# Algorithmic Algebraic Model Checking IV: Characterization of Metabolic Networks ⋆

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**Abstract.** A series of papers, all under the title of Algorithmic Algebraic Model Checking (AAMC), has sought to combine techniques from algorithmic algebra, model checking and dynamical systems to examine how a biochemical hybrid dynamical system can be made amenable to temporal analysis, even when the initial conditions and unknown parameters may only be treated as symbolic variables. This paper examines how to specialize this framework to metabolic control analysis (MCA) involving many reactions operating at many dissimilar time-scales. In the earlier AAMC papers, it has been shown that the dynamics of various biochemical semi-algebraic hybrid automata could be unraveled using powerful techniques from computational real algebraic geometry. More specifically, the resulting algebraic model checking techniques were found to be suitable for biochemical networks modeled using general mass action (GMA) based ODEs. This paper scrutinizes how the special properties of metabolic networks– a subclass of the biochemical networks previously handled–can be exploited to gain improvement in computational efficiency. The paper introduces a general framework for performing symbolic temporal reasoning over metabolic network hybrid automata that handles both GMA-based equilibrium estimation and flux balance analysis (FBA). While algebraic polynomial equations over $\mathbb{Q}[x_1, \ldots, x_n]$ can be symbolically solved using Gröbner bases or Wu-Ritt characteristic sets, the FBA-based estimation can be performed symbolically by rephrasing the algebraic optimization problem as a quantifier elimination problem. Effectively, an approximate hybrid automaton that simulates the metabolic network is derived, and is thus amenable to manipulation by the algebraic model checking techniques previously described in the AAMC papers.

## 1 Introduction

Recently, several biologists have convincingly argued for a systems level analysis, as opposed to the traditional reductionist approach of molecular biology [13, 7, 12]. When aimed at understanding the holistic properties of the dynamics of biochemical networks, this approach could not only lead to giant leaps in our elucidation of the basic science of biology, but could also contribute more directly to many practical applications, e.g., the drug and vaccine discovery process, diagnosis, agricultural and manufacturing technologies, and synthetic biology of the future. Algebraic analysis may hold the

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key to success of this venture, as it enables obtaining richer answers to deeper questions, even when both initial conditions and rate parameters can only be presented as symbolic variables. It is hoped that this methodology will expose important algebraic functional relationships among the emergent phenomena, the kinetic parameters and the initial conditions, thus revealing many fundamental unifying principles of biology. The starting point for this approach is the fundamental general mass action (GMA) law of chemical kinetics, which supplies a system of ordinary differential equations (ODEs) governing the rate of change of the concentrations of interacting biochemicals. Let $k_i$s denote the rate constants, $n_i$s the number of molecules that appear in the reactions, and $W_j$s their concentrations. Then, the continuous dynamics within each state may be described through the GMA-based ODEs [11, 27, 58]:

$$\dot{W}_h = +\{\Sigma_{j\in h_+} n_j k_j \Pi_i^j W_i\} - \{\Sigma_{j\in h_-} n_j k_j \Pi_i^j W_i\}. \quad (1)$$

Each equation above is an algebraic differential equation consisting of two affine summation terms: a positive term representing synthesis (all processes producing $W_h$) and a negative term representing degradation (all processes consuming $W_h$). The number of $W_j$s (an integer) multiplied in each term is equal to the number of molecules of reactants (and similarly, products) in that reaction (e.g., higher order terms like $100kW_i^3W_j^{10}W_k^5$ are possible[4]).

For tools analyzing GMA ODEs, their ability to handle unknown parameters or uncertainties in their estimates becomes crucial as kinetic parameters are seldom measured under ideal conditions [14]. In response to this challenge, we have extended the GMA models to the algebraic domain, by developing decidable and approximable techniques for symbolic temporal analysis within our Algorithmic Algebraic Model Checking (AAMC) framework, described in a series of publications [46, 41, 40, 6, 39, 42]. In this framework, the process of numerically integrating the differential equations and extracting a simpler examinable representation is substituted with an algebraic procedure (based on Computational Real Algebraic Geometry [37, 15, 38]) that can answer complex queries about the symbolic states of the system.

In this paper, we specialize this approach to *metabolism*, which is comprised of the complex enzyme-catalyzed pathways (excluding signal transduction and genetic regulation) that produce and consume the "metabolites" in any living cell. The system of ODEs for metabolic networks lends itself to simplification and efficient analysis because of three key properties [17]: (1) A subset of the metabolites interact with each other through reactions much faster than the rest of the system; (2) These fast reactions always reach a quasi-equilibrium state, which is local (involving only this subset of metabolites) and momentary (it is modulated by the slower reactions in the rest of the system); (3) Mass is conserved during such equilibrium recomputation, and the equilibrium configuration is completely determined by the total concentration of the metabolites. Powerful computational methods have emerged to exploit this structure of metabolic networks; in these methods, only the dynamic GMA simulation of the slow reactions are performed, while, under the assumption that the fast reactions respond quickly, the equilibria of the fast reactions are recomputed at each time-step. A

[4] Though negative and real exponents can be indirectly handled, we restrict our analyses to terms with non-negative integer exponents.

list of the most prominent methods that venture in this direction would include the following: tendency modeling [57], dynamic flux balance analysis [33], hybrid static + dynamic simulation [65], intrinsic low dimensional manifolds [52] and singular perturbation analysis [19, 32]. This paper builds upon several concepts from the earlier studies to arrive at a trichotomic characterization of the metabolites – the slow irreversible dynamic reactants $X$, the fast reversible quasi-equilibrium reactants $Y$ and the interface reactants $Z$. Their properties are elaborated below:

1. *Dynamic Reactants*: All the reactions involving these metabolites (denoted by $X$) are modeled using detailed general mass action-based differential equations. Typically, these reactions are understood to be slow and irreversible, with dynamics of the form: $\dot{X} = F(X,Z,K)$, where $K$ are the symbolic (rate) parameters.
2. *Quasi-Equilibrium Reactants*: All the reactions involving these metabolites (denoted by $Y$) are modeled in terms of their dynamic equilibria alone. They always participate in at least one reaction as a substrate and in at least one reaction as a product. Typically, these reactions are understood to be fast and reversible, with dynamics of the form: $\dot{Y} = G(Y,Z,K)$.
3. *Interface Reactants*: These reactants (denoted by $Z$) interact with both the dynamic reactants and the quasi-equilibrium reactants. Thus, their general mass action based flow equations (from slow reactions) are modified because of the fast reactions with the quasi-equilibrium reactants, giving rise to dynamics of the form: $\dot{Z} = D(X,Z,K) + P(Y,Z,K)$.

*Example 1.* Consider a simple metabolic network composed of just two reactions: a slow irreversible reaction $A + B \xrightarrow{k_s} R + S$, and a fast reversible reaction $E + S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C$. This reaction could describe an enzymatic process involving, say, an enzyme ($E$) and substrate ($S$) interacting to produce the enzyme substrate complex ($C$). We wish to study how an external slow reaction producing the substrate can control the equilibrium configuration. Let us denote the metabolite concentrations, $[E]$, $[S]$, $[C]$, by the letters $e$, $s$, and $c$, respectively. The dynamic reactants $X$ are $A$, $B$ and $R$. The quasi-equilibrium reactants $Y$ are $E$ and $C$. The interface reactant $Z$ is $S$. Their flow equations are: $\dot{a} = \dot{b} = -\dot{r} = -k_s ab$, $\dot{e} = -\dot{c} = k_r c - k_f es$ and $\dot{s} = k_s ab + k_r c - k_f es$. The dynamics are often rephrased using the flux variables $U_1 = k_s ab$, $U_2 = k_r c$ and $U_3 = k_f es$. □

The existing tools [59] for metabolic networks are all structured primarily on analyses that use numerical simulation, numerical perturbation, random sampling and parameter sweeping techniques. A list of tools in this category includes: Gepasi [35], Systems Biology Workbench [23], E-Cell [54] and BioSpice [29]. Conclusions about the behavior of the network are often made by alternating between (a) tracing specific trajectories over a suitable time frame and then (b) verifying temporal logic properties such as reachability or safety [56, 8, 2]. The slow reactions in metabolic networks are typically modeled and analyzed as per this approach. The fast reaction systems are typically subject only to a quasi-equilibrium characterization with minimal dynamic characterization. Some of the popular techniques following this strategy are: Metabolic Control Analysis (MCA) [21], Metabolic Flux Analysis (MFA) [31], Flux Balance Analysis (FBA) [28], Cybernetic approaches [43] and Metabolic Pathway Analysis (MPA) [49].

While the algebraic estimation of the equilibrium concentrations has been studied extensively [8, 36, 64, 3], in contrast, a directed effort to handle both GMA-based simulation and direct equilibrium estimates (via GMA or FBA) algebraically seems conspicuously absent.

Rather than pursuing the traditional numerical simulation based analysis, this paper suggests an entirely symbolic algorithmic algebraic framework for the unified analysis of metabolic networks. It proceeds by first mathematically characterizing the hybrid dynamical system to which metabolic networks correspond, and then integrating general mass action [11] and flux balance analysis [28] based equilibrium estimation. Next the paper shows that the algebraic equilibrium description is decidable, both using GMA and FBA. Our proof of the decidability of the algebraic approach are based on the well-established Gröbner basis and characteristic set techniques [5, 47, 62, 18] for solving polynomial equations, and the decidability of semi-algebraic[5] optimization using real quantifier elimination [55]. The paper then examines how to move from the equilibrium description to its derivative (rate of change), which can then be combined with the ODEs of the slow reactants to complete an algebraic description of the metabolic network. These steps directly lead to efficient algebraic model-checking, since, at this point, they have ensured that all the interactions operate at roughly the same time scale. Hence a bigger time-step suitable for the slow interactions is sufficient (as opposed to the smaller time-step that would have been necessary for the fast reactions), be it for simulation or algebraic temporal logic analysis, based on the techniques described in the earlier AAMC papers.

## 2 Preliminaries: Algebraic Analysis of a Biochemical Hybrid System

Biochemical systems are conveniently approximated as hybrid automata operating in one of many *discrete states* (or modes). In each state, the *continuous evolution* of different chemicals, reactions, assumptions and ODEs predominate, with *discrete transitions* to other states possible under certain *guard* conditions, leading to the variables being reassigned as per the *reset* relations. Within each state, the temporal properties of the network of interacting biochemicals are captured algebraically by the *flow* relation (from GMA-based ODEs) that relates two neighboring system-states at time instants $t$ and $t+h$, and the biochemical interactions (synthesis, degradation, multimerization, etc.) that occur in that short time interval $h$.

In the *Algorithmic Algebraic Model Checking* approach [46, 41, 40, 6, 39, 42], it was shown how most temporal logic query-answering can be expressed as a series of quantifier elimination problems over the reals. The resulting mathematical problem has been known to be decidable [55] and elementarily computable (e.g., using Qepcad [22] or Redlog [16]), though computationally expensive – time complexity, unfortunately, still remains doubly exponential in the number of variables. For such analyses to be possible, each discrete state should have only polynomial ODEs, with the guard, reset and invari-

[5] Unquantified first-order formulæ over the theory of reals (i.e., over $(\mathbb{R}, +, \times, =, <)$); see [37, 15, 38] for details.

ant relations also being semi-algebraic (Boolean combinations of polynomial equations and inequalities), thus yielding a new class of hybrid systems, as defined below:

**Definition 1. Semi-Algebraic Hybrid Automata: [46, 41]** *A k-dimensional* hybrid automaton *is a septuple, $H = (W, V, E, Init, Inv, Flow, Jump)$, consisting of the following components:*

- $W = \{W_1, \ldots, W_k\}$ *and* $W' = \{W'_1, \ldots, W'_k\}$ *are two finite sets of variables ranging over the reals* $\mathbb{R}$*;*
- $(V, E)$ *is a directed graph of discrete states and transitions;*
- *Each discrete state* $v \in V$ *is labeled by "Init"(initial), "Inv"(invariant) and "Flow" labels of the form* $Init_v[W]$*,* $Inv_v[W]$*, and* $Flow_v[W, W', t, h]$
- *Each edge* $e \in E$ *is labeled by a "Jump" condition of the form* $Jump_e[W, W'] \equiv Guard_e(W) \wedge Reset_e(W, W')$
- *Init, Inv, Flow, and Jump are all semi-algebraic.* □

Within each state of a biochemical hybrid dynamical system, the network of interacting biochemicals is modeled using variables that represent their concentrations (see *Eqn.* 1). The semi-algebraic hybrid automaton structure requires that the continuous dynamics of each discrete state $v$ be captured in the *flow* relation $Flow_v[W, W', t, h]$ that connects the symbolic state $W$ of the system at time $t$ with the symbolic state $W'$ at time $t + h$. To derive an approximate *flow* relation, the polynomial differential equations describing the continuous evolution are integrated using one of the symbolic schemes (e.g., the *Taylor* series, the linear *Euler* or the higher degree *Runge-Kutta*). The error is controlled by an upper bound on the time spent in one continuous step, as we aim for over- or under-approximating the flow equations (also see [30]). Thus, we can write the flow equations for the biochemical dynamical system as shown here[6]:

$$Flow_v[W, W', t, h] \equiv \{W' = W + h\dot{W}(W, K)\}.$$

Here, $W$ represents the vector of concentrations at time $t$, $\dot{W}$ is the vector of first temporal derivatives (from the GMA-based ODEs) expressed as a polynomial in $W$ and the rate constants $K$ (and $t$, if necessary as with many time-variant systems), and $W'$ is the approximate value of $W(t + h)$ (with $O(h^2)$ error, in the case of the Euler forward integration). Note that the incompleteness that results from following the biochemical traces using a fixed time step (chosen based on the desired integration error bound) that plagues numerical methods is not alleviated in the algebraic procedure detailed here.

Since the guard, resets and invariants are also restricted to be Boolean combinations of polynomial equations and inequalities, the complete transition relation (see *Defn. 3 – Semantics of Hybrid Automata* in [41]) of the biochemical hybrid dynamical system can be written in terms of a semi-algebraic expression. Once such a relation is derived, temporal logic analysis can be performed to algebraically characterize global and emergent dynamical properties of the biochemical network (for example, see the analysis of the Delta-Notch pathway using Timed Computation Tree Logic in the tool Tolque [41]).

[6] Without loss of generality, in this paper, we will adopt the Euler forward symbolic integration scheme [41] to compute the trajectories of the metabolic reactions.

## 3 Algebraic Analysis of Metabolic Hybrid Systems

The basic outline of our algebraic procedure is as follows:

1. Start with a complete general mass action based hybrid automaton model of the entire metabolic network, with symbolic variables (parameters) substituted in place of unknowns.
2. Within each discrete state:
   (a) Identify sub-networks of reversible fast reactions (using information from biochemistry literature).
   (b) Compute the dynamic equilibrium concentrations and fluxes of the fast sub-networks. This step can be performed accurately over the GMA model using the Gröbner basis and Wu-Ritt characteristic set techniques (see *Sec.* 3.1). Similar analysis can also be obtained from the FBA approach, using algebraic optimization (see *Sec.* 3.2). Irrespective of which algorithm is used, we formulate an algebraic description of the equilibrium state of the reactants participating in fast reactions. (In some cases, this equilibrium description might yield differential equations – see *Defn.* 5 and *Note* 1)
3. Now the entire hybrid system is ready to be simulated or analyzed using a time-step appropriate for the slow biochemical reactions, with the fast reactants in each discrete state updated as determined by the equilibrium relations (or in some cases, the new differential equations) derived in *Step* $2(b)$.

*Steps* 1, $2(a)$ and 3 are part of the standard procedure [57, 33, 65], and there is no need for a new algebraic version. This paper provides the necessary mathematical details for *Step* $2(b)$, where we wish to symbolically characterize the momentary quasi-equilibria that the fast variables (interface and quasi-equilibrium metabolites) reach in response to a change in the slow interactions (dynamic reactants) at each time-step. We first formally capture the dynamical system to which the subclass of metabolic networks corresponds, as constrained by our assumptions (see *Sec.* 1 for details).

**Definition 2.** ***Metabolic Dynamics:*** *A metabolic network comprises the slow irreversible dynamic reactants $X$, the fast reversible quasi-equilibrium reactants $Y$, the interface reactants $Z$ that participate in both slow and fast reactions, and symbolic (rate) parameters $K$, such that the following differential algebraic equations hold:*

$$\dot{X} = F(X,Z,K)\ ,\ \dot{Y} = G(Y,Z,K)\ ,\ \dot{Z} = D(X,Z,K) + P(Y,Z,K). \quad \square$$

As before, let $X$, $Y$ and $Z$ be the concentrations of the dynamic, quasi-equilibrium and interface metabolites respectively, at time $t$ — the start of the integration step. The goal is to derive the $Flow(\{X,Y,Z\},\{X',Y',Z'\},h,K)$ relation (in each discrete state[7] of the semi-algebraic hybrid automaton of the metabolic network) that expresses the algebraic values of the concentrations $X'$, $Y'$ and $Z'$ at time $t+h$ in terms of their concentrations at time $t$, the small time-step $h$ and the rate parameters $K$ (and time $t$, if required to capture

[7] The subscript $v$ denoting the discrete state and the explicit time variable $t$ are dropped for clarity from the $Flow_v$ notation.

some other external aspects of the dynamics). The flow equations of the dynamic reactants $X$ do not involve any simplification and are directly given by the Euler forward approximation as $X' = X + hF(X,Z,K)$. Thus,

$$\begin{aligned} Flow(\{X,Z,Y\},\{X',Z',Y'\},h,K) \;\equiv\; & \\ \{\; X' = X + hF(X,Z,K) \;\wedge\; Flow(\{Z,Y\},\{Z',Y'\},h,K). & \end{aligned}$$

Thus, the essence of the problem is the expression of $Flow(\{Z,Y\},\{Z',Y'\},h,K)$ — the flow of the quasi-equilibrium and interface reactants, *algebraically*. As a result of the way we have formulated the problem, the complete set of constraints, which must be true to achieve quasi-equilibrium are given by:

**Definition 3.** ***Quasi-Equilibrium Relation:***

$$\mathscr{E}(Z,Y,K) \equiv \{P(Z,Y,K) = 0 \;\wedge\; G(Y,Z,K) = 0\} \quad \square$$

GMA follows the straightforward approach of solving the quasi-equilibrium equations to obtain the exact *concentrations*. FBA instead guesses what the equilibrium *fluxes* must be by optimizing some function, without using the kinetic parameters $K$; the exact concentrations are then obtained by substituting the concentration terms for the flux variables. The algebraic versions of the two procedures and their mathematical details are further elaborated below.

### 3.1 General Mass Action based Approximation

Since the quasi-equilibrium characterization (see *Defn.* 3) involves only equalities, the relation $\mathscr{E}$ is effectively just a system of polynomial equations, which needs to be solved for $Z$ and $Y$. The issue of simultaneous solution of polynomial equations, especially in the context of biochemical networks, has been addressed before [4, 8, 36]. The well-established methods for solving such systems of simultaneous multivariate polynomial equations with symbolic parameters are to be found in the Gröbner Basis algorithm [5] and the Wu-Ritt characteristic set [47, 62] algorithm. Their many implemented forms include PoSSo [10], CoCoA [9] and Macaulay-2 [20].

In the case of metabolic dynamical systems, the system of polynomial equations can be solved more easily by exploiting the fact that the concentration of each chemical form of a metabolite at pseudo-equilibrium is dictated by the total concentration of its different chemical forms. In other words, each substrate of each reaction involving at least one interface metabolite ($Z$) also as a substrate, is associated with a mass conservation equation. As suggested in the literature [57, 33, 65], the total concentration of these substrate metabolites in their many chemical forms at equilibrium is captured using *equilibrium pool* variables $T$. The mass conservation equations $T = \mathscr{M}(Z,Y)$ have the form: $T_i = \Sigma_{j \in Pool_i} W_j$, where $Pool_i$ represents the set of the different chemical forms $W_j$, in which the $i$-th substrate metabolite exists. Effectively, as a result of the structure of metabolic pathways, the equilibrium concentrations of $Z$ and $Y$ are expressible in terms of the equilibrium pool concentrations $T$. The simplified GMA equilibrium relation may thus be expressed as:

**Definition 4.** ***GMA Equilibrium Relation:***

$$\mathscr{E}_{GMA}(Z,Y,T,K) \equiv \{Z = E_Z(T,K) \ \wedge \ Y = E_Y(T,K)\},$$

*where $E_Z$ and $E_Y$ represent the solutions obtained using the Gröbner basis technique over* $\{P(Z,Y,K) = 0 \ \wedge \ G(Y,Z,K) = 0 \ \wedge \ T = \mathscr{M}(Z,Y)\}$. $\square$

We are now ready to construct $Flow(\{X,Y,Z,T\},\{X',Y',Z',T'\},h,K)$ – the continuous flow expression, which connects the state of the system $\{X,Y,Z,T\}$ at time $t$ and the state of the system $\{X',Y',Z',T'\}$ at time $t+h$. During the quasi-instantaneous recomputation of the equilibrium point, the total concentrations of the pool variables $T$ can be assumed to remain unchanged. This assumption is justifiable because the time required for re-establishing the equilibrium is negligible compared to the time-step used for simulating the slow reactions. Consequently, the change in the concentrations attributed to the slow reactions is negligible compared to the effect of the equilibrium recomputation (which only redistributes the metabolites among the different chemical forms being added in each equilibrium pool variable). Thus, the ODEs for $Z$ and $Y$ can be directly approximated from $E_Z$ and $E_Y$ by differential calculus:

$$\dot{Z} \approx \frac{dE_Z(T,K)}{dt} = \frac{\partial E_Z(T,K)}{\partial T}.\frac{dT}{dt} = \frac{\partial E_Z}{\partial T}H,$$

where $\dot{T}_i = \Sigma_{j \in Pool_i}\dot{W}_j = H_i(X,Y,Z,K)$. The same applies to $Y$ as well. Thus we have our final result:

**Definition 5.** ***GMA-Approximated Metabolic Dynamics:***

$$\begin{aligned} Flow_{GMA}(\{X,Y,Z,T\},\{X',Y',Z',T'\},h,K) \ \equiv& \\ \{(X' = X + hF(X,Z,K)) \ \wedge \ (T' = T + hH(X,Y,Z,K) \ \wedge& \\ (Z' = Z + h\dot{Z}(T,X,Y,Z,K) \ \wedge \ (Y' = Y + h\dot{Y}(T,X,Y,Z,K))\},& \\ where: \dot{X} = F(X,Z,K) \quad , \quad \dot{T} = H(X,Y,Z,K),& \\ \dot{Z}(T,X,Y,Z,K) \approx \frac{\partial E_Z}{\partial T}H \ \ \& \ \ \dot{Y}(T,X,Y,Z,K) \approx \frac{\partial E_Y}{\partial T}H \quad \square& \end{aligned}$$

### 3.2 Flux Balance Analysis based Approximation

Flux Balance Analysis [28] aims to estimate the steady-state flux distribution using the stoichiometric matrix and the input and output fluxes of the system to constrain the solution space, without relying on any kinetic parameters. Since the number of fluxes is always greater than the number of metabolites, the system of linear flux equations is under-determined. FBA overcomes this hurdle by assuming that the biochemical network would have so evolved as to optimize certain physiologically important functions such as growth. Thus, the essence of flux balance analysis is optimizing a function under the set of equilibrium and other external constraints.

The general optimization (in its maximization formulation) problem can be rephrased as follows: *for all values $U$ that differ from the optimal value $\check{U}$ and still satisfy the constraints $\mathscr{C}(U,V)$ involving parametric variables $V$ (not being optimized), the value of*

*the function $\mathscr{F}(U,V)$ is, by definition, less than $\mathscr{F}(\check{U},V)$.* This step immediately leads to the following characterization of $\{\check{U},V\}$:

**Definition 6.** ***Optimization Relation:*** $Optimize(\check{U},\mathscr{F}(U,V),\mathscr{C}(U,V)) \equiv$
$\mathscr{C}(\check{U},V) \bigwedge \{\forall U,\ (U \neq \check{U}\ \wedge\ \mathscr{C}(U,V)) \Rightarrow (\mathscr{F}(U,V) < \mathscr{F}(\check{U},V))\ \}$ $\square$

If $\mathscr{C}$ is semi-algebraic and $\mathscr{F}$ is polynomial, then $Optimize(\check{U},\mathscr{F}(U,V),\mathscr{C}(U,V))$ is a quantified semi-algebraic set. Gröbner bases or characteristic sets cannot be used to solve this optimization problem as they can handle only equations and not inequality relations. Instead, the general technique of real quantifier elimination [55] has to be employed to perform the algebraic optimization [1]. In addition to quantifier elimination tools like Qepcad [22] and Redlog [16], specialized systems such as the Maple-based Symbolic-Numeric toolbox for Real Algebraic Constraints (SyNRAC) [63] could also be exploited for performing algebraic optimization.

Unlike the GMA-based approach which uses concentrations to describe the dynamics, FBA uses the *flux* variables: $U_j \equiv n_j k_j \Pi_i^j W_i$. For metabolic networks, the flux variables may be divided into $U_{zx} \equiv U_z \cup U_x \cup U_{z\wedge x}$ and $U_{zy} \equiv U_{z\wedge y} \cup U_y$ based on whether the reactions are fast or slow[8]: reactions in which only $Z_i$s and/or $X_i$s participate contribute the slow flux terms $U_{zx}$; reactions in which $Z_i$s and $Y_i$s interact and those in which only $Y_i$s interact contribute the fast flux terms $U_{zy}$. Thus the metabolic dynamics (see *Defn.* 2) may be rephrased as:

$$\dot{X} = F_U(U_{zx})\ ,\ \dot{Y} = G_U(U_{zy})\ ,\ \dot{Z} = D_U(U_{zx}) + P_U(U_{zy}).$$

Let $\mathscr{C}(U_{zy},U_{zx})$ represent the semi-algebraic constraints on the kinetic parameters, rates of change, bounds on parameters, energy balance equations, etc. Let $\mathscr{F}(U_{zy},U_{zx})$ represent the function that the metabolic network is assumed to be optimizing. Thus, the complete set of equations and inequalities that needs to be true at the equilibrium predicted by FBA may be represented thus:

**Definition 7.** ***FBA Equilibrium Relation:***

$$\mathscr{E}_{FBA}(\check{U}_{zy},U_{zx}) \equiv \{\ Optimize(\check{U}_{zy},\mathscr{F}(U_{zy},U_{zx}),\mathscr{C}(U_{zy},U_{zx})) \wedge G_U(\check{U}_{zy}) = P_U(\check{U}_{zy}) = 0\ \}. \quad \square$$

Consistent with the static optimization based dynamic flux balance analysis approach [33], it is assumed that at the beginning of each small time interval $h$, the fast reactions optimize growth (or some other physiological function) by re-establishing equilibrium ($U_{zy}$) based on the current concentrations of the fast and dynamic reactants ($U_{zx}$). The slow reactions are then integrated assuming that these fluxes stay constant over that time period $h$. Thus, the FBA-based dynamics can now be characterized algebraically as:

**Definition 8.** ***FBA-Approximated Metabolic Dynamics:***

$$Flow_{FBA}(\{X,Y,Z\},\{X',Y',Z'\},h,K) \equiv$$
$$\{\mathscr{E}_{FBA}(U_{z''y'},U_{zx})\ \wedge\ X' = X + hF(X,Z'',K)\ \wedge\ Z' = Z'' + hD(X,Z'',K)\}$$

*where* $U_i \equiv n_i k_i \Pi_j^i W_j$. $\square$

[8] Note that since $X$ and $Y$ do not interact, there are no $U_{x\wedge z}$ terms.

*Remark 1.* Alternatively, one could perform FBA using the concentration variables themselves. Let $\mathscr{C}(Z',Y',Z,Y,K)$ represent the semi-algebraic constraints on the kinetic parameters, rates of change, bounds on parameters, energy balance equations, etc. Let $\mathscr{O}(Z',Y',Z,Y)$ represent the function[9] that the metabolic network is assumed to be optimizing. Since FBA assumes that the kinetic parameters $K$ are unavailable, the effective set of constraints over which the optimization must be performed *may be* obtained by eliminating $K$ from the accurate equilibrium relation $\mathscr{E}$ (see *Defn.* 3). Note that if $K$ is not eliminated, the equilibrium is exactly defined by the relation $\mathscr{E}$; hence there is no room for optimization. Further, the existential quantifier captures the assumption that there exist some kinetic parameters (involved in the genetic variation, and discovered during evolution via natural selection) for which the network optimizes the physiologically relevant function (i.e., its "fitness" function). Thus, the dynamics may be approximated thus:

$$\begin{aligned}
&\mathscr{O}(Z',Y',Z'',Y) \equiv \exists K, \{\mathscr{C}(Z',Y',Z'',Y,K) \wedge \mathscr{E}(Z',Y',K)\},\\
&\mathscr{E}_{FBA}(Z'',Y,Z',Y') \equiv \mathit{Optimize}(\{Z',Y'\}, \mathscr{F}(Z',Y',Z'',Y), \mathscr{O}(Z',Y',Z'',Y)) \;\&\\
&\mathit{Flow}_{FBA}(\{X,Y,Z\},\{X',Y',Z'\},h,K) \equiv\\
&\{(X' = X + hF(X,Z,K)) \wedge (Z'' = Z + hD(X,Z,K)) \wedge \mathscr{E}_{FBA}(Z'',Y,Z',Y')\}.
\end{aligned}$$

The validity and utility of this approach need to be investigated further.

*Note 1.* In some cases, the solution after optimization and substitution with the concentration variables might be a set of polynomial equations, which can then be solved (by Gröbner basis like methods, say) to yield the general solution

$$\mathit{Flow}_{FBA}(\{Y,Z\},\{Y',Z'\},h,K) \equiv \{Z' = E_Z(Z,Y,T,K) \wedge Y' = E_Y(Z,Y,T,K)\}.$$

Then, we can write:

$$\dot{Z} = \frac{\partial E_Z}{\partial Z}\dot{Z} + \frac{\partial E_Z}{\partial Y}\dot{Y} + \frac{\partial E_Z}{\partial T}\dot{T} \;\;,\;\; \dot{Y} = \frac{\partial E_Y}{\partial Z}\dot{Z} + \frac{\partial E_Y}{\partial Y}\dot{Y} + \frac{\partial E_Y}{\partial T}\dot{T}.$$

By solving these two equations, one can obtain the general solution:

$$\dot{Y} = \frac{\frac{\frac{\partial E_Y}{\partial Z}\frac{\partial E_Z}{\partial T}}{1-\frac{\partial E_Z}{\partial Z}} + \frac{\partial E_Y}{\partial T}}{1 - \frac{\frac{\partial E_Y}{\partial Z}\frac{\partial E_Z}{\partial Y}}{1-\frac{\partial E_Z}{\partial Z}} - \frac{\partial E_Y}{\partial Y}}\dot{T} \;\;,\;\; \dot{Z} = \frac{\frac{\partial E_Z}{\partial Y}\dot{Y} + \frac{\partial E_Z}{\partial T}\dot{T}}{1 - \frac{\partial E_Z}{\partial Z}}.$$

Also note that $\{\dot{Z} = \frac{\partial E_Z}{\partial T}\dot{T} \;,\; \dot{Y} = \frac{\partial E_Y}{\partial T}\dot{T}\}$ derived in the GMA based approximation is just a special case where $\frac{\partial E}{\partial Y} = \frac{\partial E}{\partial Z} = 0$.

## 4 Example

Our approach is now illustrated on the *Example* 1 introduced earlier. Recall that $X = \{A,B,R\}$, $Y = \{E,C\}$ and $Z = \{S\}$.

[9] The primed variables may be necessary to capture relations involving the rate of change of concentrations.

### 4.1 GMA-Based Approximation

The only reaction with an interface metabolite as a substrate is $E + S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C$. The mass-conservation equations can be written for the two substrates $E$ and $S$ as $e_T = e + c$ and $s_T = s + c$, where $e_T$ and $s_T$ are the new equilibrium pool variables. At equilibrium, $\mathscr{E}(\{s\},\{e,c\},K) \equiv \{(k_f es - k_r c = 0)\}$, i.e., $k_f es = k_r c$. Rewriting in terms of the equilibrium pool variables, we get $k_f(e_T - c)(s_T - c) = k_r c$. Let $k = k_f/k_r$. In the general case, we would solve these equations using the Gröbner basis technique. Here, these quadratic equations can be solved directly, under the constraint that all concentrations are non-negative, leading to the solution:

$$\mathscr{E}_{GMA} \equiv \{c = \frac{(s_T + e_T + 1/k) - \sqrt{(s_T + e_T + 1/k)^2 - 4(s_T + e_T)}}{2} \ \wedge \\ e = e_T - c \ \wedge \ s = s_T - c\}.$$

Observe that $\dot{T}$ is: $\{\dot{s}_T = \dot{s} + \dot{c} = k_s ab, \ \dot{e}_T = \dot{e} + \dot{c} = 0\}$. Thus,

$$\hat{\dot{c}} = \frac{\partial c}{\partial T}\dot{T} = \frac{\partial c}{\partial s_T}\dot{s}_T = \frac{1}{2}(1 - \frac{2(s_T + e_T + 1/k) - 4}{2\sqrt{(s_T + e_T + 1/k)^2 - 4(s_T + e_T)}})k_s ab$$

$$\hat{\dot{e}} = -\dot{c} \ , \ \hat{\dot{s}} = k_s ab - \dot{c}. \ \textit{Thus, we get}:$$

$$\begin{aligned} Flow_{GMA} \quad & (\{\{a,b,r\},\{s\},\{e,c\},\{e_T,s_T\}\}, \\ & \{\{a',b',r'\},\{s'\},\{e',c'\},\{e'_T,s'_T\}\},h,k) \equiv \\ & \{(a' = a + h\dot{a}) \ \wedge \ (b' = b + h\dot{b}) \ \wedge \ (r' = r + h\dot{r}) \ \wedge \ (s' = s + h\hat{\dot{s}}) \ \wedge \\ & (e' = e + h\hat{\dot{e}}) \ \wedge \ (c' = c + h\hat{\dot{c}}) \wedge (e'_T = e_T + h\dot{e}_T) \wedge (s'_T = s_T + h\dot{s}_T)\}. \end{aligned}$$

### 4.2 FBA-Based Approximation

Observe that $U_{zx} = U_{z \wedge x} = \{U_1\}$ and $U_{zy} = U_{z \wedge y} = \{U_2, U_3\}$. Let $\mathscr{C}(\{U_2,U_3\},\{U_1\})$ represent the external constraints under which the network is assumed to be optimizing the function $\mathscr{F}(\{U_2,U_3\},\{U_1\})$. Thus, the equilibrium may be characterized as:

$$\begin{aligned} \mathscr{E}_{FBA}(\{\check{U}_2,\check{U}_3\},\{U_1\}) = \ & \mathscr{C}(\{\check{U}_2,\check{U}_3\},\{U_1\}) \ \wedge \\ & \{\forall U_2, U_3, \ (U_2 \neq \check{U}_2 \ \vee \ U_3 \neq \check{U}_3) \ \wedge \ \mathscr{C}(\{U_2,U_3\},\{U_1\}) \\ & \Rightarrow \ \mathscr{F}(\{U_2,U_3\},\{U_1\}) < \mathscr{F}(\{\check{U}_2,\check{U}_3\},\{U_1\})\} \\ & \wedge \check{U}_2 = \check{U}_3. \end{aligned}$$

This leads to the complete flow characterization:

$$\begin{aligned} Flow_{FBA} \ & (\{\{a,b,r\},\{s\},\{e,c\}\}, \\ & \{\{a',b',r'\},\{s'\},\{e',c'\}\},h,\{k_s,k_f,k_r\}) \ \equiv \\ & \{\mathscr{E}_{FBA}(\{U_2,U_3\},\{U_1\}) \ \wedge \\ & a' = a + h\dot{a} \ \wedge \ b' = b + h\dot{b} \ \wedge \ r' = r + h\dot{r} \ \wedge \ s' = s'' + h\dot{s}\}, \end{aligned}$$

where $U_1 = k_s ab$, $U_2 = k_f e' s''$, $U_3 = k_r c'$, $\dot{a} = \dot{b} = -\dot{r} = -k_s ab$, and $\dot{s} = k_s ab + k_r c' - k_f e' s''$.

## 5 Discussion

Several extensions of the mathematical theory [37, 15] are necessary for the approach to be more practical and useful. To improve computational complexity, it is necessary to develop more efficient, albeit less general, techniques for equilibrium estimation: for instance, applications of the Wu-Ritt characteristic set algorithm [34], resultant computation followed by eigen decomposition [60], and heuristics for choosing among them [45]. To reduce the computational overload due to the algebraic optimization involved in FBA, some less universal quantifier elimination approaches may be used [61, 26]. More recent efforts at efficient optimization include the following: constraint logic programming with first-order constraints $CLP(RL)$ [53] based on Redlog [16], systems theoretic algebraic optimization [25], and semidefinite programming [44].

In additional to the purely algebraic research described previously, several Systems Biology extensions and applications also necessitate further investigation. For instance, the relative merit of flux-based and concentration-based characterization of dynamics (see *Remark* 1) has to be further investigated, in terms of both the complexity gain and the repertoire of constraints that can be handled (such as Minimization of Metabolic Adjustment (MOMA) [50] and Regulatory On-Off Minimization (ROOM) [51]). Similarly, an integration with singular perturbation analysis-like methods [52, 19, 32] can potentially help automate the classification of the metabolic system interactions as fast and slow, and the decomposition into sub-modules of a large network. Other approximate methods to estimate the equilibrium fluxes (e.g., cybernetic modeling [43]) may also become more powerful when extended into the algebraic domain. Another important perspective comes from the mathematically rigorous approaches being developed in non-linear Control Theory [24, 48]. A related thorny problem that remains to be properly addressed is the semi-automatic (approximate) translation of a one-state biochemical dynamical system into a multi-state hybrid system.

In summary, we have exploited techniques from the AAMC approach to enable efficient analysis of metabolic networks. This paper shows how the numerical procedure for exploiting the inherent multi-time-scale quasi-equilibrium structure of metabolic networks could be extended to the algebraic domain, using techniques from Computational Real Algebraic Geometry: namely, real quantifier elimination, Gröbner bases, Wu-Ritt Characteristic sets, and algebraic optimization. Our approach is thus an algebraic generalization of numerical approaches, as typified by tendency modeling [57], dynamic flux balance analysis [33] and hybrid static + dynamic simulation [65]. The more general mathematical approaches [52, 19, 32] make fewer assumptions about the structure of metabolic networks, and can be incorporated into the proposed framework. Further, the paper provides a uniform algebraic framework to handle two distinct approaches for equilibrium estimation: (i) solving the general mass action-based polynomial equations and (ii) optimizing the flux distribution using flux balance analysis. Thus, the paper demonstrates how a standard biochemistry problem description can be automatically transformed into an entirely algebraic dynamical system specification. This algebraic framework can potentially elicit a powerful symbolic functional description of the dynamical behavior of the metabolic network, in terms of the quasi-equilibrium states of its fast reversible sub-networks. This algebraic approach is to be contrasted with the conventional analysis, which involves performing numerical integration of the ordinary

differential equations (ODEs), time-course data assimilation, visualization and model-checking of concentration-traces.

In conclusion, we note the success of Algorithmic Algebraic Model Checking project, which was initiated to integrate relevant theory in dynamical systems, model checking, hybrid automata and systems biology, in an effort to establish a sound and rigorous procedure for symbolic temporal reasoning over biochemical networks. While, in terms of building a suitable theoretical foundation, it has been successful, it has also pointed to newer theoretical and pragmatic problems that were unforeseen at the outset. One apparent shortcoming of our approach is its computational complexity; but it is hoped that this hurdle could be overcome, when the different avenues of extending these ideas are explored in the theoretical and practical realms.

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