

Graphic Analysis of Coupling in Biological Systems

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We have developed a mathematical model in which we apply the graph theory to the problem of coupling in biological systems. Coupling in biological systems is represented as a network of digraph. We introduce a new “graphic transformation method”, which is an extension of the graphic method of King & Altman (1956). We obtain the expression of the steady-state distribution of the coupled system as an extension of the Boltzmann distribution. We introduce the “coupling ratio” as a quantity which represents the direction in which chemical reaction proceeds. It is suggested that the largeness of the coupling ratio is closely related to the largeness of deviation from the Boltzmann distribution.

1. Introduction

Coupling is an essential problem in biological systems. In the literature, in general, “coupling” is understood in the following sense.

- (i) A certain path in the reaction network (path 1) involves an increase in the free energy, so this path cannot proceed by itself.
- (ii) Another path in the reaction network (path 2) involves a decrease in the free energy, so this path can proceed spontaneously.
- (iii) When the two paths (path 1 and path 2) are “coupled”, and the total free energy (path 1 + path 2) decreases, path 1 can proceed.

In the above definition, it is usually assumed that the two pathways “coupled” are in close vicinity in the reaction network. However, this is not necessarily the case, as can be seen in Fig. 1. It is to be noted that two pathways which are distant in the reaction network can also be “coupled”.

If two pathways need not be close to each other in order to be “coupled”, what is the condition for coupling to occur? “Coupling” is a concept we employ to explain the direction in which a chemical reaction proceeds. It is therefore important to understand the mechanism which determines the direction of the chemical reaction.

In this paper we first apply the graphic analysis method of King & Altman (1956) and the “graphic transformation method” (Mogi, 1992*a*) to obtain the steady-state distribution of the coupled system as an extension of the Boltzmann distribution.

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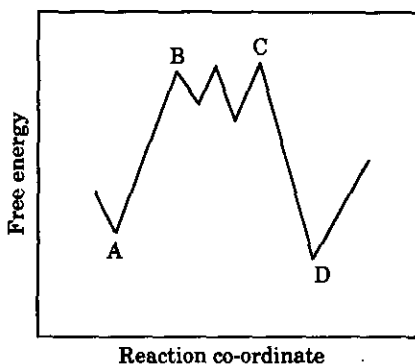


FIG. 1. Coupling as a global property of the reaction system. This figure shows that "coupling" is not necessarily a "local" mechanism involving only these steps that are in close vicinity to each other. In the figure above, the step $A \rightarrow B$ involves an increase in the free energy, and the step $C \rightarrow D$ involves a decrease in the free energy. The transition of the system is in the direction of $A \rightarrow D$, as the free energy level of D is lower than that of A. The step $A \rightarrow B$ cannot proceed by itself. However, because of the existence of the step $C \rightarrow D$, the step $A \rightarrow B$ can proceed. We can therefore say that the step $A \rightarrow B$ is "coupled" with the step $C \rightarrow D$, although the two steps are distant in the reaction network. This example shows that "coupling" is not necessarily a "local" mechanism involving only these steps that are in close vicinity to each other.

The deviation from the Boltzmann distribution is explicitly given. We then introduce the "coupling ratio" as a quantity that represents the direction in which a chemical reaction proceeds. Finally, we suggest that the "coupling ratio" and the deviation from the Boltzmann distribution are closely related.

2. Theory

FORMULATION OF THE MODEL

In this section, we present the mathematical model which can be used to analyze "coupling" in biological systems.

We prepare a two-dimensional plane with the x - and y -axes representing the two degrees of freedom that are "coupled". The chemical states are represented as points on the plane. The chemical reaction is expressed as a series of transitions between the states.

We assume that the system is provided with a large reservoir of chemical elements involved in the coupling. Under this assumption, the system returns to the same state when one unit of reaction is completed in either of the two degrees of freedom coupled in the reaction. We call this property the translational invariance of the system. Therefore, a unit of repetition can be constituted from one unit of translation in the directions of x - and y -axes. We call this unit a "cell".

In a steady state, transitions between the states can be expressed as directed edges connecting the states, with the largeness of flow assigned to it. Namely, the system is expressed as a network of directed graph (digraph) (Fig. 2).

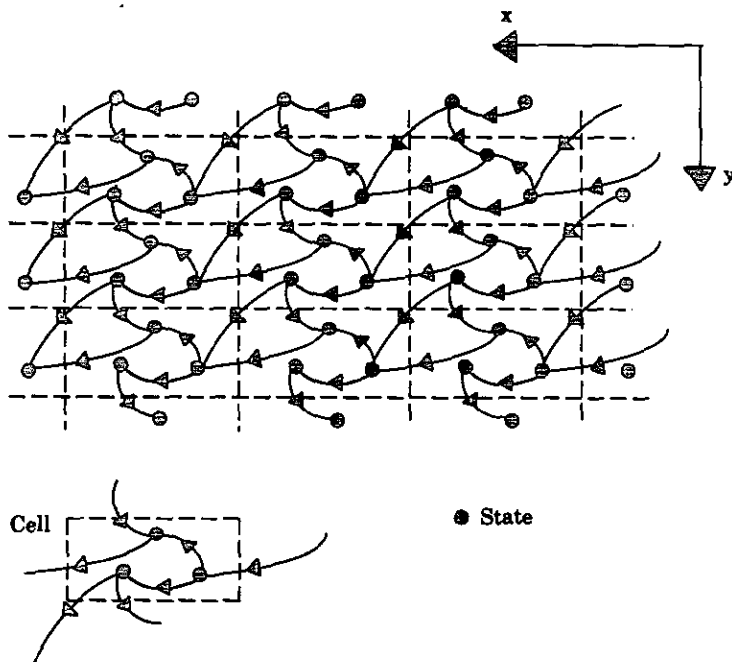


FIG. 2. Directed graph on a two-dimensional plane. We express the system as a directed graph on a two-dimensional plane. The x - and y -axes stand for the degrees of freedom that are "coupled" in the reaction. If the system is provided with a large reservoir of chemical elements involved in the coupling, we can assume that the system returns to the same state when one unit of reaction occurs in the x - or y -direction. From one unit of translation in the x - and y -axes we can construct a "cell", a unit of repetition on the plane representing the coupled reaction. The states can be expressed as points in a cell. We assume that the system is in a steady state. Transitions between the states are expressed as directed arcs that connect the points. The system is now equivalent to a directed graph (digraph). In this figure, the number of states in a cell is 3.

We assume that there are N states within a cell. To each cell, we assign a coordinate (x, y) , where x and y are integers. The states therefore can be expressed as $S(i, x, y)$. We further assume that the free energy level changes by E_x and E_y , respectively, with the translation of one unit in the x and y direction. Therefore, the state $S(i, x, y)$ has the free energy level of

$$E(i) + xE_x + yE_y. \quad (1)$$

Note that the free energy gradients E_x and E_y are functions of the concentrations of the chemical species in the reservoir.

We express the rate constants of these transitions as $K(i, j, x, y)$, which corresponds to the transition from state $S(j, z_1, z_2)$ to state $S(i, z_1 + x, z_2 + y)$, " z_1 " and " z_2 " denoting arbitrary integers.

In the following arguments, we assume that $K(i, j, x, y)$ is zero unless both x and y are within the range of $[-1, 1]$. In other words, we assume that transitions occur

only between states within the same cell or between states in adjacent cells. This is in accordance with the usual situation encountered in coupling.

Assuming that the transition rate constants between the states are Arrhenius type, we can write down the relations

$$\frac{K(i, j, x, y)}{K(j, i, -x, -y)} = \exp\left(\frac{-E(i) + E(j) - xE_x - yE_y}{kT}\right). \quad (2)$$

The states $S(i, z_1, z_2)$ (z_1 and z_2 denoting arbitrary integers) all have the same population, owing to the translational invariance of the system. We therefore write the states $S(i, z_1, z_2)$ as $S(i)$, omitting the indices z_1 and z_2 . We write the probability that the system stays in the state $S(i)$ as $P(i)$. The normalization condition is

$$\sum_i P(i) = 1. \quad (3)$$

The condition for the steady state can be written by the balance equation

$$\sum_j \sum_x \sum_y (K(i, j, x, y)P(j) - K(j, i, x, y)P(i)) = 0. \quad (4)$$

The steady-state distribution of the states is obtained as the solution for eqns (2-4).

Let us express the vector (the "steady flow vector") which represents the direction in which the reaction proceeds as

$$V = (V_x, V_y).$$

The x - and y -component of the steady flow vector can be written as

$$V_x = \sum_i \sum_j \sum_x \sum_y xK(i, j, x, y)P(j) \quad (5)$$

$$V_y = \sum_i \sum_j \sum_x \sum_y yK(i, j, x, y)P(j). \quad (6)$$

As the total free energy change must be negative, we can write the condition

$$E_x V_x + E_y V_y < 0. \quad (7)$$

Under the condition (7), it is interesting to consider the "coupling ratio", which is defined as

$$\frac{V_x}{V_y} = \frac{\sum_i \sum_j \sum_x \sum_y xK(i, j, x, y)P(j)}{\sum_i \sum_j \sum_x \sum_y yK(i, j, x, y)P(j)}. \quad (8)$$

In most biologically important coupled reactions, "coupling" is used to drive a reaction which is otherwise energetically unfavorable. We can assume that the reaction represented by x is the unfavorable reaction (i.e. $E_x > 0$) and the reaction represented by y is the reaction which drives the coupled reaction (i.e. $E_y < 0$) without a loss of generality. The coupling ratio (8) then represents the efficiency the free energy gradient in the y -degree of freedom is used to drive the reaction in the x -degree of freedom.

REPRESENTATION OF THE SYSTEM AS A GENERALIZATION OF AN EQUILIBRIUM STATE (GRAPHIC TRANSFORMATION METHOD)

Now we apply the graphic analysis method (graphic transformation method) to the system formulated in the previous section.

The system in question has a "Boltzmann distribution" (see, for example, Ishihara, 1971) when the free energy gradients E_x and E_y are zero. In the general case, the distribution satisfying eqn (4) is non-Boltzmann. However, the system approaches the equilibrium when the free energy gradients E_x and E_y approach zero. Therefore, it is interesting to ask if the system can be treated as a generalization of a system in equilibrium. Specifically, we would like to give the distribution of the states as an extension of the Boltzmann distribution.

Our task is to obtain the solution for eqns (2-4).

If we take the summations over the indices x and y and write them as

$$K(i, j) = \sum_x \sum_y K(i, j, x, y) \tag{9}$$

eqn (4) can be rewritten as

$$\sum_j K(i, j)P(j) - K(j, i)P(i) = 0. \tag{10}$$

The general solution for eqn (10) can be written by the Cramer's Rule

$$P(i) = \frac{w(i)}{\sum_i w(i)}$$

where

$$w(i) = \begin{vmatrix} \sum_j K(1, j) & -K(2, 1) & \cdots & 0 & \cdots & -K(N, 1) \\ -K(1, 2) & \sum_j K(2, j) & \cdots & 0 & \cdots & -K(N, 2) \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ 1 & 1 & \cdots & 1 & \cdots & 1 \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ -K(1, N) & -K(2, N) & \cdots & 0 & \cdots & \sum_j K(N, j) \end{vmatrix} \leftarrow \text{the } i\text{-th row.}$$

the i -th column
↓

Equation (11) is equivalent to the graphic expression by Kii_B & Altman (1956) (reviewed, for example, in Chou, 1990)

$$w(i) = \sum_m \prod_{g(i(m))} K(p, q), \tag{12}$$

where

$$\prod_{g_i(m)} K(p, q)$$

denotes the product of rate constants for the spanning in-tree $g_i(m)$, which has the state $S(i)$ as the sink. The summation is taken over $m = 1, 2, 3, \dots, n_g$, where n_g is the number of spanning in-trees which has the state $S(i)$ as the sink. We have expressed the pairs of vertices which appear in $g_i(m)$ as (p, q) .

We now apply the “graphic transformation method” (Mogi, 1992a) to obtain an expression which expresses the state distribution of the system as an extension of the Boltzmann distribution. (For an introduction to the basic concepts of graph theory, see, for example, Chou *et al.*, 1979.)

In the graphic transformation method, we “normalize” the expression (12) by the spanning in-trees which has a standard state (which is taken arbitrarily) as the sink.

How can this “normalization” be accomplished? Let us take the state $S(1)$ as the standard state for normalization. Now let us normalize the weight $w(i)$. When we compare the spanning in-trees $g_i(m)$ and $g_1(m)$, the only difference between them is in the path between $S(1)$ and $S(i)$ (Fig. 3). The edges belonging to this path have opposite directions for $g_i(m)$ and $g_1(m)$. We express the subgraph connecting the states $S(1)$ and $S(i)$ by $p_{ji}(m)$ for $g_1(m)$, and $p_{ij}(m)$ for $g_i(m)$. In order to transform $g_i(m)$ into $g_1(m)$, it is necessary and sufficient to reverse the directions of the directed edges of the subgraph $p_{ij}(m)$. In accordance with this graphic transformation, the rate constants $K(p, q)$ corresponding to the edges belonging to the subgraph $p_{ij}(m)$ in the expression (12) should be transformed.

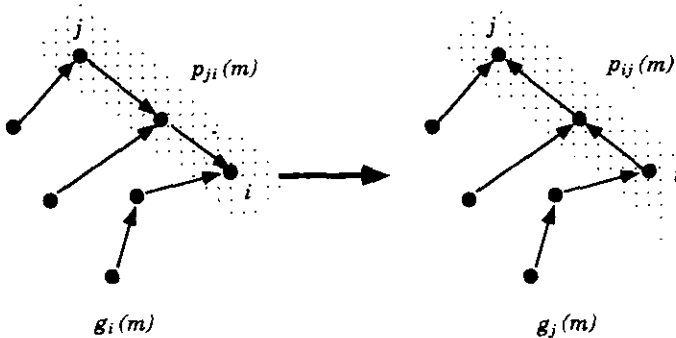


FIG. 3. Transformation of a spanning in-tree (graphic transformation method). In the graphic transformation method, the graphic expression of the weight of the states is transformed into a standard form which is expressed as the summation over spanning in-trees with the standard state as the sink. In this figure, we illustrate the transformation of a spanning in-tree $g_j(m)$, which has the state j as the sink, into $g_i(m)$; which has the state i as the sink. When we compare $g_i(m)$ and $g_j(m)$, the only difference is in the path that connects the states i and j . This path corresponds to the subgraph $p_{ij}(m)$ for $g_i(m)$, and $p_{ji}(m)$ for $g_j(m)$. As there is by definition no loop in a tree, there is only one path that connects the states i and j . The transformation of $g_i(m)$ into $g_j(m)$ is accomplished by the reversal of the directions of the directed edges of the subgraph $p_{ij}(m)$ of the spanning in-tree $g_i(m)$, to obtain $p_{ji}(m)$, which is a subgraph of the spanning in-tree $g_j(m)$.

By considering these transformations, we can obtain the general distribution of the states as an extension of the Boltzmann distribution. Details of the derivation procedure are given in Appendix A.

To conclude, the weight $w(i)$ for the i -th state is given by the graphic transformation method as

$$w(i) = \exp\left(\frac{-E(i)}{kT}\right) \frac{\sum_j \sum_m g_j(m) \prod_{p_{ij}(m)} K(p, q) \prod_{p_{ij}(m)} K_t(p, q)}{\sum_j \sum_m \prod_{g_j(m)} K(p, q)}, \quad (13)$$

where $K_t(p, q)$ are the transformed rate constants defined as

$$K_t(p, q) = \sum_x \sum_y K(p, q, x, y) \exp\left(\frac{xE_x + yE_y}{kT}\right). \quad (14)$$

The quantity

$$\frac{\sum_j \sum_m \prod_{g_j(m) - p_{ij}(m)} K(p, q) \prod_{p_{ij}(m)} K_t(p, q)}{\sum_j \sum_m \prod_{g_j(m)} K(p, q)} \quad (15)$$

gives the largeness of deviation from the Boltzmann distribution for the j -th state.

As shown in Appendix A, eqn (13) reduces to the Boltzmann distribution when E_x and E_y are zero, as expected.

In conclusion, we have derived a general solution of the state distribution of the system we are treating, which expresses the distribution as a generalization of the Boltzmann distribution.

EVALUATION OF THE COUPLING RATIO UNDER THE FREE LOAD CONDITION

In this section, we evaluate the largeness of the "coupling ratio" defined in eqn (8). The "coupling ratio", as has been already mentioned, is related to the efficiency of driving an otherwise unfavorable reaction, and is an important factor in understanding the nature of coupling.

In this paper we restrict our analysis to the special case when the distribution of states is given by the Boltzmann distribution. This case is of particular interest, as in the previous section we have expressed the distribution of states as an extension of the Boltzmann distribution.

We define the "free load condition", as the case where $E_x = 0$ (cf. Harada *et al.*, 1990). In the "free load condition", we assume that no (i.e. minimum) unfavorable free energy gradient exists for the degree of freedom driven by coupling. The "free load condition" is therefore a good testing case for investigating the efficiency of coupling.

When the distribution of the states is the Boltzmann distribution, the coupling ratio [eqn (8)] can be written as

$$\frac{V_x}{V_y} = \frac{\sum_i \sum_j \sum_x \sum_y x K(i, j, x, y) \exp\left(\frac{-E(j)}{kT}\right)}{\sum_i \sum_j \sum_x \sum_y y K(i, j, x, y) \exp\left(\frac{-E(j)}{kT}\right)} \quad (16)$$

By a mathematical procedure detailed in Appendix B, it can be shown that when the distribution of the states is given by the Boltzmann distribution, the maximum value of the coupling ratio under the free load condition is 1.

On the other hand, we can easily show that the coupling ratio under the free load condition can be at least as large as N (number of states in a cell). An example of such a case is given in Fig. 4.

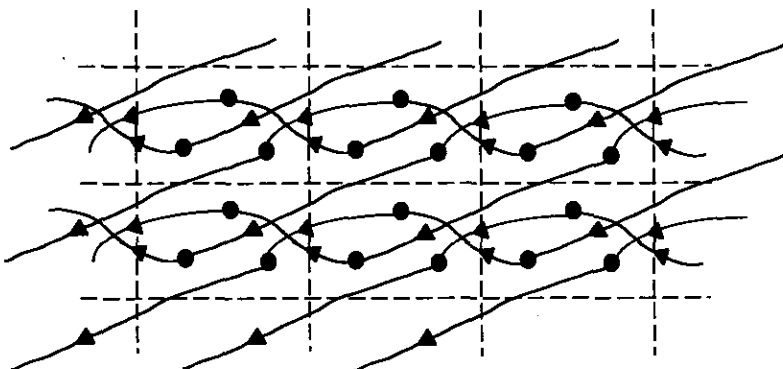


FIG. 4. An example of a directed graph with the coupling ratio $V_x/V_y = N$. This figure shows a directed graph for which the coupling ratio V_x/V_y is equal to N , the number of states in a cell. In the directed graph above, $N=3$, and the coupling ratio $V_x/V_y=3$, as can be found by tracing the directed arcs that connect the states. It is assumed that $E_x=0$ (free load condition) and $E_y < 0$.

We conclude that in the region where the coupling ratio is between 1 and N , there is a non-zero deviation of the state distribution from the Boltzmann distribution. In other words, the coupling ratio (i.e. the efficiency of the driving of an otherwise unfavorable reaction) is related to the deviation of the state distribution from the Boltzmann distribution. We are currently investigating this relation in more detail.

It is to be noted that the case $E_x=0$ and $E_y=0$ (i.e. no free energy gradient) is not the only case where a Boltzmann distribution is realized. For example, if the rate constants $K(i, j, x, y)$ can be written as the product of two independent rate constants

$$K(i, j, x, y) = Ka(i, j)Kb(x, y) \quad (17)$$

we can show that the state distribution is given by the Boltzmann distribution.

3. Discussion

In this paper we have formulated a graphic method which treats coupling in biological systems in a general formalism.

Graphic method initiated by King & Altman (1956) has been extended in various aspects. Chou (Chou *et al.*, 1979; Chou, 1989, 1990) extended the method to the analysis of non-steady-state kinetics and protein-folding kinetics, and also presented some new rules which are valuable in actually performing the calculation by the graphic method. Zhou & Deng (1984) extended the graphic rules of Chou. Mazur & Kuchinski (1992) applied the method to steady-state enzyme kinetics which avoids using the mass action law. See also Volkenstein & Goldstein (1966) and Mazur (1990). [Note also that graph theory has been applied to other areas of biology, for example, protein structure (Mitchell *et al.*, 1989).] In this paper we introduced a graphic transformation method, which is an extension of the previous works that applied graph theory to enzyme kinetics. The graphic transformation method has also been applied to the analysis of asymmetric Boltzmann machines (Mogi, 1992b).

We have shown that in the system we have formulated, the distribution of the states can be written as an extension of the Boltzmann distribution. In general, there exists a deviation from the Boltzmann distribution, and the principle of detailed balancing (Bridgman, 1928) does not hold (for a discussion on the relation between the principle of detailed balancing and the graphic method, refer, for example, to Mazur & Kuchinski, 1992). In other words, in the system examined in this paper, free energy levels of the states cannot be defined in the sense of a system in equilibrium.

It is interesting to consider the implication of these facts for the study of enzyme kinetics. In the experimental and theoretical treatments of enzyme kinetics, it is usually assumed that there are definite free energy levels for each quasistable state. The states which appear in the experimental and theoretical treatments of enzyme kinetics can be interpreted, in the formalism of this paper, to be the degenerate expression $S(i)$ standing for the states $S(i,x,y)$, where x and y are arbitrary integers. As we have seen, the free energy levels for these states cannot be defined in the general case. Therefore, the "free energy levels" as used in the experimental and theoretical treatments of enzyme kinetics should be reinterpreted from this point of view.

The quantity "coupling ratio" defined in this paper corresponds to the "step size" in muscle contraction (Yanagida *et al.*, 1985; Harada *et al.*, 1990). Our argument concerning the upper limit of coupling ratio under the free load condition is expected to be relevant to the problem of "step size" in muscle contraction.

We stress the view that "coupling" as used in the literature can sometimes be misleading. For example, in the case of muscle contraction, "coupling" cannot necessarily be located to the step of phosphate release. Although it cannot be denied that in the case of muscle contraction the phosphate release step is the most important step in the network involving the largest decrease of free energy, it is the geometrical property of the network of the directed graph representing the system that determines the direction in which reaction proceeds.

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APPENDIX A

Derivation of the State Distribution as a Generalization of the Boltzmann Distribution (Graphic Transformation Method)

In order to obtain the state distribution as a generalization of the Boltzmann distribution, we first "normalize" the graphic expression (12) of weight $w(i)$ ($i = 1, 2, 3, \dots, N$) by the spanning in-trees contributing to the graphic expression of $w(1)$. Namely, we transform the spanning in-trees $g_i(m)$ ($m = 1, 2, 3, \dots, n_g$) into spanning in-trees $g_1(m)$ ($m = 1, 2, 3, \dots, n_g$). This can be accomplished by the reversal of the directed edges belonging to $p_{1i}(m)$, which is a subgraph of $g_i(m)$ connecting $S(1)$ and $S(i)$ (Fig. 3).

Let us express the vertices belonging to $p_{1i}(m)$ by $\{a(s); s = 0, 1, 2, \dots, n_p\}$, where $a(0)$ denotes $S(i)$, and $a(n_p)$ denotes $S(1)$. We now reverse the direction of the directed edges belonging to $p_{1i}(m)$, to obtain $p_{i1}(m)$. This is always possible, as $p_{1i}(m)$ and $p_{i1}(m)$ share the same underlying graph.

The part of quantity (12) that is transformed is

$$\prod_{p_{ii}(m)} K(a(s), a(s+1)). \tag{A.1}$$

By using eqn (2), $K(a(s), a(s+1))$ can be transformed as

$$\begin{aligned} K(a(s), a(s+1)) &= \sum_x \sum_y K(a(s), a(s+1), x, y) \\ &= \sum_x \sum_y K(a(s+1), a(s), -x, -y) \\ &\quad \times \exp\left(\frac{-E(a(s)) + E(a(s+1)) - xE_x - yE_y}{kT}\right) \\ &= \sum_x \sum_y K(a(s+1), a(s), x, y) \\ &\quad \times \exp\left(\frac{-E(a(s)) + E(a(s+1)) + xE_x + yE_y}{kT}\right). \end{aligned} \tag{A.2}$$

If we define $Kl(a(s+1), a(s))$ as

$$Kl(a(s+1), a(s)) = \sum_x \sum_y K(a(s+1), a(s), x, y) \exp\left(\frac{x E_x + y E_y}{kT}\right) \tag{A.3}$$

eqn (A.2) can be written as

$$K(a(s), a(s+1)) = \exp\left(\frac{-E(a(s)) + E(a(s+1))}{kT}\right) Kl(a(s+1), a(s)). \tag{A.4}$$

The product (A.1) is then transformed as

$$\begin{aligned} \prod_{p_{ii}(m)} K(a(s), a(s+1)) &= \prod_{p_{ii}(m)} \exp\left(\frac{-E(a(s)) + E(a(s+1))}{kT}\right) Kl(a(s+1), a(s)) \\ &= \exp\left(\frac{-E(a(0)) + E(a(n_p))}{kT}\right) \prod_{p_{ii}(m)} Kl(a(s+1), a(s)) \\ &= \exp\left(\frac{-E(i) + E(1)}{kT}\right) \prod_{p_{ii}(m)} Kl(a(s+1), a(s)) \end{aligned} \tag{A.5}$$

where we have replaced $p_{li}(m)$ by $p_{ii}(m)$.

Note that the exponential terms cancel except for the states on both ends of the path $p_{ii}(m)$ [which are $S(i)$ and $S(1)$]. This is the most important property of the graphic transformation method.

Finally, $w(i)$ is expressed as

$$\begin{aligned} w(i) &= \sum_m \prod_{g_1(m)} K(p, q) \\ &= \sum_m \prod_{g_1(m)-p_{1i}(m)} K(p, q) \prod_{p_{1i}(m)} K(p, q) \\ &= \exp\left(\frac{-E(i)+E(1)}{kT}\right) \sum_m \prod_{g_1(m)-p_{1i}(m)} K(p, q) \prod_{p_{1i}(m)} Kt(a(s+1), a(s)) \quad (\text{A.6}) \end{aligned}$$

where

$$\prod_{g_1(m)-p_{1i}(m)} K(p, q)$$

is the product of rate constants over $g_1(m)$ excluding the subgraph $p_{1i}(m)$. As $g_1(m)-p_{1i}(m)$ and $g_1(m)-p_{1j}(m)$ are equivalent, we have substituted $g_1(m)-p_{1i}(m)$ by $g_1(m)-p_{1j}(m)$ in the derivation of (A.6).

Since the expression for $w(1)$ is the same as (12), the weight $w(i)$ normalized by the state $S(1)$ can be written as

$$w(i) = \exp\left(\frac{-E(i)}{kT}\right) \sum_m \prod_{g_1(m)-p_{1i}(m)} K(p, q) \prod_{p_{1i}(m)} Kt(a(s+1), a(s)). \quad (\text{A.7})$$

Equation (A.7) expresses the distribution of the states as a deviation from the Boltzmann distribution.

In order to derive an expression that is symmetric in the index i , we obtain similar expressions corresponding to (A.7) by performing the same procedure with the states $S(2), S(3), \dots, S(N)$ taken as the sinks of the standard spanning in-trees used for normalization. Then we take the sum of the N normalized expressions of $w(i)$. Finally, we obtain

$$w(i) = \exp\left(\frac{-E(i)}{kT}\right) \sum_j \sum_m \prod_{g_j(m)-p_{ij}(m)} K(p, q) \prod_{p_{ij}(m)} Kt(a(s+1), a(s)). \quad (\text{A.8})$$

We can substitute $(a(s), a(s+1))$ by (p, q) , and divide the right side of eqn (A.8) by a normalization factor to obtain

$$w(i) = \exp\left(\frac{E(i)}{kT}\right) \frac{\sum_j \sum_m \prod_{g_j(m)-p_{ij}(m)} K(p, q) \prod_{p_{ij}(m)} Kt(p, q)}{\sum_j \sum_m \prod_{g_j(m)} K(p, q)} \quad (\text{A.9})$$

where (p, q) denotes the pairs of vertices belonging to the subgraphs over which products are taken.

In conclusion, we have obtained the distribution of the states as a generalization of the Boltzmann distribution.

The distribution (A.9) should reduce to the Boltzmann distribution when we put $E_x=0$ and $E_y=0$. We can confirm this by noting that when $E_x=0$ and $E_y=0$,

$Kt(a(s+1), a(s))$ defined in eqn (A.3) reduces to

$$\begin{aligned}
 Kt(a(s+1), a(s)) &= \sum_x \sum_y K(a(s+1), a(s), x, y) \exp\left(\frac{x \cdot 0 + y \cdot 0}{kT}\right) \\
 &= K(a(s+1), a(s))
 \end{aligned}
 \tag{A.10}$$

$w(i)$ then becomes

$$\begin{aligned}
 w(i) &= \exp\left(\frac{-E(i)}{kT}\right) \frac{\sum_j \sum_m \prod_{g_j(m)-p_j(m)} K(p, q) \prod_{p_j(m)} Kt(p, q)}{\sum_j \sum_m \prod_{g_j(m)} K(p, q)} \\
 &= \exp\left(\frac{-E(i)}{kT}\right) \frac{\sum_j \sum_m \prod_{g_j(m)-p_j(m)} K(p, q) \prod_{p_j(m)} K(p, q)}{\sum_j \sum_m \prod_{g_j(m)} K(p, q)} \\
 &= \exp\left(\frac{-E(i)}{kT}\right)
 \end{aligned}
 \tag{A.11}$$

which indeed gives the Boltzmann distribution.

APPENDIX B

Evaluation of the Maximum Value of the Coupling Ratio under the Free Load Condition when the State Distribution is given by the Boltzmann Distribution

We define the rate constants $Ks(i, j, x, y)$ as

$$Ks(i, j, x, y) = K(i, j, x, y) \exp\left(\frac{-E(j)}{kT}\right)
 \tag{B.1}$$

which satisfy the relations

$$\begin{aligned}
 \frac{Ks(i, j, x, y)}{Ks(j, i, -x, -y)} &= \frac{K(i, j, x, y)}{K(j, i, -x, -y)} \exp\left(\frac{-E(j) + E(i)}{kT}\right) \\
 &= \exp\left(\frac{-xE_x - yE_y}{kT}\right).
 \end{aligned}
 \tag{B.2}$$

We transform V_x as

$$\begin{aligned}
 V_x &= \sum_i \sum_j \sum_x \sum_y xK(i, j, x, y)P(j) \\
 &= \sum_i \sum_j \sum_x \sum_y xK(i, j, x, y) \exp\left(\frac{-E(j)}{kT}\right) \\
 &= \sum_i \sum_{j \neq i} \sum_x \sum_y xKs(i, j, x, y) + \sum_i \sum_{j=i} \sum_x \sum_y xKs(i, j, x, y)
 \end{aligned}$$

$$\begin{aligned}
&= \sum_i \sum_{j < i} \sum_x \sum_y x \{ Ks(i, j, x, y) + Ks(j, i, x, y) \} \\
&\quad + \sum_i \sum_{j=i} \sum_x \sum_y x Ks(i, j, x, y) \\
&= \sum_i \sum_{j < i} \sum_x \sum_y x \{ Ks(i, j, x, y) - Ks(j, i, -x, -y) \} \\
&\quad + \sum_i \sum_{j=i} \sum_x \sum_y \frac{x}{2} \{ Ks(i, j, x, y) - Ks(j, i, -x, -y) \} \\
&= \sum_i \sum_{j < i} \sum_x \sum_y x Ks(i, j, x, y) \left\{ 1 - \exp\left(\frac{x E_x + y E_y}{kT}\right) \right\} \\
&\quad + \sum_i \sum_{j=i} \sum_x \sum_y \frac{x}{2} Ks(i, j, x, y) \left\{ 1 - \exp\left(\frac{x E_x + y E_y}{kT}\right) \right\}. \tag{B.3}
\end{aligned}$$

In the expression (B.3), we have omitted the normalization factor in $P(j)$, which is common for the expressions of V_x and V_y , as we are interested only in the ratio of V_x to V_y .

If we redefine $Ks(i, i, x, y)$ as

$$Ks(i, i, x, y) = \frac{1}{2} Ks(i, i, x, y)$$

V_x can be written as

$$V_x = \sum_i \sum_{j \leq i} \sum_x \sum_y x Ks(i, j, x, y) \left\{ 1 - \exp\left(\frac{x E_x + y E_y}{kT}\right) \right\}. \tag{B.4}$$

By a similar procedure, V_y can be written as

$$V_y = \sum_i \sum_{j \leq i} \sum_x \sum_y y Ks(i, j, x, y) \left\{ 1 - \exp\left(\frac{x E_x + y E_y}{kT}\right) \right\}. \tag{B.5}$$

Under the free load condition $E_x = 0$, the coupling ratio can be written as

$$\frac{V_x}{V_y} = \frac{\sum_i \sum_{j \leq i} \sum_x \sum_y x Ks(i, j, x, y) \left\{ 1 - \exp\left(\frac{y E_y}{kT}\right) \right\}}{\sum_i \sum_{j \leq i} \sum_x \sum_y y Ks(i, j, x, y) \left\{ 1 - \exp\left(\frac{y E_y}{kT}\right) \right\}}. \tag{B.6}$$

We define $Ks(x, y)$ as

$$Ks(x, y) = \sum_i \sum_{j \leq i} Ks(i, j, x, y). \tag{B.7}$$

Now we write V_x and V_y explicitly

$$\begin{aligned}
 V_x &= K_S(1, 1) \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} + K_S(1, -1) \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\} \\
 &\quad - K_S(-1, 1) \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} - K_S(-1, -1) \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\} \\
 V_y &= \{ K_S(1, 1) + K_S(0, 1) + K_S(-1, 1) \} \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} \\
 &\quad - \{ K_S(1, -1) + K_S(0, -1) + K_S(-1, -1) \} \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\}. \quad (B.8)
 \end{aligned}$$

In the system we are treating, we have assumed that the reaction proceeds in the direction y increases. Therefore, we can assume that $E_y < 0$ without any loss of generality. When $E_y < 0$,

$$1 - \exp\left(\frac{E_y}{kT}\right) > 0 \quad \text{and} \quad 1 - \exp\left(\frac{-E_y}{kT}\right) < 0. \quad (B.9)$$

As $K_S(x, y) \geq 0$, we can evaluate the signature of the terms in the expressions of V_x and V_y .

In the expression of V_x ,

$$K_S(1, 1) \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} \quad \text{and} \quad -K_S(-1, -1) \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\} \quad \text{are positive,}$$

$$K_S(1, -1) \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\} \quad \text{and} \quad -K_S(-1, 1) \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} \quad \text{are negative.}$$

On the other hand, the terms in the expression of V_y are all positive.

We note that the rate constants $K_S(x, y)$ are independent, as we have already paired the related rate constants and expressed them as one in the procedure from eqn (B.1) to eqn (B.7).

Therefore, coupling ratio V_x/V_y is maximum when

$$K_S(1, -1) \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\} = 0$$

$$K_S(-1, 1) \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} = 0$$

$$\begin{aligned}
 K_S(0, 1) \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} &= 0 \\
 K_S(0, -1) \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\} &= 0.
 \end{aligned}
 \tag{B.10}$$

The maximum value of coupling ratio can be obtained as

$$\begin{aligned}
 \frac{V_x}{V_y} &\leq \frac{K_S(1, 1) \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} - K_S(-1, -1) \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\}}{K_S(1, 1) \left\{ 1 - \exp\left(\frac{E_y}{kT}\right) \right\} - K_S(-1, -1) \left\{ 1 - \exp\left(\frac{-E_y}{kT}\right) \right\}} \\
 &= 1.
 \end{aligned}
 \tag{B.11}$$

In conclusion, we have shown that the maximum value of coupling ratio under free load condition is 1, when the distribution of states satisfies the Boltzmann distribution.