

A new measure of the robustness of biochemical networks

Supplemental materials

Simulation experiment 2

Consider the glycolytic-glycogenolytic pathway in perfused rat liver (Scrutton and Utter, 1968; Torres, 1994). Glycolysis and glycogenolysis are central steps of carbohydrate metabolism (White *et al.*, 1968). They yield the common intermediate glucose-6-phosphate, which can subsequently be used by the organism as a major source of energy. The main route of glycogen utilization is glycogenolysis via glycogen phosphorolysis to glucose-1 phosphate and subsequent conversion to glucose-6-phosphate via the phosphoglucomutase reaction. Glucose-6-phosphate can be used for energy production or formation of glucose. It is clear that a detailed, quantitative understanding of the kinetic and dynamic properties of glucose, glycogen, and glucose-6-phosphate is central to any assessments of the energy economy of organisms and of diseases like diabetes that are associated with the balance and imbalance of carbohydrates (Voit, 2000). The kinetic properties of the pathway are obtained as follows (Voit, 2000), which are also shown in Figure S2

$$\begin{aligned}
 \dot{X}_1 &= 0.077884314X_4^{0.66}X_6 - 1.062708258X_1^{1.53}X_2^{-0.59}X_7 & X_1(0) &= 0.07 \\
 \dot{X}_2 &= 0.585012402X_1^{0.95}X_2^{-0.41}X_5^{0.32}X_7^{0.62}X_{10}^{0.38} \\
 &\quad - 7.93456 \times 10^{-4}X_2^{3.97}X_3^{-3.06}X_8 & X_2(0) &= 0.5 \\
 \dot{X}_3 &= 7.93456 \times 10^{-4}X_2^{3.97}X_3^{-3.06}X_8 - 1.05880847X_3^{0.3}X_9 & X_3(0) &= 0.16 \\
 X_4 &= 10, X_5 = 5, X_6 = 3, X_7 = 40, X_8 = 136, X_9 = 2.86, X_{10} = 4
 \end{aligned} \tag{29}$$

where

$$\begin{array}{ll}
X_1 = \text{glucose-1-P (G1P)} & X_7 = \text{phosphoglucomutase} \\
X_2 = \text{glucose-6-P (G6P)} & X_8 = \text{phosphoglucose isomerase} \\
X_3 = \text{fructose-6-P (F1P)} & X_9 = \text{phosphofructokinase} \\
X_4 = P_i & X_{10} = \text{glucokinase} \\
X_5 = \text{glucose} & X_{11} = \text{glycogen} \\
X_6 = \text{phosphorylase } \alpha
\end{array}$$

In this case, the system matrix A_D is obtained as

$$A_D = \begin{bmatrix} -1.53 & 0.59 & 0 \\ 0.95 & -4.38 & 3.06 \\ 0 & 3.97 & -3.36 \end{bmatrix}$$

The dynamic response of the nominal glycolytic-glycogenolytic pathway in (29) is given in Figure S3(a) and the upper bound of the tolerance is given by

$$A_D A_D^T = \begin{bmatrix} 2.689 & -4.0377 & 2.3423 \\ -4.0377 & 29.4505 & -27.6702 \\ 2.3423 & -27.6702 & 27.0505 \end{bmatrix}$$

That is, if the parameter perturbation measure $\Delta A_D \Delta A_D^T$ is less than $A_D A_D^T$, i.e. the robustness matrix $R > 0$, the characteristics of the steady state will be preserved.

Suppose the glycolytic-glycogenolytic pathway suffers from a kinetic perturbation as follows

$$\begin{array}{ll}
\dot{X}_1 = 0.077884314 X_4^{0.66} X_6 - 1.062708258 X_1^{1.5007} X_2^{-0.64825} X_7 & X_1(0) = 0.07 \\
\dot{X}_2 = 0.585012402 X_1^{1.0113} X_2^{-0.6152} X_5^{0.32} X_7^{0.62} X_{10}^{0.38} & X_2(0) = 0.5 \\
\quad - 7.93456 \times 10^{-4} X_2^{3.8804} X_3^{-3.0351} X_8 & \\
\dot{X}_3 = 7.93456 \times 10^{-4} X_2^{3.8804} X_3^{-3.0351} X_8 - 1.05880847 X_3^{0.3} X_9 & X_3(0) = 0.16 \\
X_4 = 10, X_5 = 5, X_6 = 3, X_7 = 40, X_8 = 136, X_9 = 2.86, X_{10} = 4 &
\end{array} \tag{30}$$

in which the parameter perturbation is of the following form

$$\Delta A_D = \begin{bmatrix} 0.0293 & 0.05825 & 0 \\ 0.613 & -0.1156 & -0.0249 \\ 0 & -0.00896 & 0.0249 \end{bmatrix}$$

In this perturbed case, $\Delta A_D \Delta A_D^T = \begin{bmatrix} 0.0043 & -0.0049 & -0.0005 \\ -0.0049 & 0.0177 & 0.0004 \\ -0.0005 & 0.0004 & 0.0007 \end{bmatrix}$ and

$R = \begin{bmatrix} 2.6847 & -4.0328 & 2.3428 \\ -4.0328 & 29.4328 & -27.6706 \\ 2.3428 & -27.6706 & 27.0498 \end{bmatrix}$, which is a positive definite matrix with its

eigenvalues all positive (i.e., 0.0204, 2.8261, 56.3208). From our computational result and the perturbed dynamic response shown in Figure S3(b), the robustness of steady state is preserved. Suppose another parameter perturbation occurs such that the glycolytic-glycogenolytic pathway is perturbed as

$$\begin{aligned} \dot{X}_1 &= 0.077884314 X_4^{0.66} X_6 - 1.062708258 X_1^{1.3581} X_2^{-0.69463} X_7 & X_1(0) &= 0.07 \\ \dot{X}_2 &= 0.585012402 X_1^{0.80419} X_2^{-0.18704} X_5^{0.56977} X_7^{0.59214} X_{10}^{0.48904} & X_2(0) &= 0.5 \\ &\quad - 7.93456 \times 10^{-4} X_2^{3.7771} X_3^{-3.0793} X_8 & & \\ \dot{X}_3 &= 7.93456 \times 10^{-4} X_2^{4.0071} X_3^{-3.0793} X_8 - 1.05880847 X_3^{0.3} X_9 & X_3(0) &= 0.16 \\ X_4 &= 10, X_5 = 5, X_6 = 3, X_7 = 40, X_8 = 136, X_9 = 2.86, X_{10} = 4 \end{aligned} \quad (31)$$

Remark S1: The perturbed pathway is based on the GMA model (Voit, 2000) to meet the constraints of the precursor-product relationships.

In this case, we have

$$\Delta A_D = \begin{bmatrix} 0.1719 & 0.10463 & 0 \\ -0.14581 & 0.42225 & 0.0193 \\ 0 & 0.0371 & -0.0193 \end{bmatrix}, \Delta A_D \Delta A_D^T = \begin{bmatrix} 0.0405 & 0.0191 & 0.0039 \\ 0.0191 & 0.1999 & 0.0153 \\ 0.0039 & 0.0153 & 0.0017 \end{bmatrix}.$$

$R = \begin{bmatrix} 2.6845 & -4.0568 & 2.3384 \\ -4.0568 & 29.2506 & -27.6855 \\ 2.3384 & -27.6855 & 27.0488 \end{bmatrix}$ is not positive definite, because its

eigenvalues are not all positive (i.e., -0.0915, 2.7967, 56.2427). Therefore, the robustness condition is violated. From the dynamic response shown in Figure S3(c),

we can see that the steady state of the biochemical network ceases to exist.

In order to confirm the compensatory parameter variations (i.e., $\Delta g_{ij} = \Delta h_{ij}$ for all i, j such that $\Delta A_D = 0$) in Remark 2 of the text, let us consider the following compensatory parameter perturbations due to negative self-feedback regulation in metabolite X_2

$$\Delta g_{22} = \Delta h_{22} = 5 \quad (32)$$

In this high parameter perturbation case, the simulation result is shown in Figure S3(d). It is seen that the time response is almost the same as the nominal system at the steady state, though with some difference in the transient state. Obviously, the occurrence of compensatory perturbation due to negative self-feedback regulation is helpful for maintaining the robustness of biochemical networks.

Simulation experiment 3

The tricarboxylic acid (TCA) cycle in *Dictyostelium*, a soil-living amoeba, produces ATP very efficiently while decomposing pyruvate to water and CO_2 via acetyl-CoA. Under a nutrient-rich condition, the cycle is fed by ingested proteins that are broken down into amino acids. During the period of starvation, cellular proteins are used up (Newsholme, 1973; Voit, 2000). In this case, the TCA cycle model is simplified reasonably to involve the following 13 dependent metabolites and 26 enzyme-catalyzed process (Newsholme, 1973; Kelly *et al.*, 1979; Voit, 2000).

$X_1 = \text{Oxalacetata 1 (OAA 1)}$	$X_{16} = \text{Succinate dehydrogenase}$
$X_2 = \text{Oxalacetate 2(OAA 2)}$	$X_{17} = \text{Fumarase}$
$X_3 = \text{Acetyl-CoA (ACO)}$	$X_{18} = \text{Malate dehydrogenase}$
$X_4 = \text{Isocitrate (ISOC)}$	$X_{19} = \text{Malic enzyme}$
$X_5 = \text{Pyruvate (PYR)}$	$X_{20} = \text{Ala} \rightarrow \text{Pyr}$
$X_6 = \text{Glutamate(GLU)}$	$X_{21} = \text{Pyruvate dehydrogenase complex}$
$X_7 = \text{Aspartat (ASP)}$	$X_{22} = \text{Oaa 2} \rightarrow \text{Asp}$
$X_8 = \text{Alaninee (ALA)}$	$X_{23} = \text{Asp} \rightarrow \text{Oaa 2}$
$X_9 = \text{Citrate 1 (CIT 1)}$	$X_{24} = \text{Citrate synthetase}$
$X_{10} = \alpha - \text{Ketoglutarate (2KG1)}$	$X_{25} = \text{Aconitase}$
$X_{11} = \text{Succinate (SUC)}$	$X_{26} = \text{Isocitrate dehydrogenase}$
$X_{12} = \text{Fumarate (FUM)}$	$X_{27} = \text{Glu} \rightarrow \text{Suc}$
$X_{13} = \text{Malate (MAL 1)}$	$X_{28} = \text{Aspartate transaminase}$
$X_{14} = \text{Glutamate dehydrogenase}$	$X_{29} = \text{Alanine transaminase}$
$X_{15} = \alpha - \text{Ketoglutarate dehydrogenase complex}$	

The S-system model is shown as follows (Voit, 2000)

$$\begin{aligned}
\dot{X}_1 &= 0.8282 X_1^{-0.038} X_6^{-0.0204} X_7^{0.106} X_{10}^{0.114} X_{13}^{0.7} X_{18}^{0.807} X_{28}^{0.108} X_{31}^{0.0848} X_{46}^{0.599} X_{48}^{-0.181} - 1.3423 X_1 X_{30}^{0.915} X_{33}^{0.0847} \\
\dot{X}_2 &= 1.3401 X_1^{0.915} X_7^{0.0848} X_{23}^{0.0848} X_{30}^{0.915} - 17.166 X_2^{0.706} X_3^{0.0716} X_{22}^{0.0848} X_{24}^{0.915} X_{47}^{-0.0341} \\
\dot{X}_3 &= 0.3231 X_3^{-0.405} X_5^{0.156} X_{21}^{0.427} X_{35}^{0.573} X_{46}^{0.422} X_{47}^{0.405} X_{48}^{-0.418} - 9.6952 X_2^{0.376} X_3^{0.489} X_{24}^{0.554} X_{41}^{0.446} X_{47}^{-0.00206} \\
\dot{X}_4 &= X_9 X_{25} - 0.152 X_4^{0.958} X_{26} X_{46}^{0.0348} X_{48}^{-0.862} \\
\dot{X}_5 &= 1.875 X_7^{0.0274} X_8^{0.465} X_{13}^{0.336} X_{19}^{0.535} X_{20}^{0.465} - 0.01923 X_3^{-0.717} X_5^{0.413} X_6^{0.306} X_8^{-0.29} X_{10}^{-0.0883} X_{21}^{0.756} X_{29}^{0.244} X_{46}^{0.748} \\
&\quad \times X_{47}^{0.718} X_{48}^{-0.741} \\
\dot{X}_6 &= 2.459 X_1^{-0.00921} X_6^{-0.0154} X_7^{0.0162} X_{10}^{0.086} X_{11}^{0.276} X_{28}^{0.813} X_{32}^{0.276} X_{39}^{0.6413} - 1.1528 X_5^{0.0963} X_6^{1.01} X_8^{-0.204} X_{10}^{-0.062} X_{14}^{0.0518} \\
&\quad \times X_{27}^{0.277} X_{29}^{0.171} X_{45}^{0.5} X_{46}^{0.0222} X_{48}^{-0.0191} \\
\dot{X}_7 &= 2.1167 X_1^{0.129} X_2^{0.129} X_{22}^{0.129} X_{33}^{0.129} X_{34}^{0.741} - 3.4893 X_1^{-0.0187} X_6^{-0.0311} X_7^{0.868} X_{10}^{0.174} X_{23}^{0.129} X_{28}^{0.165} X_{31}^{0.129} X_{40}^{0.577} \\
\dot{X}_8 &= 0.5724 X_5^{0.111} X_6^{0.247} X_8^{-0.234} X_{10}^{-0.0713} X_{29}^{0.197} X_{38}^{0.803} - 1.9369 X_8 X_{20}^{0.375} X_{44}^{0.625} \\
\dot{X}_9 &= 16.242 X_2^{0.679} X_3^{0.0782} X_{24} X_{47}^{-0.0372} - X_9 X_{25} \\
\dot{X}_{10} &= 0.156 X_4^{0.724} X_5^{0.106} X_6^{0.259} X_8^{-0.223} X_{10}^{-0.0679} X_{14}^{0.0568} X_{26}^{0.756} X_{29}^{0.188} X_{46}^{0.0506} X_{48}^{-0.672} - 0.8063 X_1^{-0.0101} X_6^{-0.0168} \\
&\quad \times X_7^{0.0177} X_{10}^{0.99} X_{11}^{-0.879} X_{15}^{0.911} X_{28}^{0.0891} X_{46}^{0.882} X_{47}^{0.879} X_{48}^{-0.881} \\
\dot{X}_{11} &= 2.0031 X_6^{0.166} X_{10}^{0.491} X_{11}^{-0.481} X_{15}^{0.499} X_{27}^{0.166} X_{36}^{0.335} X_{46}^{0.483} X_{47}^{0.481} X_{48}^{-0.483} - 2.4373 X_{11}^{0.495} X_{12}^{-0.00542} X_{16}^{0.574} X_{32}^{0.166} X_{42}^{0.261} \\
\dot{X}_{12} &= 1.271 X_{11}^{0.106} X_{12}^{-0.00836} X_{16}^{0.885} X_{37}^{0.115} - 9.1694 X_{12}^{1.89} X_{13}^{-1.24} X_{17}^{0.911} X_{43}^{0.0893} \\
\dot{X}_{13} &= 8.289 X_{12}^{1.98} X_{13}^{-1.36} X_{17} - 0.9387 X_1^{-0.0197} X_7^{0.0196} X_{13}^{0.775} X_{18}^{0.618} X_{19}^{0.382} X_{46}^{0.458} X_{48}^{-0.139} \\
X_1(0) &= 0.003, X_2(0) = 0.003, X_3(0) = 0.065, X_4(0) = 0.01, X_5(0) = 0.32, X_6(0) = 6.63, X_7(0) = 2.035 \\
X_8(0) &= 5.313, X_9(0) = 0.0275, X_{10}(0) = 0.01, X_{11}(0) = 0.9, X_{12}(0) = 0.04, X_{13}(0) = 0.24
\end{aligned}$$

$$\begin{aligned}
X_{14} &= 0.977, X_{15} = 7610, X_{16} = 3.15, X_{17} = 25.7, X_{18} = 77.8, X_{19} = 3.08, X_{20} = 0.196 \\
X_{21} &= 258, X_{22} = 74, X_{23} = 0.1, X_{24} = 8.24, X_{25} = 80, X_{26} = 271, X_{27} = 0.133, X_{28} = 9.95 \\
X_{29} &= 2.67, X_{30} = 800, X_{31} = 0.1, X_{32} = 1, X_{33} = 74, X_{34} = 1.06, X_{35} = 2.07, X_{36} = 1.62 \\
X_{37} &= 0.36, X_{38} = 2.03, X_{39} = 1.86, X_{40} = 0.446, X_{41} = 27.2, X_{42} = 1.57, X_{43} = 7, X_{44} = 0.326 \\
X_{45} &= 0.24, X_{46} = 0.072, X_{47} = 0.1, X_{48} = 0.18
\end{aligned}$$

(33)

The TCA network in the above equation is shown in Figure S4, and the dynamic response is shown in Figure S5(a). The system matrix A_D is obtained as

$$A_D = \begin{bmatrix}
-1.04 & 0 & 0 & 0 & 0 & -0.02 & 0.106 & 0 & 0 & 0.114 & 0 & 0 & 0.7 \\
0.915 & -0.706 & -0.0716 & 0 & 0 & 0 & 0.085 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -0.376 & -0.894 & 0 & 0.156 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -0.958 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0.717 & 0 & -0.41 & -0.31 & 0.027 & 0.755 & 0 & 0.089 & 0 & 0 & 0.336 \\
-0.01 & 0 & 0 & 0 & -0.10 & -1.03 & 0.016 & 0.204 & 0 & 0.148 & 0.276 & 0 & 0 \\
0.148 & 0.129 & 0 & 0 & 0 & 0.031 & -0.87 & 0 & 0 & -0.17 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0.111 & 0.247 & 0 & -1.234 & 0 & -0.07 & 0 & 0 & 0 \\
0 & 0.379 & 0.0782 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\
0.01 & 0 & 0 & 0.724 & 0.106 & 0.276 & -0.02 & -0.223 & 0 & -1.06 & 0.879 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0.166 & 0 & 0 & 0 & 0.491 & -0.976 & 0.005 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.106 & -1.9 & 1.24 \\
0.02 & 0 & 0 & 0 & 0 & 0 & -0.02 & 0 & 0 & 0 & 0 & 1.98 & -2.14
\end{bmatrix}$$

From A_D , we can calculate the upper bound of the perturbation tolerance $A_D A_D^T$.

That means when the network is perturbed by ΔA_D such that the robustness condition (14) is violated, and the steady state of the biochemical network may not exist. Suppose there is a perturbation ΔA_D as follows

$$\Delta A_D = 10^{-2} \times \begin{bmatrix} M & N \\ P & Q \end{bmatrix} \quad (34)$$

where

$$M = \begin{bmatrix} 2.09 & 0 & 0 & 0 & 0 & 2.67 & 0.0292 \\ 2.19 & 4.12 & 0 & 0 & 0 & 0 & 0.0305 \\ 0 & -2.78 & 0 & 0 & 5.8 & 0 & 0 \end{bmatrix}, \quad N = \begin{bmatrix} 0 & 0 & 5.65 & 0 & 0 & 1.42 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

$$P = \begin{bmatrix} 0 & 0 & 1.57 & 1.57 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 6.11 & -1.98 & -0.02 \\ 0.87 & 0 & 0 & 0 & -3.44 & 1.11 & 0.01 \\ 0.039 & 0.726 & 0 & 0 & 0 & 0.492 & 0.0054 \\ 0 & 0 & 0 & 0 & 4.13 & -1.33 & 0 \\ 0 & 2.16 & 0 & 0 & 0 & 0 & 0 \\ 1.19 & 0 & 1.79 & 1.79 & -4.72 & 1.53 & 0.017 \\ 0 & 0 & 0 & 0 & 0 & 2.07 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1.24 & 0 & 0 & 0 & 0 & 0 & 0.017 \end{bmatrix},$$

$$Q = \begin{bmatrix} 0 & 1.35 & 0 & 0 & 0 & 0 \\ 0.571 & 0 & -4.18 & 0 & 0 & -1.05 \\ -0.32 & 0 & 2.35 & 1.57 & 0 & 0 \\ 0 & 0 & 1.04 & 0 & 0 & 0 \\ 0.39 & 0 & -2.82 & 0 & 0 & 0 \\ 0 & 1.49 & 0 & 0 & 0 & 0 \\ -0.44 & 0 & 3.23 & 2.15 & 0 & 0 \\ 0 & 0 & 4.38 & 2.93 & 1.03 & 0 \\ 0 & 0 & 0 & 2.39 & 0.843 & 0.9 \\ 0 & 0 & 0 & 0 & 0.79 & 0.84 \end{bmatrix}.$$

In the perturbed case, the robustness matrix R is not a positive definite matrix with its eigenvalues (-0.006, 0.0218, 0.0955, 0.3666, 0.5360, 0.8629, 0.9511, 1.3141, 2.2116, 2.4675, 2.9605, 3.9098, 13.7447) and the dynamic response of the perturbed TCA cycle is shown in Figure S5(b). Obviously, the steady state of the perturbed TCA cycle ceases to exist.

Simulation experiment 4

Periodic responses are often encountered in organisms ranging from bacteria to mammals. A periodic oscillation can be considered as one kind of steady state

phenomenon from the system point of view. A periodic network in Figure S6 is modeled as follows to produce the spontaneous oscillations in cAMP observed during the early development of *D. discoideum* (Laub and Loomis, 1998) and account for the synchronization of the cells necessary for chemotaxis (Yi *et al.*, 2000; Ma and Iglesia, 2002).

$$\begin{aligned}
X_1 &= \text{ACA} & X_5 &= \text{internal cAMP} \\
X_2 &= \text{PKA} & X_6 &= \text{external cAMP} \\
X_3 &= \text{ERK2} & X_7 &= \text{CAR1} \\
X_4 &= \text{REG A} \\
\dot{X}_1 &= 2X_7 - 0.9X_1X_2 & X_1(0) &= 1.5 \\
\dot{X}_2 &= 2.5X_5 - 1.5X_2 & X_2(0) &= 1 \\
\dot{X}_3 &= 0.6X_7 - 0.8X_2X_3 & X_3(0) &= 1.5 \\
\dot{X}_4 &= 1 - 1.3X_3X_4 & X_4(0) &= 2.5 \\
\dot{X}_5 &= 0.3X_1 - 0.8X_4X_5 & X_5(0) &= 1 \\
\dot{X}_6 &= 0.7X_1 - 4.9X_6 & X_6(0) &= 0 \\
\dot{X}_7 &= 23X_6 - 4.5X_7 & X_7(0) &= 1.5
\end{aligned}$$

From (14) or (15), we can find that if the perturbation measure $\Delta A_D \Delta A_D^T$ is large such that the robustness condition $R = A_D A_D^T - \Delta A_D \Delta A_D^T > 0$ is violated, the robustness of the steady state may not be preserved. Suppose the parameter perturbation as follows

$$\Delta A_D = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0.1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -0.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.1 & 0 \end{bmatrix}.$$

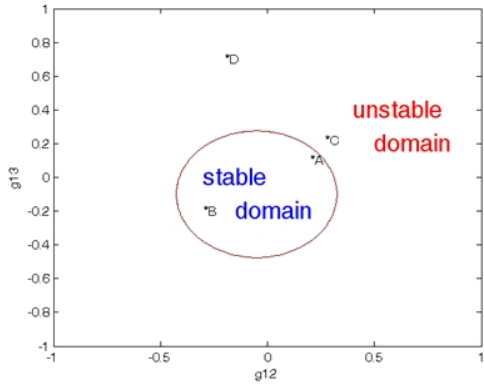
In this case, all eigenvalues of R are greater than 0, and the oscillation still exists as shown in Figure S7(b). However, when the perturbation ΔA_D is as follows

$$\Delta A_D = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0.1 \\ 0 & 0.2 & 0 & 0 & 0.1 & 0 & 0 \\ 0 & 0.2 & -0.2 & 0 & 0 & 0 & 0.1 \\ 0 & 0 & 0.2 & -0.2 & 0 & 0 & 0 \\ -0.6 & 0 & 0 & 0.1 & 0.1 & 0 & 0 \\ 0.1 & 0 & 0 & 0 & 0 & 0.02 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.1 & 0.02 \end{bmatrix},$$

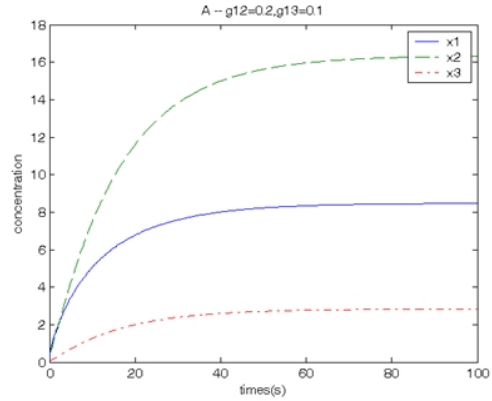
the robustness condition $R > 0$ is violated, i.e., the smallest eigenvalue of R is less than zero. The oscillation disappears and the steady state of the biochemical network ceases to exist as shown in Figure S7(c). Obviously, the proposed robustness measure is an important indicator of the robustness of biochemical networks under parameter perturbations.

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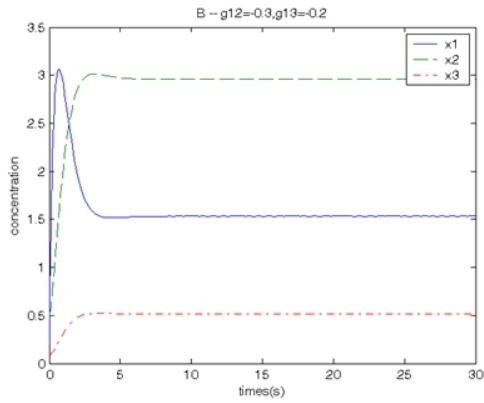
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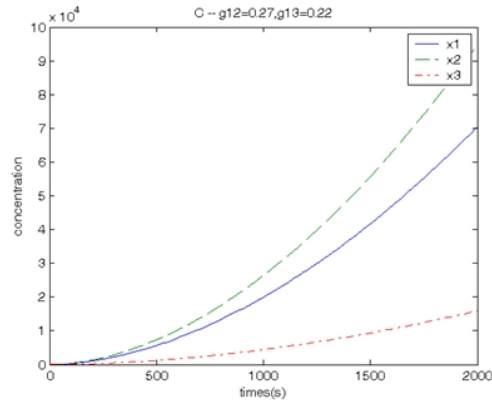
(a) The stable and unstable domains in parametric space (g_{12}, g_{13}) .



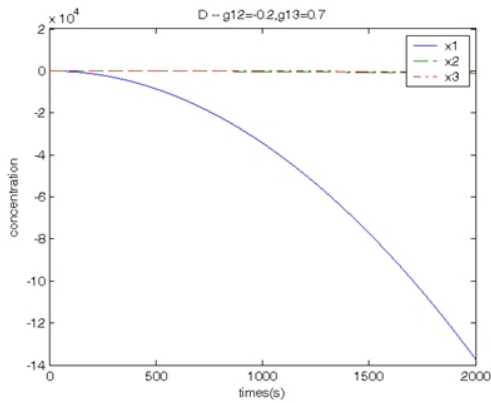
(b) The responses at parametric point A, the eigenvalues of R are 0.0193, 0.3542 and 0.7765.



(c) The responses at parametric point B, the eigenvalues of R are 0.0509, 0.3702 and 0.7788.



(d) The responses at parametric point C, the eigenvalues of R are -0.0506, 0.3305 and 0.7728.



(e) The responses at parametric point D, the eigenvalues of R are -0.3649, 0.2900 and 0.7649.

Figure S1. The stable and unstable domains in parametric space (g_{12}, g_{13}) and the dynamic responses of the four parametric points A, B, C and D. The stability robustness is confirmed by their smallest eigenvalue of robustness matrix R .

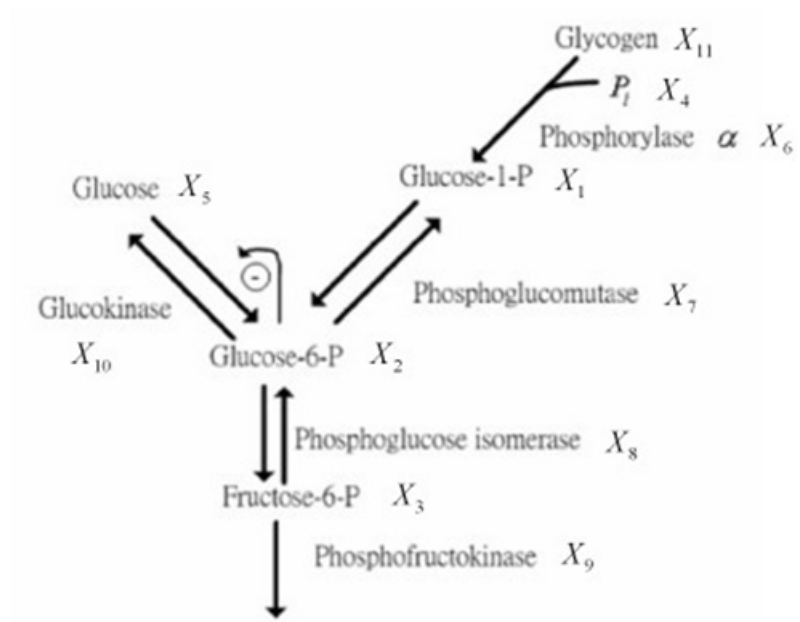
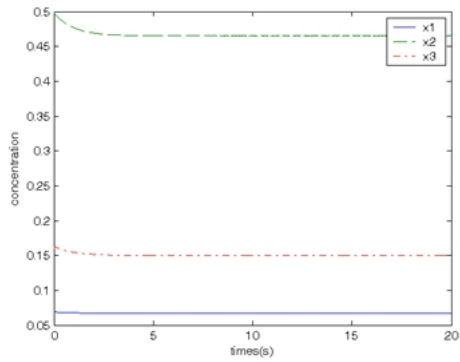
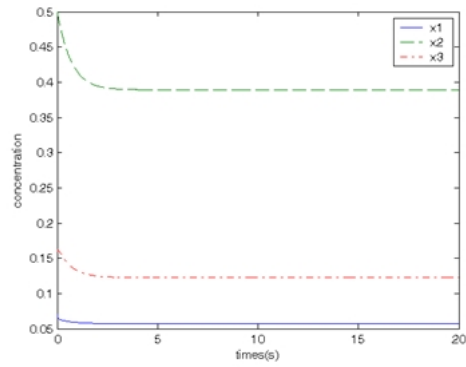


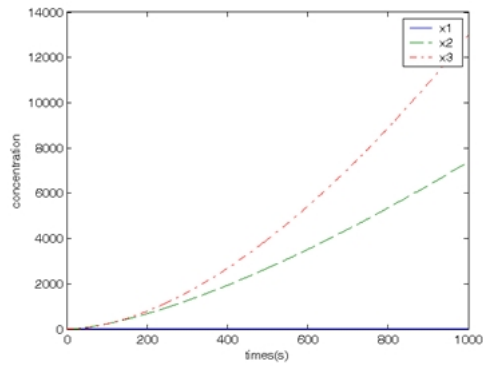
Figure S2. The glycolytic-glycogenolytic pathway in terms of biochemical names and in terms of model variables



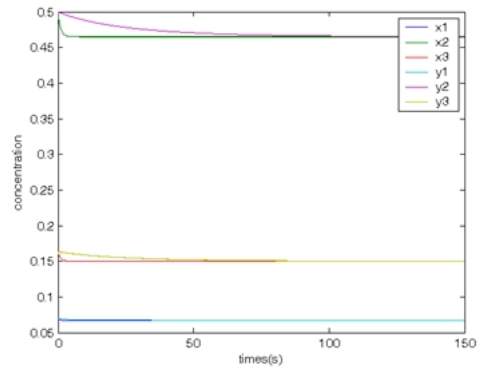
(a) The case without perturbation



(b) The case with parameter perturbation in (30)



(c) The case with parameter perturbation in (31)



(d) The case with the compensatory parameter variation in (32). y1, y2 and y3 represent the time response of the compensatory parameter perturbation case.

Figure S3. The dynamic response of the glycolytic-glycogenolytic pathway in simulation experiment 2 under different perturbative cases.

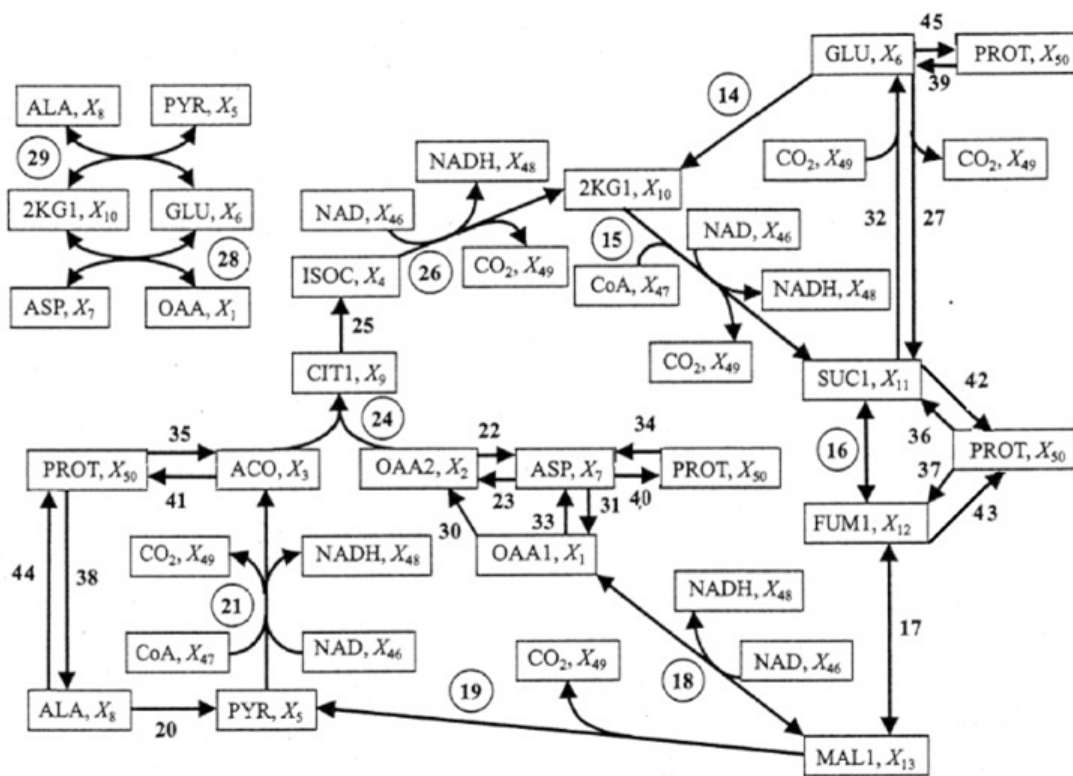
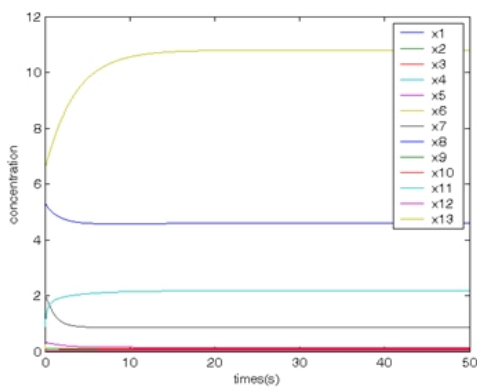
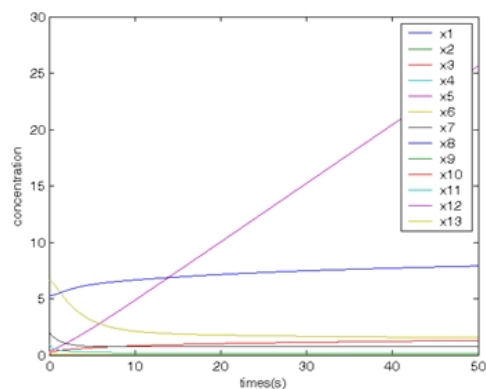


Figure S4. The network of the tricarboxylic acid (TCA) cycle in *Dictyostelium discoideum* (Voit, 2000).



(a) The case without perturbation



(b) The case with parameter perturbation in (34)

Figure S5. The dynamic response of the TCA cycle in simulation experiment 3 under the nominal case and the perturbative case.

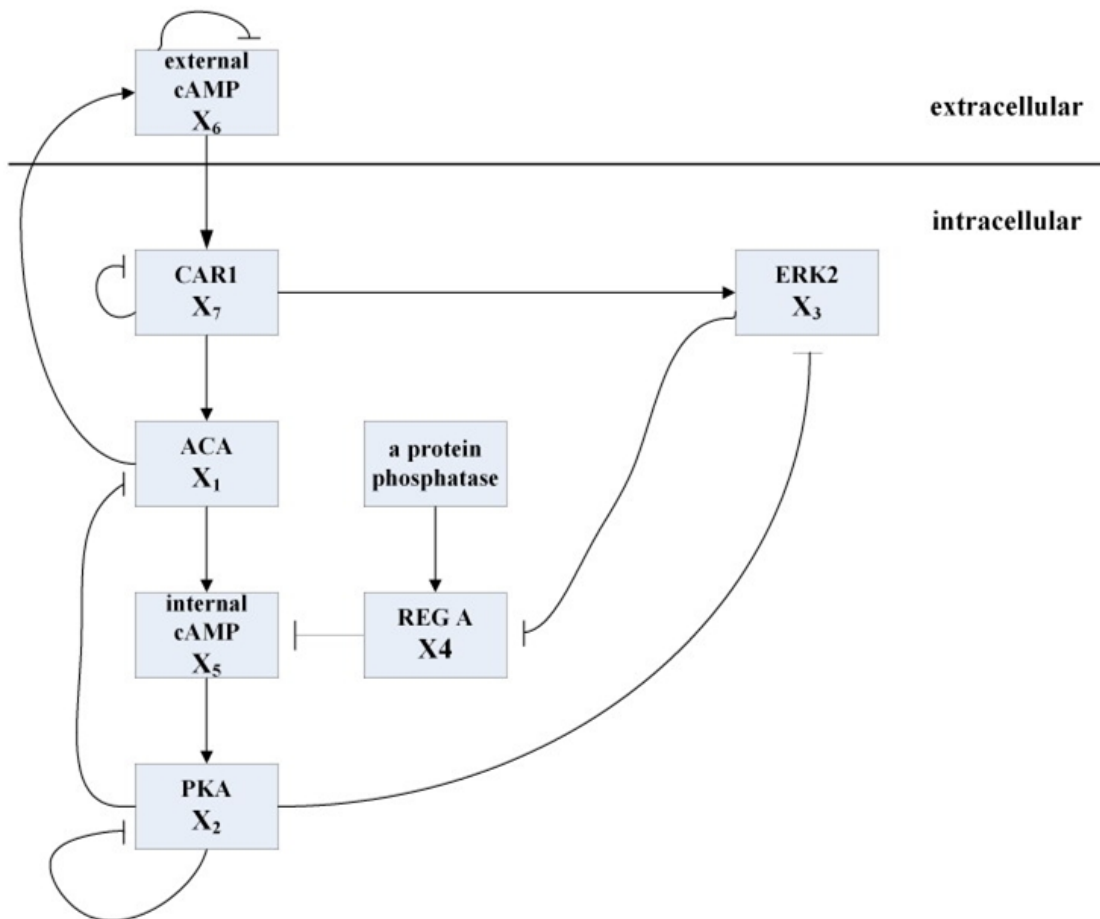
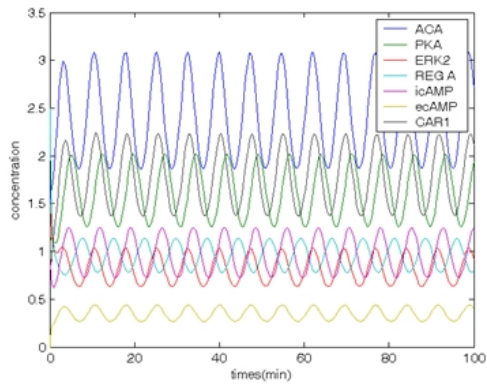
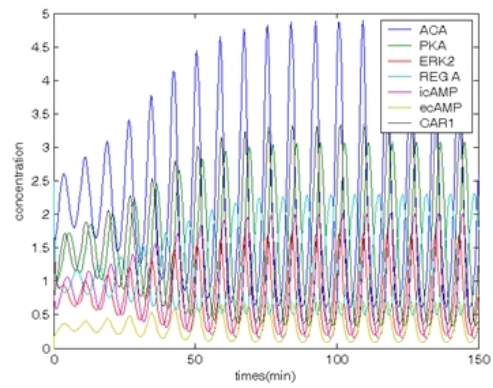


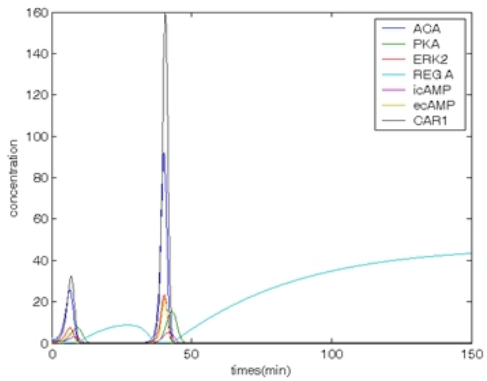
Figure S6. The cAMP oscillation network in *D. discoideum*, a soil-living amoeba.



(a) The case without perturbation.



(b) The perturbative case with the smallest eigenvalue of R as 0.0486



(c) The perturbative case with the smallest eigenvalue of R as -0.0098

Figure S7. The dynamic response of the cAMP oscillation network in simulation experiment 4 under different perturbative cases.