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Simulation of Cellular Biochemical System Kinetics

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Abstract

The goal of realistically and reliably simulating the biochemical processes underlying cellular function is achievable through a systematic approach that makes use of the broadest possible amount of *in vitro* and *in vivo* data, and is consistent with all applicable physical chemical theory. Progress will be facilitated by establishing: (1.) a concrete self-consistent theoretical foundation for systems simulation; (2.) extensive and accurate databases of thermodynamic properties of biochemical reactions; (3.) parameterized and validated models of enzyme and transporter catalytic mechanisms that are consistent with physical chemical theoretical foundation; and (4.) software tools for integrating all of these concepts, data, and models into a cohesive representation of cellular biochemical systems. Ongoing initiatives are laying the groundwork for the broad-based community cooperation that will be necessary to pursue these elements of a strategic infrastructure for systems simulation on a large scale.

1. Challenges and Goals

Simulating molecular and biochemical function at the level of cells, tissues, and organs, at a level of detail that effectively approximates real biological function, requires a systematic approach to managing data and constructing models. The overall goal is to be able to capture biological function with enough physical and physiological realism that simulations not only match experimental data used to identify the underlying models, but also predict physiological and/or pathophysiological function outside of the operating regime of carefully controlled experiments. This level of model reliability is approachable only when models are constructed and parameterized based on the widest available set of physical chemical, biochemical, and physiological principles and data.

Synthesizing all such information (at least to the extent possible) in developing and analyzing models of biochemical systems is a tremendous challenge. Yet it is a challenge that can and ought to be met. Towards this aim, Figure 1 lays out an overall organizational approach to biochemical systems modeling. Within this framework, models are constructed to match multiple diverse datasets, including data on biochemical reaction thermodynamics, and kinetics at both the single-enzyme and integrated-systems levels.

The approach outlined in the figure, where enzyme and transporter component models are constructed to match independently obtained thermophysical data on reaction and transport processes, is of particular value in analyzing *in vitro* experiments on enzyme kinetics, which is a necessary first step in the development of simulations of how enzymes function *in vivo*. This is because *in vitro* kinetics experiments can rarely (if ever) be conducted under physiological cellular conditions. Yet it is not feasible to obtain enough *in vivo* data to identify systems models of the complexity necessary to simulate, for example, cellular metabolism. In practice, data from *in vitro* experiments are typically used to determine apparent mechanisms and associated parameter values for individual enzymes and transporters in a system. A handful of recent studies have analyzed *in vitro* data on enzymes to develop models that account for distributions of biochemical species and the associated

impact on kinetics {Beard, 2008 #824; Vinnakota, 2011 #1547; Qi, 2008 #1559; Chen, 2010 #1590} and more similar studies are underway.

Models of functional components (enzymes, transporters, etc.) that are constructed in this way can be seamlessly integrated into physicochemically rigorous large-scale systems models. The theory for integrating thermodynamically balanced models of biochemical systems components that account for biochemical state and species distributions is described in recent publications [5, 6]. A critical issue is that large-scale simulations account for biochemical species distributions, and consequently, dynamic buffering of metal and hydrogen ions. Doing this requires appropriate databases of thermodynamic properties and software for building models that appropriately use these data.

As an example of this approach, consider a series of studies on oxidative ATP synthesis in the heart [7-12]. In these studies dozens of differential equations were used to simulate the dynamics of hundreds of chemical species in a cardiac muscle cell. Specifically, these models account for the dynamically changing distribution of each biochemical reactant into a number of distinct hydrogen ion- and metal cation-bound states. This dynamic state distribution influences the thermodynamics and kinetics of the biochemical reactions [6]. Accounting for these details results in kinetic models of biochemical systems of unprecedented complexity, begging the questions of whether and why such complexity in modeling is justified. Indeed, simpler mathematical models of the same system can be constructed, as illustrated in Box 1. There, the feedback control of oxidative phosphorylation in the heart is analyzed based on a simple model that by no means captures all of the available information on the biochemical reactions and transport processes involved in this system.

The significantly more complex model of Wu *et al.* [10] explains the *in vivo* data shown in Box 1 no better than the simple model developed in the box. Thus it would seem that the simple model would be preferable. However neither the model of Wu *et al.* nor the simple model of Box 1 invokes adjustable parameters to explain the data in Figure B1.1—they are both parameterized based on independent data from *in vitro* experiments on isolated enzymes and isolated mitochondria. To be sure, the complex model of Wu *et al.* took significantly more effort to develop and is much more expensive to simulate. But the payoff is that it is more useful than the simpler model. The simpler model of Box 1 may represent the minimal model able to capture the phenomena for which it was constructed to capture. But it cannot help us to understand what happens in ischemia, or acidosis, or hyperglycemia, or any number of important conditions in which variables not explicitly invoked in the model are changed. Furthermore, it cannot be integrated with models of other biochemical pathways, such as glycolysis, fatty acid oxidation, or the tricarboxylic acid cycle, because it does not explicitly invoke metabolites that are the substrates and products for those pathways. The simpler model is, in other words, a one-off: a useful analysis that serves the single purpose for which it was constructed.

The pinnacle goal illustrated in Figure 1 is a class of models that are not one-offs, that synthesize all appropriate available data and mechanisms at a consistent level of detail, and that can be used as the foundation for systematically integrating and expanding our ability to reliably simulate cellular biochemical function.

2. *In Vitro* versus *In Vivo* Settings

An influential 2000 paper by Teusink *et al.* [13] asked the question “Can yeast glycolysis be understood in terms of *in vitro* kinetics of the constituent enzymes?” One purpose of the study was to determine if kinetic models and associated parameter values determined from *in vitro* studies using purified enzyme preparations can effectively represent the observed

function of a biochemical pathway *in vivo*. A major conclusion of the study was that, even when kinetic parameters are determined under consistent controlled conditions, values determined *in vitro* must be adjusted to match *in vivo* behavior. Indeed, it is common practice that *in vitro* enzyme assays are often designed to optimize the activity of the enzyme studied and/or the activity of a coupled assay used to measure reaction progress. This means that *in vitro* assays are typically conducted at temperatures, pH values, and under ionic conditions that are non-physiological. Since all of these factors can strongly influence biochemical kinetics and thermodynamics, models and parameter values determined *in vitro* cannot in general be directly applied to *in vivo* conditions.

However, with careful analysis it is sometimes possible to re-interpret *in vitro* experimental data to obtain appropriate models and parameter values for simulation of *in vivo* systems. The key is, to the extent that it is feasible, to account for all of the various factors that effect differences between *in vitro* and *in vivo* biochemical function. For example Juel [14] reports a value of 25 ± 6.1 mM for the apparent K_m for lactate for the mammalian monocarboxylate transporter MCT. To adopt this value in a systems model, we need to know to what species concentration is this K_m relative: unbound lactate ($C_3H_5O^-_3$), protonated lactate ($C_3H_6O^0_3$), some other species, or some particular sum of chemical species. Furthermore, ideally we would know the ionic strength of the solution in which the measurements were made so that our model could be made to account for ionic screening effects on chemical activity. In fact, a detailed reanalysis by Vinnakota and Beard {Vinnakota, 2011 #1547} of the original data by Juel reveals that the observed kinetic data are consistent with at least two catalytic mechanisms for the MCT, with a dissociation constant for unbound lactate of 40.1 mM for one putative mechanism and 46.6 mM for another.

The analysis of monocarboxylate transporter kinetics of Vinnakota and Beard is representative of a series of recent works aimed at developing a consistent set of models and associated parameter values for enzymes [1, 3, 4], metabolite transporters {Vinnakota, 2011 #1547} and ion transporters and channels [15-17]. These models, which account for the effects of ionic strength, pH, and ionic composition on biochemical kinetics and thermodynamics, represent a small-scale pilot effort to establish consensus models for biochemical “components” to be used in the model building process described in Figure 1. This collection of models can be found at the URL www.biocoda.org.

This small database of mechanistic models reveals a compelling trend: for the majority of the proteins studied the consensus mechanism was found to be inconsistent with the kinetic data used to establish the consensus mechanism. In other words, if we accept the data in the literature, we cannot accept the models of enzyme and transporter mechanisms in the literature. This is not to say that every established model/mechanism is not valid. Yet clearly enough are inadequate enough that it is prudent to systematically reanalyze legacy data, conduct new experiments where necessary, and establish a set of kinetic models for enzymes and transporters that obey a consistent set of physical principles, effectively match the available data, and account for physical phenomena responsible for the differences between *in vitro* and *in vivo* kinetics. Doing this we will move toward a resolution of Teusink *et al.*'s discrepancy between *in vitro* and *in vivo* biochemistry [13]. Efforts will be aided by the establishment of standards for conducting experiments under precisely defined (and carefully reported) conditions [18]. The detailed breakdown of the intracellular milieu has been extensively characterized for certain cell types (e.g., [19]). To be of most practical value, experiments on biochemical kinetics will be conducted under conditions that mimic this environment. Making a major step in this direction, van Eunen *et al.* [20] report standard buffer conditions designed to mimic the intracellular milieu of a specific yeast strain and assay the apparent maximal fluxes of the glycolytic enzymes under those conditions.

Clearly some, but perhaps not all, discrepancies between *in vitro* and *in vivo* behavior are addressed through this systematic approach. Although potential effects of macromolecular crowding, metabolite channeling, establishment of intracellular microdomains, and related phenomena may further complicate the situation, it is suggested that in attempting to explain *in vivo* function, one first apply a self-consistent simulation framework built on the principles of standard solution chemistry before invoking more complex hypothetical phenomena.

3. Strategic Infrastructure

Figure 1 illustrates an organizational approach to computer modeling of biochemical systems that builds on extensive sets of thermodynamic and kinetic data for individual reactions to establish a set of kinetic models for individual enzymes and transporters, which are to be used in integrated models of biochemical systems. Realization of the logical connections in Figure 1 requires a specific set of resources in the following areas: (1.) Biochemical Thermodynamics; (2.) Biochemical Kinetics; (3.) Database Development; and (4.) Software. Each of these areas represents a category of strategic infrastructure that must be built on and expanded to optimally meet the main challenges and goals outlined above.

3.1. Biochemical Thermodynamics

The kinetics of biochemical reactions—operating either in isolation or as components of integrated reaction systems—are highly sensitive to the thermodynamic properties of the reactions. Most obviously, the state of a reaction that is maintained near chemical equilibrium (as a significant fraction of biochemical reactions are maintained *in vivo*) is less sensitive to kinetic parameters (e.g., Michaelis-Menten constants and/or rate constants) than to the reference Gibbs free energy of the reaction, or equivalently, the equilibrium constant. In commonly conducted experiments, such as cell isolation, and in pathological situations, such as ischemia/hypoxia, even reactions that would not normally approach equilibrium may do so.

Furthermore, the kinetic behavior of a reaction that is maintained far from equilibrium is strongly influenced by the reaction's thermodynamic properties. This is because feasible values of the parameters associated with a given catalytic mechanism are mathematically constrained by the thermodynamic properties of the catalytic cycle [21]. Finally, every reaction has a non-integer effective proton stoichiometry that is fundamentally tied to the thermodynamics. Thus the pH dynamics, biochemical thermodynamics, and reaction kinetics are all mutually dependent in a reaction system.

For all of these reasons, physically realistic kinetics models must be built on a solid reliable foundation of thermodynamics [6]. Therefore, reliable databases of thermodynamic properties for biochemical reactions are essential.

The most extensive database of derived thermodynamic properties available for biochemical reactions, including the reactions of glycolysis, the tricarboxylic acid cycle (TCA), the purine nucleotide cycle, and a number of other pathways, was developed by R. A. Alberty [22-31]. Alberty's database was constructed based on an extensive set of experimental measures of thermodynamic properties of over 1000 biochemical reactions compiled by Robert Goldberg and colleagues reactions [31-38]. We recently calculated a database of reaction and reactant properties for the reactions of glycolysis and the tricarboxylic acid cycle (25 total reactions), based on the raw data in the Goldberg database [39]. This work provides the basic data (e.g., free energies of formation of reference species) to compute thermodynamic properties of these biochemical reactions at specified temperature, pH, ionic strength, and ionic composition. Although at present the database represents only a handful

of reactions, this work is a refinement to the Alberty database in that it accounts for the ionic strength and interactions of biochemical reactants and metal cations (Mg^{2+} , Ca^{2+} , Na^+ , and K^+) in estimating the derived properties. In addition, we are making the raw data and our documented estimations of solution properties freely available electronically. This free dissemination is crucial for facilitating future extension and improvements.

Like the Alberty database and other previous efforts, our initial efforts in this area provide a database representing only a fraction of the reactions accounted in the Goldberg dataset. For a more comprehensive database, the tremendous amount of work that goes into parsing the data published in a given study into a useable form will require a community effort. The need for a centralized resource to organize a broader community effort is highlighted by the fact that since the calculated formation properties are interrelated, the database of formation properties may be rigorously extended only by re-calculating the entire database using all of the raw data. Therefore optimal progress will depend on the establishment of a resource that allows current and future researchers worldwide to openly and constructively collaborate on expanding both the raw and derived biochemical data.

3.2. Biochemical Kinetics

Given a reliable thermodynamic model for a biochemical reaction, we have a foundation for developing a model for the mechanism of an enzyme that catalyzes that reaction, making use of the following calculations encapsulated in the thermodynamic model: the means to estimate the distribution of biochemical reactants into chemical species under specific conditions; and the means to estimate the reference free energy change $\Delta_r G^0$ associated with the turnover of the reaction.

For example, if we consider the citrate synthase reaction:

then a given reactant, such as citrate, exists in solution as a mixture of several species, such as citrate³⁻, H-citrate²⁻, and Mg-citrate¹⁻. The relative concentration of these species depends on ionic strength, temperature, pH, and ionic composition. Similarly the $\Delta_r G^0$ of a chemical reference reaction associated with a given biochemical reaction depends on ionic strength, temperature, and pH.

Kinetic modeling depends on the species distribution because the elementary steps in a given catalytic mechanism involve defined species. Thus an apparent affinity or an apparent Michaelis-Menten constant measured relative to total reactant concentration does not translate from one set of environmental conditions (e.g., that of an *in vitro* experiments) to another (e.g., physiological intracellular conditions). On the other hand, a dissociation constant measured relative to the chemical activity of a defined chemical species at a given temperature may be unambiguously translated between different environmental conditions. Thus, building kinetic models for enzymes and transporters that account for multiple ionic equilibria in complex solution is the appropriate vehicle for translating from *in vitro* to *in vitro* kinetics. Furthermore, as discussed in Section 3.1, kinetic parameters depend on thermodynamic parameters. Thus, a validated thermodynamic model of a reaction is the proper precursor for a kinetic model of a given enzyme or transporter.

We have conducted a series of studies of catalytic mechanisms at this level of detail for a handful of enzymes and transporters, including the following.

Enzymes: citrate synthase [1], isocitrate dehydrogenase [3], NADH-ubiquinone oxidoreductase (Complex I) [4].

Transporters: monocarboxylate transporters MCT1 and MCT4 {Vinnakota, 2011 #1547}.

In each of these cases we have elucidated and identified models that can simultaneously explain the available kinetic and thermodynamic data. Thus, the efforts to date have targeted components of cellular energy metabolism. Even with a relatively narrow focus, models of only a very small fraction of participating enzymes and transporters have been constructed. To obtain models for a reasonable fraction of mechanisms underlying cellular metabolism, including sub-models and/or specific parameter sets for species- and tissue-specific isoforms will necessarily require a broad community effort.

3.3. Database Development

Extant public databases of thermodynamic properties and enzyme kinetics for biochemistry provide the foundation for developing the community resources necessary for the proposed systematic approach to large-scale biochemical system analysis and modeling. A pair of NIST databases (Standard Reference Database 74: Thermodynamics of Enzyme-Catalyzed Reactions [31] and Standard Reference Database 46: Critically Selected Stability Constants of Metal Complexes [40]) are enormously useful in assembling published data for estimated thermodynamic properties. Two online resources—BRENDA (<http://www.brenda-enzymes.org/>) and SABIO-RK (<http://sabio.villa-bosch.de/>)—provide structured searchable databases of functions and published kinetics data associated with thousands of enzymes.

The NIST database Thermodynamics of Enzyme-Catalyzed Reactions, compiled over several decades by Robert Goldberg and colleagues, is a unique rich resource for biochemical thermodynamics. In essence, this database synthesizes thousands of publications reporting biochemical thermodynamic data, and critically evaluates the experimental and analytic methodology. Similarly, the NIST database Critically Selected Stability Constants of Metal Complexes synthesizes thousands of measurements of biochemical species-cation dissociation constants and dissociation enthalpies that are required to parameterize a biochemical thermodynamic model. What is needed is a systematic analysis that uses the information in these databases to provide a set of estimates of thermodynamic properties of chemical species that is optimally consistent with the data. This task is essentially what Li *et al.* [39] have done on a small scale for the reactions of glycolysis and the tricarboxylic acid cycle. The difficulty in expanding this effort to cover significantly more reactions lies in the fact that selecting and parsing the raw data into a form that may be used in computational analysis requires the effort of a human expert. For example, each raw data point (reaction equilibrium mass-action ration) used by Li *et al.* has associated with it the buffer temperature, pH, ionic strength, and free ion concentrations of Na⁺, K⁺, Mg²⁺, and Ca²⁺ for the reported experimental buffer conditions. This information is not in the NIST database. Nor is it typically reported in the original literature. Instead, this information must be gleaned from analyzing the reported buffer conditions on a case-by-case basis.

There exist (at least) two rich resources for searching for information on enzyme function and kinetics. BRENDA is an online database of manually curated functional data on enzymes [41]. The BRENDA site allows a user to search for information on biochemical reactions and enzymes in a number of different complementary ways. For example, searching for information on a specific enzyme, such as isocitrate dehydrogenase (NAD-dependent) yields a great deal of information, including enzyme classification, reaction details, links to the primary literature and structural data, and kinetic parameter estimates. If

one narrows the search to a specific species, say human, then we find reported values for the apparent Michaelis-Menten K_m value for isocitrate ranging from 0.32 to 41 mM. To determine which (if any) of these estimates are reasonable choices one must go to the primary literature. Indeed, a mechanistic kinetic model explaining the data on this particular enzyme includes 15 independently identifiable kinetic parameters [3]. It is unclear which (if any) parameter names in the database correspond to which parameters in the model. Similarly, while the SABIO-RK database [42] has the ambitious design goal of being able to automatically generate computer models by integrating reactions and associated kinetic data, the underlying data is basically the same as what is included in the BRENDA database. The example of this particular parameter value, and potential sources for the wide variety of reported values, are treated in more detail in Box 2.

The lesson is that in databases such as BRENDA and SABIO-RK each unique piece of information is associated with a unique context. There is no rationale for directly adopting models and parameter values from these databases for systems models. Yet these enzyme/reaction resources provide pointers into the literature on enzyme kinetics, and in some cases reveal the consensus mechanism and apparent kinetic parameters that can be useful starting places in model building. While they serve as valuable portals for enzyme kinetics, neither of these resources provides models of catalytic mechanisms that can be integrated into the large-scale simulation and analysis framework described herein.

Thus we have only begun to develop the large-scale databases in thermodynamics and kinetics necessary for the stated goal of realistically and reliably simulating the biochemical processes underlying cellular function. Furthermore, while data on intracellular (and intracompartmental) metabolite concentrations *in vivo* are typically more difficult to obtain and thus much more sparsely available than thermodynamic and enzyme kinetic data, they are necessary for identification, validation, and application of large-scale models. The computed thermodynamic database described in Section 3.1 (and all of the raw data on which it is based) is available online [39], as is a set of simple descriptions of the enzymes and transporters listed in Section 3.2. A current goal is to progress beyond these small-scale initiatives by establishing broader community-driven resources.

3.4. Software Development

Clearly software is needed for efficiently managing data and associated models and for opening up the proposed initiatives in physically sound biochemical systems analysis to input and application by the interested community. Toward this aim, we have developed a freely distributed package for biochemical systems modeling called Biochemical Simulation Environment (BISEN) that allows users to generate models from our small-scale thermodynamic and kinetic databases. The package, along with the most current versions of the databases and a users' manual is freely available. (See [43] or contact the author for details.) Drawbacks of the BISEN package include the facts that it operates in the MATLAB environment and that it is not integrated with the community standards for cell systems modeling, limiting the portability and flexible use of the package.

Addressing these shortcomings, by linking our tools with community standards for model representation and open-source tools for cellular system simulation [44-46] is an active area of development.

Conclusions and Outlook

The field of biochemical systems analysis sits on vast and largely untapped resources of kinetic and thermodynamic data on biochemical reactions. In particular, for hundreds (if not thousands) of metabolic reactions, data on reaction thermodynamics and kinetics of multiple

enzyme isoforms exist in the literature. The reason these resources are underutilized is that, as a rule, the legacy data require expert reanalysis using a unifying consistent set of theoretical principles and a common logical framework. Relatively narrowly focused pilot studies described above have demonstrated the feasibility and utility of the approach advocated for here.

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Box 1**Feedback control of oxidative phosphorylation in the heart**

Adenosine triphosphate (ATP) is consumed in a cardiac muscle cell via a variety of essential processes, all resulting in the hydrolysis of ATP to form adenosine diphosphate (ADP) and inorganic phosphate (P_i):

In the heart ATP is synthesized from ADP and P_i primarily via oxidative phosphorylation in mitochondria. In addition, concentrations of ATP and ADP in the cytosol are influenced by two reactions that are maintained at or near chemical equilibrium under normal conditions:

(Here the following abbreviations are employed: Cr, creatine; CrP, creatine phosphate, and AMP, adenosine monophosphate.)

By providing the capacity to synthesize ATP from CrP and ADP, the creatine phosphate system provides an effective buffer against rapid changes in ATP concentration [11]. However under steady-state conditions, where the rate of ATP synthesis must equal the rate of ATP hydrolysis, there is no net turnover of either the creatine kinase or the adenylate kinase reaction. Experimental data on *in vivo* steady-state $[\text{CrP}]/[\text{ATP}]$ and $[P_i]/[\text{CrP}]$ ratios as functions of cardiac oxygen consumption (MVO_2) are plotted in Figure B1.1. As the cardiac work rate increases (and MVO_2 increases), the ratio $[\text{CrP}]/[\text{ATP}]$ decreases slightly while the ratio $[P_i]/[\text{CrP}]$ increases significantly.

The control of metabolite levels in this system may be understood based on a simple analysis as follows. First, conserved pools of adenine nucleotides, creatine, and exchangeable phosphate in the cytoplasm are defined:

$$\begin{aligned} a_1 + a_2 + a_3 &= A_o \\ c_0 + c_1 &= C_o \\ (1 + \gamma) p + a_2 + 2a_3 + c_1 &= P_o, \end{aligned} \quad \begin{array}{l} \text{(B.} \\ \text{1)} \end{array}$$

where we use the definitions $a_1 = [\text{AMP}]$, $a_2 = [\text{ADP}]$, $a_3 = [\text{ATP}]$, $c_0 = [\text{Cr}]$, $c_1 = [\text{CrP}]$, and $p = [P_i]$, and A_o and C_o are the total cytoplasmic adenine nucleotide and creatine pools, set here to physiologically reasonable values of 10 mM and 40 mM, respectively. The equation for the phosphate pool P_o is obtained from the assumption that inorganic phosphate, which is transported across the mitochondrial inner membrane, is in approximately equal concentration in the cytosolic and mitochondrial water space. The factor γ is, therefore, the ratio of mitochondrial water space to cytosolic (non-mitochondrial cytoplasmic) water space, set here to 0.5, a reasonable value for cardiac muscle cells.

In addition to the conservation equations, we have equilibrium ratios that are maintained by the adenylate kinase and creatine kinase enzymes:

$$\begin{aligned}\frac{a_1 a_3}{a_2^2} &= R_{AK} \approx 2 \\ \frac{a_3 c_0}{a_2 c_1} &= R_{CK} \approx 166\end{aligned}\quad (\text{B. } 2)$$

Together Equations (B.1) and (B.2) give five equations for six unknown concentrations in terms of the conserved pool concentrations. Thus there is one degree of freedom, which is set by the interaction between the rate of ATP synthesis (in the heart mainly in mitochondria) and the rate of ATP hydrolysis.

We may further simplify this system with a few additional approximations. First, we note that a_3 remains essentially constant in response to physiological changes in work rate and rate of oxygen consumption, and that c_1 decreases from approximately 23.5 mM at resting levels of ATP consumption to approximately 20 mM at near-maximal levels of ATP consumption in the heart [10]. Given this range, we can compute the range of ADP concentration to be approximately 42 μM (at rest) to 60 μM (at maximal ATP consumption). Introducing the superscripts 'R' and 'M' to denote rest and maximal work rate, we compute (holding a_3 constant): $c_1^R = 23.5$ mM, $c_1^M = 20$ mM, $a_1^R = 0.36$ μM , $a_1^M = 0.73$ μM , $a_2^R = 42$ μM , and $a_2^M = 60$ μM . These values yield $a_3^R - a_3^M \approx 18$ μM , validating the assumption that a_3 is effectively constant in the equilibrium ratios (B.2).

From these values we can compute the change in cytosolic inorganic phosphate concentration (from rest to maximal work) to be

$$\Delta p = p^M - p^R = (1+\gamma)^{-1} \left[2(a_2^R - a_2^M) + a_2^R - a_2^M + c_1^R - c_1^M \right] \approx 2.3 \text{ mM}, \quad (\text{B. } 3)$$

which is reasonably close to the value of $\Delta p = 2.0$ mM estimated by analysis based on a detailed kinetics model [10]. Using the value of $p^R = 0.29$ mM from Wu *et al.* [10], we have $p^M = 2.59$ μM .

From the conservation and equilibrium expressions, we can find approximate expressions for a_1 , a_3 , c_1 , and p as functions of a_2 :

$$\begin{aligned}a_1(a_2) &= R_{AK} \frac{a_2^2}{a_3} \approx R_{AK} \frac{a_2^2}{A_o} \\ a_3(a_2) &= A_o - a_1(a_2) - a_2 \\ c_1(a_2) &= \frac{C_o}{1 + K_{CK} a_2 / a_3} \approx \frac{C_o}{1 + K_{CK} a_2 / A_o} \\ p(a_2) &= (1+\gamma)^{-1} [P_0 - a_2 - 2a_3(a_2) - c_1(a_2)]\end{aligned}\quad (\text{B. } 4)$$

where $a_2 \in [a_2^R, a_2^M]$ defines the observed physiological range. For metabolic flux, we may use the simple two-parameter model for P_i^- and ADP-driven control of the rate of oxidative phosphorylation introduced by Katz *et al.* [53]:

$$W(a_2, p) = W_{\max} \frac{a_2 p / (K_{a2} K_p)}{1 + a_2 / K_{a2} + p / K_p + a_2 p / (K_{a2} K_p)}, \quad (\text{B. } 5)$$

where K_{a2} and K_p are the effective half-activation constants and $W_{\max}$ is the maximal rate. Using the values of $K_{a2} = 20$ μM and $K_p = 0.8$ mM obtained from *in vitro* experiments [54, 55], varying a_2 over the estimated physiological range for heart, applying Equation (B.4) to estimate $p(a_2)$, Equation (B.5) predicts the curves plotted in Figure B1.2. Thus this feedback-control model explains the control of the rate of oxidative ATP synthesis in the heart [10, 56].

Box 2**The difference between apparent and actual kinetic parameters**

Consider the example described in Section 3.3 where reported values for the K_m value for isocitrate for NAD-dependent isocitrate dehydrogenase range from 0.32 to 41 mM. Qi *et al.* [3] identify a model for this enzyme with the following expression for the forward steady-state flux in the absence of products

$$J = \frac{\frac{V_{mf}}{K_{iA} K_{mB}} [A] [B]}{1 + \frac{[A]}{K_{iA}} + \frac{K_{mA} [B]}{K_{iA} K_{mB}} + \frac{[A][B]}{K_{iA} K_{mB}}}, \quad (\text{B. } 1)$$

where A and B are the species NAD^- and MgICIT^- , respectively. Qi *et al.* estimate a value of 149 μM for K_{mB} , which is measured relative to the species concentration $[\text{MgICIT}^-]$, not the total reactant concentration $[\Sigma\text{ICIT}]$. In the presence of magnesium ion and in the absence of other metal cations, species and reactant concentrations are related [3]

$$[\text{MgICIT}^-] = [\Sigma\text{ICIT}] \frac{[\text{Mg}^{2+}] / K_{Mg}}{1 + \frac{[\text{H}^+]}{K_{H1}} + \frac{[\text{H}^+]^2}{K_{H1} K_{H2}} + \frac{[\text{Mg}^{2+}]}{K_{Mg}}}, \quad (\text{B. } 2)$$

where K_{H1} , K_{H2} , and K_{Mg} , are dissociation constants, $[\text{Mg}^{2+}]$ is the free magnesium ion concentration, and $[\text{H}^+]$ is the hydrogen ion activity. Defining an *apparent* Michaelis-Menten constant measured relative to $[\Sigma\text{ICIT}]$ such that $[\text{MgICIT}^-] / K_{mB} = [\Sigma\text{ICIT}] / K'_{mB}$, we obtain

$$K'_{mB} = K_{mB} \frac{1 + \frac{[\text{H}^+]}{K_{H1}} + \frac{[\text{H}^+]^2}{K_{H1} K_{H2}} + \frac{[\text{Mg}^{2+}]}{K_{Mg}}}{[\text{Mg}^{2+}] / K_{Mg}}. \quad (\text{B. } 3)$$

Figure B2.1 illustrates how the apparent kinetic parameter varies at fixed pH over a range of $[\text{Mg}^{2+}]$. We can see how it is possible to have a range in reported values of two orders of magnitude due to differences in experimental buffers.

Figure 1.

Strategic approach to biochemical systems modeling and analysis. Models are constructed based on three major data sets: biochemical thermodynamics, kinetics of enzymes *in vitro*, and systems-level data, such as from intact organelles, cells, tissues, and/or organs.

Thermodynamic data are used to construct databases of thermodynamic properties; enzyme kinetic data and thermodynamic property data are used to develop models of individual enzyme and transporter mechanisms. Individual enzyme and transporter models are integrated together to build systems-level models that are parameterized and validated using systems-level data.

Figure B1.1.

Phosphate metabolite levels in the heart *in vivo*. A: Measured ratio of [CrP]/[ATP] plotted as a function MVO₂. Measured ratio of [P_i]/[CrP] plotted as a function MVO₂. Data are adapted from: $\bigcirc$, Zhang *et al.* [47]; $\triangleleft$, Zhang *et al.* [48]; $\diamond$, Gong *et al.* [49]; $\triangle$, Ochiai *et al.* [50]; ∇ , Gong *et al.* [51]; $\square$, Bache *et al.* [52]. (Shaded data points indicate situation when P_i is below limit of detection.)

Figure B1.2.

Feedback control of oxidative phosphorylation in the heart. The rate of mitochondrial synthesis is physiologically activated by products of ATP hydrolysis, of which P_i is the most important controller, as illustrated in A. Panels B and C shows comparison of predictions of Equation (B.5) with data obtained from ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS). Data in B and C are adapted from [47-52]. Shaded data points indicate situation when P_i is below limit of detection. Model predictions use $W_{\max} = 5.14 \cdot W^R$. The factor α (set to 1.12) in panel B accounts for the fact that that ATP signal measured from ^{31}P -MRS arises from cytosolic plus mitochondrial ATP, while CrP is confined to the cytosol.

Figure B2.1.

Dependence of the apparent Michaelis-Menten constant for isocitrate for NAD-dependent isocitrate dehydrogenase on free Mg^{2+} concentration. Values assumed for dissociation constants are $K_{H1} = 2.29 \mu\text{M}$, $K_{H2} = 46.8 \mu\text{M}$, and $K_{Mg} = 1.91 \text{ mM}$; $[H^+] = 10^{-7} \text{ M}$.