pyrimidine and purine synthesis, respectively. The DNMT reaction methylates cytosines in DNA and is important for gene regulation.

Theoretical Approaches to Complex Metabolic Systems.

Cell metabolism is an extremely complex system and the large number of modeling studies on particular parts of the system can not be summarized is a short review. However, we can discuss several different theoretical approaches.

Metabolic Control Analysis (MCA). This theory, which goes back to the original papers of Kacser and Burns [21],Kacser and Burns [22], enables one to calculate "control coefficiants" that give some information about the system properties of metabolic networks. Let $x =< x_1, x_2, \dots >$ denote the substrate concentrations in a large metabolic network and suppose that the network is at a steady state $x^s(c)$, where c denotes a vector of constants that the steady state depends on. These constants may be kinetic constants like K_m or V_{max} values, initial conditions, input rates, enzyme concentra-

tions. etc.. If we assume that the constants are not at critical values where behavior changes, then the mapping $c \longrightarrow x^s(c)$ will be smooth and we can compute its partial derivatives. Since the kinetic formulas tell us how the fluxes along each pathway depend on the substrate concentrtions, we can also compute the rates of change of the fluxes as the parameters c are varied. These are called the "flux control coefficients." In practice, this can be done by hand only for very simple networks, and so is normally done by machine computation. MCA gives information about system behavior very close to a steady state. One of the major contributions of MCA was to emphasize that local behavior, for example a flux, was a system property in that it depended on all or many of the constants in c. So, for example, there is no single rate-limiting step for the rate of production of a particular metabolite, but, instead, control is distributed throughout the system.

Biochemical Systems Theory (BST). This theory, which goes back to Savageau Savageau [32], replaces the diverse nonlinear kinetic formulas for different enzymes with a common power-law formulation. So, the differential equation for each substrate concentration looks like $x'(t) = \sum_i \alpha_{ij} \prod_j x_{ij}^{\beta_{ij}} - \sum_i \gamma_{ij} \prod_j x_{ij}^{\delta_{ij}}$ In the first term the sum over i represents all the different reactions that produce x and the product over j gives the variables that influence each of those reactions. Similarly, the second sum contains the reactions that use x. The powers, β_{ij} and δ_{ij}, which can be fractional or negative, are to be obtained by fitting the model to experimental data. The idea is that one needs to know the network and the influences, but not the detailed kinetics. A representation of the detailed kinetics will emerge from determining the powers by fitting data. Note that the influences would naturally incude the long-range interactions mentioned above. From a mathematical point of view there certainly will be such a representation near a (non-critical) steady state if the variables represent deviations from that steady state. One of the drawbacks of this method is that biological data is

so variable (for the reasons discussed above) and therefore the right choice of data set for fitting may not be clear. BST has also been used to simulate gene networks and intracellular signaling networks Reinitz and Sharp [31],Sharp and Reinitz [34].

Metabolomics. With the advent of high-throughput studies in molecular biology there has been much interest in applying concepts and techniques form bio-informatics to understanding metabolic systems. The idea is that one measures the concentrations of many metabolites at different times, in different tissues, or cells. Statistical analysis reveals which variables seem to be correlated and one uses this informations to draw a network of influences. Clusters of substrates the vary together could be expected to be part of the same "function." The resulting networks can be compared, between cells or species, in an effort to understand how function arises from network properties; see for example Pepin et al [29].

Graph theory. A related approach has been to study the directed graphs that correspond to known metabolic (or gene) networks with the substrates (genes) as nodes and the directed edges representing biochemical reactions (or influences). One is interested in large scale properties of the networks, such as mean degree of nodes and the existence of large almost separated clusters. One is also interested in local properties, such as a particular small connection pattern, that is repeated often in the whole graph. It has been proposed by Alon [1] that such repeated "motifs" have specific biological functions. From the biological point of view, the graph theoretic approaches have a number of pitfalls. It is very natural to assume that graph properties must have biological function or significance. For example, to assume that a node with many edges must be "important", or clusters of highly connected nodes are all contributing to a single "function." Nevertheless, it is interesting to study the structure of the graphs in-

dependent of the dynamics and to ask what influnce or significance the graph structure has.

Deficiency zero systems. The study of graphs suggests a natural question about the differential equations that represent metabolic systems. When are the qualitative properties of the system independent of the local details? As discussed in Difficulties, the details will vary considerably from species to species, from tissue to tissue, from cell to cell, and even from time to time in the same cell. Yet large parts of cell metabolism keep functioning in the same way. Thus, the biology tells us that many important system properties are independent of the details of the dynamics. This must be reflected in the mathematics. But how? A major step to understanding the answer to this question was made by Marty Feinberg and colleagues, Feinberg [14].

Let m be the number of substrates. For each reaction in the network, we denote by ν the m-component vector of integers that indicates how many molecules of different substrates are used in the reaction; ν' indicates how many are produced by the reaction. Each ν is called a complex and we denote the number of complexes by c. The span of the set of vectors of the form $\nu - \nu'$ is called the stoichiometric subspace and it is invariant under the dynamics. We denote it's dimension by s and let ℓ denote the number of connected components of the graph. The deficiency of the network is defined as $\delta = c - s - \ell$. The network is weakly reversible if whenever a sequence of reactions allows us to go from complex ν_1 to complex ν_2 then there exists a sequence of reactions from ν_2 to complex ν_1. Feinberg formulated the deficiency zero theorem which says that a weakly reversible deficiency zero network with mass action kinetics has a unique globally stable equilibrium in the interior of each stoichiometric compatibility class. This is true independent of the choice of rate constants. Feinberg gave a proof in the case that there are no boundary equilibria on the faces of the positive orthant. Since

then, the proof has been extended to many cases that allow boundary equilibria Chavez [9],Anderson [2],Shiu and Sturmfels [35].

Stochastic Models.

There are many sources of stochasticity in cellular networks. For example, the initial conditions for a cell will be random due to the random assignment of resources at cellular division, and the environment of the cell is random due to fluctuations in such things as temperature and the chemical environment of the cell. If these were the only sources of randomness, then one would only need to modify the coefficients and initial conditions of the differential equation models to obtain reasonable models taking these stochastic effects into account. But many cellular processes involve substrates and enzymes present in the system in very small numbers, and small (random) fluctuations in these numbers may have significant effects on the behavior of the system. Consequently, it is the discreteness of the system as much as its inherent stochasticity that demands a modeling approach different from the classical differential equations.

Markov chain models. The idea of modeling a chemical reaction network as a discrete stochastic process at the molecular level dates back at least to Delbrück [12] with a rapid development beginning in the 1950s and 1960s. See, for example, Bartholomay [7, 8]; McQuarrie [27]. The simplest and most frequently used class of models are continuous-time Markov chains. The *state* $X(t)$ of the model at time t is a vector of nonnegative integers giving the numbers of molecules of each species in the system at that time. These models are specified by giving transition *intensities* (or *propensities* in much of the reaction network literature) $\lambda_l(x)$ which determine the infinitesimal probabilities of seeing a particular change or transition $X(t) \to X(t+\Delta t) = X(t) + \zeta_l$ in the next small interval of time $(t, t+\Delta t]$, that is,

$$P\{X(t+\Delta t) = X(t) + \zeta_l | X(t)\} \approx \lambda_l(X(t))\Delta t.$$

In the chemical network setting, each type of transition corresponds to a reaction in the network, and $\zeta_l = \nu_l' - \nu_l$, where ν_l is a vector giving the number of molecules of each chemical species consumed in the l_{th} reaction and ν_l' is a vector giving the number of molecules of each species produced in the reaction.

The intuitive notion of a transition intensity can be translated into a rigorous specification of a model in a number of different ways. The most popular approach in the chemical networks literature is through the master (or Kolmogorov forward) equation

$$\dot{p}_y(t) = \sum_l \lambda_l(y - \zeta_l) p_{y-\zeta_l}(t) - (\sum_l \lambda_l(y)) p_y(t), \tag{1}$$

where $p_y(t) = P\{X(t) = y\}$ and the sum is over the different reactions in the network.

Another useful approach is through a system of stochastic equations

$$X(t) = X(0) + \sum \zeta_l Y_l(\int_0^t \lambda_l(X(s))ds), \tag{2}$$

where the Y_l are independent unit Poisson processes. Note that $R_l(t) = Y_l(\int_0^t \lambda_l(X(s))ds)$ simply counts the number of times that the transition taking the state x to the state $x + \zeta_l$ occurs by time t, that is, the number of times the l_{th} reaction occurs. The master equation and the stochastic equation determine the same models in the sense that if X is a solution of the stochastic equation, $p_y(t) = P\{X(t) = y\}$ is a solution of the master equation, and any solution of the master equation can be obtained in this way. See Anderson and Kurtz [4] for a survey of these models and additional references.

The stochastic law of mass action. The basic assumption of the simplest Markov chain model is the same as that of the classical law of mass action: the system is thoroughly mixed at all times. That assumption suggests that the intensity for a binary reaction

$$A + B \to C \tag{3}$$

should be proportional to the number of pairs consisting of one molecule of A and one molecule of B, that is, $\lambda(X(t)) = kX_A(t)X_B(t)$. The same intuition applied to the binary reaction

$$2A \to C \tag{4}$$

would give an intensity

$$\lambda(X(t)) = \kappa\binom{X_A(t)}{2} = \frac{\kappa}{2}X_A(t)(X_A(t)-1) = kX_A(t)(X_A(t)-1),$$

where we replace $\kappa/2$ by k.

For unary reactions, for example $A \to C$, the assumption is that the molecules behave independently and the intensity becomes $\lambda(X(t)) = kX_A(t)$.

Relationship to deterministic models. The larger the volume of the system the less likely a particular pair of molecules is to come close enough together to react, so it is natural to assume that intensities for binary reactions should vary inversely with respect to some measure of the volume. If we take that measure N to be Avogadro's number times the volume in liters, then the intensity for (3) becomes

$$\lambda(X(t)) = \frac{k}{N}X_A(t)X_B(t) = Nk[A]_t[B]_t,$$

where $[A]_t = X_A(t)/N$ is the concentration of A measured in moles per liter. The intensity for (4) becomes $\lambda(X(t)) = k[A]_t([A]_t - N^{-1}) \approx k[A]_t^2$, assuming, as is likely, that N is large and that $X_A(t)$ is of the same order of magnitude as N (which may not be the case for cellular reactions). If we assume that our system consists of the single reaction (3), the stochastic equation for species A, written in terms of the concentrations, becomes

$$[A]_t = [A]_0 - \frac{1}{N}Y(N\int_0^t k[A]_s[B]_s ds) \approx [A]_0 - \int_0^t k[A]_s[B]_s ds,$$

where, again assuming that N is large, the validity of the approximation follows by the fact that the law of large numbers for the Poisson process implies $N^{-1}Y(Nu) \approx u$.

Analysis along these lines gives a derivation of the classical law of mass action starting from the stochastic model. See, for example, Kurtz [25], Kurtz [26], or Ethier and Kurtz [13], Chapter 10.

Simulation. Among the basic properties of a continuous time Markov chain (with intensities that do not depend on time) is that the holding time in a state x is exponentially distributed and is independent of the value of the next state occupied by the chain. To be specific, the parameter of the holding time is

$$\bar{\lambda}(x) = \sum_l \lambda_l(x),$$

and the probability that the next state is $x + \zeta_l$ is $\lambda_l(x)/\bar{\lambda}(x)$. This observation immediately suggests a simulation algorithm known in the chemical literature as *Gillespie's direct method* or the *stochastic simulation algorithm* (SSA)Gillespie [16, 17]. Specifically, given two independent uniform $[0, 1]$ random variables U_1 and U_2 and noting that $-\log U_1$ is exponentially distributed with mean 1, the length of time the process remains in state x is simulated by $\Delta = \frac{1}{\bar{\lambda}(x)}(-\log U_1)$. Assuming that there are m reactions indexed by $1 \le l \le m$ and defining $\rho_0(x) = 0$ and $\rho_l(x) = \bar{\lambda}(x)^{-1} \sum_{k=1}^{l} \lambda_k(x)$, the new state is given by

$$x + \sum_l \zeta_l \mathbf{1}_{(\rho_{l-1}(x), \rho_l(x)]}(U_2),$$

that is, the new state is $x + \zeta_l$ if $\rho_{l-1}(x) < U_2 \le \rho_l(x)$.

If one simulates the process by simulating the Poisson processes Y_l and solving the stochastic equation (2), one obtains the *next reaction* (next jump) method as defined by Gibson and Bruck [15].

If we define an Euler-type approximation for (2), that is, for $0 = \tau_0 < \tau_1 < \cdots$, recursively define

$$\hat{X}(\tau_n) = X(0) + \sum_l \zeta_l Y_l \left(\sum_{k=0}^{n-1} \lambda_l(\hat{X}(\tau_k))(\tau_{k+1} - \tau_k) \right),$$

we obtain Gillespie's τ-leap method, which provides a useful approximation to the stochastic model in situations where $\bar{\lambda}(x)$ is large for values of the state x of interest Gillespie [18]. See Anderson [3]; Anderson et al [5] for additional analysis and discussion.

Hybrid and multiscale models. A discrete model is essential if the chemical network consists of species present in small numbers, but a typical biochemical network may include some species present in small numbers that need to be modeled as discrete variables and others species present in much larger numbers that would be natural to model as continuous variables. This observation leads to hybrid or piecewise deterministic models (in the sense of Davis [11]) as considered in the chemical literature by Crudu et al [10]; Haseltine and Rawlings [19]; Hensel et al [20]; Zeiser et al [39]. We can obtain these models as solutions of systems of equations of the form

$$X_k(t) = X_k(0) + \sum_{l \in \mathcal{R}_d} \zeta_l Y_l(\int_0^t \lambda_l(X(s))ds), \qquad k \in \mathcal{S}_d,$$

$$X_k(t) = X_k(0) + \sum_{l \in \mathcal{R}_c} \zeta_l \int_0^t \lambda_l(X(s))ds = X_k(0) + \int_0^t F_k(X(s))ds, \qquad k \in \mathcal{S}_c,$$

where $\mathcal{R}_d$ and $\mathcal{S}_d$ are the indices of the reactions and the species that are modeled discretely, $\mathcal{R}_c$ and $\mathcal{S}_c$ are the indices for the reactions and species modeled continuously, and $F_k(x) = \sum_{l \in \mathcal{R}_c} \zeta_l \lambda_l(x)$.

Models of this form are in a sense "multiscale" since the numbers of molecules in the system for the species modeled continuously are typically many orders of magnitude larger than the numbers of molecules for the species modeled discretely. Many of the stochastic models that have been considered in the biochemical literature are multiscale for another reason in that the rate constants vary over several orders of magnitude as well. (See, for example, Srivastava et al [38, 37].) The multiscale nature of the species numbers and rate constants can be exploited to identify subnetworks that function naturally on different time scales and to obtain reduced models for each of the time scales. Motivated in part by Rao and Arkin [30] and Haseltine and Rawlings [19], a

systematic approach to identifying the separated times scales and reduced models is developed in Ball et al [6] and Kang and Kurtz [23].

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