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# Design of Experiments for Identification of Complex Biochemical Systems with Applications to Mitochondrial Bioenergetics

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*Abstract*— **Identification of a complex biochemical system model requires appropriate experimental data. Models constructed on the basis of data from the literature often contain parameters that are not identifiable with high sensitivity and therefore require additional experimental data to identify those parameters. Here we report the application of a local sensitivity analysis to design experiments that will improve the identifiability of previously unidentifiable model parameters in a model of mitochondrial oxidative phosphorylation and tricaboxylic acid cycle. Experiments were designed based on measurable biochemical reactants in a dilute suspension of purified cardiac mitochondria with experimentally feasible perturbations to this system. Experimental perturbations and variables yielding the most number of parameters above a 5% sensitivity level are presented and discussed.**

## I. INTRODUCTION

### A. Parameter identification in complex biochemical systems

A key problem in Systems Biology is the construction of a computational model that is parameterized for the biological system being studied. Ideally, a computational model should be parameterized for the same biochemical system that is being investigated experimentally in order to generate predictions for valid hypothesis testing [4]. However, it is often not possible to experimentally characterize the kinetics of every component of a biochemical system generally composed of many enzymes and transporters organized in various subcellular compartments. Therefore, extant models of biochemical pathways in the literature derive their model components and parameters from previously published data in the literature that may differ in experimental conditions and/or in biological species. This is a necessary step in generating a first quantitative working hypothesis of the system in question. In the present study we outline strategies for designing experiments for identification of model parameters of a specific biochemical system when a working computational model with an initial estimate of parameters is available for this system.

### B. Strategies for experiment design based on parameter sensitivities

Optimal design of experiments for system identification consists of two essential stages: 1) design of measurable experimental transients for model identification, and 2) selection of a sampling schedule for minimizing the variances of the estimated parameters [2]. The present study is focused on the former aspect of model-based experimental design. We employ local sensitivity functions as a practical metric for parameter identifiability [3]. Measurable state variables must be selected based on practical considerations for computing local sensitivities with respect to model parameters. In the current work we define the local sensitivity using a central difference formula:

$$S_{i,j} = \frac{0.5\langle|C_i(P_j - 0.1P_j, t_k) - C_i(P_j, t_k)|\rangle}{0.1\langle C_i(P_j, t_k)\rangle} + \frac{0.5\langle|C_i(P_j + 0.1P_j, t_k) - C_i(P_j, t_k)|\rangle}{0.1\langle C_i(P_j, t_k)\rangle}, \quad (1)$$

where $S_{i,j}$ is a normalized local sensitivity, $C_i$ is a state variable, $P_j$ is a parameter, $t_k$ is the $k^{th}$ time-step, and $\langle\cdot\rangle$ denotes averaging over time.

Finally, we employ the following strategies for designing an experimentally measurable time course of selected model state variables:

1) Compute local sensitivities based on existing parameter values.
2) Compute local sensitivities under different initial conditions for the concentration state variables and total metabolite pools.
3) Identify perturbations for a single experiment type that can enhance sensitivities of measurable state variables to parameters.
4) Identify combinations of experiments that can enhance sensitivities of measurable state variables to parameters.

### C. Model based experiment design for mitochondrial bioenergetics

The application presented in this study pertains to mitochondrial oxidative phosphorylation and tricarboxylic acid (TCA) cycle. We used a previously published model of these pathways by Wu *et al.* [8] with the goal of improving normalized sensitivities listed in Table 3 in [8]. This model has been parameterized using transient and steady state data from experiments on purified rat and porcine cardiac mitochondria. The *in vitro* model was also applied to *in vivo* data [7], [9] with additional cytosolic enzymes creatine kinase, ATPase, and adenylate kinase for validating the role of a key physiological feedback control signal from inorganic phosphate in regulating cardiac energetics.

In the study of Wu *et al.* [8], sensitivity was defined as the relative change in the sum of squares of the residuals

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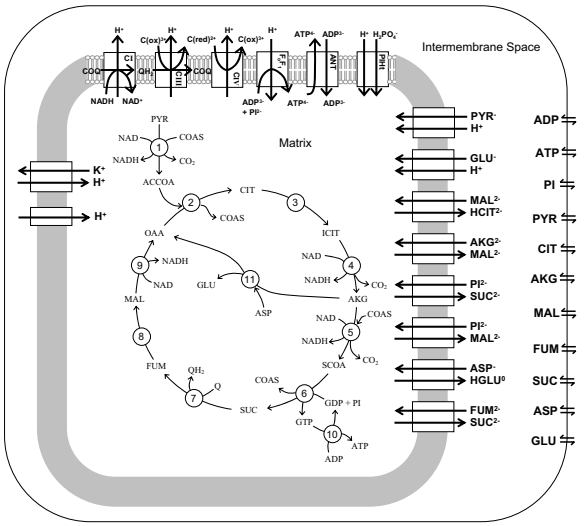


Fig. 1. Schematic of the Wu et al. [8] model for mitochondrial oxidative phosphorylation, TCA cycle, and electrophysiology. The biochemical reactants that are transported into the buffer space constitute the measurable state variables (ATP, ADP, Pi, MAL, GLU, AKG, FUM, ASP, SUC, PYR, CIT) in addition to NADH autofluorescence and dye based measurements of mitochondrial membrane potential. MAL, GLU, AKG, FUM, ASP, SUC, PYR, CIT are the seven subsrates for experiement design. The adjustable parameters are 43 enzyme and transporter activities and two kinetic regulatory parameters. Abbreviations used are explained in Wu *et al.* [8]

with respect to a 10% change in a single parameter value from the set of optimized adjustable model parameters. In order to identify the model for our experimental system, we designed experiments based on the transition of mitochondria from a resting state (State 2) to maximal stimulation using ADP (State 3) and a return to another resting state upon consumption of ADP (State 4).

## II. METHODS

A previously published computational model of mitochondrial oxidative phosphorylation, TCA cycle, and electrophysiology [8] was used to simulate experiments and compute parameter sensitivities. Model compilation and simulation was performed in a Matlab (Mathworks Inc., Natick, MA) based simulation environment BISEN [6]. A schematic representation of the Wu *et al.* [8] model applied to our experiments is illustrated in Fig. 1.

Biochemical reactants transported to the buffer space from the intermembrane space constitute the measurable state variable list in a dilute suspension of purified mitochondria, in addition to the variables that can be measured by fluorescence-based techniques, such as NAD(P)H and mitochondrial membrane potential. The experiment considered is a State 2-3-4 transition using various mitochondrial substrates under different inorganic phosphate (Pi) concentrations. Sensitivity analysis was performed by perturbing each parameter by 10% and computing the normalized local sensitivity of measurable state variables using Eq. 1. The sensitivity calculations of measurable state variables with respect to adjustable model parameters were performed for experiments with all possible dual TCA cycle substrate inputs, at different initial Pi concentrations. Specifically, the initial phosphate pool was set at high (2.5 mM), intermediate (0.5 mM), and limiting (0.1 mM) concentrations to generate data that is sufficiently sensitive to the phosphate-dependent biochemical feedback to identify related aspects of the model. For each experiment we listed all state variables with greater than 5% sensitivity to the adjustable parameters. Experiments with the highest number of such sensitivities were chosen and analyzed for implementation.

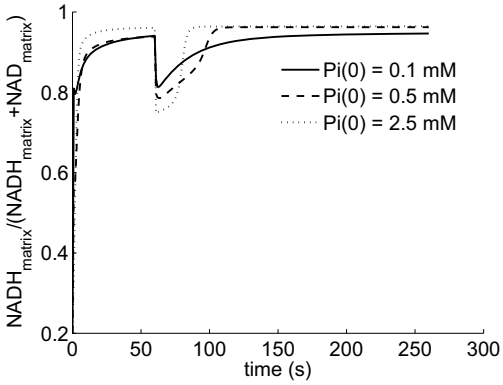


Fig. 2. Time courses of matrix NADH/(NADH+NAD) during a State-2-3-4 transition with 10 mM pyruvate and 10 mM malate as substrates at three different initial Pi concentrations. Substrates are added at 0 s followed by the addition of ADP at 60s.

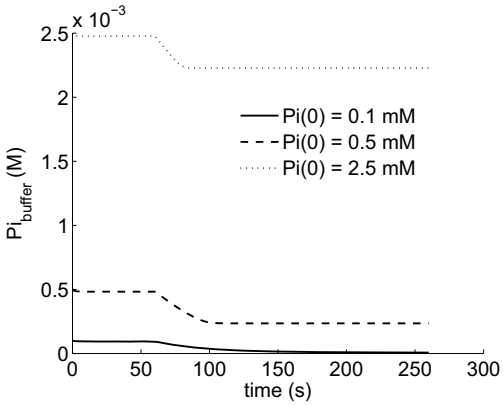


Fig. 3. Time courses of buffer phosphate concentration during a State-2-3-4 transition with 10mM pyruvate and 10 mM malate as substrates at three different initial Pi concentrations. Substrates are added at 0 s followed by the addition of ADP at 60s.

## III. RESULTS

Fig. 2 and Fig. 3 show the time courses of NADH/(NADH+NAD) in the matrix and inorganic phosphate (Pi) in the buffer during a State-2-3-4 transition at different initial buffer Pi concentrations using pyruvate and malate as the substrates. Experiments with varying initial buffer Pi concentrations were designed to estimate the sensitivities of the measured state variables to the phosphate control parameters in the Wu *et al.* [8] model.

Variation in initial buffer phosphate concentrations produces distinct changes in the time courses of the measurable state variables. Phopshorylation of 250 $\mu$M of ADP during the course of these experiments decreases the buffer phosphate concentration by the same amount as shown in Fig. 3.

The total number of experiments computed from two substrate and single substrate incubations of seven TCA cycle substrates and three different initial phosphate concentrations is 84. The number of measurable state variables is 13 and the number of adjustable parameters is 45, resulting in a total of 585 sensitivities for each experiment (see Fig. 1). Sensitivity values greater than 5% were enumerated for each of the 84 experiments defined by substrate combination and initial phosphate concentration. Analysis of sensitivity computation for all 84 experiments showed that 28 parameters are associated with sensitivities above 5%. The top five parameters associated with highest number of sensitivity values greater than 5% are the activity of proton leak, activity of Complex III, Pi sensitivity parameter of Complex III, activities of adenine nucleotide translocator and succinate dehydrogenase, which are indeed key system parameters.

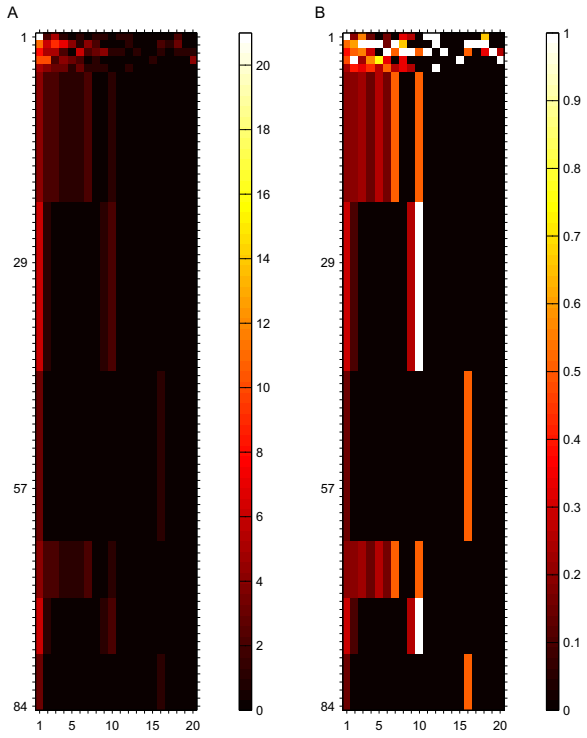


Fig. 4. Interval distribution of number of sensitivity functions above 5% in 84 experiments. In both panels the first 19 columns denote intervals from 5% to 100% in increments of 5% and 20th the column represents the interval for sensitivity greater than 100%. The 84 rows correspond to possible experiment designs at three levels of initial Pi concentration where, rows 1-28 represent experiments with 0.1 mM initial Pi; rows 29-56 represent experiments with 0.5 mM initial Pi; rows 57-84 represent experiments with 2.5 mM initial Pi. A) Number of sensitivity functions in each interval B) Number of sensitivity functions in each interval normalized to the highest number in that interval (column normalized)

A summary of the number of sensitvity functions greater than 5% is presented in Fig. 4A and Fig. 4B on a hot colormap. The first 19 columns of Fig. 4 represent intervals ranging from 5%-100% in increments of 5% and the 20th column represents the interval for sensitivity greater than 100%. The rows represent the 84 experiments, and number of sensitivity functions in each interval are mapped to a hot colormap, with a columnwise normalization in panel B. These figures indicate that the highest number of sensitivity functions above 5% are clustered in experiments 1-5. These are experiments with aspartate-glutamate(51 values), aspartate-$\alpha$-ketoglutarate (42 values), aspartate-malate (37 values), aspartate-citrate (36 values), and aspartate-pyruvate (23 values) as the substrate pairs at the lowest concentration (0.1 mM) of initial phosphate used in our analysis. All other experiments yield significantly fewer sensitivity functions (4-14 values) above 5%. Fig. 5 shows the sensitivity values for these five experiments. Only 6.5% of all sensitivity values are above 5%.

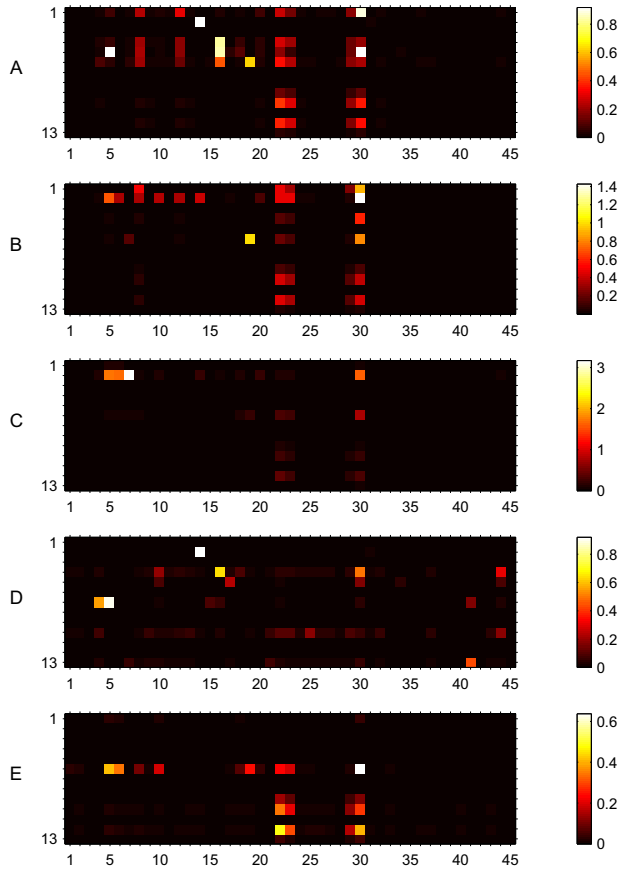


Fig. 5. Sensitivity values of measurable state variables in the 5 experiments with the highest number of sensitivity values above 5% i.e. 0.05. Panels A to E represent the sensitivity matrices corresponding to experiments 1-5 in Fig. 4. In each panel, the abscissa shows the parameter index and the ordinate shows measurable state variable indices.

Measurement of all 13 state variables is not practical in most situations, therefore we identified a subset of experiment-state variable combinations that yielded the most number of sensitivity values. We define measurement of NADH autofluorescence, ATP, ADP, Pi and two of the TCA cycle intermediates by enzymatic analysis as a feasible subset

of measurements for a given experiment i.e., 6 out of the 13 state variables. Based on the preceding definition of feasible measurements, Aspartate-citrate incubation with measurement of malate, glutamate, NADH, ATP, ADP and Pi yields the highhest number of sensitivities (36 values). In summary, only a small subset experiment-measurement combinations among the large number of possible experiments can provide good parameter sensitivity based on the present model.

## IV. DISCUSSION AND FUTURE WORK

The present study demonstrates the application of a combination of sensitivity analysis with experimental perturbations guided by prior knowledge about the system, to help select a subset of measurable state variables for parameter identification. The normalized sensitivity function is generally defined as the partial derivative of log of state variable with respect to log of a parameter. In the present study we defined an approximate sensitivity in Eq 1, in order to obtain a single number that could be used as a measure of sensitivity. Thomaseth and Cobelli [5] have derived generalized sensitivity functions that also account for the influence of model sensitivities with respect state variables, which could be useful in choosing a sampling schedule for acquiring chemical measurements of reactant time courses.

The present study was guided by prior knowledge on model parameters and physiological regulation revealed in multiple systems using the current model. The sensitivity analyses must be interpreted with the understanding that only a narrow range of parameter space was probed by perturbing one parameter at a time [3]. In the absence of a prior point estimate of the parameters from any source, experiments must be conducted to obtain an initial estimate. The initial estimate may be evaluated for precision and sensitivity, which may lead to further design upon detection of insufficiencies. These steps constitute an iterative process for experiment design to obtain reliable parameter estimates in a complex biochemical system model.

Future work will consist of locally alphabet-optimal designs computed based on the selected experiment(s) and initial point estimate of the parameters [1], and generalized sensitivity functions [5].

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