Effects of thermal fluctuation and the receptor-receptor interaction in bacterial chemotactic signaling and adaptation

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Bacterial chemotaxis is controlled by the conformational changes of the receptors in response to the change of the ambient chemical concentration. In a statistical mechanical approach, the signaling due to the conformational changes is a thermodynamic average quantity, dependent on the temperature and the total energy of the system, including both ligand-receptor interaction and receptor-receptor interaction. This physical theory suggests to biology an understanding of cooperation in ligand binding and receptor signaling problems. How much experimental support of this approach can be obtained from the currently available data? What are the parameter values? What is the practical information for experiments? Here we make comparisons between the theory and recent experimental results. Although currently comparisons can only be semiquantitative or qualitative, consistency is clearly shown. The theory also helps to sort a variety of data.

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I. INTRODUCTION

Bacterial chemotaxis refers to the phenomenon that a bacterium such as *Escherichia coli* swims towards a higher concentration of attractant or a lower concentration of repellent [1–4]. With the switching rate determined by the change of the ambient chemical concentration, the motors of the bacterium switch between counterclockwise and clockwise rotations, consequently the bacterium switches between tumbling and running. The ratio between the frequencies of the two rotation modes is determined by the rate at which kinase CheA phosphorylates CheY, which binds the base of a motor. CheA phosphorylation rate is regulated by the receptor conformational state, which is influenced by ligand binding. The receptors are dimeric and joined to a CheA dimer by a CheW dimer, furnishing a signaling complex. Hence a receptor dimer can be regarded as a basic unit, as supported by the finding that a receptor dimer with a damaged subunit can still work [5]. Because of thermal fluctuation, even in the absence of ligand binding or in a fully adapted situation, there is still a certain probability distribution for a receptor dimer to be in different conformational states; microscopically a receptor dimer stochastically flips between the two states. Attractant binding changes the probability distribution, causing the receptor dimer to be more likely in the state corresponding to the lower CheA phosphorylation rate. On a longer time scale, after an initial response to the ligand concentration change, the activity of the system returns to the prestimulus level. A careful consideration of such a basic picture already finds the ideas of statistical mechanics necessary: with the presence of thermal fluctuation, it is the probability distribution of the conformational states of the receptors that is monitored by ligand concentration change and determines the motor rotation bias. However, it seems that this point is not universally appreciated in biological literature.

The chemotactic response is very sensitive [6]. It had been conjectured that there might be cooperation between receptors or the signaling complexes so that the signal could be amplified [7,3]. The fact that most of the receptors cluster together at a pole of the cell provides further clues for cooperation between receptors [8,9]. More importantly, it was found experimentally that the clustering of receptors is not favorable for counting statistics and that the receptor cluster does not favor a special end of the cell [10]. This is an indication that there is a special reason for clustering, which may well be to make the receptor-receptor interaction possible.

With a detailed analysis on the experimental findings, I suggested the possible existence of interaction between neighboring receptor dimers and constructed a statistical mechanical theory to provide a picture of how the receptors cooperate through physical interaction and how the thermal fluctuation makes statistical mechanics important in the signaling process [11,12]. In our model, we combine cooperativity and feedback to account for the sensitivity and adaptation. As will be stressed here, the first message from our approach is an emphasis on thermal fluctuation. In a cell, the energy scale is comparable with the thermal fluctuation. Moreover, thermal fluctuation helps to distinguish different stimuli. Because of the large separation of time scales, the thermal fluctuation can be treated as quasiequilibrium, so equilibrium statistical mechanics can give a reasonable response-stimulus relation. Hence the basic elements of our theory are useful no matter whether or not there is interaction between receptor dimers. The second message of our theory is that the anticipated cooperation is just a physical receptor-receptor interaction between neighboring receptor dimers. Therefore the conformational state of a receptor dimer is not only influenced by ligand binding of itself, but also by the receptor-receptor interaction that depends on the conformations of the two neighboring receptor dimers. The third message is that the large separation of time scales leads to a complementary usage of equilibrium statistical mechanics in calculating the response on a shorter time scale and a nonequilibrium description of the adaptation on a longer time scale. Dynamics on the longer time scale determines whether

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the randomness of ligand binding is quenched or annealed on the shorter time scale of the quasiequilibrium state, as will be elaborated later on. In the high temperature limit, this does not make a difference on the average signaling.

Recently there appeared some experimental data which are more directly relevant for the many-body nature of the receptor cluster and the possible cooperation [13–15]. Therefore it is interesting and important to make comparisons between the theory and the experimental results, testing the theory on one hand, and providing some information on what experiments are wanted on the other hand. However, we do not expect the model in its current form to perfectly fit everything on this complex system. Rather, what we provide is a theoretical framework on which refinements are possible. For example, we have only considered the cooperation between the receptor dimers, while extensions to possible cooperation among other components at later stages of the signaling process, for example, CheA, CheY, CheZ, and the switch complex, are straightforward if sufficient information is available. The idea of a receptor-receptor interaction broadens the view on cooperation, which previously largely meant the existence of more than one binding site, as described by the model presented by Hill a century ago [16]. For simplicity, we try to preserve the scenario of one binding site, while an extension to the situation with more binding sites is straightforward if necessary. Our strategy is to start with the minimum model, which yet explains the most essential features.

With improvement and simplification, we first describe the theory. Then we make comparisons with the experimental results, followed by a summary and discussions.

II. THEORY

Consider a lattice of receptor dimers, as shown in Fig. 1. Let the coordinate number be ν, which is 6 for a honeycomb lattice and 4 for a square lattice. The exact coordinate number in reality is subject to experimental investigations. The behavior of the system is determined by its energy function, or Hamiltonian, which can be written as

$$\mathcal{H}(t)=-\sum_{\langle ij\rangle} T_{ij}V_iV_j-\sum_i H_iV_i+\sum_i W_iV_i. \qquad (1)$$

V_i is a variable characterizing the conformation of receptor dimer i. It may be interpreted as the position of the receptor molecule with respect to a certain equilibrium position. In the popular two-state approach, V_i assumes one of two values V^0 or V^1. H_i is the influence, or force, due to ligand binding and the modulation of methylation level. $H_i=0$ if there is no ligand binding, while $H_i=H$ if there is a ligand binding. $-H_iV_i$ is the energy due to ligand binding, hence ligand binding causes the energy difference between the two conformations to make a shift of $H(V^1-V^0)$. $W_i(V^0-V^1)$ is the original energy difference between the two conformations. $\langle ij\rangle$ denotes nearest-neighboring pairs and $-T_{ij}V_iV_j$ is the interaction energy between the neighboring receptor dimers.

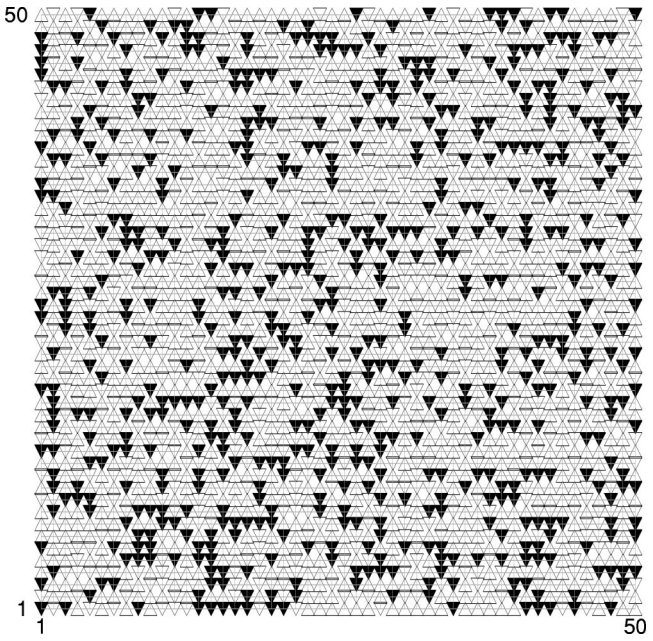

FIG. 1. An illustrative snapshot of the configuration of receptor dimers on a 50×50 square lattice. An up triangle represents the conformation state $S_i=1$, a down triangle represents $S_i=-1$, a filled triangle represents ligand binding, and an empty triangle represents no ligand binding.

For convenience, defining $S_i=2(V_i-V^0)/\Delta V-1$, with $\Delta V=V^1-V^0$, one transforms the Hamiltonian to

$$\mathcal{H}(t)=-\sum_{\langle ij\rangle} J_{ij}S_iS_j-\sum_i B_i(t)S_i+\sum_i U_iS_i, \qquad (2)$$

where $S_i=1,-1$ represents the two conformational states of the receptor dimer at site i, $J_{ij}=T_{ij}\Delta V^2/4$, $B_i=H_i\Delta V/2$, $U_i=\Delta VW_i/2-\Delta V^2\Sigma_jT_{ij}$. We refer to B_i as "field." For simplicity, it is assumed that $J_{ij}=J$ and $U_i=U$ are independent of i and j. $B_i=0$ if there is no ligand binding, while $B_i=B=H\Delta V/2$ if there is a ligand binding. Hence the energy difference due to ligand binding between the two conformations are $2B$. US_i represents the original energy in the absence of ligand binding. Equations (1) and (2) can be justified as follows. It is reasonable to assume an interaction energy proportional to $(V_i-V_j)^2$, which can be reduced to $-T_{ij}V_iV_j$, with constant terms neglected and the terms proportional to S_i or S_j included in $\Sigma_iU_iS_i$. This assumption is simple enough to allow a feasible treatment which yet captures the essential features.

From now on, we focus on Eq. (2). Suppose that before time $t=0$, there is no ligand binding the system, or the system is fully adapted though it is bound to ligands. Hence $B_i(t<0)=0$. Afterwards, at time $t=0$, the occupancy, i.e., the fraction of receptor dimers with ligands bound, changes to c. Hence the occupancy change is $\delta c=c$. This means $B_i(t=0)=B_i^0$, with

$$B_i^0=\begin{cases} B & \text{with probability } c \\ 0 & \text{with probability } 1-c. \end{cases} \qquad (3)$$

The occupancy c is determined by the ligand concentration L, $c=L/(L+K_d)$, where the dissociation constant K_d is on a time scale during which the receptor has undergone many flips between different conformations, hence it is an average and phenomenological quantity.

On the other hand, through the modulation of the methylation level by CheB and CheR, there is a negative feedback from the receptor state S_i to the field B_i, with a time delay t_r. A simple quantitative representation of this feedback is

$$\frac{dB_i(t)}{dt}=-\sigma[S_i(t-t_r)-m_0], \tag{4}$$

where $\sigma>0$ and m_0 is the prestimulus average of S_i. Precise forms of both the energy function and the feedback are, of course, subject to experimental investigations. It seems that in the biological world, feedback is a ubiquitous way to achieve adaptation and preserve sensitivity of response.

A remarkable feature of this system is the large separation of time scales. Ligand binding and conformational change occur within only a millisecond, while the overall time needed to complete the adaptation, through the slow modulation of the methylation level, is on the time scale of many seconds to minutes [18,2]. We note that in most cases, ligand debinding is on a much longer time scale than ligand binding, seen as follows. Consider the kinetics of the following reaction:

$$L+R\rightleftharpoons R_L, \tag{5}$$

where R represents the receptor without ligand binding while R_L represents the liganded receptor. k_+ and k_- are reaction rates for the binding and debinding, respectively. The ratio between the time scales of debinding and binding is $k_+L/k_-\equiv L/K_d$, where K_d is the dissociation constant. A typical value is $K_d\sim 1.2\ \mu M$ [2]. Usually, L is much larger, so the debinding time scale is much longer than the time scale of ligand binding and receptor conformational change. In extreme cases, when L is comparable with K_d, the debinding time scale is comparable to the binding time scale.

With the large separation of time scales, the treatment within the above framework becomes easier. One may discretize the time on the scale of adaptation, according to the feedback delay time. t is thus replaced by an integer τ, which is the integer part of t/t_r. On the other hand, *each instant* τ *is still very long compared with the time scale of conformational change*. Hence the activity at each τ is an average quantity $m(\tau)$, which can be calculated from the Hamiltonian in Eq. (2) by standard methods of equilibrium statistical mechanics. The average activity m is just on the time scale of the measurement of the macroscopic quantities such as motor bias, longer than the very short period in which the receptor is in either of the two conformations, but shorter than the adaptation time. In making the average, an important thing is that the randomness of the field is usually quenched since $L\gg K_d$, and is annealed otherwise. In fact we obtain a generalized version of the so-called random-field Ising model; in the conventional random-field Ising model, the average field vanishes, but it is generically nonzero in our model. On the long time scale, the field changes because of feedback. It can be expressed as $B_i(\tau)=B_i^0+M(\tau)$, where $M(\tau)$ is an induced field due to methylation modulation,

$$M(\tau)=-\sigma\sum_{k=0}^{\tau-1}[m(k)-m_0]. \tag{6}$$

Before stimulation, $m(\tau<0)=m_0$ is determined by U. If and only if $U=0$, $m_0=0$, which means that each receptor dimer is in either of the two conformational states with equal probability.

In most cases, the randomness of B_i^0 is quenched, the general relation between $m(\tau)$ and δc is then

$$m(\tau)=\frac{2\delta c}{1+\exp\left[-2\beta\left(\nu Jm(\tau)-\theta(\tau-1)\sigma\sum_{k=\tau_0}^{\tau-1}[m(k)-m_0]+U+B\right)\right]}+\frac{2(1-\delta c)}{1+\exp\left[-2\beta\left(\nu Jm(\tau)-\theta(\tau-1)\sigma\sum_{k=\tau_0}^{\tau-1}[m(k)-m_0]+U\right)\right]}-1, \tag{7}$$

where $\beta=1/k_BT$, the step function $\theta(x)$ is 1 if $x\geqslant 0$, and is 0 otherwise. On the other hand, when ligand concentration is comparable with K_d, the randomness of B_i^0 is annealed. Then it can be found that

$$m=\frac{\delta c[e^{\beta[f(m)+B]}-e^{-\beta[f(m)+B]}]+(1-\delta c)[e^{\beta f(m)}-e^{-\beta f(m)}]}{\delta c[e^{\beta[f(m)+B]}+e^{-\beta[f(m)+B]}]+(1-\delta c)[e^{\beta f(m)}+e^{-\beta f(m)}]}, \tag{8}$$

where $f(m)=\nu Jm-\theta(\tau-1)\sigma\Sigma_{k=0}^{\tau-1}m(k)+U$.

$m(\tau)$ vs the c relation corresponds to the response-stimulus relation. After the step increase at $\tau=0$, $m(\tau)$ always decreases towards the prestimulus value m_0. This explains the robustness of exact adaptation [19]. In practice the adaptation time is obtained when $m-m_0$ reaches the detection threshold m^*.

The results can be simplified under the condition that the thermal fluctuation is so strong that $\beta\nu J$ and βB are not large. Then both Eqs. (7) and (8) can be simplified to

$$m(\tau\geqslant 0)-m_0=\frac{\beta B\,\delta c}{1-\beta\nu J}\left(1-\frac{\beta\sigma}{1-\beta\nu J}\right)^{\tau}, \tag{9}$$

with

$$m_0=\frac{\beta U}{1-\beta\nu J}. \tag{10}$$

$1-\beta\nu J$ represents the enhancement of the response compared with the noninteracting scenario.

One may obtain the adaptation time t^*, after which $m-m_0$ is less than the detection threshold m^*:

$$\tau^*=\frac{\log\delta c+\log\left(\frac{\beta B}{1-\beta\nu J}\right)-\log m^*}{-\ln\left(1-\frac{\beta\sigma}{1-\beta\nu J}\right)}. \tag{11}$$

m^* can be related to the lower bound of detectable occupancy change, δc^*, by

$$m^*=\frac{\beta B\,\delta c^*}{1-\beta\nu J}, \tag{12}$$

hence

$$\tau^*=\frac{\log\delta c-\log\delta c^*}{-\ln\left(1-\frac{\beta\sigma}{1-\beta\nu J}\right)}. \tag{13}$$

At exact adaptation, setting $m(\tau)=m_0$, one may obtain the total induced field due to methylation modulation as $M^*=Bc$. Then for the next stimulus, suppose that the occupancy changes from δc to $\delta c+\Delta c$ at a later time τ_1, it can be found that the result with the occupancy $\delta c+\Delta c$ and the induced field M^* is the same as that for the situation in which the occupancy is Δc and there is no induced field. That is to say, the previous occupancy change has been canceled by the induced filed M^*, therefore the full adaptation with ligand binding is equivalent to no ligand binding. So $m(\tau\geqslant\tau_1)$ is given by the above relevant equations with τ changed to $\tau-\tau_1$, and δc substituted by Δc. One can thus simply forget the preadaptation history and restart the application of the above formulation with τ_1 shifted to 0. The cancellation holds exactly only under the assumption of small $\beta\nu J$ and βB, which is likely the reality. The finiteness of the detection threshold further widens the practical range of its validity.

III. COMPARISONS BETWEEN EXPERIMENTS AND THE THEORY

A. Clustering

The clustering has recently been experimentally studied in greater detail [15]. The observed clustering of receptors and the colocalization of the CheA, CheY, and CheZ with the receptors is a favor for the effects of interactions. An *in vitro* receptor lattice formation was also observed [17].

B. Response-stimulus relation

A basic prediction of our theory is the response-stimulus relation. Note that the time scale of the response, corresponding to m in our theory, is longer than the very short lifetime of a specific conformation, but is only transient on the time scale of the adaptation process. An interesting thing is that m in our theory is measurable. Motor rotation bias was measured [13]. From this result we can obtain m, as follows. The motor bias is

$$b=f_{ccw}/(f_{ccw}+f_{cw}), \tag{14}$$

where f_{ccw} and f_{cw} are rates of counterclockwise and clockwise rotations, respectively. Suppose the value of b is r_1 for conformational state 1, and is r_{-1} for conformational state -1. Then the average bias is

$$\overline{b}=r_1x+r_{-1}(1-x), \tag{15}$$

where x is the average fraction of receptors with state 1. x is related to m by $m=x-(1-x)=2x-1$. So if we know r_1 and r_{-1}, we can obtain m from $\overline{b}$. However, there seems to be no investigation on r_1 and r_{-1}. A simple assumption which is often implicitly assumed is that $r_1=1$, $r_{-1}=0$, i.e., state 1 corresponds to counterclockwise rotation while state -1 corresponds to clockwise rotation. We follow this assumption here. But it should be kept in mind that an experimental investigation on r_1 and r_{-1} would be very valuable. Therefore, for the time being, we use

$$\overline{b}=\frac{m+1}{2}. \tag{16}$$

Thus from the prestimulus value of $\overline{b}$, one may determine m_0, and thus βU. An empirical formula is $\overline{b}=1-0.0012(rcd-360)$, where rcd is the absolute angular rate of change of direction of the cell centroid in deg s^{-1} [13,23]. From [23], the prestimulus value of rcd is known as ~ 600, so the prestimulus value of $\overline{b}$ is ~ 0.712. Hence

$$m_0=\frac{\beta U}{1-\beta\nu J}\approx 0.424. \tag{17}$$

The occupancy change used in [14] was calculated from the ligand concentration under the assumption that the ligand randomly binds one of two possible binding sites: in addition to the site with $K_d\sim 1.2\ \mu M$, as widely acknowledged [18], there is another site with $K_d\sim 70\ \mu M$. This was based on an earlier attempt to have a better fitting for the adaptation time

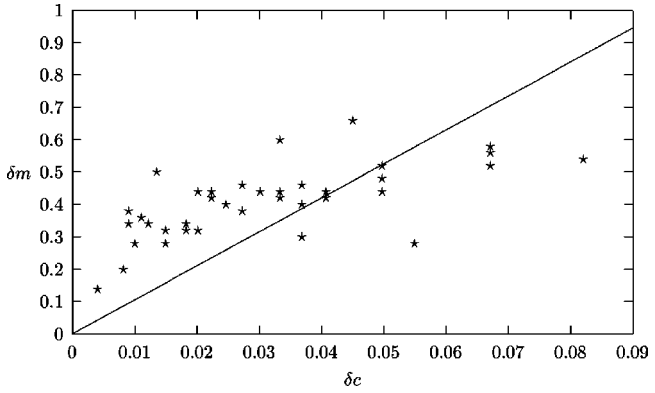

FIG. 2. Response-stimulus relation δm vs δc. The data points are transformed from those read from [13] with computer software. The range of receptor occupancy change is too small, so only qualitative comparison is possible. The straight line is the least squares fitting $\delta m = 10.49 \delta c$.

[20]. However, as said above, we try to make things as simple as possible in the first instance, so we prefer to preserve the scenario of one binding site with $K_d \sim 1.2\ \mu M$. Actually with one binding site, as discussed later on, it seems that our theory can fit the adaptation time by choosing appropriate parameter values and thus improve the coherence among various data. So we should first transform the values of the occupancy change in [13] to the values one would have obtained without coherence without the assumption of two binding sites. One has

$$c_J = \frac{1}{2}(c_1 + c_2), \tag{18}$$

where c_J represents the occupancy used by Jasuja *et al.*, c_1 corresponds to the dissociation constant $K_1 = 1.2\ \mu M$, and c_2 corresponds to the dissociation constant $K_2 = 70\ \mu M$. From $c_l = L/(L + K_l)$, $l = 1,2$, one obtains the change of the occupancy

$$\delta c_l = \frac{K_l \delta L}{(L + \delta L + K_l)(L + K_l)}, \tag{19}$$

where δL is the change of ligand concentration. Since $\delta L \ll L$, one may obtain $\delta c_1 = 2\delta c_J/(1+\alpha)$, where $\alpha \approx K_1(L + K_1)^2/K_2(L + K_2)^2$. With $L \approx 10\ \mu M$, $\alpha \approx 1$, one has $\delta c_1 \approx \delta c_J$. Therefore under this condition, we may simply use the occupancy change in [13]. Equation (16) leads to the relation between the initial change of m and that of the motor bias, δb,

$$\delta m = 2\,\overline{\delta b}, \tag{20}$$

where $\delta m = m(\delta c, \tau = 0) - m_0$.

So the data in Fig. 3 of [13] can be transformed to the δm vs δc relation as shown in our Fig. 2. Unfortunately, it is notable that the data is limited to very low values of occupancy change. Nevertheless, a qualitative fitting can be made. According Eq. (9), setting $\tau = 0$, we fit the data with a straight line $\delta m = a\,\delta c$, where

$$a = \frac{\beta B}{1 - \beta \nu J} \approx 10.49 \tag{21}$$

is the slope of the fitting line.

C. Adaptation time

Equation (19) tells us that with the same concentration change, the occupancy change and thus the response *decreases* with the increase of the prestimulus ligand concentration. This is verified by Fig. 7 of [20]. Equation (11) predicts that the adaptation time increases linearly with, but not proportional to, the logarithm of occupancy change. It had been thought that the adaptation time is proportional to the occupancy change [21,22,20]. We found that a logarithmic relation is also consistent with the currently available data. As an example, we examine the better set of data, the left plot (D-ribose), in Fig. 4 of [22]. For accuracy, the two data points at the highest and lowest concentration changes are dropped. This is because they are at the detection limits, and they have no recognizable differences in adaptation time with the data points closest to them respectively, although the values of the concentration change are quite different. Moreover, the adaptation time is recorded to be zero for the two smallest values of the concentration change, so the data point with the smallest concentration change should be ignored. Using $K_d = 3 \times 10^{-7}$ (no unit was given, but should be the same as that of the concentration, so there is no problem in using it), we transform the concentration to the occupancy. The transformed data is shown in our Fig. 3, with Fig. 3(a) the normal-normal plot and Fig. 3(b) the normal-logarithmic plot. While there could be a fitting with a proportional relation, as usually assumed, it is at least reasonable to fit them with a logarithmic relation, $t^*/0.354\ \text{s} \equiv \tau^* t_r/0.354\ \text{s} = (g \log_{10}\delta c + h)/0.354\ \text{s}$, with $g = 95.151 \times 0.354\ \text{s} = 33.7\ \text{s}$ and $h = 124.0574 \times 0.354\ \text{s} = 43.9\ \text{s}$. The factor 0.354 s comes from the data normalization in [22], which is the percentage of one of three maximum recovery times, $0.56m$, $0.58m$, and $0.62m$, i.e., 35.4 s on average. From Eq. (11), we have

$$\frac{t_r}{-\log_{10}\left(1 - \dfrac{\beta\sigma}{1 - \beta\nu J}\right)} = g \tag{22}$$

and

$$\frac{t_r[\log_{10}\delta c^*]}{\log_{10}\left(1 - \dfrac{\beta\sigma}{1 - \beta\nu J}\right)} = h. \tag{23}$$

Using $\delta c^* \approx 0.004$ [21], and assuming $t_r \approx 0.1$ s, one finds

$$\frac{\beta\sigma}{1 - \beta\nu J} \approx 0.0068 \quad \text{to} \quad 0.013, \tag{24}$$

where the first value is estimated by using Eq. (22) and the second by using Eq. (23). They are close to each other, as an indication of the consistency of the theory.

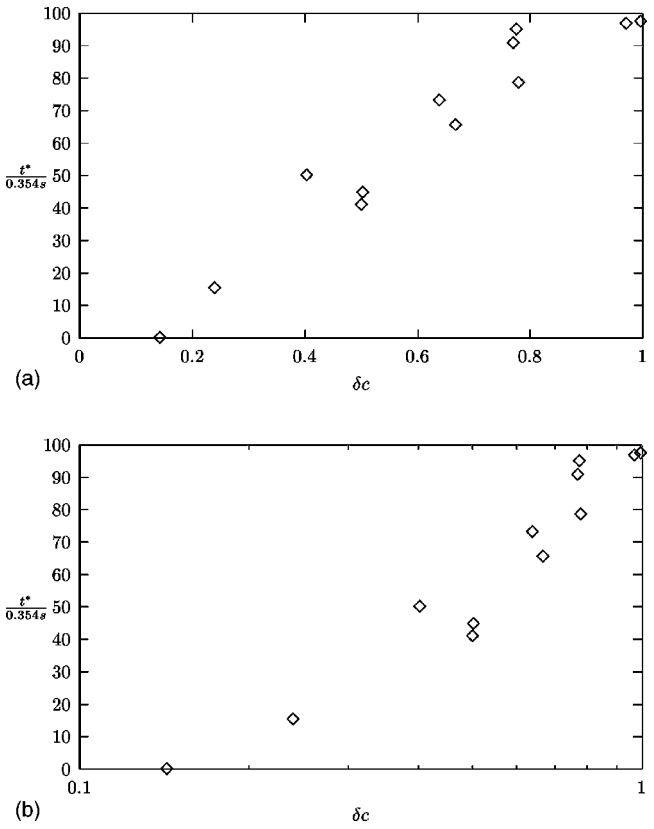

FIG. 3. (a) Normal-normal plot of the relation between the adaptation time t^* and the occupancy change δc. The data points are adopted from [22] by using computer software, with the concentration transformed to occupancy. (b) Normal-logarithmic plot of the same data, showing that they can be fitted to a logarithmic relation.

Furthermore, our predicted logarithmic relation may explain the discrepancy in the analysis of the data in Fig. 4 of [20] about a relation between the adaptation time and the concentration. The logarithm can simply decrease the predicted value of adaptation time, without resorting to the assumption of the existence of two binding sites. We have tried to make a quantitative fitting for the data in Fig. 4 of [20]. Using $K_d = 1.2\ \mu M$, we transform the ligand concentration to the occupancy, as shown in our Fig. 4. To make better use of the data, we ignore the data point for $\delta c > 0.95$ because the finiteness of detection threshold may cause uncertainty in deciding the adaptation time; the data for $\delta c > 0.95$ show too large a variation for so close values of δc. The fitting straight line is $t^* \equiv \tau^* t_r = g \log_{10} \delta c + h$, with $g = 156.3513$ and $h = 114.9912$. Using Eqs. (22) and (23), and the same values of δc^* and t_r as above, one finds

$$\frac{\beta\sigma}{1-\beta\nu J} \approx 0.0015 \quad \text{to} \quad 0.0047. \tag{25}$$

Again, these two numbers are close to each other. Moreover, Eqs. (24) and (25) are of the same order of magnitude, though they are obtained from different sets of data. Even closer numbers may be obtained by tuning the vale of t_r.

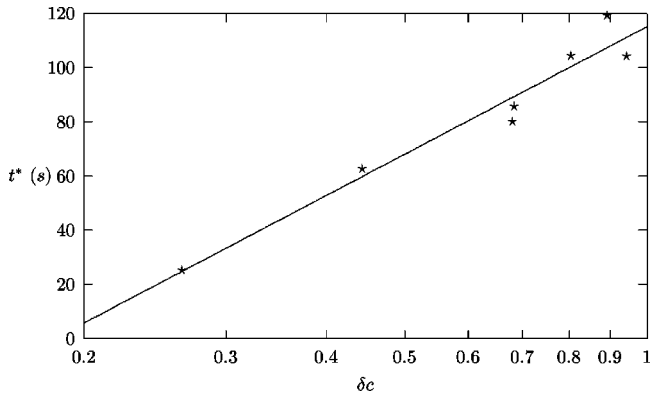

FIG. 4. Relation between the adaptation time t^* and the occupancy change δc. The data points are adopted from [20] by using computer software, with the concentration transformed to occupancy. The straight line is the least squares fitting $t^* = 156.3513 \log_{10} \delta c + 114.9912$.

D. CheA activity

Another interesting and important experimental result is on the relative CheA activity, which has been analyzed by using the Hill model with a noninteger coefficient [14]. Here we examine the data from the perspective of our theory.

Suppose $S = 1, -1$ correspond respectively to CheA activity A_1 and A_{-1}. Then the average CheA activity is $\frac{1}{2}(A_1 + A_{-1}) + (m/2)(A_1 - A_{-1})$. Consequently the relative CheA activity, as measured in [15], is

$$R = \frac{(A_1 + A_{-1}) + (A_1 - A_{-1}) m(\delta c)}{(A_1 + A_{-1}) + (A_1 - A_{-1}) m(\delta c = 0)} = 1 - F \frac{L}{L + K_d}, \tag{26}$$

where $F = a/[E + a(U/B)]$, with $E = (A_{-1} + A_1/(A_{-1} - A_1) > 0$. Note that $A_{-1} > A_1$. It is constrained that for the attractant binding, $F \leqslant 1$, since $R \geqslant 0$. Setting $F = 0.95$ and $K_d = 20\ \mu M$, we obtain a reasonable fitting to Fig. 1 of [14], as shown in our Fig. 5. Therefore

$$E \approx a\left(\frac{1}{0.95} - \frac{U}{B}\right), \tag{27}$$

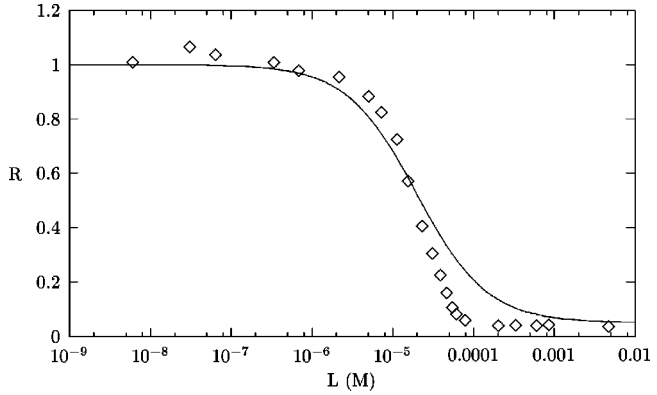

FIG. 5. Relation between the relative CheA autophosphorylation rate R and ligand concentration L. The data points are adopted from [14] by using computer software. The theoretical curve is $R = 1 - FL/(L + K_d)$, with $F = 0.95$ and $K_d = 20\ \mu M$.

which, combined with Eqs. (17) and (21), implies that the ratio between the two levels of CheA activity is $A_{-1}/A_1 \approx 164.77$. *Very interestingly, this result of deduction is in good consistency with the available experimental information that this ratio is more than 100* [2]. Again, this is an indication of the consistency of the theory.

We note that there is discrepancy in the fitting. This may be because of some other factors not considered here, especially because the correspondence between the receptor conformational state and CheA activity is more complicated in connection with r_1 and r_{-1} discussed above.

IV. SUMMARY AND DISCUSSIONS

We suggest that statistical mechanics is useful and important in understanding receptor signaling and adaptation. We have made semiquantitative comparisons between the theory and recent experiments to obtain estimations of parameter values. The thermal fluctuation in a cell is very strong, $k_BT \approx 4$ pN nm≈ 0.025 eV, comparable to the energy scale. So we simplify the formulation by using the high temperature approximation. Then Eqs. (9) and (10) essentially contain all the information we need. $1-\beta\nu J$ characterizes the enhancement of signaling by receptor-receptor interaction. With this simplified formulation, we look at recent experimental results. Unlike a clean system usually studied in physics, for such a complex system we do not expect the fitting to be quantitatively perfect. From the data on a prestimulus motor rotation bias [23], we obtain the prestimulus activity, as in Eq. (17), implying that there are approximately 70% receptor dimers at the state corresponding to the lower rate of CheA autophosphorylation. Although the data on response-stimulus relation are very limited, they are used to estimate that $\beta B/(1-\beta\nu J)\approx 10.49$, which compares the effect of ligand binding with that of cooperation. We study adaptation time for two different sets of data [22,20]. Assuming the delayed time in feedback to be 0.1 s, it is found that the feedback strength compared with coupling, $\beta\sigma/(1-\beta\nu J)$, is approximately 0.0068 to 0.013 or 0.0015 to 0.0047, respectively. These numbers obtained from different data and by using different methods are of the same order of magnitude, a sign of the consistency of theory. Precise information on the feedback delay time can improve this determination. From the data on the relative CheA activity [14], we obtain Eq. (27), which gives the relation between the two levels of CheA activity corresponding to the two conformations of the receptor dimer. Combined with other results, it tells that the ratio between the two levels of CheA activity is $A_{-1}/A_1 \approx 164.77$, in good consistency with the available experimental information on this ratio. We note that the fitting is not perfect. This may be partly due to the simple nature of the minimum model and further simplified treatment, and partly due to insufficient experimental information. However, with a working framework proposed, we anticipate more experimental and theoretical discoveries stimulated by the current attempt. On the other hand, it would not be satisfactory for us to have a good fitting of the data by simply tuning parameters without a clear physical picture.

We need improvement on the available experimental results, as well as new experimental information, to provide a basis for the extension and refinement of the theory. For example, we need a significant broadening of the range of occupancy change in the response-stimulus relation. We also need a clearer relation between the adaptation time and the occupancy change. The relation between CheA activity and the receptor conformational state and CheA activity, as well as the relative rate of the two rotation modes, is vital for going beyond the simple treatment here. More accurate results on A_{-1}/A_1 is also important. Independent determination of the dissociate constant is of fundamental importance. Most exciting experiments might be direct measurements of the conformational states V^0, V^1, and the coupling coefficient T_{ij}, as well as the energy change or force induced by the ligand binding. A clarification on whether the conformational change is a rotation or a vertical displacement is interesting. For the former, V^0 and V^1 are angles, while H, the effect of ligand binding, is a torque. For the latter, V^0 and V^1 are positions, while H is a force. The receptor-receptor interaction can be determined by measuring the relation of force or torque on one receptor dimer and the conformations of its neighbors. This would be a direct test of the conformation-dependent interaction. A determination of the geometry of the lattice is also interesting, from which one can obtain the value of $\beta\nu J$, and consequently other parameter values.

Our theory is entirely different from the Hill model. An integer Hill coefficient is understood as the number of ligands bound to a receptor. A noninteger Hill coefficient, as often used, does not seem clear conceptually, although it could be tuned to fit the data. Nonetheless, from the mean field point of view, the effect of the receptor-receptor interaction could be viewed as an effective additional ligand binding. Therefore, from this perspective the conclusion on limited cooperativity in [14] is consistent with strong thermal fluctuation in our theory.

Here we specialize in chemotactic receptors; however, the theory may also apply to many other receptor systems. For example, state-dependent co-inhibition between transmitter-gated cation channels was observed [24]. The clustering of $GABA_A$ receptors and the decrease of affinity was also studied [25], which was also analyzed in terms of the Hill model in a similar way to [14], thus it can also be explained by our theory as an indication of receptor-receptor interaction and thermal fluctuation. In many receptor systems clustering, called oligomerization, together with signaling, occurs as a response to stimulus. Theoretical investigation on this situation is presented elsewhere.

To complete this paper, let us list some experiments anticipated from the point of view of this theory. (1) More clarifications on conformational change induced by ligand binding, and the determination of conformational change due to interaction with another receptor dimer. (2) Direct measurement of the forces generated by ligand binding and by the conformational change of the neighboring receptor dimer. (3) An independent determination of a dissociate constant using other methods. (4) Investigations on the responses corresponding to fixed conformational states, and hence r_1 and r_{-1} as discussed above. (5) Direct measure-

ments on CheA and CheY activities. (6) More clarification on the relation between the receptor conformational state and CheA activity. (7) Increasing the range of occupancy change in response-stimulus relations, and a more accurate determination of prestimulus occupancy and occupancy change. (8) More accurate determination of adaptation time as a function of the occupancy change. (9) Precise determination of the form of energy function. (10) Determination of the details of feedback due to the change of the methylation level, including the delay time.

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