Supplementary Material to

Reduced-Order Modeling of Biochemical Networks: Application to the GTPase-Cycle Signaling Module

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Mano Ram Maurya[*], Scott J. Bornheimer[†], Venkat Venkatasubramanian[‡] and Shankar Subramaniam[*, †, ¶]

[*]*San Diego Supercomputer Center, 9500 Gilman Drive MC 0505, La Jolla CA 92093*

[†]*Departments of Chemistry & Biochemistry and Cellular & Molecular Medicine*
University of California, San Diego, 9500 Gilman Dr La Jolla, CA 92093

[‡]*Laboratory for Intelligent Process Systems, School of Chemical Engineering,*
Purdue University, West Lafayette, IN 47907, USA

[¶]*Department of Bioengineering*
University of California, San Diego, 9500 Gilman Dr La Jolla, CA 92093

E-mail: mano@sdsc.edu, sbornheimer@ucsd.edu, venkat@ecn.purdue.edu, shankar@ucsd.edu

Corresponding Author:

Shankar Subramaniam
Department of Bioengineering
University of California San Diego
9500 Gilman Drive
La Jolla, CA 92093
Phone: (858) 822-0986
Fax: (858) 822-5722
Email: shankar@ucsd.edu

1 Description of the GTPase-cycle module

Bornheimer et al. [1] have constructed a computational model of the GTPase-cycle module that is based on experimental data and a detailed biochemical reaction scheme (Fig. 3 of the main article).

The detailed model consists of 17 ordinary differential equations (ODEs) and 48 parameters. There are 24 reversible reactions, named as R1, R2, etc., in the network. For the reversible reaction A1, the association and dissociation rate constants are denoted by A+1 and A-1, respectively, and the equilibrium constant (i.e., ratio of the rate constant of the forward reaction to that of reverse reaction) is denoted by 1/A1 (other reactions also follow this convention). Note that some forward reactions are association reaction (such as A+i, R+i, T+i where i = 1, 2, 3, etc.) while some other forward reactions are dissociation reactions (such as P-i, D-i). The unit of the rate constant for association reactions is M^{-1} s^{-1} and that for dissociation reactions is s^{-1}. The ordinary differential equations (ODEs) are derived by applying mass-balance to various species in the network (the 12 shown nodes in Fig. 3 and R, A, T, D and P). For example,

$$\begin{aligned} d[G]/dt = {} & [GA]*A\text{-}1 + [G*T]*T\text{-}1 + [RG]*R\text{-}1 + [GD]*D\text{-}1 - [G]*[A]*A+1 - \\ & [G]*[T]*T+1 - [G]*[R]*R+1 - [G]*[D]*D+1. \end{aligned} \quad (1)$$

There are 15 distinct faces in the three cubes – each of which gives a thermodynamic constraint (corresponds to the thermodynamic cycle with 4 reversible reactions), e.g., the constraint for the face involving G, GA, G*AT and G*T is A1*T2 = T1*A2 (involves 8 rate constants). 4 additional thermodynamic constraints are generated from the thermodynamic cycles corresponding to the four horizontal paths (cycles with 3 reversible reactions) based upon free energy change ($\Delta G°$) for GTP hydrolysis (for example, $P1*D1*T1 = 1/K_{eq} = 1/(1.457 \times 10^9)$ where $\Delta G° = -RT.\ln(K_{eq})$ = kcal/mole (T = 25°C = 298.15 K), is one of the 4 constraints). Out of 19 constraints, only 13 are linearly independent which are used to calculate 13 parameters (see next section) using

the values of other 35 parameters. Seven parameters are fixed. The calculated parameters are required to be within their bounds by adding a penalty term to the objective function. Apart from these, four additional constraints (A2/A3 > 100; A5/A6 > 100; 5.0×10^{-8} < 1/A2 and 1/A5 < 1.5×10^{-7}) that are based on experimental data are used to restrict the search space. Thus, 28 parameters are estimated using the hybrid genetic-algorithm (GA)-based optimizer [2] to fit the experimental data.

2 Parameter calculation procedure

As is well known, parameters of reactions of thermodynamic cycles are related by the second law of thermodynamics. Such relations can be used to calculate the value of one parameter based on the values of others and are therefore constraints on the parameter set. The constraints of this type present in the detailed model, and the way they were maintained during parameter optimization, is described below.

The methodology is explained for the detailed model. Based upon the second law of thermodynamics, totally 19 thermodynamic constraints can be derived for the model shown in Fig. 3. The 19 constraints are: A1T2 = T1A2; A4T4 = T3A5; R1T3 = T1R2; R4T4 = T2R5; R2A5 = A2R5; R1A4 = A1R4; A2P2 = P1A3; A5P4 = P3A6; R2P3 = P1R3; R5P4 = P2R6; A3R6 = R3A6; A3D2 = D1A1; A6D4 = D3A4; R3D3 = D1R1; R6D4 = D2R4; P1D1T1 = $1/(1.457\times10^{9})$; P2D2T2 = $1/(1.457\times10^{9})$; P3D3T3 = $1/(1.457\times10^{9})$; P4D4T4 = $1/(1.457\times10^{9})$. In the last 4 constraints, the factor $1/(1.457\times10^{9})$ = $1/K_{eq}$, where K_{eq}, the equilibrium constant for GTP hydrolysis, is calculated from the relationship $\Delta G° = - RT.\ln(K_{eq}) = -12.5$ kcal/mol at T = 298.15 K (25 °C).

Although these constraints (also referred to as equations hereafter) involve nonlinear terms, on a log-scale (or log10-scale) they are linear algebraic equations in terms of log of various rate constants and log of $1/K_{eq}$. Hence, the equations can be represented as AX = b where X is a column vector containing all the parameters involved

in all the equations and the entries of A are the appropriate coefficients (0, 1 or –1). Out of the 19 (= N) equations, only 13 are linear independent (rank(A) = rank([A | b]) = 13). Thus, a maximum of 13 rate constants can be calculated using these equations. Thus, one needs to select 13 parameters out of 48 parameters, such that these 13 parameters would be calculated using the values of the other 35 parameters. The details of the calculation procedure are as follows.

The essential idea is to calculate those parameters for which the least information is known; remaining parameters, the values of which were constrained but not fixed by experimental data, should be optimized. To do this, the list of parameters in each of the 13 equations is sorted in the order of decreasing UB/LB and the parameter to be calculated by each equation is recursively assigned. For sorting, the user can also specify that certain parameters should (preferably) be calculated and that certain parameters must be optimized. For example, it was required that the parameters A+2, A+5, T+3, T+4, R+3, R+6 must be optimized and that the parameters P+1, P+2, P+3, P+4, R+1, R-1, R+4, R-4 should (preferably) be calculated (see below). The algorithm tries to find such an assignment. If such an assignment is not found over all combinations, then the user is prompted to assign a weight to the parameters that must be calculated. For such parameters for sorting purposes, the effective range is amplified by this weight to increase its chances of being calculated. It must be mentioned that for the models described in this article, this situation did not arise. Based upon this assignment, the equations are reordered and rearranged so that the assigned variables appear first in the equations in monotonically increasing order (i.e., parameter x_i is matched with the i^{th} equation). Then A and X are partitioned as $A = [A_1\ A_2]$ and $X = [X_1\ X_2]^T$ so that X_1 consists of N (=19) parameters that can be potentially calculated (the test for consistency and independence is not yet done), X_2 consists of the remaining parameters from X, and the matrices A_1 and A_2 comprise of the corresponding columns from A. Essentially, the matrix equation becomes $A_1X_1 = -A_2X_2 + b$. The procedure for extracting the maximal set of consistent and independent equations from the full set of N equations that can be used (manipulated) to derive the expressions for to-be-calculated variables is explained below.

From the full set of N equations, a maximal set of consistent equations, C = {C_1, C_2, … C_k} (that satisfies rank($A_{1,C}$) = rank([$A_{1,C}$ [-$A_{2,C}$ b_C]]), where the subscript 'C' is used to indicate appropriate sub-matrices corresponding to the equations C (the matrix $A_{2,C}$ consists of columns from A that are not in $A_{1,C}$) are extracted (starting with equation 1, equations 2, 3, etc., are added to the set C (one at a time)) and tested for consistency and independence). From these consistent equations, consistent and independent equations, I = {I_1, I_2, … I_l} that satisfy rank($A_{1,I}$) = rank([$A_{1,I}$ [-$A_{2,I}$ b_I]]) = l are selected (starting with the first equation in the set C, the 2nd, 3rd equation, etc., are added to the set I (one at a time) and tested for consistency and independence). This procedure identifies the consistent and independent equations in conjunction with the parameters that are assigned to them. If l = rank(A), a maximal set has been found and the procedure stops, else, another combination (matching) is selected and tested. It is possible that some of the parameters "desired to be calculated" may not be calculated and they could be optimized instead. This is because set I is a subset of the original matching between equations and variables. Now, the equations in set I are written as $A_{1,I}X_{1,I} = -A_{2,I}X_{2,I} + b_I$ (one can assume b_I to be part of $A_{2,I}$ assuming that last component of $X_{2,I}$ is 1). The matrix $P = (A_{1,I})^{-1}([-A_{2,I} \quad b_I])$ is computed which contains the coefficients that are needed to expresses $X_{1,I}$ in terms of $X_{2,I}$ (including the last component in 1). Thus, $X_{1,I} = PX_{2,I}$ on log-scale. Finally, by exponentiation (or $10^{p_{ij}}$), the symbolic expressions are calculated on normal-scale.

For the GTPase-cycle module, a maximal set of (comprising of 13) consistent-and-independent equations (and the corresponding 13 parameters) is identified. By solving these 13 consistent-and-independent equations, the symbolic expressions (in terms of other 35 parameters) for the 13 parameters to be calculated are derived. Every time the 28 parameters to be optimized are assigned a value, the 13 parameters are calculated using these expressions. For completeness, the 13 calculated parameters and the corresponding expressions are given below.

A-1 = 1.0/T+1 * A+1 * T+2 * 1.0/T-2 * T-1 * 1.0/A+2 * A-2

A-4 = A+4 * T-3 * T+4 * 1.0/T-4 * 1.0/T+3 * 1.0/A+5 * A-5

R-1 = R+1 * 1.0/T+1 * 1.0/T-3 * T-1 * 1.0/A+2 * A-2 * T+3 * A+5 * 1.0/A-5 * 1.0/R+5 * R-5

R-4 = R+4 * 1.0/T+2 * T-2 * T+4 * 1.0/T-4 * 1.0/R+5 * R-5

R+2= A+2 * 1.0/A-2 * 1.0/A+5 * A-5 * R-2 * R+5 * 1.0/R-5

P+1 = 1.0/A-3 * 1.0/T-3 * 1.0/A+2 * A-2 * T+3 * A+5 * 1.0/A-5 * 1.0/R+5 * R-5 * P-1 * A+3 * 1.0/A+6 * A-6 * R+6 * 1.0/R-6 * D-3 * 1.0/D+3 * 6.8634×10^{-10}

P+3 = 1.0/T-3 * T+3 * P-3 * D-3 * 1.0/D+3 * 6.8634×10^{-10}

R-3 = A-3 * 1.0/A+3 * A+6 * 1.0/A-6 * R+3 * 1.0/R+6 * R-6

P+4 = 1.0/T-3 * T+3 * A+5 * 1.0/A-5 * P-4 * 1.0/A+6 * A-6 * D-3 * 1.0/D+3 * 6.8634×10^{-10}

D+2= T-3 * T+2 * 1.0/T-2 * 1.0/T+3 * 1.0/A+5 * A-5 * R+5 * 1.0/R-5 * A+6 * 1.0/A-6 * 1.0/R+6 * R-6 * D-2 * 1.0/D-3 * D+3

D+1= A-3 * T+1 * T-3 * 1.0/T-1 * A+2 * 1.0/A-2 * 1.0/T+3 * 1.0/A+5 * A-5 * R+5 * 1.0/R-5 * 1.0/A+3 * A+6 * 1.0/A-6 * 1.0/R+6 * R-6 * D-1 * 1.0/D-3 * D+3

P+2 = 1/T-3 * T+3 * A+5 * 1.0/A-5 * 1.0/R+5 * R-5 * P-2 * 1.0/A+6 * A-6 * R+6 * 1.0/R-6 * D-3 * 1.0/D+3 * 6.8634×10^{-10}

D+4= T-3 * T+4 * 1.0/T-4 * 1.0/T+3 * 1.0/A+5 * A-5 * A+6 * 1.0/A-6 * D-4 * 1.0/D-3 * D+3;

It is interesting to note that except for the constant multiplying factors, a parameter X appears as X or 1/X (and not in fractional powers of X) because the elements of the matrix P are either 0, 1 or -1 in all but the last column. Also, except for R+2 and R+4, all the parameters desired to be calculated are calculated.

For the case of reduced-order models, the methodology remains the same but some of the constraints become irrelevant and hence the assignment between the remaining constraints and the parameters to be calculated changes. Thus, this pairing is identified for every candidate reduced-order model (ROM) individually.

3 Experimental data, simulation of experiments, and the values of parameters

The experimental data used to estimate the parameters for the reduced-order models is the same as the data used to estimate the parameters for the detailed model (Table 2). These experiments were carried out in Professor Elliott M. Ross's laboratory (The

University of Texas Southwestern Medical Center) and consist of data from 5 different scenarios. Data from three scenarios (data sets) are shown repeatedly in three separate plots in each of Figs. 4-9 (4*B*, 5*B*, 6*B*, 7*B*, 8*B* and 9*B*) and consist of measurements of *v* (GTP-hydrolysis rate) at different concentrations of GTP or GAP and in the presence or absence of active receptor as specified in Table 2 [3]. Data from two additional scenarios (one data point in each) were used for all optimizations but not shown in Fig. 4-9. These data are *Z* (fraction of active G-protein) in the presence of active receptor, with or without GAP present [4] (Table 2). In these, the GAP is PLC-β1 at its IC50 concentration; the maximal GAP activity of PLC-β1 (15 s^{-1}) is similar to RGS4 (25 s^{-1}). In all reduced-order models, the predicted value of *Z* in these two scenarios fit well to the experimental value listed in Table 2.

Bornheimer et al. [1] provided extensive information about how some parameter-values were fixed, and others were constrained within upper and/or lower bounds, by data available in the literature. These same bounds (listed in Table 3) and constraints are used in this study, except for the following difference.

For data sets 1 and 3 (Table 2), there is uncertainty in the measurement of [R]. Hence, for the detailed model, for data sets 1 and 3, [R] is optimized between 1.5 – 3.5 nM and 4.0 – 7.0 nM, respectively, along with other parameters to fit the experimental data. Based upon optimization, it was found that I.C. for [R] for data sets 1 and 3 were confined in narrow ranges around 2.0 nM and 5.0 nM, respectively. So, for the ROMs they are optimized between 2.0 nM – 2.24 nM and 5.0 nM – 5.5 nM, respectively.

4 Parameter estimation using a hybrid Genetic Algorithm

Genetic Algorithm-based optimization

Genetic algorithm (GA) is based upon the Darwin's theory of natural selection and survival of the fittest [5, 6] and has been successfully used in many optimization problems. In an optimization problem, an objective function is to be minimized by manipulating the values of the free parameters within certain bounds (search space). GA

is a population-based search technique. The population consists of members. Each member corresponds to a particular set of parameters values. Thus, an objective-value is associated with each member. For a minimization problem, a member with lower objective-value is considered fitter as compared to a member with higher objective-value. In the classical GA [5], the parameters in each member are represented through a binary string (called genome) though for real-parameter optimization problems, a floating-point representation has been recently proposed by Wolf and Moros [7] and later used by Katare et al. [2]. The number of bits reserved for each parameter depends on the allowed range (upper bound (UB) – lower bound (LB)) of the parameter and the desired accuracy in its solution. For example, if LB = 10, UB = 50, and the required precision is 0.1 then the required number of bits >= *ceil*(log2((50-10)/0.1+1)) = 9 bits where *ceil* is the ceiling function. The search starts by randomly choosing a population of initial guesses. Depending upon the range (small or large), the values of the parameters in the initial population can be chosen either uniformly (for small ranges) or on a logarithmic-scale (for large ranges). Then, the objective function is evaluated for each member and rank-ordered according to decreasing objective. Thus, the fittest member is the last in the sorted population. Each member is assigned a fitness value based upon its objective and the objective for other members. The best member (which fits the data best) has the highest fitness. Next, crossover and mutation operators are used to generate offsprings from parents. The more fit members get more chance to undergo crossover (mating) and hence they are more likely to pass their genes to the next generation. Either both crossover and mutation can be applied or only crossover or mutation can be applied. In crossover, there is high possibility of exchanging large parts of the genomes. In mutation, only few bits get mutated randomly. Crossover brings large changes whereas mutation results in small changes in most cases though the actual change depends upon the location of the bit in the string. A crossover probability of 90% is quite common. Thus, if a random number between 0 and 1 is less than 0.9 then the chosen parents mate, otherwise, offsprings are generated by mutating the parents. Mutation probability is kept low (e.g., 1-2%). There are several rules for choosing the parents for crossover and mutation [8]. In the present implementation, one parent is chosen on the basis of rank in the population and the other is chosen on the basis of fitness [2, 7]. In some applications,

some of the best members of the current population are directly transferred to the next generation as decided by elitism fraction [2, 8]. Usually, the population size (about 5-10 times as compared to the number of unknowns (parameters to be estimated)) is kept fixed for each generation. Thus, an appropriate number of new members are generated by applying the mutation and crossover operators to the parents. The process of going from the current generation to the next generation is called evolution. GA works on the premises that the members in the next generation are on an average fitter than in the current generation. In other words, it is likely that some of the members of the next generation fit the data better as compared to the members in the current generation. Usually, the objective-value for the best member in the next generation is not always better than the best member of the current generation. Thus, the best member improves in discrete steps. Elitism ensures that the best member of the next generation is at least as good as the best member of the current generation [2]. The process of evolution continues for a fixed number of generations, or several generations until a solution with the desired objective-value is found, or most of the members of the population become similar (based upon a genome-similarity measure). Sometimes, GA-based search can get trapped in local-minima; to avoid this, the mutation rate can be increased. In fact, depending upon the diversity of the population, one may schedule the mutation rate to ensure enough diversity. It is also advisable to carry out several runs of GA starting with different initial guesses. At the end of GA, one can either accept the member with the lowest objective-value as the solution or analyze/post-process several members with the objective-value close to the best member. For parameter-estimation problems the latter is beneficial [2] since the user can then judge the collection of models having low objective-value; this is useful because the lowest objective-value may not correspond to the best model due to the difficulty of designing a flawless objective function and to noise or errors in the data.

In the present method of hybrid genetic algorithm, all the members of the final generation (many of which are good) and the best members of each generation are collected (duplicates are removed). They represent the potentially promising regions of the search space [2]. To explore the search space around these promising regions, these candidates are chosen as starting points for local-search-based optimization. Thus, if there is a local

minimum in the vicinity (which may possibly be a global minimum) the corresponding member will move to the local minimum at the end of local-optimization. Essentially, by using the hybrid GA, several local optima across the search space are identified. Based upon tests on several problems, many of the local optima are close to the global optima in location or objective-value or both [2].

Despite the details presented above, the reader unfamiliar with GA is not expected to be able to use GA-based optimization directly and is referred to above mentioned references to gain further and working knowledge about GA. The public-domain GA code, GAlib, available from MIT (http://lancet.mit.edu/ga/) can also serve as a primer.

Application to the parameter estimation problem

In order to use GA-based optimization for parameter estimation, one needs to choose an objective function and appropriately handle constraints as discussed below.

Selection of objective function:

As suggested in the main manuscript ("Methods"), in the problem of parameter estimation using experimental data, the simplest objective function can be the fit-error (sum of squared-error (SSE)) between the experimental data and the model predictions. If several data sets (curves) are used for estimating the parameters, the SSE on each data set can be weighted by the inverse of the variance of noise or measurement error (if estimates are available). Various points on each curve (data set) can also be weighted (say, by inverse of the square of the corresponding point-wise error-estimates). Additional weighting schemes can be used. For example, if the concentration of a substrate or enzyme-like species is increased, starting with a low value, to saturation, then the data corresponding to half-saturation (K_M (Michaelis-Menten parameter) or EC50 (enzyme concentration at half-maximum) or IC50 (inhibitor concentration at half-maximum)) and saturation may be more important. Hence, these points may be given more weight. A biologist or statistician may suggest additional normalization and scaling schemes. In the case study presented in this manuscript, no error-estimates were available. The number of data points was not enough for noise estimation. In fact, the

data is nearly noise-free. Hence, in a chosen data set, all data points except the middle point were given uniform weight. The weight assigned to the middle point was 5 times higher. Thus, in the data sets 1, 2 and 3, which consisted of 10, 9 and 10 data points, respectively, error at the points 5, 4 and 5, respectively, were magnified (5 times). Next, to normalize for variation in the values across different data sets, the first data set (i.e., the experimental data in plot 1 of Fig. 4 *C* and data set 1 in Table 2) was chosen as a reference and the error (experimental value – predicted value) in other data sets (Table 2) were scaled by the ratio of the maximum value in plot 1 to the maximum value in the respective data sets. Data sets 4 and 5 consist of single data points (at IC50 value of the GAP). Since these correspond to a different GTPase system (the GAP is PLC-β1), to avoid much effect due to these two data points, the chosen IC50 weight is unity. An expression for the objective function can be written as follows. Let $v_{i,j}$ and $Z_{i,j}$ denote the predicted value of v and Z, respectively, corresponding to j^{th} data point of i^{th} data set. Let $\bar{v}_{i,j}$ and $\bar{Z}_{i,j}$ denote the corresponding experimental value of v or Z. Let $w_{i,j}$ denote the weight assigned to them. Further, let $v_{i,max}$ and $Z_{i,max}$ denote the maximum value of v and Z, respectively, in data set i. Then the objective function is:

$$obj = \sum_{j=1}^{10}[(v_{1,j} - \bar{v}_{1,j}) * w_{1,j}]^2 + \sum_{j=1}^{9}[(v_{2,j} - \bar{v}_{2,j}) * w_{2,j} * \left(\frac{\bar{v}_{1,\max}}{\bar{v}_{2,\max}}\right)]^2 +$$

$$\sum_{j=1}^{10}[(v_{3,j} - \bar{v}_{3,j}) * w_{3,j} * \left(\frac{\bar{v}_{1,\max}}{\bar{v}_{3,\max}}\right)]^2 + [(Z_{4,1} - \bar{Z}_{4,1}) * \left(\frac{\bar{v}_{1,\max}}{\bar{Z}_{4,1}}\right)]^2 + [(Z_{5,1} - \bar{Z}_{5,1}) * \left(\frac{\bar{v}_{1,\max}}{\bar{Z}_{5,1}}\right)]^2 \quad (2)$$

where $w_{1,5} = w_{2,4} = w_{3,5} = 5$ (all others are 1).

Handling constraints:

Since GA-based optimization cannot handle constraints explicitly a penalty term is added to the objective function corresponding to each constraint that must be satisfied by the solution. The new objective that is minimized by GA is:

$$obj_{GA} = obj + \sum_k \phi_k + \sum_l \varphi_l \quad (3)$$

where $\phi_k = W_k * |h_k(p,...)|$ denotes the penalty corresponding the equality constraint $h_k(p,...) = 0$, and $\phi_l = V_l * \max(0, g_l(p,...))$ denotes the penalty corresponding to the

inequality constraint $g_l(p,...) \leq 0$ [9]. p refers to the parameter vector with respect to which the objective function is to be minimized (p is to be estimated so as to fit the data well). The weights W_k and V_l can possibly differ to prioritize the constraints. Depending upon the problem under consideration, other types of constraints may be used. If all constraints are satisfied, then $obj = obj_{GA}$, a solution to the original constrained-optimization problem has been found.

The constraints used in the GTPase-cycle module case study are as follows:

Bound constraints on the calculated parameters:

For the detailed model, penalty corresponding to the bounds on the 13 calculated parameters (denoted as p_i below) are calculated through the following expression where $p_{i,LB}$ and $p_{i,UB}$ are the lower bound (LB) and upper bound (UB) for parameter p_i:

$$\varphi_i = \begin{cases} 10 * \log(p_{i,LB} / p_i) / \log(p_{i,UB} / p_{i,LB}) \; if \;\; p_i < p_{i,LB} \\ 10 * \log(p_i / p_{i,UB}) / \log(p_{i,UB} / p_{i,LB}) \; if \;\; p_i > p_{i,UB} \\ 0, otherwise \end{cases} \quad (4)$$

The index ‘i’ has been used just for convenience.

Ratio constraints:

4 ratio constraints are also used. These are related to the ratio of the equilibrium constant for reaction A3 to A2, A6 to A5, and the equilibrium constant for reactions A5 and A2, respectively. Let $r_1 – r_4$ be defined as follows:

r_1 = (A-3/A+3)/(A-2/A+2)
r_2 = (A-6/A+6)/(A-5/A+5)
r_3 = (A-5/A+5)
r_4 = (A-2/A+2)

Further, let $r_{1,LB}$, $r_{2,LB}$, $r_{3,LB}$, $r_{3,UB}$, $r_{4,LB}$ and $r_{4,UB}$ be appropriate bounds on $r_1 - r_4$.

Then, the penalty corresponding to the ratio constraints r_1 and r_2 are:

$$\varphi_i = \begin{cases} V_i * (r_{i,LB} - r_i) / r_{i,LB} \; if \; r_i < r_{i,LB} \\ 0, otherwise \end{cases} \tag{5}$$

where $V_1 = 0.5$, $V_2 = 0.1$, $r_{1,LB} = r_{2,LB} = 100.0$ (index 'i' used only for convenience). The penalty corresponding to the ratio constraints r_3 and r_4 are:

$$\varphi_i = \begin{cases} V_i * (r_{i,LB} / r_i) / (r_{i,UB} / r_{i,LB}) \; if \; r_i < r_{i,LB} \\ V_i * (r_i / r_{i,UB}) / (r_{i,UB} / r_{i,LB}) \; if \; r_i > r_{i,UB} \\ 0, otherwise \end{cases} \tag{6}$$

where $V_3 = 0.1$, $V_4 = 0.02$, $r_{3,LB} = 5.0 \times 10^{-8}$, $r_{3,UB} = 1.5 \times 10^{-7}$, $r_{4,LB} = 5.0 \times 10^{-8}$, and $r_{4,UB} = 1.5 \times 10^{-7}$ (index 'i' used only for convenience).

The equality constraints were already used to calculate the 13 parameters. That is why bound-constraints are used on the 13 calculated parameters. It can be noted that for the reduced-order models, if any of the parameters involved in a particular constraint is eliminated, then that constraint becomes irrelevant (within the scope of the reduced-order model) and hence, that constraint is not used. In fact all the constraints become irrelevant for all the reduced-order models discussed in the manuscript (except for the 40 parameter model shown in Fig. 9).

5 The best set of parameter-values for various models

To verify that the traditional approaches of model simplification such as reversible reactions and quasi-steady-state assumption on enzyme-substrate complexes are not applicable for the GTPase cycle system, using one set of parameter-values for the

detailed model, dynamic simulation is carried out starting with various concentrations of the receptor and the GAP (see also Section 4.1 of the manuscript). Fig. 10 shows the comparison of the concentrations of various species across the entire range of the initial values of [R] and [GAP] (at t = 0). At t = 10 sec, value of R is doubled to its initial value to emulate perturbation from a steady state.

For all the models discussed in the manuscript, the values of the parameters in the best set (with best visual fit and/or minimum fit-error), and ranges (MIN, MAX) across the good sets are listed in tables as described below. For the retained parameters, a comparison of their ranges with the corresponding ranges in the detailed model is also shown in various figures as described below. For models in which none of the parameter-value sets fit the data well (the textbook model (Fig. 6) and the model with 40 parameters (Fig. 9)), neither multiparametric variability analysis (MPVA) nor comparison of ranges is meaningful. However, for completeness, for such models, MPVA has been performed by choosing a threshold on the fit-error to reflect similar difference between the minimum fit-error and the fit-error threshold as for the detailed model. If too few sets were obtained then somewhat larger threshold has been used. The minimum fit-error and the fit-error threshold are listed in the tables. This strategy has also been used for models in which the visual fit to the data is good for some sets but the numerical value of the fit-error is quite different as compared to the fit-error for the detailed model. It is important to realize that these results should be used only for qualitative interpretation.

The tables for the best set of parameter-values and the MIN and MAX:

- For the detailed model: Table 4.
- The ROM of Fig. 4 (21 parameter ROM): Table 5.
- The ROM of Fig. 5 (17 parameter ROM): Table 6.
- The textbook model shown in Fig. 6: Table 7.
- The ROM of Fig. 7 (Biddlecome et al. [4] model and 10 parameter ROM): Table 8.
- The ROM of Fig. 8 (15 parameter ROM): Table 9.
- The model of Fig. 9 (40 parameter model): Table 10.

The values shown in Table 4-10 are on normal-scale. Fixed parameters are shown in bold-face. *Calculated parameters* (if any) are shown in italics with smaller font-size. The optimized parameters are shown in normal style.

The figures for the comparison of MIN, MAX for ROMs with the MIN, MAX for the detailed model:

- The ROM of Fig. 5 (17 parameter ROM): Fig. 11.
- The textbook model shown in Fig. 6: Fig. 12.
- The ROM of Fig. 7 (Biddlecome et al. [4] model and 10 parameter ROM): Fig. 13.
- The ROM of Fig. 8 (15 parameter ROM): Fig. 14.
- The model of Fig. 9 (40 parameter model): Fig. 15.

6 Obtaining a copy of the prototype software for model-reduction

The prototype software for the model-reduction framework, most parts of which are highly customized for the GTPase-cycle module case study (for semi-automatic generation of C++ code) and hence, may require substantial effort by the user for another case study, can be obtained by sending an e-mail to the corresponding author. The code for the hybrid-genetic-algorithm (hybrid-GA) would be made available upon request. Any GA code available in the public-domain (such as GAlib from MIT, http://lancet.mit.edu/ga/) can be used with the model-reduction framework.

7 References

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Figure Legends:

Figure 10: Comparative analysis of dynamic-response during initial transients. Initial condition of [R] was varied from 1.0E-15 M to 1.0e-6 M and that of [A] was varied from 1.0E-12 M to 1.0E-6 M (both in multiplicative steps of 1000). R is reset to twice of its initial condition at t = 10 s. (a) panels in the first row: dependence of the concentration of the major product to the reactant for the dissociation reactions D1 – D4, (b) panels in the 2^{nd} and 3^{rd} rows: dependence of the enzyme-substrate concentration on the substrate concentration for the enzymatic reactions involving GAP, i.e reactions A1 – A6, and (C) panels in the 4^{th} and 5^{th} rows: dependence of the concentration of Receptor-G-protein complex on the concentration of the substrate (GDP or GTP bound G-protein or its complexes with GAP) for the reactions R1 – R6.

Figure 11. Comparison of ranges (MIN, MAX) for the ROM shown in Fig. 5 (17 parameter model).

Figure 12. Characteristics of the model shown in Fig. 6: (A) Prediction of limiting signaling regimes (LSRs), and (B) Comparison of ranges (MIN, MAX).

Figure 13. Characteristics of the ROMs shown in Fig. 7: (A) Biddlecome et al. [4] model: prediction of limiting signaling regimes, (B) Comparison of ranges, (C) the 10 parameter ROM: limiting signaling regimes, and (D) Comparison of ranges.

Figure 14. Comparison of ranges (MIN, MAX) for the model shown in Fig. 8 (15 parameter ROM).

Figure 15. Comparison of ranges (MIN, MAX) for the ROM shown in Fig. 9 (40 parameter model).

Table 2. Experimental data. Columns 1-5 specify initial conditions (M). Column 6 is turnover rate (v). d[D]/dt = d[T]/dt = d[P]/dt = 0 in all data sets.

GD	R	GAP (A)	GTP (T)	GDP (D)	v (1/s)
Data set 1: 10 data points; [D] = 0, I.C. for T varies					
1.00E-08	2.00E-09	4.00E-06	1.00E-09	0.00E+00	0.00083
1.00E-08	2.00E-09	4.00E-06	3.16E-09	0.00E+00	0.00167
1.00E-08	2.00E-09	4.00E-06	1.00E-08	0.00E+00	0.00833
1.00E-08	2.00E-09	4.00E-06	3.16E-08	0.00E+00	0.01667
1.00E-08	2.00E-09	4.00E-06	1.00E-07	0.00E+00	0.03833
1.00E-08	2.00E-09	4.00E-06	3.16E-07	0.00E+00	0.08333
1.00E-08	2.00E-09	4.00E-06	1.00E-06	0.00E+00	0.18833
1.00E-08	2.00E-09	4.00E-06	3.16E-06	0.00E+00	0.3
1.00E-08	2.00E-09	4.00E-06	1.00E-05	0.00E+00	0.40833
1.00E-08	2.00E-09	4.00E-06	3.16E-05	0.00E+00	4.50E-01
Data set 2: 9 data points; [D] = [A] = 0, d[A]/dt = 0					
1.00E-08	3.00E-09	0.00E+00	1.00E-09	0.00E+00	3.33E-04
1.00E-08	3.00E-09	0.00E+00	3.16E-09	0.00E+00	8.33E-04
1.00E-08	3.00E-09	0.00E+00	1.00E-08	0.00E+00	3.33E-03
1.00E-08	3.00E-09	0.00E+00	3.16E-08	0.00E+00	6.00E-03
1.00E-08	3.00E-09	0.00E+00	1.00E-07	0.00E+00	9.50E-03
1.00E-08	3.00E-09	0.00E+00	3.16E-07	0.00E+00	1.10E-02
1.00E-08	3.00E-09	0.00E+00	1.00E-06	0.00E+00	0.015
1.00E-08	3.00E-09	0.00E+00	3.16E-06	0.00E+00	0.01317
1.00E-08	3.00E-09	0.00E+00	1.00E-05	0.00E+00	0.01367
Data set 3: 10 data points; [D] = 0; I.C. for A varies.					
1.00E-08	5.00E-09	3.00E-10	1.00E-05	0.00E+00	0.01667
1.00E-08	5.00E-09	1.00E-09	1.00E-05	0.00E+00	0.01667
1.00E-08	5.00E-09	3.00E-09	1.00E-05	0.00E+00	0.05
1.00E-08	5.00E-09	1.00E-08	1.00E-05	0.00E+00	0.08333
1.00E-08	5.00E-09	3.00E-08	1.00E-05	0.00E+00	0.16667
1.00E-08	5.00E-09	1.00E-07	1.00E-05	0.00E+00	0.43333
1.00E-08	5.00E-09	3.00E-07	1.00E-05	0.00E+00	0.7
1.00E-08	5.00E-09	1.00E-06	1.00E-05	0.00E+00	0.88333
1.00E-08	5.00E-09	3.00E-06	1.00E-05	0.00E+00	0.98333
1.00E-08	5.00E-09	1.00E-05	1.00E-05	0.00E+00	1
GD	**R**	**GAP (A)**	**GTP (T)**	**GDP (D)**	***Z***
Data set 4: no GAP.					
2.00E-09	2.00E-10	0.00E+00	1.00E-06	0.00E+00	0.62
Data set 5: GAP is present.					
2.00E-09	2.00E-10	2.00E-07	1.00E-05	0.00E+00	0.12

Table 3. Lower bound (LB) and upper bound (UB) for parameters [1]. The units of k_{+1} are 1/(M.s) and that of k_{-1} are 1/s.

	k_{+1} (association rate constant)		k_{-1} (dissociation rate constant)	
Reaction	LB	UB	LB	UB
A1	10000	1.00E+10	0.000001	5000
A2	200000	2.00E+07	0.005	0.5
A3	10000	1000000	0.0001	5000
A4	10000	1.00E+10	0.000001	1000
A5	200000	2.00E+07	0.005	0.5
A6	10000	1000000	0.01	5000
R1	1000	1.00E+10	0.000001	100
R2	1000	1.00E+10	0.01	100
R3	5000000	1.00E+08	0.000001	1
R4	1000	1.00E+10	1.00E-07	100
R5	1000	1.00E+10	0.01	100
R6	5000000	1.00E+08	0.000001	1
T1	1	1.00E+08	1.60E-09	0.001
T2	1	1.00E+08	1.60E-09	0.001
T3	100000	1.00E+07	0.001	0.1
T4	100000	1.65E+07	0.001	0.1
P1	1.07E-11	11	0.013	0.013
P2	1.64E-08	16400	25	25
P3	1.00E-20	1	0.013	0.013
P4	3.91E-08	39100	25	25
D1	1	1000000	0.0001	0.0001
D2	1	1000000	0.0001	0.0001
D3	360000	1.00E+07	2	2
D4	1000	5.00E+07	2	5

Table 4: The best set of parameter-values and the MIN and MAX for the detailed model.

Parameter	Best value	MIN	MAX
Minimum fit-error = 0.020410, Cut-off = 0.025			
D-3	**2.00E+00**	**2.00E+00**	**2.00E+00**
D+3	1.47E+06	1.47E+06	7.73E+06
T+3	8.53E+05	6.82E+05	8.53E+05
T-3	4.68E-03	1.99E-03	4.94E-02
A+2	3.86E+05	3.86E+05	5.35E+05
A-2	4.08E-02	2.46E-02	6.40E-02
P-2	**2.50E+01**	**2.50E+01**	**2.50E+01**
P+2	*2.44E-01*	*1.86E-02*	*1.79E+00*
A+3	6.41E+04	1.63E+04	8.14E+04
A-3	9.50E-01	2.88E-01	9.88E-01
P-1	**1.30E-02**	**1.30E-02**	**1.30E-02**
P+1	*9.03E-07*	*6.96E-08*	*6.41E-06*
R+3	9.47E+07	9.44E+07	1.00E+08
R-3	*2.27E-03*	*2.40E-04*	*1.22E-02*
A+1	8.78E+06	1.17E+06	8.98E+06
A-1	*8.00E+00*	*1.74E-01*	*3.88E+01*
A+4	7.43E+04	1.42E+04	9.68E+04
A-4	*5.73E-03*	*5.57E-04*	*9.32E-02*
A+5	6.31E+06	5.50E+06	9.90E+06
A-5	4.78E-01	3.11E-01	4.95E-01
A+6	1.30E+04	1.07E+04	6.77E+04
A-6	6.85E-01	1.33E-01	1.62E+00
R+1	6.36E+08	2.11E+07	9.24E+08
R-1	*1.79E-02*	*2.76E-05*	*3.60E-02*
R+5	6.20E+06	3.43E+06	1.06E+07
R-5	4.33E-02	2.40E-02	8.80E-02
R+6	4.94E+07	2.28E+07	9.97E+07
R-6	4.21E-03	1.17E-03	8.80E-03
T+1	5.29E+05	2.83E+05	8.66E+05
T-1	8.38E-06	3.24E-07	8.38E-06
T+2	4.48E+04	4.01E+03	1.01E+05
T-2	8.23E-08	3.36E-08	9.74E-08
T+4	1.62E+06	8.93E+05	2.83E+06
T-4	8.75E-03	2.44E-03	8.83E-03
P-3	**1.30E-02**	**1.30E-02**	**1.30E-02**
P+3	*2.22E-09*	*9.93E-11*	*2.23E-09*
P-4	**2.50E+01**	**2.50E+01**	**2.50E+01**
P+4	*2.97E-03*	*7.66E-05*	*5.14E-03*
D-1	**1.00E-04**	**1.00E-04**	**1.00E-04**
D+1	*6.23E+01*	*9.67E+00*	*1.60E+04*
D-2	**1.00E-04**	**1.00E-04**	**1.00E-04**
D+2	*3.83E+00*	*1.44E+00*	*5.10E+01*
D-4	2.75E+00	2.55E+00	3.61E+00
D+4	*2.94E+03*	*2.94E+03*	*1.26E+05*
R+4	2.28E+05	2.28E+05	9.56E+05
R-4	*5.43E-07*	*3.70E-07*	*5.15E-05*
R+2	*1.32E+08*	*8.39E+07*	*6.77E+08*
R-2	1.29E+00	1.03E+00	5.76E+00

Table 5: The best set of parameter-values and the MIN and MAX for the ROM shown in Fig. 4 (21 parameter ROM).

Parameter	Best value	MIN	MAX
Minimum fit-error = 0.015216, Cut-off = 0.025			
D-3	**2.00E+00**	**2.00E+00**	**2.00E+00**
T+3	7.41E+05	4.88E+05	1.07E+06
A+2	4.25E+05	2.66E+05	6.39E+05
A-2	3.62E-02	1.79E-02	9.18E-02
P-2	**2.50E+01**	**2.50E+01**	**2.50E+01**
A-3	6.05E+02	7.29E-02	1.00E+03
P-1	**1.30E-02**	**1.30E-02**	**1.30E-02**
R+3	9.98E+07	7.59E+07	1.00E+08
A+5	2.46E+06	8.24E+05	8.59E+06
A-5	1.31E-01	6.10E-02	4.30E-01
R+5	5.06E+08	1.64E+08	2.76E+09
T+1	9.85E+02	2.36E+00	7.98E+05
T-2	5.48E-05	8.78E-09	9.88E-04
T+4	1.47E+06	1.01E+06	2.74E+06
P-3	**1.30E-02**	**1.30E-02**	**1.30E-02**
P-4	**2.50E+01**	**2.50E+01**	**2.50E+01**
D-1	**1.00E-04**	**1.00E-04**	**1.00E-04**
D-2	**1.00E-04**	**1.00E-04**	**1.00E-04**
D-4	2.46E+00	2.22E+00	3.54E+00
R+4	3.96E+09	1.62E+06	9.76E+09
R-2	2.28E+01	5.77E+00	1.00E+02

Table 6: The best set of parameter-values and the MIN and MAX for the ROM shown in Fig. 5 (17 parameter ROM).

Parameter	Best value	MIN	MAX
Minimum fit-error = 0.049962, Cut-off = 0.052			
D-3	**2.00E+00**	**2.00E+00**	**2.00E+00**
T+3	8.42E+05	7.16E+05	8.96E+05
A+2	4.52E+05	4.52E+05	5.05E+05
P-2	**2.50E+01**	**2.50E+01**	**2.50E+01**
A-3	8.21E+01	1.37E+01	9.90E+01
P-1	**1.30E-02**	**1.30E-02**	**1.30E-02**
R+3	9.93E+07	9.73E+07	1.00E+08
A+5	8.83E+05	6.79E+05	2.04E+06
T+1	6.84E+04	1.01E+04	9.86E+04
T+4	1.28E+06	9.50E+05	1.46E+06
P-3	**1.30E-02**	**1.30E-02**	**1.30E-02**
P-4	**2.50E+01**	**2.50E+01**	**2.50E+01**
D-1	**1.00E-04**	**1.00E-04**	**1.00E-04**
D-2	**1.00E-04**	**1.00E-04**	**1.00E-04**
D-4	2.59E+00	2.55E+00	2.82E+00
R+4	2.01E+06	2.23E+05	8.13E+06
R-2	2.82E+01	2.23E+01	6.68E+01

Table 7: The best set of parameter-values and the MIN and MAX for the textbook model shown in Fig. 6.

Parameter	Best value	MIN	MAX
Minimum fit-error = 0.629271, Cut-off = 0.632			
D-3	**2.00E+00**	**2.00E+00**	**2.00E+00**
D+3	1.86E+06	4.52E+05	9.97E+06
T+3	1.50E+06	1.11E+06	2.00E+06
A+2	8.04E+05	6.81E+05	9.48E+05
P-2	**2.50E+01**	**2.50E+01**	**2.50E+01**
A-3	8.53E+02	5.15E+01	1.00E+03
P-1	**1.30E-02**	**1.30E-02**	**1.30E-02**
R+3	1.00E+08	9.93E+07	1.00E+08
T+1	9.86E+04	1.71E+03	2.47E+06
D-1	**1.00E-04**	**1.00E-04**	**1.00E-04**
D+1	7.46E+02	2.44E+00	9.98E+02
R-2	9.94E+01	5.19E+01	1.00E+02

Table 8: The best set of parameter-values and the MIN and MAX for the ROM shown in Fig. 7 (Biddlecome et al. [4] model and 10 parameter ROM).

Parameter	Best value	MIN	MAX
Biddlecome et al. [4] model: Minimum fit-error = 0.021563, Cut-off = 0.025			
D-3	**2.00E+00**	**2.00E+00**	**2.00E+00**
T+3	8.24E+05	6.61E+05	9.26E+05
P-1	**1.30E-02**	**1.30E-02**	**1.30E-02**
R+3	1.00E+08	8.58E+07	1.00E+08
A+5	1.43E+07	3.62E+06	1.92E+07
A-6	1.03E-02	1.00E-02	5.93E-02
T+1	1.47E+02	1.91E+00	9.97E+02
T+4	1.37E+06	9.27E+05	1.56E+06
P-4	**2.50E+01**	**2.50E+01**	**2.50E+01**
D-1	**1.00E-04**	**1.00E-04**	**1.00E-04**
D-4	2.20E+00	2.10E+00	2.46E+00
R-2	5.86E+00	9.67E-01	6.96E+00
10 parameter ROM: Minimum fit-error = 0.118451, Cut-off = 0.12			
D-3	**2.00E+00**	**2.00E+00**	**2.00E+00**
T+3	1.71E+06	1.48E+06	1.99E+06
P-1	**1.30E-02**	**1.30E-02**	**1.30E-02**
R+3	1.00E+08	9.57E+07	1.00E+08
A+5	2.00E+07	1.98E+07	2.00E+07
A-6	9.85E+02	1.31E+02	9.99E+02
T+1	4.07E+05	2.86E+03	9.97E+06
P-4	**2.50E+01**	**2.50E+01**	**2.50E+01**
D-1	**1.00E-04**	**1.00E-04**	**1.00E-04**
R-2	5.81E-02	5.35E-02	6.21E-02

Table 9: The best set of parameter-values and the MIN and MAX for the ROM shown in Fig. 8 (15 parameter ROM).

Parameter	Best value	MIN	MAX
Minimum fit-error = 0.026256, Cut-off = 0.028			
D-3	**2.00E+00**	**2.00E+00**	**2.00E+00**
T+3	8.30E+05	7.01E+05	9.80E+05
A+2	2.00E+05	2.00E+05	2.53E+05
P-2	**2.50E+01**	**2.50E+01**	**2.50E+01**
A-3	2.35E+02	4.17E+01	1.55E+03
P-1	**1.30E-02**	**1.30E-02**	**1.30E-02**
R+3	9.98E+07	9.63E+07	1.00E+08
A+5	6.57E+05	4.72E+05	1.47E+06
A-6	2.25E-02	1.56E-02	5.99E-02
T+1	9.01E+05	5.66E+02	9.98E+05
T+4	1.45E+06	1.25E+06	1.68E+06
P-4	**2.50E+01**	**2.50E+01**	**2.50E+01**
D-1	**1.00E-04**	**1.00E-04**	**1.00E-04**
D-4	2.52E+00	2.43E+00	2.67E+00
R-2	7.01E-01	5.86E-01	1.24E+00

Table 10: The best set of parameter-values and the MIN and MAX for the model shown in Fig. 9 (40 parameter model).

Parameter	Best value	MIN	MAX
Minimum fit-error = 0.659865, Cut-off = 0.67			
D-3	**2.00E+00**	**2.00E+00**	**2.00E+00**
D+3	5.34E+06	6.88E+05	9.23E+06
T+3	2.13E+06	1.13E+06	2.13E+06
T-3	*5.16E-02*	*9.13E-03*	*9.01E-02*
A+2	7.99E+05	6.71E+05	9.90E+05
A-2	8.05E-02	4.06E-02	9.94E-02
P-2	**2.50E+01**	**2.50E+01**	**2.50E+01**
P+2	6.61E-01	2.82E-01	9.66E-01
A+3	2.25E+05	2.10E+05	9.78E+05
A-3	*8.81E+00*	*8.81E+00*	*6.90E+02*
P-1	**1.30E-02**	**1.30E-02**	**1.30E-02**
P+1	*8.86E-07*	*3.80E-08*	*1.37E-06*
R+3	9.93E+07	9.84E+07	1.00E+08
R-3	4.52E-03	4.48E-03	4.98E-02
A+1	7.10E+05	5.73E+04	9.72E+05
A-1	*1.25E+02*	*6.16E-04*	*1.25E+02*
A+4	8.10E+04	2.38E+04	9.87E+04
A-4	*5.34E+00*	*1.22E+00*	*1.08E+01*
A+6	1.77E+04	1.10E+04	1.92E+04
A-6	9.60E+00	3.89E+00	9.94E+00
R+1	3.70E+09	9.11E+08	9.74E+09
R-1	*6.10E+00*	*3.16E-04*	*6.10E+00*
R+6	8.22E+07	3.45E+07	9.48E+07
R-6	5.18E-02	2.13E-02	9.63E-02
T+1	*7.16E+02*	*7.16E+02*	*3.22E+06*
T-1	9.79E-08	4.23E-09	9.98E-08
T+2	7.87E+05	1.21E+05	9.99E+05
T-2	6.17E-08	9.06E-09	9.28E-07
P-3	**1.30E-02**	**1.30E-02**	**1.30E-02**
P+3	*1.38E-10*	*5.34E-11*	*5.45E-10*
D-1	**1.00E-04**	**1.00E-04**	**1.00E-04**
D+1	7.37E+00	7.37E+00	9.90E+04
D-2	**1.00E-04**	**1.00E-04**	**1.00E-04**
D+2	*3.31E+01*	*1.92E+00*	*1.34E+02*
D-4	2.71E+00	2.00E+00	4.91E+00
D+4	8.80E+05	3.00E+05	9.00E+05
R+4	4.24E+06	1.03E+05	9.42E+06
R-4	*2.62E-03*	*1.21E-04*	*7.72E-02*
R+2	3.31E+07	3.11E+07	9.05E+07
R-2	9.67E+00	8.65E+00	9.96E+00

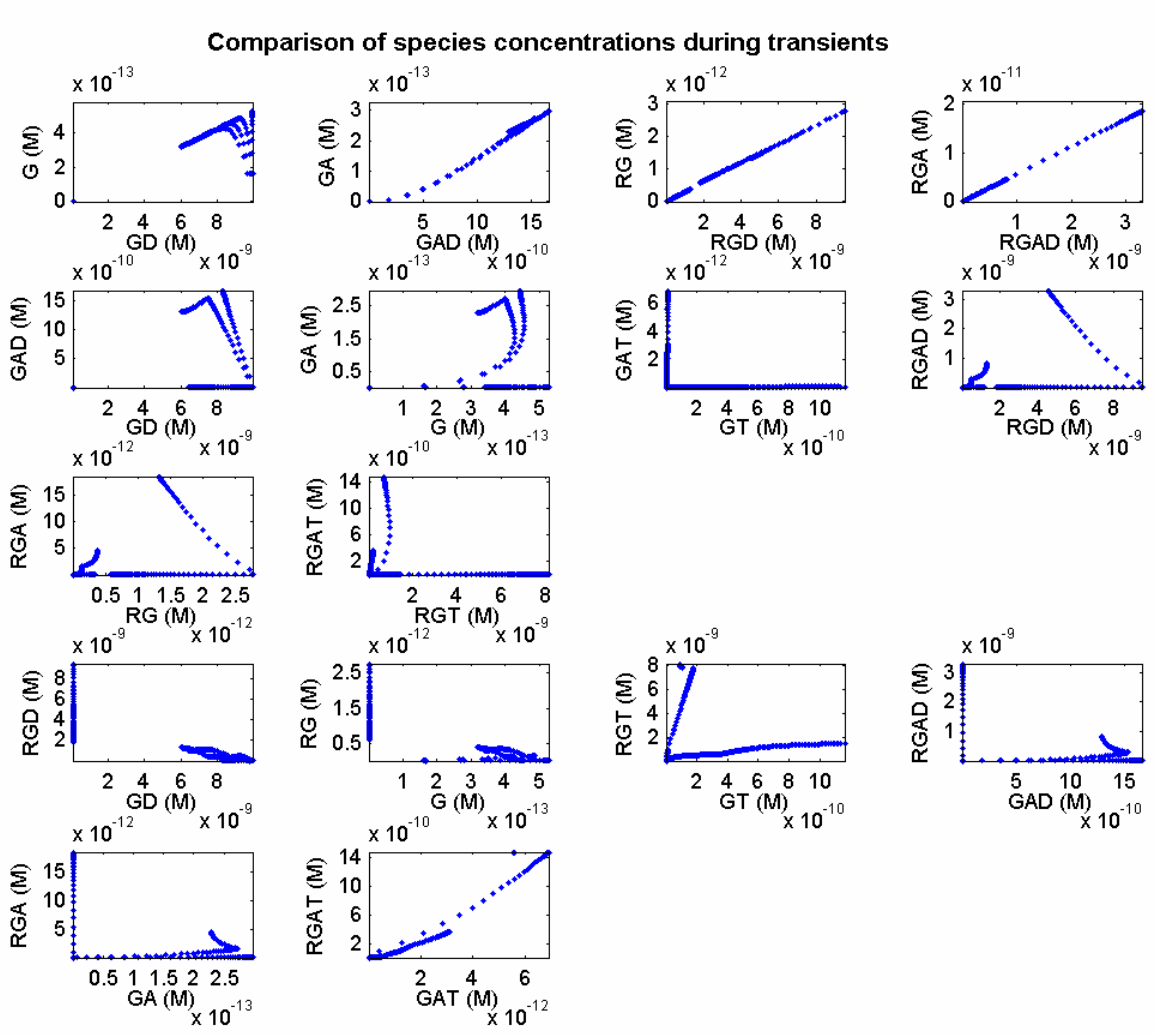

Figure 10: Comparative analysis of dynamic-response during initial transients. Initial condition of [R] was varied from 1.0E-15 M to 1.0e-6 M and that of [A] was varied from 1.0E-12 M to 1.0E-6 M (both in multiplicative steps of 1000). R is reset to twice of its initial condition at t = 10 s. (a) panels in the first row: dependence of the concentration of the major product to the reactant for the dissociation reactions D1 – D4, (b) panels in the 2nd and 3rd rows: dependence of the enzyme-substrate concentration on the substrate concentration for the enzymatic reactions involving GAP, i.e reactions A1 – A6, and (C) panels in the 4th and 5th rows: dependence of the concentration of Receptor-G-protein complex on the concentration of the substrate (GDP or GTP bound G-protein or its complexes with GAP) for the reactions R1 – R6.

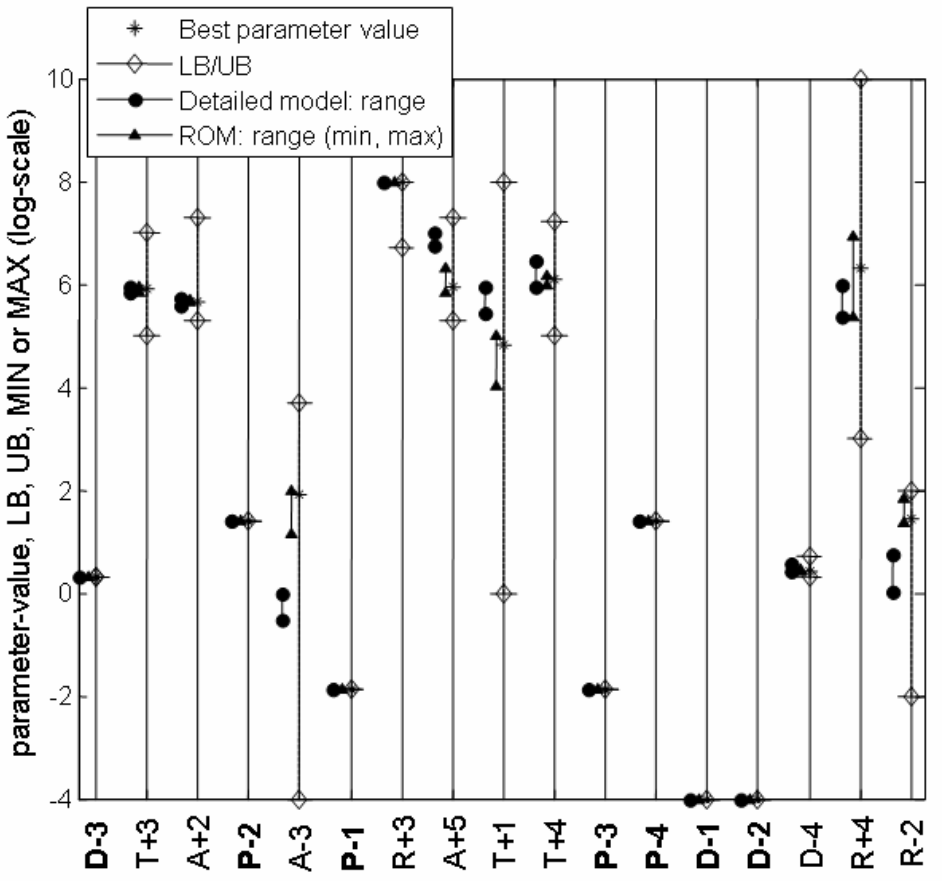

Figure 11. Comparison of ranges (MIN, MAX) for the ROM shown in Fig. 5 (17 parameter model).

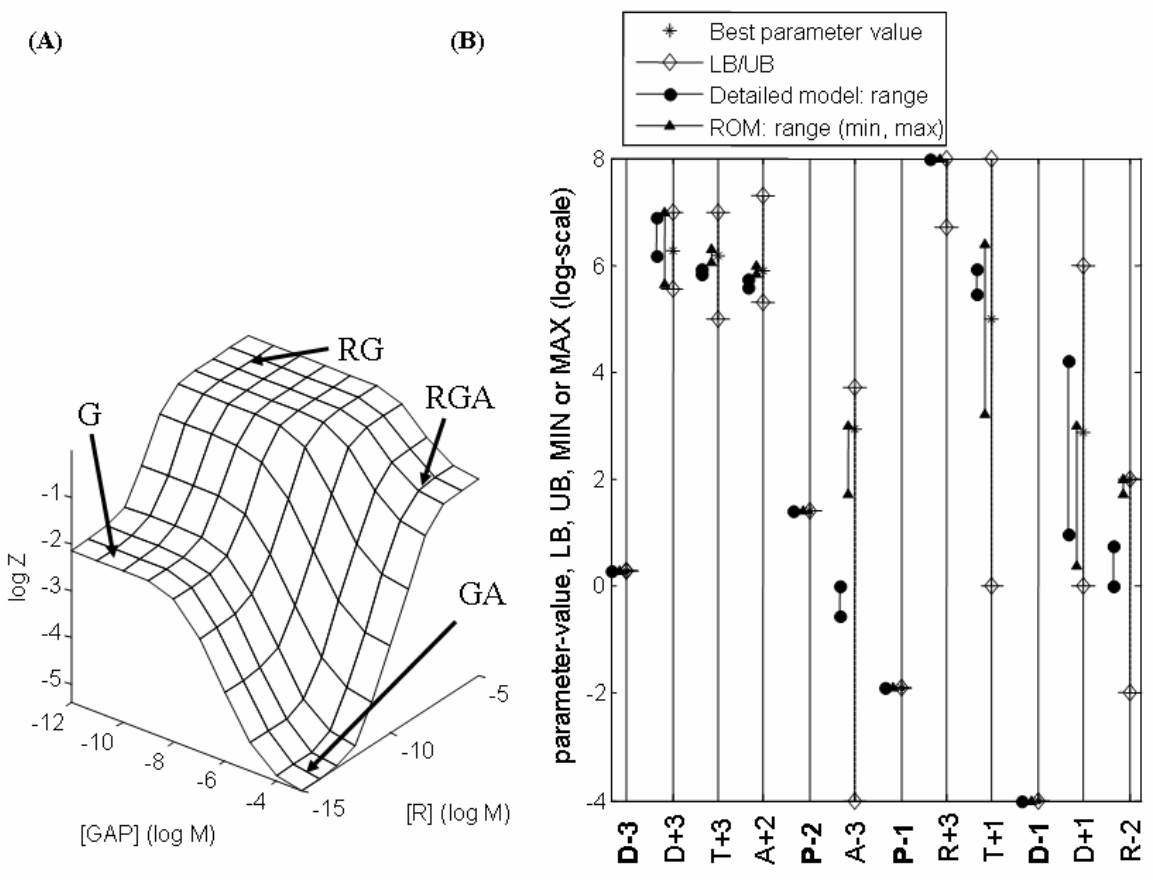

Figure 12. Characteristics of the model shown in Fig. 6: (A) prediction of limiting signaling regimes (LSRs), and (B) comparison of ranges (MIN, MAX).

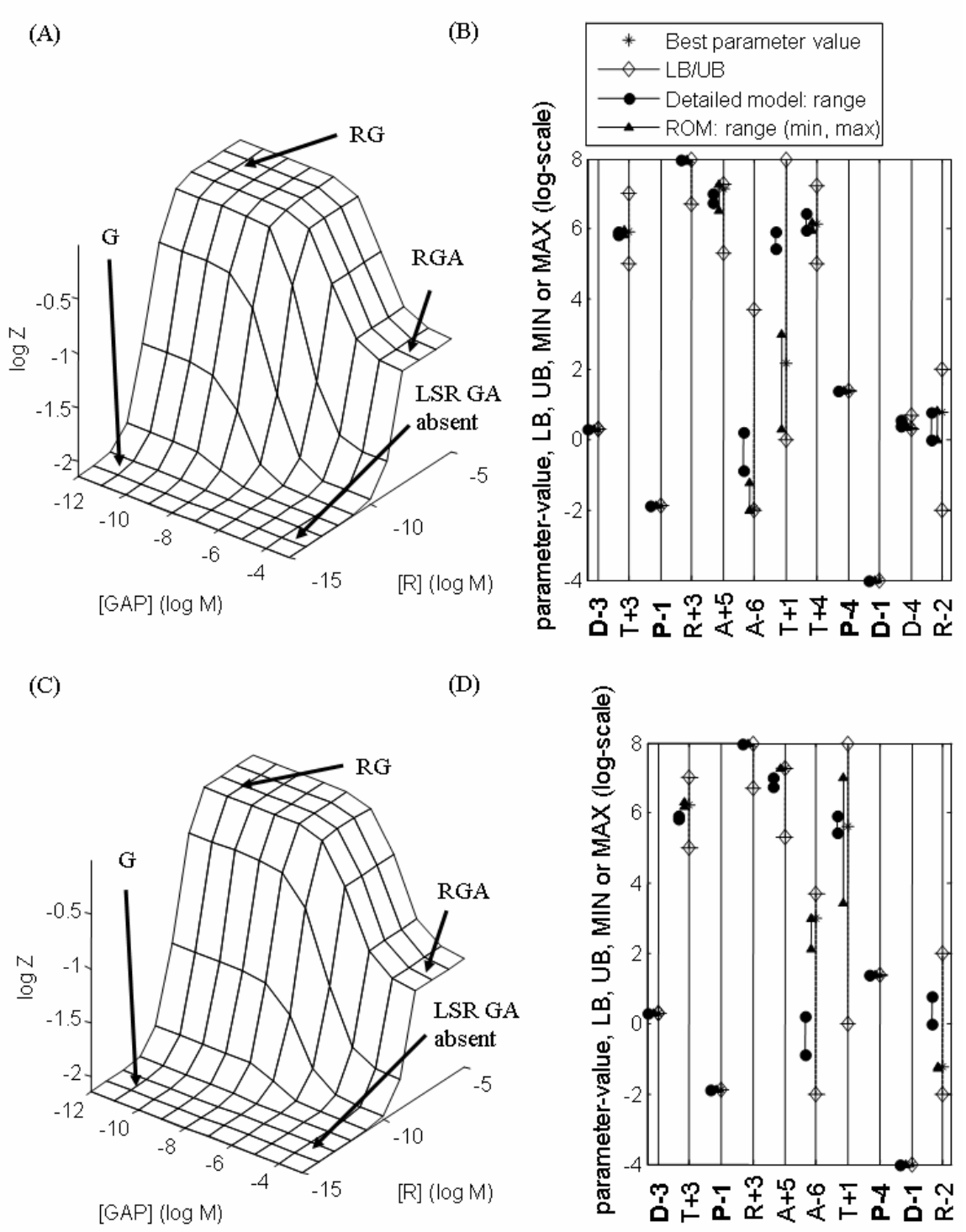

Figure 13. Characteristics of the ROMs shown in Fig. 7: (A) Biddlecome et al. [4] model: prediction of limiting signaling regimes, (B) Comparison of ranges, (C) the 10 parameter ROM: limiting signaling regimes, and (D) Comparison of ranges.

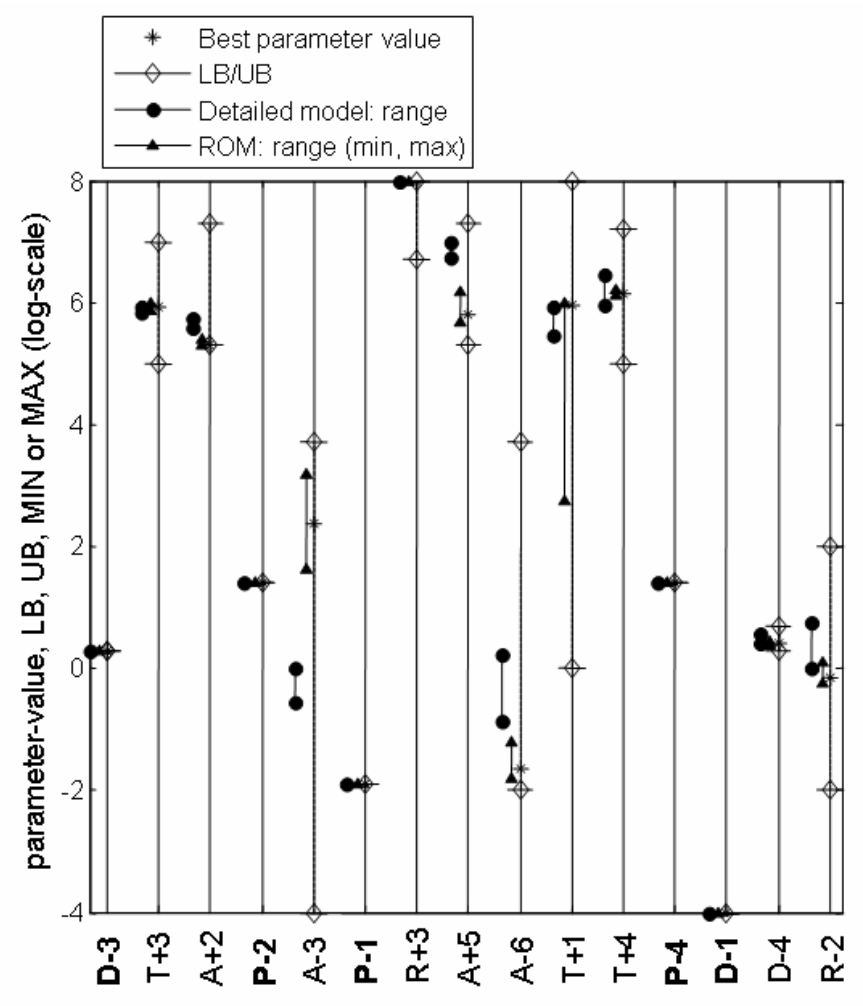

Figure 14. Comparison of ranges (MIN, MAX) for the model shown in Fig. 8 (15 parameter ROM).

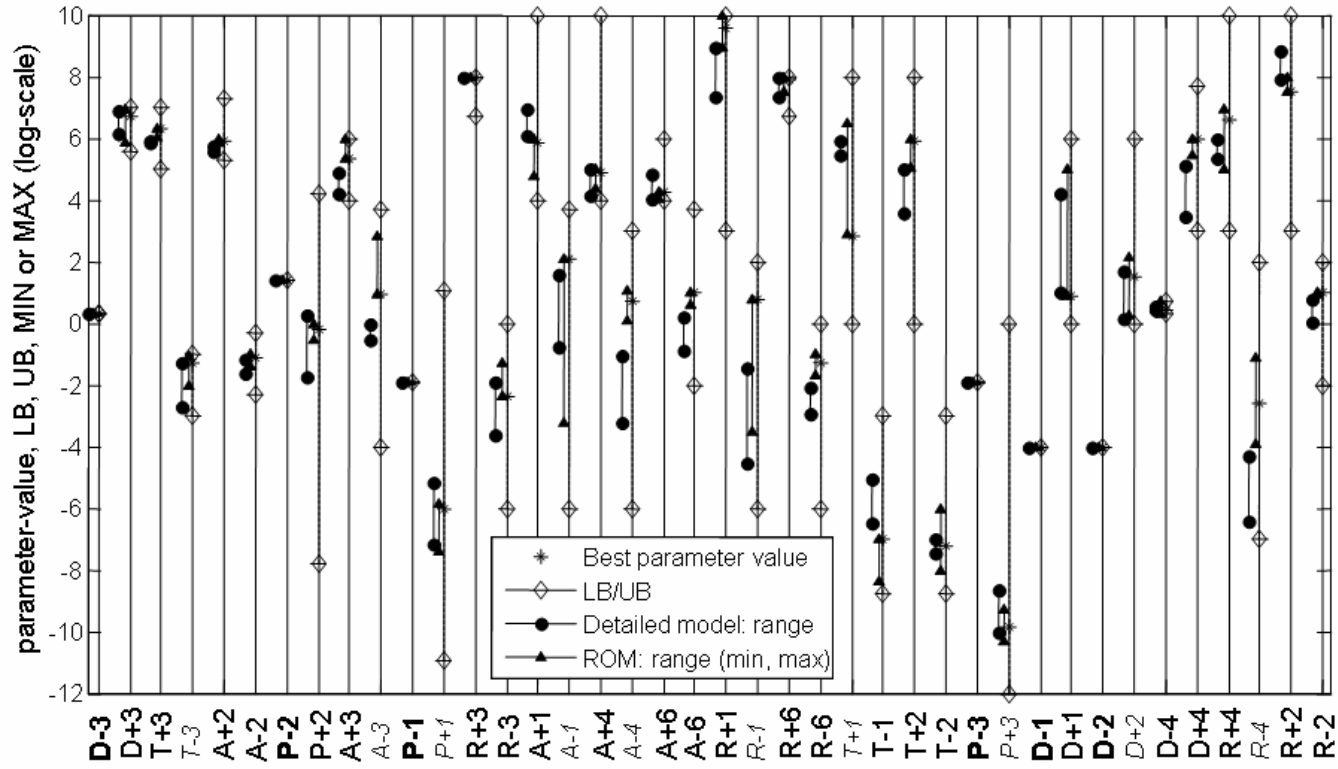

Figure 15. Comparison of ranges (MIN, MAX) for the ROM shown in Fig. 9 (40 parameter model).