Increased accuracy of ligand sensing by receptor internalization

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Many types of cells can sense external ligand concentrations with cell-surface receptors at extremely high accuracy. Interestingly, ligand-bound receptors are often internalized, a process also known as receptor-mediated endocytosis. While internalization is involved in a vast number of important functions for the life of a cell, it was recently also suggested to increase the accuracy of sensing ligand as the overcounting of the same ligand molecules is reduced. Here we show, by extending simple ligand-receptor models to out-of-equilibrium thermodynamics, that internalization increases the accuracy with which cells can measure ligand concentrations in the external environment. Comparison with experimental rates of real receptors demonstrates that our model has indeed biological significance.

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I. INTRODUCTION

Biological cells can sense and respond to various chemicals in their environment. However, the precision with which a cell can measure the concentration of a specific ligand is negatively affected by many sources of noise [1–5]. Most noticeable is external noise from the random arrival of ligand molecules at the cell-surface receptors by diffusion. Nonetheless several examples exist in which measurements are performed with surprisingly high accuracy. In bacterial chemotaxis, for instance, fast moving bacteria such as Escherichia coli can respond to changes in concentration as low as 3.2 nM [6]. This value is remarkable, since cells have only about one second between “tumbles” to evaluate the ligand concentration [7]. Furthermore, this concentration value corresponds to only about three ligand molecules in the volume of the cell, assumed to be one femtoliter, suggesting single molecule detection. High accuracy is observed also in spatial sensing by single cell eukaryotic organisms. Best characterized are the slime mold Dictyostelium discoideum, which is able to sense a concentration difference of 1−5% across the cell diameter [8], as well as Saccharomyces cerevisiae (budding yeast), able to orient growth in a gradient of α-pheromone mating factor down to estimated 1% receptor occupancy difference across the cell [9]. Spatial sensing is also efficiently performed by lymphocytes, neutrophils and other cells of the immune system [10], as well as by growing synaptic and tumor cells.

Previously, the fundamental physical limits to the accuracy of sensing as set by ligand diffusion have been calculated [11–15]. Recent work based on simplifying models indicates that, if a cell effectively acts as an absorber of ligand, the accuracy is significantly increased [16]. Such an increase in accuracy can be explained with the fact that absorption prevents ligand molecules from unbinding the receptors. Hence, the same ligand molecule can only be counted once by a receptor, avoiding a source of measurement uncertainty. However, whether cells with realistic receptors can act as absorbers and increase the accuracy of sensing is unknown.

Motivated by these observations, in this paper we analyze the role of receptor-mediated endocytosis, i.e. the internalization of either bound or unbound receptors from the cell membrane into the cell interior, often observed in eukaryotic cells [17, 18]. Internalization of ligand-bound receptors effectively leads to the absorption of ligand molecules and is therefore expected to draw the cell nearer to the physical limit of the perfect absorber [16]. Using simple models for the ligand-receptor dynamics, we find that the effect of receptor-mediated endocytosis indeed increases the accuracy of sensing ligand concentration, if internalization of ligand competes with ligand unbinding. Comparison of our results to the available literature of experimental rate constants shows that receptors often work in this limit, indicating biological relevance of our results.

The paper is organized as follows: In Section II we review the results regarding the accuracy of sensing for a single, immobile receptor without internalization. In Section III, we study the role of internalization using a model of ligand-receptor dynamics with internalization. While ligand-receptor binding and unbinding is described by equilibrium thermodynamics, as previously developed in Refs. [12, 20], internalization clearly introduces non-
The steady-state solution for the concentration of ligand where the ligand dissociation constant. The kinetics for the occupancy of the receptor are therefore given by

\[ \frac{\partial n(t)}{\partial t} = k_+ c [1 - n(t)] - k_- n(t), \]

where the concentration of ligand \( c(x, t) = \bar{c} \) is assumed uniform and constant. The steady-state solution for the receptor occupancy is given by

\[ \bar{n} = \frac{\bar{c}}{\bar{c} + K_D} \]

with \( K_D = k_- / k_+ \) the ligand dissociation constant. The rates of binding and unbinding are related to the (negative) free energy of binding through detailed balance

\[ \frac{k_+ \bar{c}}{k_-} = e^\beta \]

with \( T \) the temperature in energy units. In the limit of very fast ligand diffusion, \( i.e. \) when a ligand molecule is immediately removed from the receptor after unbinding, the dynamics of the receptor is effectively decoupled from the diffusion of ligand molecules and hence, diffusion does not need to be included explicitly.

Following Bialek and Setayeshgar [12], the accuracy of sensing is obtained by applying the Fluctuation Dissipation Theorem (FDT) [19], which relates the spectrum of the fluctuations in occupancy to the linear response to a perturbation in the receptor binding energy. Furthermore, at equilibrium the fluctuations in occupancy can be directly related to the uncertainty in ligand concentration using Eq. (2). For a measurement performed on a time scale \( \tau \) much larger than the correlation time of the binding and unbinding events, the fluctuations of the occupancy \( \langle (\delta n)^2 \rangle \) are obtained from the zero-frequency spectrum divided by \( \tau \). Using \( \langle (\delta n)^2 \rangle \), one then obtains the uncertainty in measuring ligand concentration \( \bar{c} \) [12, 20]

\[ \frac{\langle (\delta c)^2 \rangle_\tau}{\bar{c}^2} = \frac{k_+ \bar{c} (1 - \bar{n})}{2 k_+ \bar{c} (1 - \bar{n}) \tau} + \frac{1}{\pi s D_3 \bar{c} \tau} \]

where the first term on the right-hand side of Eq. (6a) is the same as in Eq. (4), while the second term is the increase in uncertainty due to diffusion. This term accounts for the additional measurement uncertainty from rebinding of previously bound ligand to the receptor. For diffusion-limited binding, one obtains Eq. (6a) [20].

Comparison of Eqs. (4) and (6a) shows that removal of previously bound ligand by fast diffusion increases the accuracy of sensing, since the same ligand molecule is never measured more than once.
III. EFFECT OF RECEPTOR INTERNALIZATION

Here, we consider the case of receptor internalization. As depicted in Fig. 2, receptors at \( x = \bar{x}_0 \) can bind and unbind ligand with given rates. Furthermore, a bound receptorStren can be internalized at rate \( k_i \), while an unbound receptor can be internalized at rate \( k_0^i \). Hence, the kinetics of the fractions of occupied receptors \( n(t) \) and unoccupied receptors \( m(t) \) are given by

\[
\frac{\partial n(t)}{\partial t} = k_+ c m(t) - (k_- + k_i) n(t) \quad \text{(7a)}
\]

\[
\frac{\partial m(t)}{\partial t} = -k_+ c m(t) - k_0^i m(t) + k_- n(t) + k_r. \quad \text{(7b)}
\]

Imposing a single receptor at \( x = \bar{x}_0 \) at any time via

\[ n(t) + m(t) = 1, \quad \text{(8)} \]

Eq. (7) becomes redundant. As shown in Fig. 3 this condition implies that an internalized receptor is immediately replaced by a new, unoccupied receptor with rate \( k_r(t) = k_0^i m(t) + k_i n(t) \). Furthermore, rate \( k_0^i \) of incoming ligand compensates for internalized ligand.

A. Limit of fast diffusion

We first consider the case of fast diffusion, i.e. when ligand unbound from the receptor is immediately removed. In this case the kinetics for the occupancy \( n(t) \) of the single receptor is described by

\[
\frac{\partial n(t)}{\partial t} = k_+ c [1 - n(t)] - (k_- + k_i) n(t)
= k_+ c [1 - n(t)] - k_- n(t), \quad \text{(9)}
\]

where \( k_- = k_+ + k_i \) is the combined rate of unbinding and internalization. The steady-state solution for the receptor occupancy is given by

\[
\bar{n}_i = \frac{\bar{c}}{\bar{c} + k_-/k_+} = \frac{\bar{c}}{\bar{c} + K_M}, \quad \text{(10)}
\]

where \( K_M = k_-/k_+ \) is a Michael-Menten-type constant, and subscript \( i \) is used to indicate the steady-state value for the occupancy of the receptor in presence of internalization (cf. Eq. (2)).

While Eq. (9) could be solved immediately by analogy to Eq. (1), we adopt here, for the fast diffusion case, the method of the effective temperature, which allows us to solve the general case in section III B. Similar to the equilibrium case, at the non-equilibrium steady state the rates can formally be related to the binding free energy

\[
\frac{k_+ \bar{c}}{k_-} = \frac{k_+ \bar{c}}{k_- (1 + k_i/k_-)} = \frac{e^{F/T}}{1 + k_i/k_-} = e^{F/T_e}, \quad \text{(11)}
\]

where we introduced the effective temperature

\[
T_e = \frac{T}{1 - \frac{k_i + k_r}{\ln(k_r/k_i)}}. \quad \text{(12)}
\]

Hence, the effective temperature maps the non-equilibrium steady state to an effective equilibrium, allowing the generalization of the FDT to out-of-equilibrium phenomena \([21,24]\) with applications in modeling biological processes \([22]\). Conceptually, an effective temperature larger than the environment temperature \((T_e > T)\) corresponds to a decrease in the receptor occupancy, approximately reflecting internalization in the non-equilibrium steady state.

In order to calculate the spectrum of the fluctuations in receptor occupancy, we follow Refs. \([12,20]\) and consider small fluctuations around the stationary solution

\[ n(t) = \bar{n}_i + \delta n(t). \]

In order to apply the generalized FDT (gFDT), we introduce fluctuations in the conjugate variable of the receptor occupancy, i.e. the free energy \( F \), via fluctuations of the binding and unbinding rates

\[
\frac{\delta F}{T_e} = \frac{\delta k_+}{k_+} - \frac{\delta k_-}{k_-}, \quad \text{(13)}
\]

where we approximate \( T_e \) as a parameter.

Linearization of Eq. (13) leads to

\[
\frac{\partial [\delta n(t)]}{\partial t} = -(k_+ \bar{c} + k_-) \delta n(t) + k_+ \bar{c} (1 - \bar{n}_i) \frac{\delta F(t)}{T_e}, \quad \text{(14)}
\]

where we used Eq. (13) to replace the fluctuations in the rate constants with fluctuations in the free energy, as well as steady state solution Eq. (10).

Fourier Transforming Eq. (14) yields the susceptibility

\[
\chi(\omega) = \frac{\delta \bar{n}_i(\omega)}{\delta F(\omega)} = \frac{1}{T_e} \frac{k_+ \bar{c} (1 - \bar{n}_i)}{k_+ \bar{c} + k_- - i\omega}, \quad \text{(15)}
\]
describing the linear response of the receptor occupancy to a perturbation in the free energy. We now use the gFDT to calculate the spectrum $S_n(\omega) = \langle |\delta n(\omega)|^2 \rangle$ of the fluctuations in $n(t)$

$$S_n(\omega) = \frac{2T_r}{\omega} \text{Im}[\chi(\omega)] = \frac{2k_+c(1 - \bar{n}_i)}{(k_+c + \kappa_-)^2 + \omega^2} = 2\langle (\delta n)^2 \rangle \frac{\tau_C}{1 + (\omega t_C)^2},$$  \tag{16}

where the correlation time $\tau_C = (k_+c + \kappa_-)^{-1}$ and the total variance

$$\langle (\delta n)^2 \rangle = \int_{-\infty}^{+\infty} \frac{d\omega}{2\pi} S_n(\omega) = \frac{k_+c(1 - \bar{n}_i)}{k_+c + \kappa_-} = \bar{n}_i(1 - \bar{n}_i)$$  \tag{17}

have been introduced. Using Eq. (10), we calculate the uncertainty in ligand concentration from fluctuations in occupancy

$$\delta c = \frac{(c + K_\M)^2}{K_\M} \delta n = \frac{K_\M}{(1 - \bar{n}_i)^2} \delta n = \frac{\bar{c}}{\bar{n}_i(1 - \bar{n}_i)} \delta n.$$  \tag{18}

From Eq. (17) the normalized variance can be obtained

$$\langle (\delta c)^2 \rangle = \frac{1}{\bar{n}_i(1 - \bar{n}_i)},$$  \tag{19}

corresponding to an instantaneous measurement.

In the more realistic case, in which a measurement is performed during an averaging time $\tau \gg \tau_C$, the error in the occupancy is linked to the low frequency spectrum via

$$\langle (\delta n)^2 \rangle_{\tau} \simeq \frac{S_n(0)}{\tau} = \frac{2\bar{n}_i^2(1 - \bar{n}_i)}{k_+c\tau}. $$  \tag{20}

Using Eqs. (17) and (20), the accuracy of sensing is given by

$$\langle (\delta c)^2 \rangle_{\tau} = \frac{2}{k_+c(1 - \bar{n}_i)\tau} \rightarrow \frac{1}{2\pi D_\M c s \tau}.$$  \tag{21}

Eq. (21) is identical to the result in Eq. (4) without internalization, except that $\bar{n}_i < \bar{n}$ due to internalization. In fact, the removal of unbound ligand by fast diffusion at equilibrium is equivalent to removal of bound ligand by internalization at the non-equilibrium steady state. This equivalence can be readily shown from Eq. (3), which is indistinguishable from simple unbinding with rate $\kappa_- = k_- + k_i$. Hence, the effective temperature applied here is in fact exact.

\section*{B. Solution near equilibrium}

When considering ligand diffusion, the above procedure still applies with the exception that the concentration of ligand is allowed to vary due to binding and unbinding. The kinetics of the receptor occupancy and ligand concentration is described by

$$\frac{\partial n(t)}{\partial t} = k_+c(x_0, t)[1 - n(t)] - \kappa_- n(t) \tag{22a}$$

$$\frac{\partial c(\vec{x}, t)}{\partial t} = D_\M \nabla^2 c(\vec{x}, t) - \delta(\vec{x} - \vec{x}_0) \left[ \frac{\partial n(t)}{\partial t} + k_i n(t) \right] + k_\infty \delta(\vec{x} - \vec{x}_\infty), \tag{22b}$$

where $\kappa_- = k_- + k_i$ is used as before. Furthermore, a source of ligand with rate $k_\infty$ is considered at location $\vec{x}_\infty$ so as to compensate the loss of ligand molecules due to internalization.

We note that the steady-state solution for the concentration is not spatially uniform, but is depleted near the receptor due to internalization. This leads to the anomaly that we mathematically evaluate the rate of binding using the ligand concentration at $\vec{x}_0$ in Eq. (22a), while physically the diffusive flux and hence binding of ligand is determined by the ligand concentration $\vec{c}$ far away from the receptor (see section III C). This is remedied by linearizing the ligand concentration around $\vec{c}$ in the following. Furthermore, Eq. (8) is again assumed valid, and therefore an additional equation describing the unoccupied receptor fraction $m(t)$ with rates $k_0^i$ and $k_r$ is redundant.

Linearizing Eqs. (22a) and (22b) leads to

$$\frac{\partial(\delta n(t))}{\partial t} = k_+c(x_0, t)[1 - n(t)] - \kappa_- \delta n(t)$$  \tag{23a}

$$\frac{\partial[\delta c(\vec{x}, t)]}{\partial t} = D_\M \nabla^2 \delta c(\vec{x}, t) - \delta(\vec{x} - \vec{x}_0) \left[ \frac{\partial[\delta n(t)]}{\partial t} \right] + k_i \delta n(t).$$  \tag{23b}

By applying the quasi-equilibrium picture with the effective temperature $T_r$ introduced in the previous subsection, we use Eq. (10) to introduce fluctuations in the free energy, and obtain
Fourier Transforming Eqs. (24a) and (24b), we obtain
\[ \delta \hat{c}(\vec{q}, \omega) = e^{i \vec{q} \cdot \vec{x}_0} \frac{i \omega - k_i}{D_3 q^2 - i \omega} \delta \hat{n}(\omega), \] (25)
which can be inverse-Fourier Transformed in \( \vec{x}_0 \)
\[ \delta \hat{c}(\vec{x}_0, \omega) = (i \omega - k_i) \delta \hat{n}(\omega) \int \frac{d^3q}{(2\pi)^3} \frac{1}{D_3 q^2 - i \omega}. \] (26)
Inserting Eq. (26) in the Fourier-Transformed Eq. (24a), we obtain
\[ \chi(\omega) = \frac{\delta \hat{n}(\omega)}{\delta F(\omega)} = \frac{1}{T_C} \frac{\kappa - \bar{n}_i}{k_i + \bar{c} + \kappa_- + (k_i - i \omega) \Sigma_1(\omega)} - i \omega, \] (27)
where \( \Sigma_1(\omega) \) is given by
\[ \Sigma_1(\omega) = \int \frac{d^3q}{(2\pi)^3} \frac{k_i (1 - \bar{n}_i)}{D_3 q^2 - i \omega} = \int_0^\Lambda \frac{dq}{2\pi^2} \frac{q^2 [k_i (1 - \bar{n}_i)]}{2\pi^2 D_3 q^2 - i \omega} \approx \frac{k_i (1 - \bar{n}_i)}{2\pi^2 D_3}, \] (28)
Here \( \Lambda \approx \pi/s \) is a cut-off due to the finite size \( s \) of the receptor, introduced to regularize the integral in Eq. (28).
As before, we apply the gFDT to derive the spectrum of the fluctuations \( \delta \hat{n}(\omega) \)
\[ S_n(\omega) = \frac{2T_C}{\omega} \text{Im}[\chi(\omega)] \]
\[ = \frac{2k_i \bar{c} (1 - \bar{n}_i) [1 + \Sigma_1(\omega)]}{[k_i \Sigma_1(\omega) + k_i \bar{c} + \kappa_- + \omega^2 (1 + \Sigma_1(\omega))^2]}, \] (29)
where we used Eq. (27) for the susceptibility. In the realistic case, in which the measurement is time averaged over duration \( \tau \) much larger then the correlation time of the fluctuations, the relevant part of the spectrum is the zero frequency limit
\[ S_n(\omega \simeq 0) \approx \frac{2k_i \bar{c} (1 - \bar{n}_i) [1 + \Sigma_1(0)]}{[k_i \Sigma_1(0) + k_i \bar{c} + \kappa_-]^2} \]
\[ = 2 \langle (\delta n)^2 \rangle \frac{(1 + \Sigma_1(0)) \tau_C}{(1 + k_i \Sigma_1(0) \tau_C)^2} \]
\[ \simeq 2 \langle (\delta n)^2 \rangle \frac{(1 + \alpha \Sigma_1(0)) \tau_C}{(1 + \alpha \Sigma_1(0))^2}, \] (30)
where \( \alpha = 1 - 2k_i \tau_C < 1 \), and higher order terms in \( \Sigma_1(0) \) are neglected for sufficiently fast diffusion. As before, \( \langle (\delta n)^2 \rangle = \bar{n}_i (1 - \bar{n}_i) \) and \( \tau_C = (k_i \bar{c} + \kappa_-)^{-1} \).

Using Eq. (15), the normalized variance of the concentration is given by
\[ \frac{\langle (\delta c)^2 \rangle}{\tau^2} = \frac{\langle (\delta n)^2 \rangle}{\tau^2} = \frac{1}{\tau} \] (31)
where \( \tau \) is the averaging time. Using Eq. (30) for the power spectrum, we finally obtain for the accuracy of sensing with ligand internalization and diffusion
\[ \frac{\langle (\delta c)^2 \rangle}{\tau^2} = \frac{2}{k_i \bar{c} (1 - \bar{n}_i) \tau} + \frac{\alpha}{\pi D_3 \bar{c} \tau} \] (32a)
\[ \rightarrow \frac{1 + 2\alpha}{2\pi D_3 \bar{c} \tau}. \] (32b)

The following conclusions can be drawn by comparison with the result Eq. (6) without internalization: (i) Receptor internalization mainly reduces the second term in Eq. (4), demonstrating for the first time that internalization increases the accuracy of sensing by reducing the uncertainty from rebinding of previously bound ligand. The first term is only reduced by replacing \( \bar{n} \) by \( \bar{n}_i \), with \( \bar{n}_i < \bar{n} \). (ii) In the limit \( k_i \rightarrow 0, \alpha \rightarrow 1 \) and the equilibrium result Eq. (6) without internalization is recovered. (iii) As Eq. (32) becomes unphysical in the limit of \( k_i \rightarrow \infty \), i.e. does not approach Eq. (24) without readding, our result can only be regarded an approximation valid near equilibrium.

C. Comparison with perfect absorber

The perfect absorber is here defined as a receptor, which internalizes a ligand immediately once it is bound. Following Ref. [16], the accuracy of sensing can be calculated from the Poisson statistics of the number of binding events \( N \) in time \( \tau \)
\[ \frac{\langle (\delta c)^2 \rangle}{\tau^2} = \frac{\langle (\delta N)^2 \rangle}{\langle N^2 \rangle} = \frac{1}{4\pi D_3 \bar{c} \tau^2}. \] (33)
obtained from the diffusive flux of ligand to an absorbing sphere of radius \( s \). Comparison with Eq. (24) for diffusion-limited binding without readding shows that the perfect absorber is yet more accurate by a factor 2. This is due to the fact that the fluctuations in occupancy in Eq. (24) stem from the random binding and unbinding/internalization events, while the uncertainty in Eq. (33) solely stems from the random binding events (see also factor 2 in Eq. (A2), as well as Ref. [20] for further explanation).
IV. IMPLICATIONS FOR BIOLOGY

In the previous section we showed that internalization increases the accuracy of sensing by reducing the measurement uncertainty from rebinding of previously bound ligand. In this section we review some receptors of known rate constants. We specifically would like to determine if the rate of internalization $k_i$ is fast enough, i.e. comparable to the unbinding rate $k_-$, in order to effectively increase the accuracy of sensing.

In Table I, we summarize experimental values for rate constants, including internalization, of various receptors. Most G-protein coupled receptors (GPCR) undergo internalization $\alpha$. The Ste2 receptor in haploid yeast cells of α-mating type is involved in α pheromone sensing and signal transduction, leading to cell polarization, 'shmoo' formation and mating. The folate receptor (FR) in Dictyostelium, likely a GPCR $\dagger$, is used to sense and hunt bacteria. (The folate-binding protein in mammalian cells is a diagnostic marker for various cancers, and its internalization is exploited for drug delivery into cancerous human cell $[29]$. ) However, the cAR1 receptor in Dictyostelium, used for sensing of cAMP under starvation, is not internalized $[30]$. The epidermal growth factor receptor (EGFR), a tyrosine kinase, is another important example of a receptor which is internalized $[31]$. This receptor is involved in cell growth, proliferation and differentiation $[31] [32]$. Another class of internalized receptors is involved in uptake. Transferrin receptor (TfR) is used for iron uptake from extracellular space and plays therefore an important role in blood cells $[31]$. Transferrin binds to TfR, is internalized and releases its iron load through ion pump-induced pH reduction. The ligand-bound TfR is then recycled back to cell surface. Another example is the low density lipoprotein receptor (LDLR) $[31]$. When bound to LDL-cholesterol via adaptin, LDLR is internalized via clathrin-coated vesicles $[31]$. Furthermore, the vitellogenin receptor (VtgR) is involved in oogenesis (egg-formation) $[31]$. Once internalized, vitellogenin is turned into yolk proteins. Ligand-free receptors are recycled back to cell surface. Table I shows that in most cases $k_i$ is of the same order or larger than $k_-$, except for FR, where internalization is much slower than the unbinding of the ligand from the receptor.

| Receptor | Function | $k_-$ (min$^{-1}$) | $K_D$ (nM) | $k_i$ (min$^{-1}$) | $k^\dagger$ (min$^{-1}$) |
|----------|----------|-----------------|-----------|-----------------|-----------------|
| FR       | chemotaxis: feeding | 0.096 $[34]$ | 20.0 $[34]$ $^\dagger$ | 9.6×10$^{-4}$ $[29]$ $^*$ | – |
| Ste2     | mating   | 0.06 $[32]$ | 22.1 $[35]$ | 0.24 $[36]$ | 0.024 $[36]$ |
| EGFR $[31]$ | development | 0.108 $[37]$ | 6.0 $[27]$ | 0.156 $[38]$ | 0.0156 $[38]$ |
| TIR $[31]$ | uptake: iron | 0.24 | 2.47 | 0.15 | 0.02 |
| LDLR $[31]$ | cholesterol | 0.09 | 29.8 | 0.6 | 0.6 |
| VtgR $[31]$ | vitellogenin | 0.04 | 14.3 | 0.195 | 0.195 |
|          |          | 0.07 | 1300 | 0.108 | 0.108 |

TABLE I: Summary of experimental data for relevant receptor rates discussed in the main text. $^\dagger$ However, other values have been reported as well $[41]$. $^*$ This rate is measured for folate-binding protein in cancerous mice cells, not in Dictyostelium.

Figure 4 visualizes the contribution of internalization to the accuracy of sensing for the receptors from Table I. The faster the internalization, the larger the increase of the accuracy of sensing. However internalization can only reduce the second term in Eq. $[15]$ from rebinding of previously bound ligand, not the first term from random binding and unbinding. To illustrate the relative contribution of the two terms, we plot the square root of their ratio in Fig. 4 (filled bars). This shows that in most cases, in which internalization occurs, the noise ratio of the two terms is significant, ranging from few hundredths to order of unity. Hence, internalization can lead to a substantial increase in the accuracy of sensing. However, measured rate constants are substantially uncertain (see below) and diffusion coefficients of small ligand molecules range from 0.1μm$^2$/s in the synaptic cleft between neurons $[32]$ to 1 – 10μm$^2$/s in blood $[40]$ to 300μm$^2$/s in water $[16]$. In order to avoid this uncertainty in parameters, we also plot the upper limit of the noise ratio for diffusion-limited binding equal to $\sqrt{2}$ (dashed line in Fig. 4). Removal of the second noise term in this limit by internalization would increase the accuracy in Eq. $[6b]$ by a factor 3. In Fig. 4, we also show the strength of internalization, defined by the ratio of internalization and unbinding rates (open bars).

How reliable are the measured values for the rate constants? Rate constants are generally obtained through radioactive labeling of ligand, with the isotope choice targeted to each specific case (the isotope $^{125}$I giving the most accurate measurements) $[42]$. In order to mea-
sure the unbinding and the binding rates, i.e. $k_-$ and $k_+ = k_- / K_D$, the receptors on the membrane must be separated by filtration or centrifugation from the soluble ligand. If the ligand-unbinding process is slow compared to separation, then the measurement of the amount of bound ligand through the radioactive label can be easily carried out; in the case of fast unbinding, measurements are less accurate. For the internalization rate, the ratio between the intensity at the surface and inside the cell is measured and from the slope of the time variation of this ratio, $k_i$ is determined. This method, though, does not take into account recycling (diacytosis) of the receptor, which is a recurrent feature of the internalization process, also included in our model. These shortcomings, as well as the variability associated with different cell preparations, lead to a large error in the rate determination ($\sim 20\%$) and variability between different measurements ($20\%-90\%$). Other measurement methods employed in experiments include protease sensitivity assays and destination assays.

Many examples in fact suggest a direct relation between ligand internalization and the accuracy of sensing, measured by the sensitivity of cell polarization or cell movement in shallow chemical gradients. These include sensing of α-factor by budding yeast, folate by Dictyostelium, and PDGF by fibroblasts. Other examples relate to embryonic development. In zebrafish, primordial germ cells migrate toward chemokine SDF-1a that binds and activates the receptor CXCR4b. It was recently shown that ligand-induced CXCR4b internalization is required for precise arrival of germ cells at their target destination. During Drosophila oogenesis, border cells perform directional migration. EGFR, together with two other receptor tyrosine kinases, is the main guidance receptor. Recent work in this system provided compelling evidence that guided cell movement also requires spatial control of signaling events by endocytic dynamics.

V. DISCUSSION AND CONCLUSIONS

In this paper we analyzed the role of receptor internalization in the accuracy of sensing ligand concentration. By extending equilibrium single receptor models to nonequilibrium thermodynamics introduced by internalization, we derived expressions for the uncertainty in sensing ligand concentration. As expected, internalization of ligand-bound receptors makes the cell act similarly to an absorber and increases the accuracy of sensing. We then analyzed relevant experimental data, summarized in Table I, and concluded that in most cases, the contribution of receptor internalization to the increase in accuracy of sensing is non-negligible. However, a perfect absorber is yet more accurate as its uncertainty only stems from random binding events, not from additional random unbinding and internalization events. Whether cells have developed mechanisms to approach the limit of the perfect absorber, e.g. by internalization, is not clear yet. However, since the accuracy of concentration sensing can always be improved by increasing the averaging time, one might expect that receptor internalization becomes increasingly important when time is of the essence. In addition to chemotaxis, embryonic development may be a biological system for which receptor internalization is important. Specifically, the Fg8 morphogen, which regulates tissue differentiation and morphogenesis in zebrafish, forms exponential concentration gradients by diffusion and degradation, the latter being achieved precisely by receptor internalization. However, based on our results, internalization may also be used for the accurate readout of the gradient in the short amount of time dictated by cell division.

Cells generally have many receptors to estimate external concentrations of chemicals, leading to a spatial averaging and consequently further increase in the accuracy of sensing. However, even the employment of many receptors cannot increase the accuracy of sensing beyond the physical limit of the perfect absorber. In fact a cell only needs a relatively small number of receptors to achieve an accuracy comparable to the physical limit. On the downside, if a cell uses many receptors, it needs to integrate this information in signaling pathways. If this process is fundamentally limited by noise as well, then this noise provides an upper limit on the overall estimation performance.

Although we analyzed the role of receptor internalization in increasing the accuracy of sensing, it is important to stress that internalization fulfills several other known purposes in cells. Among them are (i) redistribution of receptors to different locations on the cell membrane, (ii) uptake of nutrients and chemicals, (iii) signaling by...
ligand in cell interior, and (iv) turning off persistent signal as part of adaptation. All these aspects would need to be considered to fully characterize the working of a receptor.

In order to derive the accuracy of sensing with internalization, we made a number of simplifying assumptions. In our model we neglected other possible sources of fluctuations such as fluctuations in receptor density, in order to relate our results to the single, immobile receptor. Furthermore, we introduced the effective temperature $T_e$ to generalize the FDT to non-equilibrium thermodynamics. While $T_e$ is well defined for well separated time scales, a potential time or frequency dependence of $T_e$ was neglected here. However, our non-equilibrium result with internalization is consistent with the equilibrium result in the fast diffusion limit. Removal of ligand by fast diffusion at equilibrium is equivalent to removal of bound ligand by internalization at the non-equilibrium steady state, providing confidence in our method.

Internalization is not the only mechanism, by which a cell can act as an absorber and increase its accuracy of sensing. Other potential mechanisms include enzymatic degradation of ligand at the cell surface, e.g. degradation of cAMP by mPDE in Dictyostelium and of mating pheromone by Bar1 in budding yeast. Furthermore, at excitatory neural synapses, fast diffusion of AMPA receptors on the post-synaptic membrane surface has an important role in the sensing of neurotransmitter glutamate. Ligand-bound, desensitized receptors diffuse away and are replaced by fresh receptors, leading to fast recovery and readiness for the next action potential and release of neurotransmitter. By the same mechanism, the accuracy of sensing may be increased, since ligand-bound receptors diffuse away and release ligand far away from region of signaling, thus preventing an overcounting of same ligand molecules.

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Appendix A: Langevin approach

As an alternative derivation, here we provide the solution for the accuracy of sensing for internalization using a Langevin approach. We first consider fast diffusion. We start from Eq. (9) but add a noise term, $\xi_n(t)$

$$\frac{\partial n(t)}{\partial t} = k_+c[1-n(t)] - \kappa_- n(t) + \xi_n(t), \quad (A1)$$

where we assume

$$\langle [\xi_n(\omega)]^2 \rangle = k_+c(1-\bar{n}_i) + \kappa_-\bar{n}_i = 2k_+c(1-\bar{n}_i) \quad (A2)$$

due to Poisson statistics. Linearizing and Fourier Transforming Eq. (A1), assuming the rates $k_+$ and $\kappa_-$ constant, leads to

$$\delta \hat{n}(\omega) = \frac{\hat{\xi}_n(\omega)}{k_+c + \kappa_- - i\omega} \quad (A3)$$

Hence, the power spectrum of the fluctuations in receptor occupancy is given by

$$S_n(\omega) = \langle [\delta \hat{n}(\omega)]^2 \rangle = \frac{\langle [\dot{\xi}_n(\omega)]^2 \rangle}{(k_+c + \kappa_-)^2 + \omega^2} = \frac{2k_+c(1-\bar{n}_i)}{(k_+c + \kappa_-)^2 + \omega^2}, \quad (A4)$$

where in the last step the property Eq. (A2) was used. Eq. (A1) is indeed equivalent to result Eq. (16) in the main text.

For the general solution, we start from the Fourier-Transformed Eq. (24) and Eq. (26), i.e.

$$\langle \delta \hat{n}(\omega) \rangle = \frac{\xi(1-\bar{n}_i)}{k_+c(1-\bar{n}_i)} \hat{\xi}(\bar{x}_0,\omega) + \xi(\bar{\omega}), \quad (A5)$$

and

$$\delta \hat{\chi}(\bar{x}_0,\omega) = \frac{(i\omega - k_1)\Sigma_1(\omega)}{k_+c(1-\bar{n}_i)} \delta \hat{n}(\omega) + \xi(\bar{\omega}), \quad (A6)$$

respectively, where $\xi_n(\omega)$ and $\xi(\omega)$ are additive noise terms, and $\Sigma_1(\omega)$ is given by Eq. (28). Inserting Eq. (A6) in Eq. (A5) and solving for $\delta \hat{n}(\omega)$ leads to

$$\delta \hat{n}(\omega) = \frac{\xi_n(\omega) + k_+c(1-\bar{n}_i)\xi(\bar{\omega})}{k_+c + \kappa_- + k_1\Sigma_1(\omega) - i\omega(1 + \Sigma_1(\omega))}, \quad (A7)$$

from which the following expression for $\langle [\delta \hat{n}(\omega)]^2 \rangle$ ensues

$$\langle [\delta \hat{n}(\omega)]^2 \rangle = \frac{k_+c(1-\bar{n}_i)^2 + \langle [\xi(\bar{\omega})]^2 \rangle}{k_+c + \kappa_- + k_1\Sigma_1(\omega)} + \frac{\langle [\xi_n(\omega)]^2 \rangle}{\omega(1 + \Sigma_1(\omega))} \quad (A8)$$

In the limit $\omega \to 0$, using Eq. (A2) as in the previous case, we obtain

$$\langle [\delta \hat{n}(\omega)]^2 \rangle \to 0 \Rightarrow \frac{k_+c(1-\bar{n}_i)^2 + 2k_+c(1-\bar{n}_i)}{k_+c + \kappa_- + k_1\Sigma_1(0)} \quad (A9)$$

Following [58], we set

$$\langle [\xi_n(\omega)]^2 \rangle \sim S^{\text{4D}}_c(\omega \to 0) \sim \frac{\bar{c}}{D_{4\text{s}}} \sim \frac{\Sigma_1(0)}{k_+(1-\bar{n}_i)}, \quad (A10)$$

in Eq. (A9), and obtain for the power spectrum

$$\langle [\delta \hat{n}(\omega)]^2 \rangle = \frac{2\langle [\xi_n(\omega)]^2 \rangle[1 + \Sigma_1(0)]\tau_c}{[1 + k_\Sigma_1(0)c]^2} \quad (A11)$$

Eq. (A11) is identical to result Eq. (30), obtained with the gFDT in main text.
Appendix B: Numerical values

In this section we provide the numerical values used for plotting Fig. 4. Noise ratio and internalization strengths are respectively given by: 0.1/0.01 (FR), 0.08/4 (Ste2), 0.47/0.625 (EGFR), 0.08/6.6 (TfR), 0.08/4.875 (LDLR), 0.011/1.54 (VtgR). Specifically, to calculate the noise ratio, the first and the second term in Eq. (6a) are given by (in units of $\tau$): 2500/27.6 (FR), 4000/25.1 (Ste2), 1000/224 (EGFR), 2667/18.6 (TfR), 6000/38.72 (LDLR), 3428/0.426 (VtgR). We have used $s = 1nm$, $\bar{n} = 1/2$, i.e. setting $\bar{e} = K_D$ from Table I, and $D_s = 1 \mu m^2/s$.

[1] A. Raj and A. van Oudenaarden, Cell 135, 216 (2008).
[2] R. C. Yu et al., Nature 456, 755 (2008).
[3] M. S. Samoilov, G. Price, and A. P. Arkin, Sci. STKE 2006, re17 (2006).
[4] T. Gregor et al., Cell 130, 153 (2007).
[5] V. Shahrezaei and P.S. Swain, Curr. Opin. Biotech. 19, 369 (2008).
[6] H. Mao, P.S. Cremer, and M. D. Manson, Proc. Natl. Acad. Sci. USA 100, 5449 (2003).
[7] H. C. Berg, Random Walks in Biology, Princeton University Press (1993).
[8] R. A. Arkovitz, Trends Cell. Biol. 9, 20 (1999).
[9] J. E. Segall, Proc. Natl. Acad. Sci. USA 90, 8332 (1993).
[10] S. H. Zigmund, J. Cell. Biol. 75, 606 (1977).
[11] H. C. Berg and E. T. Purcell, Biophys. J. 20, 193 (1977).
[12] W. Bialek and S. Setayeshgir, Proc. Natl. Acad. Sci. USA 102, 10040 (2005).
[13] W. Rappel and H. Levine, Proc. Natl. Acad. Sci. USA 105, 19720 (2008).
[14] W. Rappel and H. Levine, Phys. Rev. Lett. 100, 228101 (2006).
[15] K. Wang, W. Rappel, R. Kerr, and H. Levine, Phys. Rev. E 75, 061905 (2007).
[16] R. G. Endres and N. S. Wingreen, Phys. Rev. E 75, 061905 (2008).
[17] S. Mukherjee, R. N. Ghosh, and F.R. Maxfield, Phys. Rev. E 77, 750 (1997).
[18] S. S. F. Ferguson, Pharmacol. Rev. 53, 1 (2001).
[19] R. Kubo, Rep. Prog. Phys. 29, 255 (1966).
[20] R. G. Endres and N.S. Wingreen, Progr. Biophys. Mol. Biol., 100, 33 (2009).
[21] L. F. Cugliandolo, J. Kurchan, and L. Politi, Phys. Rev. E 55, 3898 (1997).
[22] Th. M. Nieuwenhuizen, Phys. Rev. Lett. 80, 5580 (1998).
[23] A. Crisanti and F. Ritort, J. Phys. A 36, R181 (2003).
[24] L. Leuzzi, J. of Non-Cryst. Solids 355, 686 (2009).
[25] T. Lu, J. Hasty, and P. G. Wolynes, Biophys. J. 91, 84 (2006).
[26] R.G. Endres and N.S. Wingreen, Phys. Rev. Lett. 103, 158101 (2009).
[27] J. L. Rifkin, Cell Motil. Cytoskeleton 48, 121 (2001).
[28] F. Kesbeke et al., J. Cell. Sci. 96, 669 (1990).
[29] C. M. Paulos et al., Mol. Pharm. 66, 1406 (2004).
[30] M. J. Catarina, D. Hereld, and P.N. Devreotes, J. Biol. Chem. 270, 4418 (1995).
[31] H. Shankaran, H. Resat, and H. S. Wiley, PLoS Comp. Biol. 3, e101 (2007).
[32] A. Bianco et al., Nature 448, 362 (2007).
[33] P. Duchek and P. Rorth, Science 291, 131 (2001).
[34] S. G. Nandini-Kishore and W. A. Frazier, Proc. Natl. Acad. Sci. USA 78, 7299, (1981).
[35] S. K. Raths, F. Naider and J. M. Becker, J. Biol. Chem 263, 17333 (1988).
[36] T. Yi, H. Kitano, and M. I. Simono, Proc. Natl. Acad. Sci. USA 100, 10765 (2003).
[37] D. Jennes, A. C. Burkholder, and L. H. Hartwell, Mol. and Cell. Biol. 6, 318 (1986).
[38] L. Hiche, B. Zanolari and H. Riezman, J. of Cell Biology 141, 349 (1998).
[39] T. A. Nielsen, D. A. Di Gregorio and R. A. Silver, Neuron 42, 757 (2004).
[40] U. Olga, V. Kurtcuoglu and D. Poulikakos, Am. J. Physiol. Heart Circ. Physiol. 294, H909 (2008), R. C. Roberts, D. G. Makey and U. S. Seal, J. of Biol. Chem. 241, 4907 (1966), R. G. Thorne, S. Hrabetova and C. Nicholson, J. Neurophysiol. 92, 3471 (2004).
[41] J. E. Segall et al., J. Cell. Sci. 91, 479 (1998).
[42] A. Levitzki, Receptors: a quantitative approach, Benjamin/Cummings Pub. Co. (1984).
[43] L. Opresko and H. S. Wiley, J. Biol. Chem. 262, 4109 (1987).
[44] K. A. Schandel and D.D. Jenness, Mol. Cell. Biol. 14, 7245 (1994).
[45] K. J. Blumer and J. Thonnese, Proc. Natl. Acad. Sci. USA 87, 4363 (1990).
[46] K. Kawada et al., Mol. and Cell. Biol. 29, 4508 (2009).
[47] S. Minina, M. Reichman-Fried, and E. Raz, Curr. Biol. 17, 1164, (2007).
[48] G. Jékely, H. Sung, C. M. Luque, and P. Rorth, Dev. Cell 9, 197 (2005).
[49] C. Le Roy and J. L. Warn, Dev. Cell 9, 167 (2005).
[50] S.R. Yu et al., Nature, 461, 533, (2009).
[51] I. Mellman, Ann. Rev. Cell Dev. Biol 12, 575, (1996).
[52] J. B. Hicks and I. Herskowitz, Nature 260, 246 (1976).
[53] M. Barkai, M.D. Rose, and N.S. Wingreen, Nature, 396, 422 (1998).
[54] D. Choquet and A. Triller, Nature Rev. Neurosci. 4, 251 (2003).
[55] G. Aquino and R. G. Endres, in preparation.
[56] P. Dettwiler et al., Biophysics. J 79, 2801, (2000).