Cooperativity, Sensitivity, and Noise in Biochemical Signaling

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Cooperative interactions in the binding of multiple signaling molecules is a common mechanism for enhancing the sensitivity of biological signaling systems. It is widely assumed this increase in sensitivity of the mean response implies the ability to detect smaller signals. Extending the classic work of Berg and Purcell [Biophys. J. **20**, 193 (1977)] on the physical limits of chemoreception, we show that the random arrival of diffusing signaling molecules at receptor sites constitutes a noise source that is not reduced by cooperativity. Cooperativity makes reaching this limit easier, but cannot reduce the limit itself.

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In biological systems, many signals are carried by changes in the concentration of various molecules, from the small, such as cyclic nucleotides to the large, such as transcription factors regulating gene expression. A striking feature of these systems is their sensitivity: concentrations can be quite small in absolute terms, and small fractional changes in concentration can have a significant effect. Thus, many transcription factors achieve their half maximal effect at nanomolar concentrations [1], and recent studies indicate expression levels can be regulated to an accuracy of $\sim 10\%$. Similarly, bacteria respond to small changes in concentrations of molecules in their environment. These signals are transduced into changes in levels of phosphorylated proteins; changes in the concentration of a particular phosphorylated protein by less than $\sim 10\%$ can produce reliable differences in the output of the bacterial flagellar motor [2], and so on. A natural question concerns the origins of this sensitivity and its ultimate limits.

A central theme in biological regulation is that of cooperativity or allostery [3–5]: multiple ligands bound at different sites on a single protein molecule can interact so that the mean occupancy of these sites depends steeply on ligand concentration. Indeed, conventional models predict that cooperative effects among many sites can produce arbitrarily high sensitivity. But there is a difference between sensitivity in the mean response and reliable response to small changes—there are limits to measurement that cannot be evaded just by having an amplifier of higher gain. Here we consider the ''noise floor'' for detection of small concentration changes by receptors exhibiting cooperativity, or more generally, multiple internal states in response to binding of multiple ligands. We find that while cooperativity can reduce the effective noise level against which small signals are discriminated, there is a physical limit set by the size of the receptor complex, the diffusion constant of the signaling molecule and its absolute concentration. Cooperativity can make it easier to approach this limit (as with high gain amplifiers), but does not lead to a fundamentally lower noise floor.

The classic discussion of noise in biochemical signaling is by Berg and Purcell [6] . Their arguments were heuristic, suggesting one should think of receptors with size ℓ as counting molecules in a volume $\sim \ell^3$. Poisson statistics then sets a limit to the counting precision that can be reduced by averaging over time, but allowing a time $\sim \ell^2/D$ for the volume to be cleared by diffusion between genuinely independent measurements. Thus, by averaging for a time τ, one can detect fractional changes in concentration

$$\frac{\Delta c}{\bar{c}} \sim \frac{1}{\sqrt{D\ell\bar{c}\tau}}. \quad (1)$$

Bacterial chemotaxis seems to operate close to this limit [6]. The discussion of noise has been invigorated by experiments that measure directly the noise in the regulation of gene expression [7]; much theoretical work aims to connect these data to specific kinetic models [8]. Recently we argued that even for these more complex systems, there is a limit analogous to that derived by Berg and Purcell, which should be independent of the (often unknown) kinetic details [9]. Our argument was based on the idea that binding of signaling molecules to receptor sites usually is an equilibrium process, so that fluctuations in the occupancy of the sites becomes a form of thermal noise that can be analyzed using the fluctuation-dissipation theorem. We considered multiple noninteracting binding sites, showing how correlations among site occupancies leads to behavior that approximates a single larger receptor. Here we extend this to the case of cooperatively interacting binding sites. We begin with a general analysis, and then work it out for a specific model.

Consider a receptor molecule or complex that has many states labeled by an index i; the population of each state is p_i. The free energy of state i, with n_i signaling molecules bound, is $F_i = E_i - n_i\mu$, where μ is the chemical potential of the signaling molecule at the location of the receptor. At low concentration, $\mu = k_B T \ln c$, with c in appropriate units. The chemical kinetics of this system (which we do

not specify in detail) determine the linear response of the populations to changes in the F_i's. In the frequency domain,

$$\delta\tilde{p}_i(\omega) = \sum_j \alpha_{ij}^{(0)}(\omega)\delta\tilde{F}_j(\omega) \tag{2}$$

$$= \sum_j \alpha_{ij}^{(0)}(\omega)\left[\delta\tilde{E}_j(\omega) - n_j k_B T \frac{\delta\tilde{c}(\mathbf{x}_0;\omega)}{\bar{c}}\right], \tag{3}$$

where all (un)binding of signaling molecules is taken to occur at a single location $\mathbf{x}_0$ of the receptor.

The susceptibility $\alpha_{ij}^{(0)}(\omega)$ can encode interactions among different binding sites, as it describes all possible states of the receptor complex. For example, given two binding sites that can be empty or occupied, four possible states exist, labeled $i \equiv \{00, 01, 10, 11\}$. Positive cooperativity in this simplest of models results when the energy decrease on binding the second ligand is greater than for the first, $E_{11} - E_{10} < E_{10} - E_{00}$. More complex, realistic models ascribe cooperativity not to direct interaction between binding sites but rather to an interaction between the binding and other conformational degrees of freedom of the complex, as in the specific model discussed later.

To continue, we must count signaling molecules bound to the receptor complex. Changes in this number act as a source or sink for diffusion. The total number of bound ligands is $\sum_i n_i p_i$, and hence the diffusion equation for the signaling molecule becomes

$$\frac{\partial c(\mathbf{x},t)}{\partial t} = D\nabla^2 c(\mathbf{x},t) - \delta(\mathbf{x}-\mathbf{x}_0)\sum_i n_i \frac{dp_i}{dt}. \tag{4}$$

We solve explicitly for the response to small changes in the populations, again in the frequency domain:

$$\delta\tilde{c}(\mathbf{x}_0;\omega) = i\omega\left[\int \frac{d^3k}{(2\pi)^3}\frac{1}{-i\omega + Dk^2}\right]\sum_i n_i \delta\tilde{p}_i(\omega). \tag{5}$$

The term in brackets is ultraviolet divergent because we treat the receptor complex as a point object. As in Ref [9] we remove the divergence by cutting off the k integrals at a scale $k_{\max} \sim \pi/\ell$, where ℓ is the size of the complex. In the low frequency limit $\omega \ll D/\ell^2$,

$$\delta\tilde{c}(\mathbf{x}_0;\omega \to 0) = \frac{i\omega}{2\pi D\ell}\sum_i n_i \delta\tilde{p}_i(\omega). \tag{6}$$

Equation (6) tells us how the concentration responds to changes in the population of the different states of the receptor complex, while Eq. (3) tells us how the populations respond to changes in concentration. Denoting α^{-1} as the matrix inverse to α, they yield

$$\delta\tilde{E}_i(\omega) = \sum_j \alpha_{ij}^{-1}(\omega)\delta\tilde{p}_j(\omega), \tag{7}$$

$$\alpha_{ij}^{-1}(\omega) = \alpha_{ij}^{(0)-1}(\omega) + n_i n_j \frac{i\omega k_B T}{2\pi D\ell\bar{c}}. \tag{8}$$

We see the effect of coupling to diffusion is to add a self-energy term to the inverse susceptibility.

The fluctuation-dissipation theorem (FDT) holds that fluctuations in occupancy of the states can be viewed as the response to fluctuations in conjugate energies E_i, the spectrum of which is determined by [10]

$$\langle\delta\tilde{E}_i(\omega)\delta\tilde{E}_j(\omega')\rangle = 2\pi\delta(\omega+\omega')\frac{2k_B T}{\omega}\mathrm{Im}[\alpha_{ij}^{-1}(\omega)]. \tag{9}$$

We define the noise power spectrum through

$$\langle\delta\tilde{E}_i(\omega)\delta\tilde{E}_j(\omega')\rangle = 2\pi\delta(\omega+\omega')\mathcal{N}_{ij}(\omega), \tag{10}$$

$$\mathcal{N}_{ij} = \mathcal{N}_{ij}^{(0)} + A n_i n_j, \tag{11}$$

where $A = (k_B T)^2/(\pi D\ell\bar{c})$, and $\mathcal{N}_{ij}^{(0)}$ is the noise obtained from the kinetics described by the bare susceptibility $\alpha_{ij}^{(0)}$.

From Eq. (3) we see that a change in concentration is equivalent to a coordinated change in the energies of all the states,

$$\Delta\tilde{E}_i(\omega) = -n_i k_B T \Delta\tilde{c}(\mathbf{x}_0;\omega)/\bar{c}. \tag{12}$$

Thus, in general there is no single state of the receptor complex that can be monitored to provide the optimal readout of the concentration. However, if downstream mechanisms have access to all the states, and construct a readout from an appropriately weighted average of the populations, the maximum achievable signal-to-noise ratio at each frequency [11] is,

$$\mathrm{SNR}(\omega) = \sum_{ij}\Delta\tilde{E}_i(\omega)\mathcal{N}_{ij}^{-1}(\omega)\Delta\tilde{E}_j^*(\omega). \tag{13}$$

In the limit of detecting a slow change in concentration, where we are willing to average for a time τ, the total signal-to-noise ratio is given by $\tau\mathrm{SNR}(\omega \to 0)$. Defining detectability as the point where τSNR reaches unity, the threshold for detection is

$$\frac{\Delta c}{\bar{c}} = \left[\tau(k_B T)^2\sum_{ij} n_i \mathcal{N}_{ij}^{-1}(\omega=0)n_j\right]^{-1/2}. \tag{14}$$

The sum in Eq. (14) can be written as [12]

$$\mathbf{n}^T\cdot\mathcal{N}^{-1}\cdot\mathbf{n} = \frac{\mathbf{n}^T\cdot\mathcal{N}^{(0)-1}\cdot\mathbf{n}}{A\mathbf{n}^T\cdot\mathcal{N}^{(0)-1}\cdot\mathbf{n}+1}, \tag{15}$$

where $\mathbf{n}^T$ denotes the transpose of $\mathbf{n}$. Thus we obtain

$$\left(\frac{\Delta c}{\bar{c}}\right)^2 = \frac{1}{\pi D\ell\bar{c}\tau} + \frac{1}{\tau(k_B T)^2[\mathbf{n}^T\cdot\mathcal{N}^{(0)-1}\cdot\mathbf{n}]}. \tag{16}$$

Crucially, the second term is positive: $\mathcal{N}^{(0)}$ is a matrix of noise power spectra; therefore it is positive definite (symmetric with all positive eigenvalues), as must be $\mathcal{N}^{(0)-1}$, $\mathbf{n}^T\cdot\mathcal{N}^{(0)-1}\cdot\mathbf{n} > 0$. Thus, as in Ref. [9], the first term gives a lower bound to the smallest detectable signal. Up to

a factor of π, it is exactly the expression in Eq. (1), derived by Berg and Purcell [6].

To illustrate these ideas, we invoke a specific model, the Monod-Wyman-Changeux (MWC) model of cooperativity [3]. This model has been widely used, first for allosteric enzymes, then for the paradigmatic example of cooperative oxygen binding to hemoglobin [5], and more recently for studies of switching between rotational states of the bacterial flagellar motor, denoted by T (clockwise) and R (counterclockwise) [13–15]. In this model, ligands bind independently to multiple sites, but the binding energy depends on whether the whole complex is in the R or T state.

The states of the system are defined by the binary variable R/T and the number n of ligands bound to a total of N_r sites. The free energy of the R state with n ligands bound is

$$F_R(n) = F_R(0) - nk_BT \ln(c/K_R), \tag{17}$$

and similarly for the T states, where c is the ligand concentration as above. If the rate for ligand-binding to the R state [i.e., for the transition $(R, n) \to (R, n+1)$] is $k_+^R c$ and the rate of unbinding is k_-^R, then $k_-^R/k_+^R = K_R$. If the transition $(R, n) \to (T, n)$ occurs at rate $k_f(n)$, and the reverse $T \to R$ transition at rate $k_b(n)$, then by detailed balance we must have

$$\frac{k_f(n)}{k_b(n)} = \frac{k_f(0)}{k_b(0)}\left(\frac{K_R}{K_T}\right)^n. \tag{18}$$

To complete the kinetic model we follow Ref. [14] and assume the activation energies for the transition rates $k_{f,b}(n)$ are themselves linear in n, so that

$$k_s(n) = k_s(0)(K_R/K_T)^{\xi n}, \tag{19}$$

where $s = (f, b)$ and $\xi = (\gamma, \gamma - 1)$, respectively; the change in the activation energy of switching from R to T upon binding a single ligand molecule is $-k_BT\gamma \ln(K_R/K_T)$, where γ is a constant.

In this model, the probability that the motor is in the T state with n bound ligand molecules is

$$p_T(n) = C(N_r, n) \exp[-F_T(n)/k_BT]/Z, \tag{20}$$

where $C(N_r, n)$ is the binomial coefficient, Z is the partition function given by $Z = Z_R + Z_T$, and $Z_{R,T} = \sum_{n=0}^{N_r} C(N_r, n) \exp[-F_{R,T}(n)/k_BT]$. The equilibrium probability of being in the T state, obtained by summing $p_T(n)$ over n, is

$$\bar{p}_T = \left[1 + \frac{1}{L}\left(\frac{1 + c/K_R}{1 + c/K_T}\right)^{N_r}\right]^{-1}, \tag{21}$$

where $L = \exp[-(F_T(0) - F_R(0))/k_BT]$. In the limit of many sites ($N_r \to \infty$) where binding to the T state is much stronger than to the R state ($K_R \gg K_T$), p_T approaches a step function dependence on concentration. Thus, the MWC model allows for arbitrarily high sensitivity if there are enough binding sites that can cooperate.

In the (plausible) limit where the conformational transition is slow but the binding and unbinding of the ligands is fast, the dynamics reduces to a two-state system (R and T). We compute the transition rates as averages of $k_{f,b}(n)$ over the equilibrium distribution of n given that the system is in either the R or T state. We then couple the transitions of this effective two-state system to diffusion of the ligand as before, and follow the same steps [9] through the FDT to the effective noise level for concentration measurements. We outline the argument here and give details elsewhere.

The average rates $\bar{k}_f$ and $\bar{k}_b$ for transitions from R to T, and T to R, respectively, obtained by averaging $k_{f,b}(n)$ with respect to $p_{R,T}(n)$ are

$$\bar{k}_s = k_s(0)\left[\frac{K_S + c(K_R/K_T)^{\xi}}{K_S + c}\right]^{N_r}. \tag{22}$$

where $s = (f, b)$, $S = (R, T)$, and $\xi = (\gamma, \gamma - 1)$, respectively. Then, the dynamics of the T state population is given by

$$\frac{dp_T}{dt} = \bar{k}_f(1 - p_T) - \bar{k}_b p_T. \tag{23}$$

For the two states, the mean number of ligands bound is just $\bar{n}_{R,T} = N_r f_{R,T}$, so that transitions from the T to R state are associated with the release of $N_r(f_T - f_R)$ molecules into the surrounding solution. Hence the diffusion equation analogous to Eq. (4) becomes

$$\frac{\partial c(\mathbf{x}, t)}{\partial t} = D\nabla^2 c(\mathbf{x}, t) - \delta(\mathbf{x} - \mathbf{x}_0)N_r(f_T - f_R)\frac{dp_T}{dt}. \tag{24}$$

Solving Eq. (24) to compute the response of c to small changes in p_T follows the same steps as for Eq. (4), with the result that in the limit $\omega \ll D/\ell^2$

$$\delta\tilde{c}(\mathbf{x}_0; \omega \to 0) = \frac{i\omega}{2\pi D\ell} N_r(f_T - f_R)\delta\tilde{p}_T(\omega). \tag{25}$$

As in Eq. (6), ℓ is the effective size of the cluster.

The analysis of Eq. (23) is more subtle because the concentration c and the thermodynamic force conjugate to p_T are hidden in the rate constants $\bar{k}_{f,b}$. The conjugate force is the free energy difference between the T and R states, and is linked to the rate constants through detailed balance. Thus if we imagine changing the rate constants $\bar{k}_{f,b}$ by small amounts $\delta\bar{k}_{f,b}$, we must have (see also Ref. [9])

$$\frac{\delta\bar{k}_f}{\bar{k}_f} - \frac{\delta\bar{k}_b}{\bar{k}_b} = \frac{\delta F}{k_BT} + N_r(f_T - f_R)\frac{\delta c}{\bar{c}}, \tag{26}$$

as is verified in detailed computations from Eq. (22) for $\bar{k}_f$ and $\bar{k}_b$, identifying $F = F_R(0) - F_T(0)$. Linearizing Eq. (23) about the equilibrium occupancy of the T state yields

$$\frac{d\delta p_T}{dt} = -(\bar{k}_f + \bar{k}_b)\delta p_T + \bar{k}_f(1-\bar{p}_T) \times \left[\frac{\delta F}{k_B T} + N_r(f_T - f_R)\frac{\delta c}{\bar{c}}\right]. \quad (27)$$

To find the susceptibility of p_T to its conjugate force δF we transform Eq. (27) and substitute from Eq. (25):

$$\alpha^{-1}(\omega) = \frac{\delta\tilde{F}(\omega)}{\delta\tilde{p}_T(\omega)} = \frac{k_B T}{\bar{k}_f(1-\bar{p}_T)}(\bar{k}_f + \bar{k}_b) - i\omega\left[\frac{k_B T}{\bar{k}_f(1-\bar{p}_T)} + \frac{k_B T N_r^2(f_T - f_R)^2}{2\pi D\ell\bar{c}}\right]. \quad (28)$$

The FDT, as in Eq. (9) but now with the more standard sign convention (see [10]), tells us that

$$\langle\delta\tilde{F}(\omega)\delta\tilde{F}(\omega')\rangle = -2\pi\delta(\omega+\omega')\frac{2k_B T}{\omega}\mathrm{Im}[\alpha^{-1}(\omega)]. \quad (29)$$

Fractional changes in concentration are equivalent to changes in free energy through

$$\frac{\delta c}{\bar{c}} = \frac{1}{N_r(f_T - f_R)}\frac{\delta F}{k_B T}, \quad (30)$$

so if we can compute the noise in δF we can compute the equivalent noise in the concentration, as before,

$$\left(\frac{\Delta c}{\bar{c}}\right)^2 = \frac{1}{\pi D\ell\bar{c}\tau} + \frac{2}{N_r^2(f_T - f_R)^2}\frac{1}{\bar{k}_f\tau(1-\bar{p}_T)}. \quad (31)$$

This is exactly the result of our general analysis, where now the second term in Eq. (16) is explicit. Notably, as the number of cooperative sites N_r becomes large, this term diminishes, and the physical limit set by diffusion alone becomes dominant [16].

To summarize, we have found that the physical limit to biochemical signaling first suggested by Berg and Purcell is surprisingly general. Even allowing for arbitrarily complex internal states and multiple ligand-binding sites, the equivalent noise level against which concentration changes must be detected has two terms: the Berg-Purcell limit plus a positive contribution from the details of the chemical kinetics. A cooperatively interacting receptor cluster, like a noninteracting cluster [9], is ultimately limited in its measurement of the ligand concentration by the diffusive counting noise inherent to a device of size ℓ, given by the effective cluster size. Cooperative interactions serve to suppress the second term, and perhaps this is crucial in allowing any real biological system to approach the physical limit.

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[10] The sign of the noise spectrum here is opposite to what one finds in textbooks. The reason is that the energy of the system is $+\sum_i p_i E_i$, whereas in terms of forces and displacements the energy is $-\sum_i x_i f_i$.

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[16] By treating the receptor cluster as a point object in Eqs. (4) and (24), we are neglecting spatial effects arising from the discreteness of individual receptors. In the limit considered here where ligand-binding is fast compared to the rates of transition between the states of the cluster, the discreteness of individual receptors can be treated as in [9]. In doing so, it can be shown that the diffusive lower bound is given by that of a noninteracting cluster of receptors, which is ultimately limited by the effective linear dimension of the cluster as N_r becomes large.