# A Systematic Approach to Analysis of Robustness in Oscillatory Networks

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## Abstract

Robustness as a system-level property is mainly determined by structural characteristics rather than fine-tuning parameter values. The relative contribution of network components or interactions to robustness remains little studied. By decomposing an overall network into smaller subnetworks and then analyzing effects of the interactions between them, relative importance of the network components for robustness property of oscillations can be derived. Moreover, the improvement of robustness against perturbations can be also made through modification of the structural characteristics or regulatory interactions of the network. Our analysis focuses on a molecular network that produces spontaneous oscillations in *Dictyostelium discoideum* cells.

## 1 introduction

Robustness is a property that allows a system maintains its functions despite external or internal perturbations and uncertainty [1]. It is a key to understanding cellular complexity and elucidating design principles. Owing to intimate links to cellular functions, robustness properties of many oscillatory networks through negative or interlocked feedback loops have been extensively investigated [2]. Investigation of robustness for general oscillators may focus on the persistence of regular oscillations, which does not prelude quantitative changes in period or amplitude to occur. While for circadian oscillators, it may focus on the period and amplitude sensitivities to evaluate their precise time-keeping ability with respect to noises or parameter variations. Most works mainly assess robustness as a system-level property. The relative contribution of network components or interactions to robustness remains little studied.

## 2 A linear analysis approach to robustness

We model a biochemical network as

$$\dot{x} = f(x, p), \tag{1}$$

where $x$ is the state vector containing the concentration or activity of all components in the network and $p$ are the parameters. Since our goal is to consider the relative importance of interactions for robustness, we decompose the overall network (1) into smaller subnetworks $\Gamma_i$ consisting of single components modelled by

$$\dot{x}_i = f_i(x_i, u_i, p). \tag{2}$$

Each subnetwork $\Gamma_i$ has components $x_i$ as its only internal state and output, while all other components are treated as inputs $u_i$.

The linearization of system (1) around an equilibrium $x_0$ is given by

$$\Delta\dot{x}(t) = A\Delta x(t) \quad \text{with} \quad A = \frac{\partial f(x(t), p)}{\partial x}|_{x_0}, \tag{3}$$

where $\Delta x(t) = x(t) - x_0$ denotes deviation of the concentrations or activities from the equilibrium $x_0$. The decomposition of the linearized system (3) into one-component subnetworks $\Gamma_i$ is as follows

$$\Delta\dot{x}(t) = \tilde{A}\Delta x(t) + (A - \tilde{A})\Delta u, \tag{4}$$

where $\tilde{A}$ is a diagonal matrix containing the diagonal entries of $A$. In this way, the linear biochemical network (3) can be seen as an open-loop interaction free network (4). In other words, we analyze the whole network by breaking the feedback loop at each step, viewing the effects of all other components on each $x_i$ as input signals, and after analyzing the relative importance of each component on the robustness properties of the oscillations, we do re-close the loop by letting $\Delta u = \Delta x$.

The key to our approach is standard feedback control theory. Following Laplace transformation, the linear open-loop system (4) then transforms to an algebraic equation in $s$

$$\Delta x(s) = L(s)\Delta u(s), \tag{5}$$

where $L(s) = (sI - \tilde{A})^{-1}(A - \tilde{A})$. The element $L_{ij}(s)$ corresponds to the transform function from the component $j$ to component $i$ in the absence of any feedback effects, i.e. the ratio of the output $x_i$ to the input $x_j$.

According to the generalized Nyquist criteria, for a stable open-loop network $L(s)$, a sufficient condition for instability under positive feedback is that one characteristic locus $\lambda_i(L(j\omega))$ crosses the real axis to the right of the point 1 at a single frequency $\omega = \omega_{\rm crit}$. A perturbation applied to element $L_{ij}(i\omega_{\rm crit})$ such that this $\lambda_i(L(j\omega))$ moves to the point 1 on the real axis corresponds to a stabilizing perturbation.

A perturbation that moves one characteristic locus $\lambda_i(L(j\omega))$ at $\omega = \omega_{\rm crit}$ to the point on the real axis corresponds to making the return difference $I - L(j\omega)$ singular at the $\omega = \omega_{\rm crit}$, that is,

$$\det(I - L_p(j\omega_{\rm crit})) = 0, \tag{6}$$

where $L_p$ is the perturbed open-loop system.

The required perturbation $\Delta_{ij}$ is given by

$$\Delta_{ij}(\omega_{\rm crit}) = -\frac{1}{[{\rm RGA}(I - L(j\omega_{\rm crit}))]_{ij}}, \tag{7}$$

where ${\rm RGA}(M) = M \times (M^{-1})^{\rm T}$ and the $\times$ symbol denotes element by element multiplication (Hadamard or Schur product). Thus, elements with relative small values of stabilizing perturbations $|\Delta_{ij}|$ correspond to pairwise interactions that have a large influence on stability and play an important role in destabilizing the equilibrium. In other words, elements with relative small values of stabilizing perturbations $|\Delta_{ij}|$ are sensitive to perturbations, while elements with relative large values of stabilizing perturbations are more robust to perturbations. If all stabilizing perturbations are large, the oscillations of the network will be more robust.

## 3 Results

Adenosine $3', 5'$-cyclic monophosphate (cAMP) oscillations in *Dictyostelium discoideum* cells are necessary for chemotaxis and further development of *Dictyostelium* cells. The model, based on the network depicted in Fig.1, induces spontaneous oscillations in

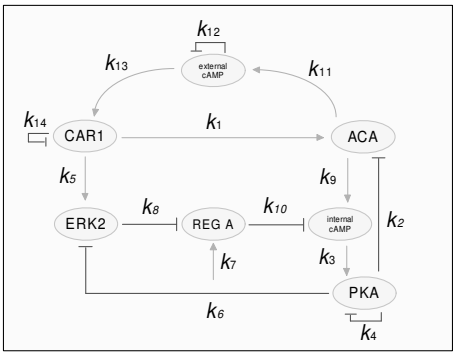


Figure 1: Molecular interactions generating cAMP oscillations in *Dictyotelium discoideum* cells. Components are connected by regulatory interactions that are direct or indirect. Arrows and bar heads indicate positive and negative regulation, respectively. Pulses of cAMP are produced when adenylate cyclase (ACA) is activated after the binding of extracellular cAMP to the surface receptor CAR1. When cAMP accumulates internally, it activates the protein kinase PKA. Ligand-bound CAR1 also activates the MAP kinase ERK2. ERK2 is then inactivated by PKA and no longer inhibits the cAMP phosphodiesterase REG A. A protein phosphatase activates REG A such that REG A can hydrolyse internal cAMP. When REG A hydrolyses the internal cAMP, PKA activity is inhibited by its regulatory subunit, and the activities of both ACA and ERK2 go up.

cAMP observed during the early development of *D. discoideum* [3]. The deterministic dynamics is governed by the following equations:

$$\begin{aligned}
&{\rm d[ACA]}/{\rm d}t = k_1[{\rm CAR1}] - k_2[{\rm ACA}][{\rm PKA}],\\
&{\rm d[PKA]}/{\rm d}t = k_3[{\rm internal\ cAMP}] - k_4[{\rm PKA}],\\
&{\rm d[ERK2]}/{\rm d}t = k_5[{\rm CAR1}] - k_6[{\rm PKA}][{\rm ERK2}],\\
&{\rm d[REG\ A]}/{\rm d}t = k_7 - k_8[{\rm ERK2}][{\rm REG\ A}],\\
&{\rm d[internal\ cAMP]}/{\rm d}t = k_9[{\rm ACA}] - k_{10}[{\rm REG\ A}][{\rm internal\ cAMP}],\\
&{\rm d[external\ cAMP]}/{\rm d}t = k_{11}[{\rm ACA}] - k_{12}[{\rm external\ cAMP}],\\
&{\rm d[CAR1]}/{\rm d}t = k_{13}[{\rm external\ cAMP}] - k_{14}[{\rm CAR1}],
\end{aligned} \tag{8}$$

where $k_i$ $(i = 1, \cdots, 14)$ are kinetic constants. The model is based on the common positive and negative control elements.

For the oscillatory network shown in Fig.1, from the linearization around the underlying equilibrium we obtain $\omega = \omega_{\rm crit} = 0.8560$ rad/min, at which the critical characteristic locus $\lambda_{\rm crit}(L(j\omega))$ crosses the real axis to

the right of the critical point $(1, 0)$. The rank-ordered stabilizing perturbations are shown in Fig.2(a). Even the largest amplitude of stabilizing perturbations is small ($< 0.035$), and it shows the poor robustness properties of the model. Such result is also supported in [4].

Different from the parameter sensitivity analysis used in [2], by which the clues on the importance of individual regulatory processes on the oscillations and relative importance of individual regulatory processes can be directly derived by the linear analysis approach. The parameter sensitivity analysis needs a large amount of computing, as shown in [2], while the relative importance can be easily obtained by the linear analysis approach, as shown in Fig.2(a). For instance, the network shows a higher sensitivity toward perturbations affecting external cAMP. It has shown that constant high levels of external cAMP lead to attenuation, whereas a brief pulse of cAMP can advance or delay the phase such that interaction cells become entrained [3]. The regulation of PKA inhibiting ERK2 enhances the robustness properties, although its role in enhancing robustness is poor. The results show that different regulatory mechanisms are of different importance for the robustness of the network.

From the Lyapunov's indirect method, it follows that local stability of an equilibrium can be determined from the linearization of the system around the equilibrium. The system is locally unstable at the equilibrium if its Jacobian has some eigenvalues in the open right-half plane. Since oscillations can be traced to destabilization of an underlying equilibrium, linear stability analysis can be used to identify mechanisms causing the oscillations by analyzing the destabilizing mechanisms of the underlying equilibrium. Linear analysis, therefore, can also be used to determine the mechanistic basis of the robustness property due to the direct connection between robustness and functionality.

### 3.1 Improvement of robustness

The PKA holoenzyme is composed of two tightly bound regulatory subunits R and two catalytic subunits C. Different from the linear kinetics used in the original model [3], we use second-order kinetics as an approximation of the interactions of two molecules of internal cAMP on each of the two regulatory subunits. Thus, the rate of accumulation of the disassociated catalytic subunit is proportional to the square of the amount of internal cAMP. The catalytic subunits rebind with PKA independently, and hence their rate of removal is assumed to be proportional to the amount

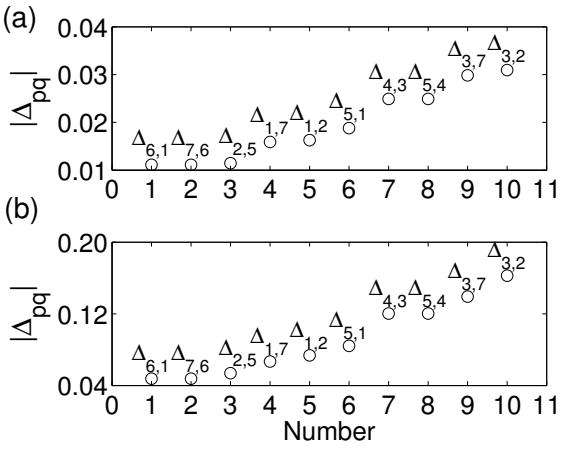


Figure 2: The magnitude of the relative perturbation $|\Delta_{pq}|$ required in element $L_{pq}$ of the open-loop model, i.e. the effect of component $q$ on component $p$ in absence of feedback interactions, so stabilize the closed-loop system, where $x = [x_1, \cdots, x_7]$ represents the concentrations of the seven proteins: $x_1$=[ACA], $x_2$=[PKA], $x_3$=[ERK2], $x_4$=[REG A], $x_5$=[internal cAMP], $x_7$=[external cAMP], and $x_7$=[CAR1]. (a) At the nominal parameter values. (b) At $k_2 = 1.5$ $\text{Mol}^{-1}\text{min}^{-1}$ and $k_3 = 1.6$ $\text{min}^{-1}$, all other parameters at their nominal values.

of catalytic subunit present. The modified form of the dynamics for PKA thus reads

$$\text{d[PKA]}/\text{d}t = k_3[\text{internal cAMP}]^2 - k_4[\text{PKA}]. \quad (9)$$

Note that the changes in the mathematical description capture the specific interactions between the internal cAMP and PKA. Equation (9) and other equations in Equations (8) except the second one define the structure of the modified model.

Still using the monotone control theory, we obtain that the modification of the structural characteristics does not change the number of equilibria. We still use the two sets of parameter values, because the modified model can produce similar oscillations at these values, and obtain $\omega_{\text{crit}} = 1.0545$ rad/min at the nominal parameter values. The perturbations required to stabilize the underlying equilibrium for the modified model at the same two sets of parameter values are shown in Fig.3. We can see that the relative perturbations required are largely increased due to the modification of the structural characteristics. A direct comparison of Fig.2 and Fig.3 indicates that the slight modification of the structural characteristics has a large impact on the robustness property. Even the smallest perturba-

tion required for the modified model is greater than the largest one for the original model.

For the original model, although the magnitude of the perturbations at the second set of parameter values is relatively larger than at the nominal ones, the order keeps unchanged. Although the order changes due to the parameter variations, the order of the most important pairwise interactions corresponding to $L_{2,5}$, $L_{6,1}$, $L_{7,6}$, $L_{5,1}$, and $L_{1,2}$, which involve the components ACA, PKA, REK2, internal cAMP, external cAMP, and CAR1, does not change despite the parameter variations. These components are instrumental to generate oscillations and relatively sensitive to perturbations. The large difference between the magnitude of the relative perturbations for the original and modified models further confirms that the structural characteristics is the major determining factor for robustness properties, although sometimes parameter variations can also have some contribution to them.

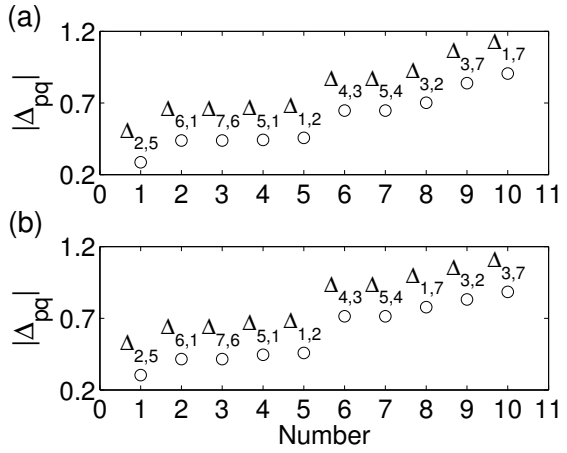


Figure 3: The magnitude of the relative perturbations $|\Delta_{pq}|$ required for the modified model. (a) At the nominal parameter values. (b) At $k_2 = 1.5$ $\text{Mol}^{-1}\text{min}^{-1}$ and $k_3 = 1.6$ $\text{min}^{-1}$, all other parameters at their nominal values.

The improvement of robustness properties by the modification of structural characteristics can be also supported by single parameter robustness analysis. The bifurcation points and degrees of robustness for the modified model are also shown in Table 1. We can see that all of them are largely increased due to the the modification of structural characteristics. it is clear that the single parameter intervals in which stable oscillations occur are largely increased.

Table 1: Comparisons of DOR for the two models

| P | NV | original model | | | modified model | | |
|---|---|---|---|---|---|---|---|
| | | $k_i^l$ | $k_i^u$ | DOR | $k_i^l$ | $k_i^u$ | DOR |
| $k_1$ | 2.0 | 1.92 | $k_{1,\max}$ | 0.04 | 0.64 | 28.22 | 0.68 |
| $k_2$ | 0.9 | 0.72 | 1.60 | 0.20 | 0.08 | 20.18 | 0.91 |
| $k_3$ | 2.5 | $k_{3,\min}$ | 2.76 | 0.09 | $k_{3,\min}$ | 38.96 | 0.94 |
| $k_4$ | 1.5 | $k_{4,\min}$ | 1.58 | 0.05 | $k_{4,\min}$ | 4.64 | 0.68 |
| $k_5$ | 0.6 | 0.54 | $k_{5,\max}$ | 0.10 | $k_{5,\min}$ | $k_{5,\max}$ | 1.00 |
| $k_6$ | 1.0 | 0.10 | 0.86 | 0.07 | 0.00 | 3.24 | 0.75 |
| $k_7$ | 1.0 | $k_{7,\min}$ | 1.10 | 0.09 | $k_{7,\min}$ | 9.64 | 0.90 |
| $k_8$ | 1.3 | 1.18 | $k_{8,\max}$ | 0.09 | 0.34 | $k_{8,\max}$ | 0.74 |
| $k_9$ | 0.3 | 0.60 | 0.32 | 0.06 | $k_{9,\min}$ | 1.18 | 0.75 |
| $k_{10}$ | 0.8 | 0.00 | 0.88 | 0.09 | $k_{10,\min}$ | 7.72 | 0.90 |
| $k_{11}$ | 0.7 | 0.68 | $k_{11,\max}$ | 0.03 | $k_{11,\min}$ | $k_{11,\max}$ | 1.00 |
| $k_{12}$ | 4.9 | 2.64 | 5.18 | 0.05 | 1.52 | 13.38 | 0.63 |
| $k_{13}$ | 23.0 | 22.22 | $k_{13,\max}$ | 0.03 | 9.32 | $k_{13,\max}$ | 0.59 |
| $k_{14}$ | 4.5 | 2.58 | 4.78 | 0.06 | 1.46 | 12.42 | 0.64 |

Remarks. (1): $k_{i,\min} = 0$ and $k_{i,\max} = 90$, $i = 1, \cdots, 14$. (2): Abbreviations: P, parameters; NV, nominal values. (3) $\text{DOR}_i = 1 - \max\left\{p_i^l/p_i, p_i/p_i^u\right\}$, stable limit cycles occur parameter range $(p_i^l, p_i^u)$.

## 4 Conclusion

A linear analysis approach was proposed to study the relative importance of components for robustness. A modification scheme which captures the specific interactions between the internal cAMP and PKA was developed to enhance robustness.

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