

How biochemical resources determine fundamental limits in cellular sensing

Christopher C. Govern¹ and Pieter Rein ten Wolde¹

¹*FOM Institute AMOLF, Science Park 104, 1098 XG Amsterdam, The Netherlands.*

Living cells deploy many resources to sense their environments, including receptors, downstream signaling molecules, time and fuel. However, it is not known which resources fundamentally limit the precision of sensing, like weak links in a chain, and which can compensate each other, leading to trade-offs between them. We show by modeling that in equilibrium systems the precision is limited by the number of receptors; the downstream network can never increase precision. This limit arises from a trade-off between the removal of extrinsic noise in the receptor and intrinsic noise in the downstream network. Non-equilibrium systems can lift this trade-off by storing the receptor state over time in chemical modification states of downstream molecules. As we quantify for a push-pull network, this requires i) time and receptors; ii) downstream molecules; iii) energy (fuel turnover) to drive modification. These three resource classes cannot compensate each other, and it is the limiting class which sets the fundamental sensing limit. Within each class, trade-offs are possible. Energy allows a power-speed trade-off, while time can be traded against receptors.

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Biochemical networks are the information processing devices of life. Like any device, they require resources to be built and run. Components are needed to construct the network, space is required to accommodate the components, time is needed to process the information, and energy is required to make the components and operate the network. These resources constrain the design and performance of any biochemical network. Yet, it is not clear which resources are indispensable, thus fundamentally limiting the performance of the network, and which resources might trade-off against each other. Here we consider the interplay among cellular resources, network design, and performance in a canonical biochemical function, namely sensing the environment.

Living cells can measure chemical concentrations with extraordinary precision (1–3), raising the question what sets the fundamental limit to the accuracy of chemical sensing (1). Cells measure chemical concentrations via receptors on their surface. These measurements are inevitably corrupted by noise that arises from the stochastic arrival of ligand molecules by diffusion and from the stochastic binding of the ligand to the receptor. Berg and Purcell pointed out that the sensing error is fundamentally bounded by this noise extrinsic to the cell, but that cells can reduce the error by taking multiple independent measurements, mitigating the risk that any one is corrupted by a noisy fluctuation (1). One way to increase the number of measurements is to add more receptors to the surface (1, 4). Another is to take more measurements per receptor over time; in this approach, the cell infers the concentration not from the instantaneous number of ligand-bound receptors but rather from the time-average receptor occupancy over an integration time T (1, 4–10).

This time integration has to be performed by the signaling networks that transmit the information from the surface of the cell to its interior (10). To reach the fundamental limit on the accuracy of sensing, these networks

have to remove the extrinsic noise in the receptor state as much as possible. Signaling networks, however, are also stochastic in nature, which means that they will also add noise to the transmitted signal. Most studies on the accuracy of sensing have ignored this intrinsic noise of the signaling network. They essentially assume that the intrinsic noise can be made arbitrarily small and that the extrinsic noise in the input signal can be filtered with arbitrary precision by simply integrating the receptor signal for longer. Yet, the extrinsic and intrinsic noise are not generally independent (11). Indeed, what resources are required to simultaneously remove the extrinsic and intrinsic noise is not understood.

While the work of Berg and Purcell and subsequent studies identify time and the number of receptors as resources that limit the accuracy of sensing, the fundamental limits that have emerged ignore the cost of making and operating the signaling network. Making proteins is costly; producing proteins that confer no benefit to the cell has been shown to slow down bacterial growth (12). They also take up valuable space that might be used for other important processes, either on the membrane or inside the cytoplasm. Both are highly crowded, with proteins occupying 25 – 75% of the membrane area (13) and 20 – 30% of the cytoplasmic volume (14). Moreover, many signaling networks must be driven out of thermodynamic equilibrium by the continuous turnover of fuel molecules such as ATP, leading to the dissipation of heat. Fuel is essential for network functions such as bistability, oscillations, and kinetic proofreading (15–17), and can be important for adaptation (18, 19). However, whether there exists a fundamental relationship between energy and sensing, independent of the design of the signaling network inside the cell, remains unclear (20–22).

In this manuscript we derive how the accuracy of sensing depends on not only time and the number of receptors, but also on the resources required to build and op-

erate the downstream signaling network: the copies of signaling molecules and fuel. This allows us to address the following questions: How do the sensing limits set by the latter resources compare to the canonical limit of Berg and Purcell, which is set by the resources time and the number of receptors? How does the limit set by one resource depend on the levels of the other resources? Can resources compensate each other to achieve a desired sensing precision, leading to trade-offs between them, or are the limits set by the respective resources fundamental, *i.e.* independent of the levels of the other resources? And how do the limits depend on the design of the signaling network? The relationship between the accuracy of sensing, the design of the network, and the resources required to build and operate it—time, energy and protein copies—underlies the design principles of biochemical sensing systems.

We first study the relationship between sensing precision, network design, and resources, for systems that are not driven out of thermodynamic equilibrium, consuming no fuel. We find that these equilibrium networks can time-integrate the receptor signal to remove the extrinsic noise in it, analogous to the mechanism described by Berg and Purcell. Clearly, fuel is not a fundamental resource for sensing or removing extrinsic noise. However, using the fluctuation-dissipation theorem, we will show that equilibrium networks face a fundamental trade-off between the removal of extrinsic noise in the receptor state and the suppression of intrinsic noise in the processing network: decreasing one source of noise necessarily increases the other. As a result, the accuracy of sensing is fundamentally limited by the number of receptors; in equilibrium networks, adding downstream components can never improve the sensing precision.

To improve the sensing accuracy beyond the limit set by the number of receptors, it is essential to break the trade-off between extrinsic and intrinsic noise. As we show, this requires a fundamentally different sensing mechanism. Instead of using the receptors to harvest the energy of ligand binding, as in the equilibrium sensing mode, the receptors should be used as catalysts to modify downstream read-out molecules. This non-equilibrium strategy, however, uses not only receptors but requires also time, copies of downstream read-out molecules, and fuel turnover.

We quantify the limits that arise from each of the resources—copies of receptors and downstream molecules, time, and fuel—for a canonical signaling motif, a receptor that drives a Goldbeter-Koshland push-pull network (23). Push-pull networks are ubiquitous in prokaryotic and eukaryotic cell signaling (24): examples include GTPase cycles, as in the Ras system (25), and phosphorylation cycles, as in mitogen-activated-protein-kinase (MAPK) cascades (26) or in two-component systems like the chemotaxis system of *Escherichia coli* (27). We find that the resource limitations of these systems

emerge naturally when the signaling networks are viewed as devices that discretely, rather than continuously, sample the receptor state via collisions of the signaling molecules with the receptor proteins. This analysis reveals that three classes of resources are required: i) time and receptors; ii) copies of downstream molecules; and iii) fuel. Indeed, these classes cannot compensate each other: each imposes a sensing limit, and it is the limiting class that imposes the fundamental limit on the accuracy of sensing. However, there can be trade-offs within each class of resources. Receptors and time trade off against each other in achieving a desired sensing accuracy and power and response time trade off against each other to meet the energy requirement for taking a measurement. We end by discussing how our findings apply to specific signaling systems and how our results on push-pull networks generalize to more complex networks involving cascades, and positive and negative feedback. In particular, our analysis leads to a concrete prediction for the optimal resource allocation, which we test for the *E. coli* chemotaxis system.

RESULTS

Consider a cell with R_T receptors on its surface that independently bind ligand, $R + L \rightleftharpoons RL$. The cell senses the ligand concentration c based on the instantaneous level of a downstream read-out molecule x at some time. Via error propagation, the cell's uncertainty about c is then (5):

$$\left(\frac{\delta c}{c}\right)^2 = \frac{1}{c^2} \frac{\sigma_x^2}{\left(\frac{d\bar{x}}{dc}\right)^2} = \frac{\sigma_x^2}{\left(\frac{d\bar{x}}{d\mu_L}\right)^2}, \quad (1)$$

where $\mu_L = \mu_0 + \log c$ is the ligand's chemical potential. The uncertainty is low if the average read-out level \bar{x} responds sensitively to changes in ligand concentration, as measured by the gain $d\bar{x}/dc$, but is not noisy, as measured by the variance σ_x^2 .

If the receptor-ligand complex itself is taken as the read-out, then the error is:

$$\left(\frac{\delta c}{c}\right)^2 = \frac{1}{p(1-p)} \frac{1}{N_I} = \frac{1}{p(1-p)} \frac{1}{R_T}, \quad (2)$$

since $\sigma_{RL}^2 = \frac{dRL}{d\mu_L} = R_T p(1-p)$, where p is the probability a receptor is bound to ligand. Indeed, $1/(p(1-p))$ is the “instantaneous error”, *i.e.* the sensing error based on a single concentration estimate via a single receptor. Because each receptor provides an independent concentration measurement (4), the total number of independent measurements is $N_I = R_T$. Clearly, the sensing error is limited by the total number of receptors on the membrane.

TRADE-OFFS IN EQUILIBRIUM SENSING

Cells can reduce the error in Eq. 2 with downstream networks that time-integrate over the history of receptor states (1). Key to the ability of networks to time-integrate is a memory of these past states, implemented, for example, by a long-lived molecular species or a signaling cascade that delays the signal (10). Equilibrium systems can have these and hence have memory of the past receptor states. Thus, we might expect that equilibrium networks can reduce the sensing error past the bound set by the number of receptors at the expense of downstream signaling molecules.

We consider cytoplasmic read-out molecules x that bind ligand-free receptors: $R + L \rightleftharpoons RL$, $R + x \xrightleftharpoons[k_r]{k_f} Rx$. Solving the associated Langevin equations (*Materials and Methods*) shows that the dynamics of the output around its mean \bar{x} is given by the time-integrated fluctuations $\delta RL(t)$ in the receptor state plus noise $\eta(t)$ due to the receptor-read-out binding:

$$\delta x(t) = \int_{-\infty}^t dt' e^{-(t-t')/\tau_I} [\beta \delta RL(t') + \eta(t')], \quad (3)$$

where $\beta = k_f \bar{x}$ and $\tau_I = \frac{1}{k_f(\bar{x} + \bar{R}) + k_r}$ is the integration time. The latter can be made arbitrarily large by slowing down the read-out dynamics, i.e. by lowering k_f and k_r . This suggests that equilibrium networks can completely filter the extrinsic noise in the receptor states and reduce the sensing error to zero. However, the idea that the sensing error can be reduced to zero ignores the fact that in these equilibrium systems ligand-receptor binding and receptor-read-out binding are coupled. In this specific system, these reactions are coupled because the read-out and the ligand compete for binding to the receptor.

To elucidate how the coupling between receptor-ligand binding and receptor-read-out binding compromises sensing in equilibrium networks, we determine the total sensing error. From Eq. 3, the variance of the output $\sigma_x^2 = \langle \delta x \rangle^2$ can be written as the sum of the extrinsic noise $\sigma_{\text{ex}}^2 \equiv \beta^2 K_{\delta RL, \delta RL}$ and the intrinsic noise $\sigma_{\text{in}}^2 \equiv \beta K_{\delta RL, \eta} + K_{\eta, \eta}$, where $K_{A,B} = \int_{-\infty}^t \int_{-\infty}^t e^{-(t-t_1)/\tau_I} C_{A,B}(t_1, t_2) e^{-(t-t_2)/\tau_I} dt_1 dt_2$ with the correlation function $C_{AB}(t_1, t_2) = \langle A(t_1)B(t_2) \rangle$. Combining σ_x^2 with the gain $d\bar{x}/dc$ gives the sensing error for this network (Eq. 1). Analytically minimizing the result, we find that it is never lower than the bound set by the number of receptors (i.e. $N_I \leq R_T$ in Eq. 2), regardless of the integration time or other parameters of the network (*SI Text*). This raises the paradox of a network that time-integrates the receptor fluctuations yet cannot reduce the sensing error with it. The resolution of the paradox is that in equilibrium systems the intrinsic and extrinsic noise are not independent, precisely because receptor-ligand and receptor-readout binding are

coupled. As a result, the fluctuations in the receptor state and the read-out become correlated; $C_{\delta RL, \eta}(t_1, t_2)$ is not zero (11). Because of these correlations, equilibrium networks face a fundamental trade-off between the removal of extrinsic noise in the receptor state and the suppression of intrinsic in the downstream signaling network. In an optimally designed network that minimizes the sensor error, increasing the integration time reduces the extrinsic noise, but also increases the intrinsic noise by at least the same amount.

Signaling networks are usually far more complicated than a single read-out molecule that binds the receptor, and it has been shown that additional network layers can reduce the sensing error (10). This raises the question whether a more complicated equilibrium network can overcome the limit set by the number of receptors. Searching over all possible network topologies to systematically address this question is difficult, if not impossible. However, equilibrium systems are fundamentally bounded by the laws of equilibrium thermodynamics, regardless of their topology. One such law is the fluctuation-dissipation theorem. Just as a decrease in the viscosity of a fluid increases both the noise in a particle's Brownian motion and the sensitivity of its response to an applied force, so too do modifications in equilibrium networks affect both the noise in the read-out and the sensitivity of its response to changes in the ligand concentration, i.e. the gain.

Specifically, for any read-out x in an equilibrium system, the fluctuation-dissipation theorem implies that the gain $\frac{d\bar{x}}{d\mu_L}$ is equal to the covariance $\sigma_{x, RL}^2$ of the fluctuations in the read-out and the ligand-bound receptor: $\frac{d\bar{x}}{d\mu_L} = \sigma_{x, RL}^2$ (28). Then, the sensing error from any read-out is (Eq. 1): $\left(\frac{\delta c}{c}\right)_x^2 = \frac{\sigma_x^2}{(\frac{d\bar{x}}{d\mu_L})^2} = \frac{\sigma_x^2}{(\sigma_{x, RL}^2)^2}$. If the receptors themselves are taken as the read-out, the sensing error is $\left(\frac{\delta c}{c}\right)_{RL}^2 = \frac{1}{\sigma_{RL}^2}$. By combining these expressions, it follows that no read-out is better for sensing than the receptors:

$$\left(\frac{\delta c}{c}\right)_x^2 = \frac{\sigma_x^2 \sigma_{RL}^2}{(\sigma_{x, RL}^2)^2} \left(\frac{\delta c}{c}\right)_{RL}^2 \geq \left(\frac{\delta c}{c}\right)_{RL}^2 \quad (4)$$

since the correlation coefficient $|\rho_{x, RL}| = |\sigma_{x, RL}| / \sqrt{\sigma_x^2 \sigma_{RL}^2} \leq 1$.

This relation leads to quantitative bounds on the sensing capacity of equilibrium networks. In general, the variance σ_{RL}^2 , and hence $(\delta c/c)_{RL}^2$, depends on the particular network. However, for any network, $\sigma_{RL}^2 \leq R_T^2/4$ since $0 \leq RL \leq R_T$. Thus, for equilibrium systems, the fundamental lower bound on the fractional error in the concentration estimate is:

$$\left(\frac{\delta c}{c}\right)_x^2 \geq \frac{4}{R_T^2}. \quad (5)$$

This proves that in equilibrium systems, which are not driven by fuel turnover, the precision of sensing is fundamentally limited by the number of receptors (Fig. 1, upper box); a downstream signaling network can never improve the accuracy of sensing.

Networks in which the receptors cooperatively bind the ligand can achieve the bound of Eq. 5 (*SI Text*). For networks without cooperative ligand binding, as in the simple example above, the sensing error is worse: $\sigma_{RL}^2 \leq \text{MIN}(R_T, R_T^2/4)$, so $(\frac{\delta c}{c})_x \geq \text{MAX}(\frac{1}{R_T}, \frac{4}{R_T^2})$ (*SI Text*). The sensing error for independent receptor binding is most easily understood for receptors with identical affinity for the ligand, as in our simple example (Eq. 2), but holds generally: different affinities do not break this bound.

The different species in a network can also be viewed as nodes through which information about the ligand flows. We can show that the data processing inequality (29) also guarantees, for an equilibrium system, that no read-out has more information about the ligand than the receptors at any given time: $I(x; \mu_L) \leq I(RL; \mu_L) \leq \log_2(R_T + 1)$, where I is the mutual information between the instantaneous levels of the arguments (*SI Text*). The history of receptor states does contain more information about the ligand concentration than the instantaneous receptor

state, but our results show that an equilibrium signaling network cannot exploit this: its output contains only as much information as the instantaneous receptor state; it does not encode the history of receptor states in any informative way, whether by time-integration or any other method.

Ultimately, equilibrium systems sense by harvesting the energy of ligand binding. This energy is used to propagate the signal through the downstream network; in the simple system studied here, for example, the energy of ligand binding is used to expel the read-out molecule from the receptor. However, detailed balance then dictates that the receptor-read-out binding also influences receptor-ligand binding, thus perturbing the signal. Indeed, the trade-offs faced by equilibrium networks are all different manifestations of their time-reversibility (30). The only way for a time-reversible system to “integrate” the past is for it to integrate—perturb—the future. Concomitantly, in a time reversible system, there is no sense of “upstream” and “downstream”, concepts which rely on a direction of time. Although we have referred to the molecule x as a “readout” of the ligand concentration, the ligand is just as much a readout of x . While in equilibrium systems the read-out encodes the receptor state, the read-out is not a stable memory that is decoupled from changes in the receptor state. It merely passively lags. In an equilibrium system, the sensing error, like any static quantity, can only depend on ratios of time scales, which is another way of seeing that increasing the “integration time” cannot improve sensing.

These results show that in an equilibrium system each receptor provides at most one independent measurement of the ligand, regardless of how much information is encoded in the history of the receptor state, how complicated the signaling machinery is downstream, how many molecules are devoted to signaling downstream, or how long the apparent integration time of the network is. Energy dissipation—fuel turnover—is required to break the trade-offs between noise and sensitivity, between intrinsic and extrinsic noise, and, ultimately, between the accuracy of sensing and space on the membrane.

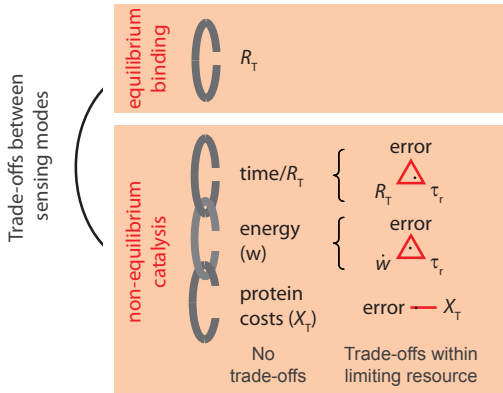


FIG. 1: The relationship among network design, resources, and the precision of biochemical sensing. Cells can use two distinct modes of sensing and signaling, the equilibrium mode based on protein binding and sequestration, and the non-equilibrium mode based on protein modification driven by fuel turnover. Upper box: In equilibrium sensing, the sensing accuracy is fundamentally limited by the number of receptors, regardless of the design of the downstream network. Lower box: In non-equilibrium sensing, the sensing precision is fundamentally limited by time and receptor copies, energy, and copies of downstream readouts. These three classes of resources cannot compensate each other, and it is the limiting resource that sets the fundamental limit to the precision of sensing. Within each class, however, trade-offs are possible: Power can be traded against speed to meet the energy requirement for reaching a desired sensing accuracy, while time can be traded against the number of receptors.

NON-EQUILIBRIUM SENSING AT THE MOLECULAR LEVEL

Networks that can reduce error via time-integration must be non-equilibrium systems. To understand the resources required to reduce the sensing error in these systems, we need to understand how they sense at the molecular level. Berg and Purcell pointed out that by integrating the receptor signal over a time T , the cell can take as many as $T/(2\tau_c)$ independent samples of the receptor state (1), where τ_c is the receptor correlation time. We will show that the cost of sensing depends on how many of these samples the cell actually takes. We

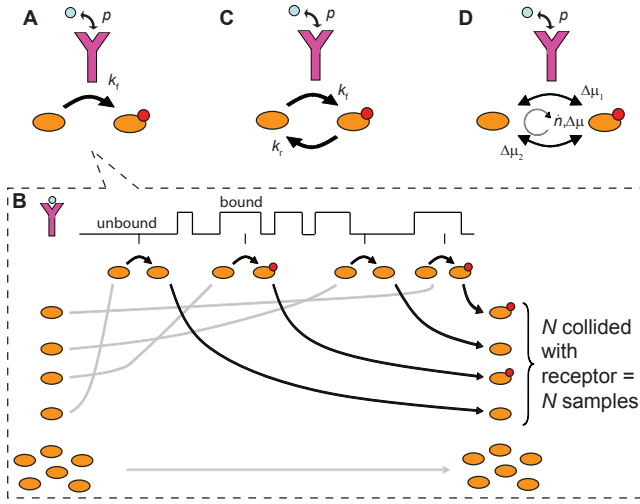


FIG. 2: Sensing at the molecular level. (A) The ligand-bound receptor drives the modification of a downstream read-out (i.e the push-pull network $RL + x \rightarrow RL + x^*$). (B) The biochemical network in (A) discretely samples the receptor state, illustrated for one receptor. The states of the receptor over time are encoded in the states of the N molecules that collided with it: the readout is modified if the receptor is bound; otherwise it is unmodified. Molecules that collide with the unbound receptor are indistinguishable from those that have never collided, leading to an additional error. (C) Active molecules can be degraded. Some samples are erased, and the remaining samples are, on average, further apart (more independent.) (D) All reactions are in principle reversible, compromising the encoding of the receptor state into the readout. The sensing error is determined by parameters that describe the energy flow in the network, including the flux \bar{n} and the free-energy drops $\Delta\mu_1$ and $\Delta\mu_2$ across the activation and deactivation reactions of the readout, respectively.

therefore view the downstream network, which consists of discrete components, as a system that discretely samples the receptor state, rather than integrating it.

To gain intuition about the resources required to build and operate these networks, we construct step by step a model of a receptor that drives a push-pull network, which is a canonical non-equilibrium motif in prokaryotic and eukaryotic cell signaling (24). In these systems, the receptor itself or the enzyme associated with it, such as CheA in bacterial chemotaxis (24), catalyzes the (chemical) modification of a read-out protein. The general principle is that these networks take samples of the receptor by storing its state in the stable modification states of the read-out molecules (Fig. 2A, B). Each read-out molecule that interacted with the receptor provides a memory of the ligand-occupation state of that receptor molecule; collectively, the read-out molecules encode the history of the receptor states. Quantitatively, if there are N receptor-readout interactions, then the cell has N samples of the receptor state and the error, $\delta c/c$, is reduced by a factor of \sqrt{N} , as in Eq. 2, or less if the

samples are not independent. By building up the model step by step, we seek to understand how different features of the network affect the number of samples, their independence, and their accuracy. One feature is that molecules can be deactivated (Fig. 2C), which we will show is equivalent to discarding or erasing samples. Additionally, reactions are microscopically reversible (Fig. 2D), which means that read-out modifications can occur independently of the receptor and receptor-mediated modifications can occur in the wrong direction; both effects reduce the reliability of a sample. Energy is needed to break time-reversibility and to protect the coding. We arrive at an expression for the sensing error that combines these effects. It reveals trade-offs between cellular resources and performance: speed, accuracy, energy, and the number of receptor and downstream molecules.

Base model

For intuition, we first consider a cell that responds after a time T to a change in a ligand's concentration at some time $t = 0$, based on the output x^* of the simple reaction network $x + RL \xrightarrow{k_f} x^* + RL$ (Fig. 2A). We assume that the cell starts with a large pool of inactive read-out molecules x and that activated molecules x^* are never deactivated. For descriptive ease, we assume the reaction is diffusion-limited, so that each collision between an inactive molecule x and a ligand-bound receptor leads to activation of x . The resulting sensing error can be derived via Eq. 1 from the Master Equation, which describes fluctuations in the network (see Materials and Methods).

However, to understand the required resources, we calculate the error instead by viewing the molecular network as one that discretely samples the receptor state. At the molecular level, readout molecules collide with the receptor over time and are modified depending on the ligand-occupation state of the receptor. The total rate at which inactive molecules collide with receptor molecules in any state is $r = k_f x R_T \approx k_f X_T R_T$ for a large readout pool, and the total number of such collisions after time T is N , with $\bar{N} = rT$ on average. If a receptor molecule is bound to ligand at the time of a collision, the read-out molecule is converted to its active form, while if it is not the read-out remains unchanged. In this way, the state of the receptor at the time of a collision is encoded in the state of the read-out molecule that collided with it, and the history of the receptor states is encoded in the states of the read-out molecules at the time T (Fig. 2B). The read-out molecules that collided with the receptor thus constitute samples of the receptor state. The average number of samples after time T is $\bar{N} = rT = k_f X_T R_T T$ —the product of the total number of receptors R_T and the number of samples per receptor $k_f X_T T$ during the integration time T .

The sensing error can then be derived by viewing the

system as one that employs the sampling process described above, estimating the average receptor occupancy from samples of the receptor state taken at the times of readout-receptor collisions, *i.e.* as $\hat{p} = x^*/\bar{N}$ (*SI Text*). This yields for the sensing error:

$$\left(\frac{\delta c}{c}\right)^2 = \frac{1}{p(1-p)} \frac{1}{\bar{N}_I} + \frac{1}{(1-p)^2} \frac{1}{\bar{N}}. \quad (6)$$

This expression has a clear interpretation in terms of sampling. The first term is exactly the error expected from \bar{N} stochastically taken samples of the receptor over the time T . Specifically, it is the error of an estimate based on a single sample, $1/(p(1-p))$, divided by the average number of samples that are independent, \bar{N}_I , where \bar{N}_I is the total number of samples \bar{N} times the fraction f_I that is independent:

$$\bar{N}_I = f_I \bar{N} = \frac{1}{1 + \frac{2\tau_c}{\Delta}} \bar{N}, \quad (7)$$

when $T \gg \tau_c$. Clearly, f_I depends on the receptor correlation time τ_c and on the time interval $\Delta \equiv T/(\bar{N}/R_T) = 1/(r/R_T) = 1/(k_f X_T)$ between samples of the same receptor; samples farther apart are more independent. This expression shows that the finite sampling rate r reduces the number of independent samples below the Berg-Purcell factor $R_T T/2\tau_c$, the maximum number of independent samples that can be taken during T . The latter is reached only when the sampling rate is infinite (e.g. the number of downstream molecules $X_T \rightarrow \infty$), so that $\bar{N} \rightarrow \infty$ and $\Delta \rightarrow 0$.

The second term in Eq. 6 accounts for the fact that the cell cannot distinguish between those molecules x that have collided with an unbound receptor (and hence provide information on the receptor occupancy), and those that have not collided with the receptor at all (Fig. 2B). If the cell could distinguish between those molecules, it could estimate the average receptor occupancy from $\hat{p} = x^*/N$ rather than $\hat{p} = x^*/\bar{N}$; then the second term would be zero (*SI Text*). Indeed, the second term arises from the biochemical noise that makes the actual number of samples, N , different from its average, \bar{N} . However, when p is small and/or \bar{N} is large, the second term is small compared to the first and the sensing error is given by the error of a single measurement, $1/(p(1-p))$, divided by the average number of independent measurements, \bar{N}_I .

Deactivation

The error in Eq. 6 decreases with the time T , suggesting that the cell can sense perfectly if it waits long enough before responding to a change in its environment. However, modification states of molecules decay, and their finite lifetime, τ_ℓ , limits sensing, regardless of how long

the cell waits. To explore this at the molecular level, we consider the network in the previous paragraph augmented with the deactivation reaction $x^* \xrightarrow{k_x} x$, $k_x = 1/\tau_\ell$ (Fig. 2C). We consider the sensing error after long times ($T \gg \tau_\ell$), in steady state, again for a large pool of inactive read-out molecules. For pedagogical clarity, we imagine the deactivation is mediated by a phosphatase and that the reaction is diffusion-limited.

We calculate the sensing error by solving the master equation or by viewing the system as one that discretely samples the receptor state, as before (*SI Text*). We find that also with deactivation the sensing error is given by Eqs. 6 and 7, yet with fewer samples, $\bar{N} = r\tau_\ell < rT$, spaced effectively farther apart, $\Delta = 2\tau_\ell/(\bar{N}/R_T) = 2/(k_f X_T) > 1/(k_f X_T)$. The molecular picture of sampling provides a clear interpretation. As before, the readout molecules encode the state of a receptor and serve as samples of the receptor state. With deactivation, however, only those readout molecules which have collided with the receptor more recently than with the phosphatase reflect the receptor state. At any given time, the average number of such readout molecules, and hence samples, is $\bar{N} = r\tau_\ell$; the lifetime τ_ℓ thus sets an effective integration time. As without deactivation, the fraction f_I of samples that are independent is determined by the effective spacing Δ between them, see Eq. 7. Though the time between the creation of samples is still $1/(k_f X_T)$, *i.e.* the spacing without readout deactivation, some of the samples are erased via collision with the phosphatase. We therefore expect that the spacing between remaining samples is larger. Indeed, calculating the effective spacing between samples taking this effect into account yields $\Delta = 2/(k_f X_T)$, which is twice that without decay (*SI Text*). The fact that the remaining samples are more independent explains a previously noted correspondence (7, 20) between the sensing error in a system with deactivation, $(\frac{\delta c}{c})^2 = \frac{1}{p(1-p)} \frac{1}{\tau_\ell/\tau_c}$, and that in a system without deactivation, $(\frac{\delta c}{c})^2 = \frac{1}{p(1-p)} \frac{1}{T/(2\tau_c)}$, in the infinite sampling limit: they are equal for $T = 2\tau_\ell$, and not for $T = \tau_\ell$ as would be expected if their samples were just as independent.

Finite pool of read-out molecules

The copy numbers of signaling molecules are often small. To take this into account, we compute the sensing error from Eq. 1 for a finite number of read-out molecules X_T using the linear-noise approximation to the Master Equation describing the biochemical fluctuations (*Materials and Methods*), and compare the result with Eq. 6. This defines an effective number of samples, $\bar{N} = r\tau_r$, where τ_r is the relaxation time of the network. For this network, $\tau_r = 1/(k_f p R_T + k_x)$. In essence, cells count only those samples created less than a relaxation time in

the past; nothing that happened earlier can influence the current state, including its ability to sense. The fraction of samples that is independent is given by Eq. 7 with $\Delta = 2\tau_r/(\bar{N}/R_T) = 2/(k_f\bar{x})$, analogously to the previous section.

Reversibility

All reactions are in principle microscopically reversible. Taking this into account, we recognize that active molecules that collide with the bound receptor sometimes become inactive, $x^* + RL \rightarrow x + RL$, and that inactive molecules that collide with the phosphatase are sometimes activated, $x \rightarrow x^*$ (Fig. 2D). These reverse reactions compromise the encoding of the receptor state into the read-out: an active x^* molecule no longer encodes the ligand-bound state of the receptor at a previous time with 100% fidelity, since it can also result from a collision with the phosphatase; similarly, x , rather than x^* , may reflect a collision with the ligand-bound receptor.

We compute the sensing error for the reversible network from Eq. 1 using the linear-noise approximation to the master equation (see *Materials and Methods*). As before, it can be written as Eqs. 6 and 7. The effective number of independent samples \bar{N}_I is a complicated expression of the 8 fundamental variables in the system: the 6 rate constants describing the forward and reverse rates of the 3 reactions (including ligand-receptor binding), and the total copy numbers X_T and R_T . However, the expression has a particularly simple and illuminating form in terms of variables that describe, as we will show, the resource limitations of the cell. In addition to variables already defined (p , τ_c , R_T , and τ_r), these include: the flux \dot{n} of x^* across the cycle in which it is created by the receptor and deactivated via the phosphatase; and the average free-energy drops, $\Delta\mu_1$ and $\Delta\mu_2$, across the receptor-catalyzed pathway and the phosphatase-catalyzed pathway, respectively, in units of $k_B T$ (Fig. 2D). Each of these variables depends in a complicated way on the fundamental parameters of the system, the rate constants and the copy numbers. In particular, the free-energy drops are related to the propensities ν_i and ν_{-i} of the reactions in the forward and backward directions, respectively: $\Delta\mu_i = \log \frac{\nu_i}{\nu_{-i}}$ (31). However, the variables can all be varied independently, except that $\Delta\mu_1 = \Delta\mu_2 = \dot{n} = 0$ in equilibrium.

In terms of these variables, the effective number of independent samples taken by the push-pull network is:

$$\bar{N}_I = \underbrace{\frac{\bar{N}}{\dot{n}\tau_r} \frac{(e^{\Delta\mu_1} - 1)(e^{\Delta\mu_2} - 1)}{e^{\Delta\mu} - 1}}_{\bar{N}_{\text{eff}}} \underbrace{\frac{1}{(1 + 2\tau_c/\Delta)}}_{f_I}, \quad (8)$$

where $\Delta\mu = \Delta\mu_1 + \Delta\mu_2$ is the total free-energy drop

across the cycle; $e^{\Delta\mu}$ is also known as the affinity of the cycle (32).

Eq. 8 is our principle result for non-equilibrium systems. It takes into account readout deactivation, the finite number of readout molecules, and the reversibility of reactions. The equation has a clear interpretation. The product $\dot{n}\tau_r$ is the number of cycles of read-out molecules involving collisions with ligand-bound receptor molecules during the system's relaxation time τ_r . The quantity $\dot{n}\tau_r/p$ is the total number of read-out cycles involving collisions with receptor molecules, be they ligand bound or not; it is thus the total number of receptor samples taken during τ_r , \bar{N} . The factor q , involving $\Delta\mu_1, \Delta\mu_2$, reflects the quality of each sample. When $\Delta\mu = \Delta\mu_1 = \Delta\mu_2 = 0$, an active read-out molecule is as likely to be created by the ligand-bound receptor as by the phosphatase and there is no coding and no sensing; indeed, in this limit, $q = 0$ and the effective number of samples $\bar{N}_{\text{eff}} = 0$. Note also that when the backward reactions are faster than the forward reactions, corresponding to $\Delta\mu$ becoming negative, x encodes the ligand-bound receptor instead of x^* . This symmetry is reflected by the symmetry of Eq. 8: the number of samples is the same when the signs of \dot{n} , $\Delta\mu_1$, and $\Delta\mu_2$ are all flipped. The effective number of accurate samples is $\bar{N}_{\text{eff}} = \bar{N}q$, less than the total number \bar{N} taken. The fraction of the \bar{N}_{eff} samples that are independent is, as before, $f_I = 1/(1 + 2\tau_c/\Delta)$ with $\Delta = 2\tau_r/(\bar{N}_{\text{eff}}/R_T)$ reflecting the time interval between effective samples.

TRADE-OFFS IN NON-EQUILIBRIUM SENSING

Eq. 8 reveals trade-offs among the different resources for sensing, and between these resources and the accuracy of sensing.

Time/receptor copy numbers

There is no fundamental relationship between receptor copy number and sensing, as in equilibrium systems. Essentially, the error is determined by the total number of samples, and it does not matter, as long as the samples are independent, whether these samples are from the same receptor over time or from many receptors at the same time. An independent sample of the same receptor can be taken roughly every $2\tau_c$ (Eq. 7). Naturally, samples can be taken more frequently. In fact, cells can time-integrate: if $X_T \rightarrow \infty$, the receptors are sampled infinitely fast and $\Delta \rightarrow 0$ and $\bar{N}_{\text{eff}} \rightarrow \infty$ (Eq. 7); then the number of independent samples \bar{N}_I taken over τ_r reaches its maximum, the Berg-Purcell factor $R_T\tau_r/\tau_c$, and the error can achieve its minimum, $4/(R_T\tau_r/\tau_c)$. However, the benefit of sampling faster by increasing X_T in reducing the sensing error is rapidly diminishing, while the

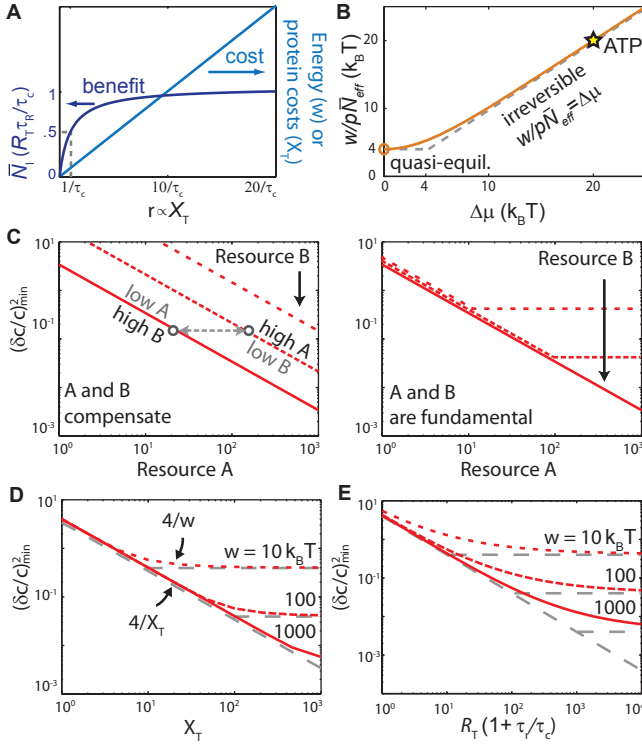


FIG. 3: Trade-offs in non-equilibrium sensing. (A) Sampling more than once per correlation time requires more resources, while the benefit is marginal. As the sampling rate r is increased by increasing the readout copy number X_T , the number of independent measurements \bar{N}_I saturates at the Berg-Purcell limit $R_T\tau_r/\tau_c$, but the energy consumption and protein cost ($\propto X_T$) continue to rise. (B) The energy requirements for sensing. In the irreversible regime ($\Delta\mu > 4k_B T$), the work to take one sample of a ligand-bound receptor, $w/(p\bar{N}_{eff})$, equals $\Delta\mu$, because each sample requires the turnover of one fuel molecule, consuming $\Delta\mu$ of energy. In the quasi-equilibrium regime ($0 < \Delta\mu < 4k_B T$), each effective sample of the bound receptor requires $4k_B T$, which defines the fundamental lower bound on the energy requirement for taking a sample. When $\Delta\mu = 0$, the network is in equilibrium and both w and \bar{N} are 0. ATP hydrolysis provides $20k_B T$, showing that phosphorylation of read-out molecules makes it possible to store the receptor state reliably. The results are obtained from Eq. 8 with $\Delta\mu_1 = \Delta\mu_2 = (1/2)\Delta\mu$. (C) When two resources A and B compensate each other, one resource can always be decreased without affecting the sensing error, by increasing the other resource; concomitantly, increasing a resource will always reduce the sensing error. When both resources are instead fundamental, the sensing error is bounded by the limiting resource and cannot be reduced by increasing the other resource. (D, E) The three resources time/receptor copies, copies of downstream molecules, and energy are all required for sensing, with no trade-offs among them (see Fig. 1B). The minimum sensing error obtained by minimizing Eq. 6 is plotted for different combinations of (D) X_T and w , and (E) $R_T(1 + \tau_r/\tau_c)$ —the number of samples were X_T and w infinite—and w . The curves track the bound for the limiting resource indicated by the grey lines, showing that the resources do not compensate each other. The plot for the minimum sensing error as a function of $R_T(1 + \tau_r/\tau_c)$ and X_T is identical to that of (E) with w replaced by X_T .

total protein and energetic costs increase (see Fig. 3A).

Downstream read-out molecules

While the effect of copy number on intrinsic noise has been studied extensively, how copy numbers of read-out signaling molecules affect the fundamental sensing limit has not been elucidated. In Eq. 8 the factor containing the chemical potentials is always less than 1; also, $\dot{n}\tau_r < X_T$ because the system has relaxed when all read-out molecules have completed an activation-deactivation cycle. Hence, Eq. 8 shows that the number of samples of a ligand-bound receptor, $p\bar{N}$, is always less than the number of downstream molecules, X_T . Each read-out molecule provides at most one sample, because at any given time it exists in only one modification state, regardless of how many times it has collided with the receptor or how long the integration time τ_r is. There is no mechanistic sense in which a single molecule "integrates" the receptor state. As a consequence, no matter how the network is designed, how much time or energy it uses, or how many receptors it has, cells are fundamentally limited by the pool of read-out molecules: the sensing error $(\delta c/c)^2 \geq 4/X_T$, obtained by analytically minimizing Eq. 6 at fixed X_T .

Energy

The free-energy drop across a cycle, $\Delta\mu$, must be provided by a fuel molecule such as ATP. This free energy represents the maximum work the fuel molecule could do if used by an ideal engine. Eq. 8 defines three regimes of sensing with respect to the energy consumption of the network.

When $\Delta\mu = 0$ the system is in equilibrium and the sensing error diverges (20), as discussed above; indeed, this system employs a fundamentally different signaling strategy than equilibrium systems use to sense. Two other regimes are defined by the work that the fuel molecules need to do in order to take a sample of the receptor. The power, the rate at which the fuel molecules do work, is $\dot{w} = \dot{n}\Delta\mu$ and the total work performed during the relaxation time τ_r is $w \equiv \dot{w}\tau_r$. This work is spent on taking samples of receptor molecules that are bound to ligand, because only they can modify downstream read-out molecules. The total number of effective samples of ligand-bound receptors obtained during τ_r , is $p\bar{N}_{eff}$. Hence, the work needed to take one effective sample of a ligand-bound receptor is $w/(p\bar{N}_{eff})$, with \bar{N}_{eff} given by Eq. 8. Fig. 3B shows this quantity as a function of $\Delta\mu$. Two regimes can be observed.

When $\Delta\mu \gtrsim 4k_B T$, the work to take one effective sample of a ligand-bound receptor is simply $w/(p\bar{N}_{eff}) = \Delta\mu$, independent of kinetic time scales. In this regime, the

read-out reactions are essentially irreversible and the sample quality factor q in Eq. 8 reaches unity, meaning that each read-out molecule reliably encodes the receptor state at an earlier time. The effective number of samples therefore equals the total number taken, and is given by that of the irreversible case already studied, $\bar{N}_{\text{eff}} = \bar{N} = \dot{n}\tau_r/p = r\tau_r = k_f \bar{x} R_T \tau_r$. The work per sample of a ligand-bound receptor, $w/(p\bar{N}_{\text{eff}})$, equals $\Delta\mu$, because each sample requires the turnover of one fuel molecule, using $\Delta\mu$ of energy. The total number of samples \bar{N}_{eff} is thus limited by the work as $\bar{N}_{\text{eff}} = \dot{n}\tau_r/p = w/(p\Delta\mu)$. In this regime, energy limits sensing not because it limits the reliability q of each sample, but because it limits the total number of samples \bar{N}_{eff} that could be taken during τ_r by limiting the receptor sampling frequency, the flux \dot{n} : increasing \dot{n} necessarily requires more work. Inserting this expression into Eq. 6 and optimizing puts a lower bound on the sensing error:

$$\left(\frac{\delta c}{c}\right)^2 \geq \frac{1}{\dot{n}\tau_r} = \frac{\Delta\mu}{w}. \quad (9)$$

Intriguingly, Eq. 9 suggests that for a fixed amount of energy, w , spent during the relaxation time τ_r , the sensing error can be reduced to zero by reducing $\Delta\mu$ to zero. However, the lower bound in Eq. 9 is only achievable (and Eq. 9 thus only applies), when $\Delta\mu \gtrsim 4k_B T$.

When $\Delta\mu \lesssim 4k_B T$, the system transitions to a quasi-equilibrium regime in which each fuel molecule provides a small but nonzero amount of energy. In this regime, the system can still consume significant amounts of energy when the fuel molecules are consumed at a rapid rate \dot{n} by many distinct read-out molecules. In the limit that $\dot{n} \rightarrow \infty$ and $\Delta\mu \rightarrow 0$ at fixed $\dot{w} = \dot{n}\Delta\mu$, the effective number of samples given by Eq. 8 reduces to

$$\bar{N}_{\text{eff}} \rightarrow \frac{w}{4p}. \quad (10)$$

In the quasi-equilibrium regime, each readout-receptor interaction corresponds to an increasingly noisy measurement of the receptor state ($q \rightarrow 0$), but many noisy measurements ($\bar{N} = \dot{n}\tau_r/p \rightarrow \infty$) contain the same information as 1 perfect measurement – provided that collectively at least $4k_B T$ was spent on them. Indeed, as Fig. 3B shows, $4k_B T$ is the fundamental lower bound on the work needed to take one accurate sample of a ligand-bound receptor. It puts another lower bound on the sensing error: inserting Eq. 10 into Eq. 6 and optimizing shows that:

$$\left(\frac{\delta c}{c}\right)^2 \geq \frac{4}{w}. \quad (11)$$

This power-law bound relates energy to information. The bound can be reached when time and X_T are not limiting, and $\Delta\mu \lesssim 4k_B T$. When $\Delta\mu \gtrsim 4k_B T$, the lower bound is higher and given by Eq. 9.

Eqs. 9 and 11 show that the sensing precision increases with the work done in the past relaxation time, $w = \dot{w}\tau_r$, setting up a trade-off among speed, power, and accuracy, as found in adaptation (18). The trade-off emerges naturally from a molecular picture of sensing. When the response needs to be rapid, τ_r needs to be small and the power demand is high: the samples, which require energy, must be taken close together in time. However, when the cell can wait a long time τ_r before responding, the power \dot{w} required to make w large can be infinitesimal: the samples can be created far apart in time. There is no minimum power requirement for sensing.

No trade-offs among time/receptors, readout molecules, and energy

The above analysis shows that each of the fundamental resource categories — time/receptor copy number, downstream read-out molecules, and power/time (fuel) — has its own trade-off with sensing accuracy (Figs. 3C,D,E). To a good approximation, the worst bound is active: $\bar{N}_I \leq \text{MIN}(R_T \tau_r / \tau_c, X_T / p, \dot{w} \tau_r / 4p)$, corresponding to $(\delta c/c)^2 \geq \text{MAX}(4/(R_T \tau_r / \tau_c), 4/X_T, 4/(\dot{w} \tau_r))$. Indeed, one of the most important conclusions of our analysis is that increasing a single resource (e.g. w) cannot reduce the sensing error indefinitely. The sensing accuracy will eventually plateau, namely when it becomes fundamentally limited by another resource (e.g. X_T). Clearly, there is no trade-off among these classes of resources: no amount of one resource can overcome a limiting amount of another, as illustrated in Figs. 3D,E. The reason is clear: taking a sample requires time and receptors, read-out molecules, and fuel. Adding receptors and read-out molecules does not improve sensing if not enough energy is available to take the samples (Fig. 3D). Similarly, waiting more time to take another sample is not beneficial if the cell has no more read-out molecules left to write the sample to, or cannot expend energy fast enough to accomplish the writing (Fig. 3E).

The picture that emerges from our analysis is summarized in the lower box of Fig. 1. The resource classes time/receptors, downstream readout molecules, and energy, act like weak links in a chain that cannot compensate each other in achieving a required sensing precision. Within these classes trade-offs are possible: time can be traded against the number of receptors to reach a required number of measurements, while power can be traded against speed to meet the energy requirement for a desired sensing accuracy.

TRADE-OFFS BETWEEN DIFFERENT MODES OF SENSING

To increase the number of measurements, equilibrium networks must increase the number of receptors. Non-equilibrium networks may instead use more downstream readouts and energy to take more measurements with the same receptors over time. Which sensing strategy is better? The strategy adopted by the cell will depend on the relative fitness costs of the different resources. If the resources are of similar costs, then, quantitatively, our analysis predicts that an equilibrium strategy will be adopted if its minimum error, $4/R_T$ for non-cooperative receptors, is less than that of the non-equilibrium strategy, $\text{MAX}(4/(R_T\tau_r/\tau_c), 4/X_T, 4/(\dot{w}\tau_r))$ (Fig. 1). For example, when the accuracy of the non-equilibrium strategy is limited by energy, meaning that $(\delta c/c)^2 \geq 4/(\dot{w}\tau_r)$, the predicted transition between the two strategies occurs when the work per receptor $\dot{w}\tau_r/R_T \approx 1k_B T$. To address this, we have considered a network that combines both strategies. The read-out binds the ligand-bound receptor, which can then boot off the read out in a modified or unmodified state (*SI Text*): $RL + x \rightleftharpoons RLx$, $RLx \rightleftharpoons RL + x^*$, $x^* \rightleftharpoons x$. This system combines both modes of sensing, because the chemical modification of the readout enables non-equilibrium sensing, while se-

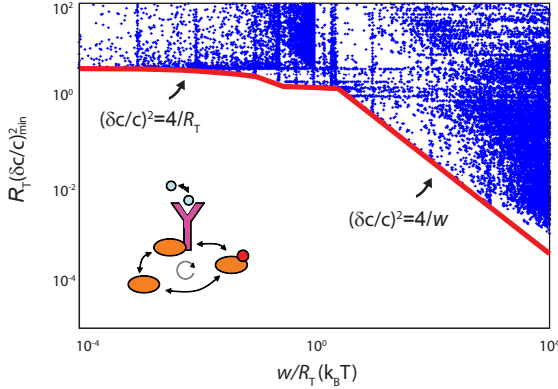


FIG. 4: The different resource requirements for equilibrium and non-equilibrium sensing lead to a trade-off between these two modes. The trade-off is illustrated for a network that combines both modes: $RL + x \rightleftharpoons RLx$, $RLx \rightleftharpoons RL + x^*$, $x^* \rightleftharpoons x$. The blue dots show the sensing error for different parameter values and the red guideline shows the minimum sensing error (*SI text*). When the energy per receptor w/R_T is less than a few $k_B T$, the optimized system employs the equilibrium strategy of sequestration, achieving the bound $4/R_T$. If the energy input is higher, it uses the non-equilibrium strategy of catalysis to transmit the signal, achieving the bound $4/w$. There is an intermediate regime around $1 k_B T$ per receptor in which the network modestly outperforms both full catalysis and full binding by partially utilizing the receptor-readout state.

questration of the unmodified readout by the receptor upon ligand binding enables equilibrium sensing. Optimizing this system over all parameters confirms that when the energy per receptor is less than a few $k_B T$, the optimized system employs the equilibrium strategy of sequestration, while if it is higher it uses the non-equilibrium strategy of catalysis to transmit the signal (Fig. 4). In addition, the networks that optimize sensing in these two regimes are the networks that we have studied; a network that combines the two sensing modes does not perform better than the two individually.

DISCUSSION

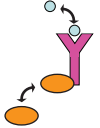
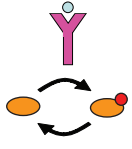
Fundamentally there are only two distinct mechanisms to transmit the information from the receptor to the downstream readout (Fig. 1). These two mechanisms, described as equilibrium and non-equilibrium sensing, have different resource requirements (Table I). Cells face a trade-off with respect to their resources in choosing between these two distinct sensing strategies.

In the equilibrium strategy the signal is transmitted from the receptor to the read-out via sequestration reactions, in which binding of an upstream component causes unbinding of a downstream component, or via adaptor proteins, which bind the up- and downstream component simultaneously. Both motifs are ubiquitous in cellular signaling. G-protein-coupled receptor (GPCR) signaling employs protein sequestration (33), while Ras signaling uses adaptor proteins like Grb2 (34).

Equilibrium systems do not require fuel turnover. They respond to changes in the environment by harvesting the energy of ligand binding, thereby capitalizing on the work that is performed by the environment to change the ligand concentration. While the response speed is determined by the rate constants, the accuracy of sensing is only limited by their ratio; there is no trade-off between speed and accuracy. The sensing precision is, however, limited by the number of receptors. This is because the energy of receptor-ligand binding is used to expel or bind the messenger protein, thus coupling receptor-ligand binding to receptor-readout binding. This inevitably leads to correlations between the extrinsic noise in the receptor and the intrinsic noise of the processing network (11). These correlations lead to a fundamental trade-off between these sources of noise in equilibrium systems.

In nonequilibrium sensing, fuel turnover allows the receptor to transmit information as a catalyst. This makes it possible to remove the correlations and the concomitant trade-off between extrinsic and intrinsic noise, and reach a sensing precision that is not limited by the number of receptors. Arguably the most important signaling motif that relies on fuel turnover is the Goldbeter-Koshland push-pull network studied here. This motif is

TABLE I: Two strategies for sensing the environment

	Equilibrium sensing	Nonequilibrium sensing
		
<i>Example implementation</i>		
<i>Biochemical mechanism</i>	sequestration/adaptor proteins	catalysis
<i>Sampling strategy</i>	measurements with more receptors	measurements of same receptors over time
<i>Fundamental resources</i>	receptors	receptors/time, fuel, readout molecules
<i>Energy source</i>	harvests energy of ligand binding	energy from fuel
<i>Intrinsic and extrinsic noise linked?</i>	yes	no
<i>Minimum error</i>	$4/R_T$ (without cooperativity) $4/R_T^2$ (with cooperativity)	$\text{MAX}(4/(R_T\tau_r/\tau_c), 4/X_T, 4/w)$
<i>Example</i>	one-component signaling	two-component signaling

used in most, if not all, signal transduction pathways.

Our analysis reveals why it may be beneficial to use this energy consuming motif: it makes it possible to store the history of the receptor state in stable chemical modification states of downstream molecules. In equilibrium sensing the stability of the downstream signaling proteins relies on physical interactions with the receptor molecules, which means that the state of the readout molecules reflects the instantaneous state of the receptor. In contrast, in non-equilibrium sensing the energy to change the state of the signaling proteins is not provided by the physical interactions with the receptor, but by the chemical fuel. The receptor catalyzes the modification of the read-out. After modification, however, the receptor and read-out become decoupled and each read-out molecule provides a stable memory of the receptor state when it was modified. It is this feature that allows these non-equilibrium systems to take samples of the receptor state over time and perform a discrete time integration. This increases the number of measurements per receptor, making it possible to beat the equilibrium sensing limit set by the number of receptors.

Taking samples fundamentally requires time so that the samples are independent; downstream molecules to store the samples; and energy to store them reliably, to protect the coding. We find that at least $4k_B T$ is needed for reliable encoding, quantifying a relationship between energy and information. One of the most widely used coding strategies is phosphorylation, which requires ATP. *In vivo*, ATP hydrolysis provides about $20k_B T$. This is sufficient to take one receptor sample essentially irreversibly (Fig. 3B), which means that the quality factor q reaches unity. Readout phosphorylation thus makes it possible to store the receptor state reliably.

Non-equilibrium networks can exhibit more complicated features than those of the simple push-pull mo-

tif, as in the MAPK cascade. The molecular picture for time-integration suggests that our results for the push-pull network hold generally, even in these more complicated systems. Indeed, we find the same or more severe resource limitations in cascades and networks with simple negative or positive feedback (*SI Text*). Although cascades can increase the response time (10), which increases information transfer, they do not make sensing more efficient in terms of energy or readout molecules.

One- and two-component signaling networks provide a case study for the trade-off between equilibrium and non-equilibrium sensing. One-component systems consist of adaptor proteins which can bind an upstream ligand and a downstream effector, while two-component systems are similar to the push-pull network studied here, consisting of a kinase (receptor) and its substrate. Interestingly, some adaptor proteins, like RocR, contain the same ligand-binding domain as the kinase and the same effector-binding domain as the substrate of a two-component system, *i.e.* NtrB-NtrC (35). It has been suggested that one-component systems have evolved into two-component systems to facilitate transfer of signals from the membrane to the nucleus (35). However, equilibrium networks can also transmit signals across space (Table I). Our results thus suggest that these one-component systems are really alternative, equilibrium solutions to the problem of signal transduction, selected because of different resource selection pressures.

Which resource sets the fundamental limit for non-equilibrium sensing? Although it has often been assumed that time/receptors are limiting (1, 4–10), our results, in contrast, show how the accuracy of sensing can instead be limited by energy or downstream copy numbers. Interestingly, experiments suggest that some key networks are not time/receptor limited. Cheong *et al.* have measured the information transmission of several important

networks, and have shown that all transmit about 1 bit of information, or less (36). This amount is far less than the networks would transmit if they were time/receptor limited (see *SI Text*). This suggests that another resource, such as copy numbers of signaling components or energy, limits sensing. In such scenarios, characterizing the response time of the network is less important for understanding sensing than characterizing protein expression levels and their energy usage.

It seems natural to expect that the resources which are limiting sensing are those that affect cell growth or fitness, while the resources that are in excess and thus wasted are those that do not significantly affect cell growth or fitness. This prediction could be tested experimentally, for example by studying the growth and chemotactic performance of bacterial populations with different expression levels of functionally and non-functionally signaling proteins (12). To the extent that all resources affect growth, evolutionary pressure should tend to drive systems so that no resource is wasted, which occurs when all are equally limiting. Resource-optimal systems sample the receptor about once per correlation time and use just enough fuel and downstream molecules to do so. Quantitatively, all resources are equally limiting when

$$R_T \tau_r / \tau_c \approx X_T \approx w. \quad (12)$$

In an optimal sensing system, the number of independent concentration measurements $R_T \tau_r / \tau_c$ equals the number of readout molecules X_T that store these measurements and equals the work (in units of $k_B T$) to create the samples.

In two-component signaling systems, including that of bacterial chemotaxis, the downstream component is typically in excess of the receptor (37–39). For the *E. coli* chemotaxis system, $X_T / R_T \approx 3 - 4$ (38, 39). Eq. 12 thus predicts that $\tau_r / \tau_c \approx 3 - 4$. This prediction can be tested, assuming that the correlation time τ_c of the receptor-CheA complex is that of receptor-ligand binding. In *E. coli*, the lifetime of the active (phosphorylated) readout, CheYp, is $\tau_l \approx 100\text{ms}$ (2), which means that $\tau_r \approx \tau_l / 3 \approx 30\text{ms}$, since about a third of the total amount of CheY is phosphorylated in steady-state. Eq. 12 thus predicts that $\tau_c \approx 10\text{ms}$. To test this prediction, we estimate τ_c from the receptor-ligand dissociation rate k_{off} as $\tau_c \simeq 1 / (2k_{\text{off}})$, ($p \approx 0.5$). The dissociation constant of Tar-aspartate (receptor-ligand) binding $K_D \approx 0.1 - 1\mu\text{M}$ (40) and with an association rate $k_{\text{on}} \approx 10^9\text{M}^{-1}\text{s}^{-1}$ (41), this yields $k_{\text{off}} \approx 100 - 1000\text{s}^{-1}$ and an estimated correlation time $\tau_c \approx 1 - 10\text{ms}$, in line with the prediction of Eq. 12.

Eq. 12 also predicts that the fundamental resources should vary proportionally to each other across different networks. For example, the relation predicts that the lifetime τ_r of the modified state of a readout molecule should increase, *ceteris parabus*, with its expression level X_T . Two-component systems can provide a large-data

set for testing these predictions once kinetic data and protein expression levels for many of them become available (42).

Our results are also important for synthetic biology, which uses two-component signaling networks as a building block (43). The design principles instruct how such networks should be constructed at the molecular level to minimize resource consumption. In turn, synthetic networks may provide a platform for testing key predictions.

A major question in cell signaling is to what extent the design of signaling pathways is shaped by the same limits that apply to other sorts of machines, and to what extent they face unique limitations because they are constructed out of molecular networks. The process of sampling a time series, like the receptor state over time, defines a specific, familiar computation that could be conducted by any machine; it is instantiated in the biochemical system by the readout-receptor pair. We find that the free-energy drops across the “measurement” and “erasure” steps, $\Delta\mu_1$ and $\Delta\mu_2$, should be identical to minimize the energetic cost, even though the fuel molecule need only be involved in one of the reactions, preparing a non-equilibrium state that relaxes via the other. This allocation of energy differs from that typically considered in the computational literature, in which only the erasure step requires energy (44). In the cellular system both steps are computational erasures: though only the “erasure” step erases memory of the receptor state, both steps erase the state of the molecule involved in the collision. Interestingly, when $p = 0.5$, the average work to measure the state of the receptor is $2k_B T$, which is perhaps surprisingly close to the Landauer bound, $k_B T \ln(2)$ (44).

MATERIALS AND METHODS

Calculating the sensing error for a biochemical network

From Eq. 1 in the main text, the sensing error for a biochemical network depends on the gain and the variance of the readout molecule. For all networks studied in this paper, we have calculated the gains using a mean-field approximation for the steady state level of the readout, which is exact for linear networks (the base model of the main text and the base model plus deactivation). Except where otherwise noted, we have calculated all variances using a linear-noise approximation (45), which is, again, exact for the linear networks. For nonlinear networks, the quality of the approximation improves with system size; it can already be quite good for systems with only 10 copies of each molecule (46). For the base model and the base model with deactivation, we have used tools of discrete stochastic processes to independently calculate the error by viewing the signaling network as a system

that samples the receptor state (see *SI Text*)

The linear-noise approximation gives the covariance matrix Σ for stationary fluctuations in species' levels as the solution to the Lyapunov equation:

$$A\Sigma + \Sigma A^T + B = 0 \quad (13)$$

where $A = S^T \nabla \nu$ and $B = S^T \text{Diag}(\nu) S$ in terms of the stoichiometric matrix S and the reaction propensity vector ν . The stoichiometric matrix describes how many molecules of each species are consumed or produced in each reaction, and the propensity vector describes the propensity (rate) of each reaction. For a network out of steady state (the base model), a non-stationary version must be used (45).

Langevin approximation to the dynamics of a biochemical network

The Langevin approximation to the dynamics of a biochemical network draws on the same framework as the linear-noise approximation (45). It expresses the fluctuations in species copy numbers \mathbf{N} as:

$$\frac{d\mathbf{N}}{dt} = A\mathbf{N} + \eta(t) \quad (14)$$

where \mathbf{N} is a vector containing the copy numbers of all species and $\eta(t)$ are Gaussian noises, uncorrelated in time, with covariance B . A and B are the matrices defined in the section "Calculating the sensing error for a biochemical network." The equation can be solved (e.g. by integrating factors; 45), yielding the result in the main text, Eq. 3, for the biochemical network considered there.

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SUPPLEMENTARY MATERIAL

Minimum sensing error of the simple equilibrium binding system

In the main text, we considered a simple equilibrium system in which the read-out binds the unbound receptor: $R + L \rightleftharpoons RL$, $R + x \rightleftharpoons Rx$. Here, we show that the sensing error for this network is limited by the number of receptors on the surface of the cell, as stated in the

main text. Calculating the variance as described in the main text (or directly via the linear-noise approximation) yields for the sensing error:

$$\left(\frac{\delta c}{c}\right)^2 = \frac{(R + RL)(Rx(x) + R(Rx + x) + RL(Rx + x))}{(RL^2)(Rx)(x)}$$

where we have written the result (following the Lagrange multiplier approach from, for example, 47) in a form that makes it easy to show that the error is bounded by the number of receptors; a direct expression in terms of the rate constants is quite complicated. Indeed, minimizing the expression over R , Rx , RL , and X_T such that all are positive and $R + RL + Rx = R_T$ and $x + Rx = X_T$ shows the result in the main text, that the error is always greater than $\frac{1}{p(1-p)} \frac{1}{R_T}$ (Eq. 2 of the main text).

Cooperativity achieves the fundamental equilibrium bound and is necessary to achieve it

First we show that cooperative binding of the ligand to the receptors can achieve the fundamental equilibrium bound. One way in which receptors can cooperatively bind ligand is when the receptors are in clusters. Consider C_T clusters, each containing n receptors that co-operatively bind n ligand molecules, $C + nL \rightleftharpoons CL^n$. The number of bound clusters, CL^n , is binomially distributed, giving variance $C_T p(1-p)$, where p is the probability a cluster is bound. The fluctuation-dissipation theorem gives the gain as $nC_T p(1-p)$, since each cluster binds n ligand molecules. The sensing error is then (Eq. 1 in the main text): $\left(\frac{\delta c}{c}\right)^2 = \frac{1}{p(1-p)} \frac{1}{n^2 C_T}$. When all the receptors are in a single cluster ($n = R_T$, $C_T = 1$), this can be as low as $4/R_T^2$, achieving the fundamental bound.

Equilibrium systems without positive cooperativity at the level of the receptors cannot achieve this bound, at least under the linear-noise approximation. We prove this in the general case that multiple different receptor species, R_i , can bind the ligand, possibly with different affinities – but not cooperatively. The fluctuation-dissipation theorem guarantees that the best readout is the total number of bound receptors, $RL = \sum R_i L$, since that is the variable conjugate to the chemical potential of the ligand. In general, the variance σ_{RL}^2 is just the sum of the variances of the species, plus corrections for the correlations between the species:

$$\sigma_{RL}^2 = \sum_i \sigma_{R_i L}^2 + \sum_i \sum_j \sigma_{R_i L, R_j L}^2 \leq \sum_i \sigma_{R_i L}^2 \quad (15)$$

where the inequality follows from the lack of (positive) cooperativity. (Negative cooperativity can emerge naturally in equilibrium networks due to competition of downstream molecules for binding to the receptors.) For an

equilibrium system, the variance of a species is always less than the mean level of that species, at least under the linear-noise approximation, so:

$$\sigma_{RL}^2 \leq \sum_i \overline{R_i L} \leq R_T. \quad (16)$$

Thus:

$$\left(\frac{\delta c}{c}\right)^2 = \frac{1}{\sigma_{RL}^2} \geq \frac{1}{R_T} \quad (17)$$

Combining this bound with the general bound for all equilibrium systems, $(\delta c/c)^2 \geq 4/R_T^2$, yields the result in the main text: $(\delta c/c)^2 \geq \max(1/R_T, 4/R_T^2)$. When R_T is large, systems without cooperativity perform worse than the fundamental bound by about $1/R_T$.

These arguments show that the absolute bound for equilibrium systems, $(\delta c/c)^2 \geq \frac{4}{R_T^2}$, can only be achieved in systems which cooperatively bind the ligand or in which multiple ligand bound to a single receptor cooperatively activate the receptor. Without cooperativity, the bound is given by $(\delta c/c)^2 \geq \max(1/R_T, 4/R_T^2)$.

Information theoretic analysis of equilibrium systems

We consider an arbitrary equilibrium biochemical network in which receptors bind ligand and the cell uses a read-out x to sense the environment. We denote the copy numbers of the N_s species in the system by the vector \mathbf{N} . The copy numbers of R , RL , and the read-out x are elements of this vector, along with any other species in the network. Since only the receptor binds the ligand, the distribution for species' copy numbers \mathbf{N} in the equilibrium system with N_s species is given in general by (48) $P(\mathbf{N}) = \frac{e^{-\mu_L RL} \mathcal{Q}(\mathbf{N})}{\Xi}$, where $\mathcal{Q}(\mathbf{N}) = \prod_{i=1}^{N_s} \frac{z_i^{N_i}}{N_i!}$ is the (canonical) partition function in terms of the molecular partition functions z_i . The grand canonical partition function, Ξ , normalizes the distribution by summing the numerator over all possible states consistent with the stoichiometric constraints \mathcal{C} :

$$\Xi = \sum_{\mathbf{N}' \in \mathcal{C}} e^{-\mu_L RL'} \mathcal{Q}(\mathbf{N}') \quad (18)$$

From this distribution, it is clear that $P(x, RL, \mu_L) = P(x|RL, \mu_L)P(RL|\mu_L)P(\mu_L) = P(x|RL)P(RL|\mu_L)P(\mu_L)$ for any read-out x , so that $\mu_L \rightarrow RL \rightarrow x$ forms a Markov chain. That is, the chemical potential of the ligand affects the read-out only via the instantaneous state of the receptors. The data processing inequality then leads to the conclusion in the main text, $I(x; \mu_L) \leq I(RL; \mu_L)$.

The information the number of bound receptors, RL , has about the chemical potential of the ligand is easily bounded, since one of the few restrictions we have

imposed on the equilibrium system is that the number of receptors is finite (less than R_T). For any random variables X and Y , $I(X; Y) \leq H(X)$ where H is the entropy of a random variable. Furthermore, the maximum entropy distribution on a bounded support is the uniform distribution and the entropy of a discrete uniform distribution is $H(X) = \log_2(n)$ where n is the number of possible states for the variable. Thus, $I(RL; \mu_L) \leq H(RL) \leq \log_2(R_T + 1)$.

The extensions to these proofs when multiple types of receptors bind the ligand or when each receptor molecule binds multiple ligand molecules are straightforward. Then, the quantity R_T is replaced in the proofs above by the total number of ligand molecules that can be bound to receptors at any time, L_T . If multiple types of receptors can bind ligand, L_T is just the total number of receptors of any type. If each receptor molecule binds more than one ligand molecule, L_T is just the total number of receptors times the number of ligand molecules each receptor can bind.

Sensing error of biochemical networks viewed as discretely sampling the receptor state

In this section we show how the sensing error of the biochemical network can be calculated by viewing the network as a discrete sampling process. The important quantities in a sampling protocol are the number of samples taken and the spacing between them, in addition to the properties of the sampled signal. By viewing the biochemical process as a sampling process, we mean that the underlying parameters of the biochemical network affect the sensing error only insofar as they affect these quantities, or the stochasticity in these quantities. The benefit of viewing the network as a sampling process is that the number of samples and the spacing between them have intuitive, and well-known, effects on the sensing error: the more samples, the lower the error; the further apart the samples are, the more independent they are. Perhaps less well known are the effects on the sensing error of stochasticity in the number of samples or the spacing between samples; these effects emerge in the process of determining the error for a discrete sampling protocol, which we do below.

We consider the biochemical networks described by the base model in the main text and the base model plus deactivation – the push-pull network. For the base model, we identified the molecules that had collided with the receptors as samples, since these molecules' states reflect the receptor states at the times of their collisions with the receptor. For the base model with deactivation, we identified the molecules that collided with the receptor more recently than with the phosphatase as samples. When we refer to the number of samples, we mean the number of these molecules; when we refer to the times of the sam-

ples, we mean the times at which these molecules collided with the receptor.

We begin by rewriting the equation for the sensing error in a form that makes the connection to discrete sampling explicit, Eq. 22 below. The cell senses its environment through the level of its readout x^* . However, this is no different from estimating the ligand concentration from $\hat{p} = x^*/\bar{N}$:

$$\left(\frac{\delta c}{c}\right)^2 = \frac{\sigma_{\hat{p}}^2}{\left(\frac{d\hat{p}}{d\mu_L}\right)^2} = \frac{\sigma_{x^*}^2}{\left(\frac{dx^*}{d\mu_L}\right)^2} \quad (19)$$

since \bar{N} is a constant, independent of μ_L . Note that the gain $d\bar{p}/d\mu_L$ is $d\bar{p}/d\mu_L = p(1-p)$.

We first consider the effect of the stochasticity in the total number of samples, N . The law of total variance allows us to decompose the variance in the estimate \hat{p} into terms arising from different sources:

$$\sigma_{\hat{p}}^2 = E[\text{var}(\hat{p}|N)] + \text{var}[E(\hat{p}|N)] \quad (20)$$

The first term of Eq. 20 reflects the mean of the variance in \hat{p} given the number of samples N ; the second term reflects the variance of the mean of \hat{p} given the number of samples N .

The mean and variance of \hat{p} given the number of samples N are more familiar quantities than their unconditioned counterparts, as we see below. Since, by definition, the samples reflect the state of the receptor at the times t_i of their collisions with the receptor, we can write the number of x^* at the final time as:

$$x^* = \sum_{i=1}^N n_i(t_i) \quad (21)$$

where $n_i(t_i)$ denotes the value of the i^{th} sample — the state of the receptor involved in the i^{th} collision at the time t_i of that collision, 1 if bound to ligand, 0 otherwise. In the following, we consider a single receptor, $R_T = 1$ and $n = n_i$. The results generalize to multiple receptors.

We can then rewrite Eq. 20:

$$\sigma_{\hat{p}}^2 = E\left[\frac{N^2}{\bar{N}^2} \text{var}\left(\frac{\sum_{i=1}^N n(t_i)}{N} \middle| N\right)\right] + \text{var}\left[\frac{N}{\bar{N}} E\left(\frac{\sum_{i=1}^N n(t_i)}{N} \middle| N\right)\right] \quad (22)$$

The equation is a bit complicated, but what is important is that it fully specifies the sensing error in terms of the number of samples, the spacings between them, and the stochasticity in these quantities. That is, this equation shows that the sensing error is the error of a sampling process. We can use it to calculate the sensing error independently from, for example, the master equation or the linear-noise approximation.

The first term describes the error of a very standard sampling process, one with a fixed number of samples. We recognize the variance

$$\text{var}\left(\frac{\sum_{i=1}^N n(t_i)}{N} \middle| N\right) \quad (23)$$

as the error of a statistical sampling protocol in which exactly N samples are taken at random times t_i . This is shown explicitly in the section “Error of discrete sampling protocols with a fixed number of samples.” In that section, it is shown that the error for such a sampling protocol is:

$$\text{var}\left(\frac{\sum_{i=1}^N n(t_i)}{N} \middle| N\right) = p(1-p) \frac{1}{f_I N} \quad (24)$$

where f_I is the fraction of the samples that are independent, as given by Eq. 7 in the main text. Then the first term in Eq. 22 is just:

$$E\left[\frac{N^2}{\bar{N}^2} \text{var}\left(\frac{\sum_{i=1}^N n(t_i)}{N} \middle| N\right)\right] = E\left[\frac{N^2}{\bar{N}^2} p(1-p) \frac{1}{f_I N}\right] = p(1-p) \frac{1}{f_I \bar{N}} \quad (25)$$

That is, the first term in Eq. 22 is the error of a discrete sampling protocol with exactly \bar{N} samples, as stated in the main text. The only effect of the expectation in the first term is to swap \bar{N} for N . Dividing by the squared gain (see Eq. 19), $d\bar{p}/d\mu_L = p(1-p)$, gives the first term in Eq. 6 in the main text.

We now turn to the second term in Eq. 22. From the law of total variance, this term describes how stochasticity in the number of samples, N , contributes to the sensing error. Because the number of samples N is Poisson with mean and variance equal to \bar{N} :

$$\text{var}\left[\frac{N}{\bar{N}} E\left(\frac{\sum_{i=1}^N n(t_i)}{N} \middle| N\right)\right] = \text{var}\left[\frac{N}{\bar{N}} p\right] \quad (26)$$

$$= \frac{p^2}{\bar{N}^2} \text{var}[N] \quad (27)$$

$$= \frac{p^2}{\bar{N}} \quad (28)$$

where the probability a receptor is bound is $E[n(t_i)] = p$. Dividing by the squared gain gives the second term in Eq. 6 in the main text. Thus, we have derived Eq. 6 in the main text as the result of a discrete sampling protocol.

The derivations leading to Eq. 22 show that the sampling error for the sampling protocol must be the same as the sensing error for the biochemical network. To check this, we can calculate the sensing error for the biochemical network, Eq. 6 in the main text, in a more standard way, determining the gain and the variance of the output x^* and using Eq. 1 in the main text. We do

this for the base model with deactivation; results for the base model follow similarly. The mean level of x^* is just $\bar{x}^* = k_f p \tau_\ell$. The variance in x^* can be calculated using standard methods (e.g. the linear-noise approximation; see section “Calculating the sensing error for a biochemical network”):

$$\sigma_{x^*}^2 = \frac{(k_f \tau_\ell)^2 p(1-p)}{1 + \frac{\tau_\ell}{\tau_c}} + \bar{x}^* \quad (29)$$

The gain is:

$$\frac{d\bar{x}^*}{d\mu_L} = p(1-p)k_f \tau_\ell \quad (30)$$

Assembling the results, Eq. 6 in the main text follows, just as it did from the sampling protocol.

The origin of the second term in Eq. 6 in the main text

The second term in Eq. 6 in the main text emerges in the derivations above as a consequence of the stochasticity in the number of samples N . However, it is more fundamentally a consequence of the fact that the cell does not distinguish between samples of the unbound receptor from blank samples that do not represent a receptor state – i.e. it does not distinguish x molecules that collided with the unbound receptor from those that never collided with the receptor in any state. A more standard sampling procedure would distinguish between these, and so would estimate \hat{p} as $\hat{p} = x^*/N$, not $\hat{p} = x^*/\bar{N}$, as above. As we show below, this procedure gives rise to only the first term of Eq. 6 in the main text, allowing us to interpret the second term as the price the cell pays for not distinguishing readout molecules that collide with the unbound receptor from those that have never collided with the receptor in any state.

One way to arrive at this conclusion is to imagine that all collisions with the receptor lead to modifications of x . Yet, while the ligand-bound receptor modifies x into state x^* , the unbound receptor modifies x into another state x^\dagger . Hence, in addition to the reaction $x + RL \rightarrow x^* + RL$ we consider the reaction $x + R \rightarrow x^\dagger + R$. Then, $N = x^* + x^\dagger$. Analogously to Eq. 1 in the main text, we can then estimate the variance of $\hat{p} = x^*/N = x^*/(x^* + x^\dagger)$ by expanding to first order:

$$\delta \hat{p} \approx g_{\hat{p}, x^*} \delta x^* + g_{\hat{p}, x^\dagger} \delta x^\dagger \quad (31)$$

where the gains are:

$$g_{\hat{p}, x^*} = \frac{d\hat{p}}{dx^*} = \frac{x^*}{(x^* + x^\dagger)^2} \quad (32)$$

$$g_{\hat{p}, x^\dagger} = \frac{d\hat{p}}{dx^\dagger} = -\frac{x^\dagger}{(x^* + x^\dagger)^2} \quad (33)$$

The variance is then:

$$\sigma_{\hat{p}}^2 = g_{\hat{p}, x^*}^2 \sigma_{x^*}^2 + g_{\hat{p}, x^\dagger}^2 \sigma_{x^\dagger}^2 + 2g_{\hat{p}, x^*} g_{\hat{p}, x^\dagger} \sigma_{x^*, x^\dagger}^2 \quad (34)$$

where the last term accounts for the covariance. The variances can be calculated in many ways since the system is linear. For example, they can be calculated exactly via the linear-noise approximation. The result is the first term of Eq. 6 in the main text, as claimed. Indeed, there is no second term for the model described here. This is precisely because with this scheme the number of samples N is known. While in the scheme of the main text (see Fig. 2), the system cannot discriminate between the molecules that have collided with an unbound receptor and the molecules that have not collided with the receptor at all, in this scheme the system knows exactly how many collisions there have been with the receptor: $x^* + x^\dagger$.

Error of discrete sampling protocols with a fixed number of samples

In this section, we derive the first term of Eqs. 20 and 22, corresponding to Eq. 6 in the main text, as the error of a discrete sampling protocol with a fixed number of samples N taken of receptor states over time. The average receptor occupancy is estimated as:

$$\hat{p} = \frac{1}{N} \sum n_i(t_i) \quad (35)$$

where $n_i(t_i)$ is the state of the receptor involved in the i^{th} sample at the time of that sample, 1 if the receptor was bound at time t_i and 0 otherwise. In what follows, we consider a single receptor, $R_T = 1$ and $n(t_i) = n_i(t_i)$. The results generalize to multiple receptors. The times t_i of the samples represent the times at which the molecules that store the samples of the receptor collided with the receptor. Therefore, we choose the distribution of times between the samples to match the distribution of times between those collisions, which depends on the particular network under consideration, described below. We count time backwards from the present time, $t = 0$. The number of samples N and the distribution of times at which they were taken specifies a sampling protocol, independent of the chemical implementation.

The variance in the estimate of receptor occupancy is:

$$\sigma_{\hat{p}}^2 = \text{var} \left(\frac{\sum_{i=1}^N n(t_i)}{N} \right) \quad (36)$$

$$= \frac{\text{var} \left(\sum_{i=1}^N n(t_i) \right)}{N^2} \quad (37)$$

$$= \frac{\sigma^2}{N} + \frac{N(N-1)}{N^2} E[\text{cov}(n(t_i), n(t_j))] \quad (38)$$

since N is fixed, where $\sigma^2 = p(1-p)$ is the variance of the instantaneous occupancy of a single receptor.

Base model: We first consider a statistical sampling protocol that matches the distribution of receptor-collision times of samples in the base model. The collisions occur at random times in the interval $[0, T]$, so we model N randomly placed samples. The time $\tilde{\Delta}$ between a randomly chosen pair of uniformly distributed samples, not necessarily consecutive, is distributed as:

$$p(\tilde{\Delta}) = \frac{2}{T} - \frac{2\tilde{\Delta}}{T^2}. \quad (39)$$

Changing variables from t_i and t_j to $\tilde{\Delta} = |t_j - t_i|$, we have $\text{cov}(n(t_i), n(t_j)) = \sigma^2 e^{-\tilde{\Delta}/\tau_c}$. The expectation of the covariance is then:

$$E[\text{cov}(n(t_i), n(t_j))] = \sigma^2 \int e^{-\tilde{\Delta}/\tau_c} p(\tilde{\Delta}) d\tilde{\Delta} \quad (40)$$

Assembling the equations above yields the first term in Eq. 6 in the main text with $\Delta = T/N$ ($R_T = 1$), where we have simplified the result with the standard assumption that $T \gg \tau_c$ and $N \gg 1$ (it does not make sense to discuss the spacing between a single sample).

Deactivation: To take into account deactivation, we consider sampling times which match the distribution of the receptor collisions of only those N molecules storing samples. We thus have to take into account that some of the samples that have been taken are thrown away due to the deactivation process. We begin with an alternative expression for the expected covariance:

$$E[\text{cov}(n(t_i), n(t_j))] = \sigma^2 \int \int e^{-|t_j - t_i|/\tau_c} p(t_i, t_j) dt_i dt_j \quad (41)$$

To match the biochemical network, the sample times t_i, t_j of two samples must be independent from each other, since the collisions of different molecules with the receptor and phosphatase are uncoupled. Therefore, $p(t_i, t_j) = p(t_i)p(t_j)$. The marginal probability $p(t_i)$ is the probability that the collision time with the receptor of a given molecule storing a sample was t_i , i.e. $p(t_i|\text{sample})$. This can be written in terms of $p(\text{sample}|t_i)$, the probability that there was a collision with the receptor at the time t_i times the probability that, given a collision at that time, the associated molecule did not subsequently collide with the phosphatase:

$$p(\text{sample}|t_i) dt = r dt e^{-t_i/\tau_\ell} \quad (42)$$

Then:

$$p(t_i|\text{sample}) = \frac{p(\text{sample}|t_i)p(t_i)}{\int p(\text{sample}|t_i)p(t_i)dt_i} = \frac{1}{\tau_\ell} e^{-t_i/\tau_\ell} \quad (43)$$

since $p(t_i)$ is uniform.

Assembling results:

$$E[\text{cov}(n(t_i), n(t_j))] = \sigma^2 \int \int e^{-|t_j - t_i|/\tau_c} \frac{e^{-t_i/\tau_\ell}}{\tau_\ell} \frac{e^{-t_j/\tau_\ell}}{\tau_\ell} dt_i dt_j \quad (44)$$

It is instructive to change variables, defining $\tilde{\Delta} = |t_j - t_i|$, as before. Then:

$$E[\text{cov}(n(t_j), n(t_i))] = \int_0^\infty e^{-\tilde{\Delta}/\tau_c} \frac{e^{-\tilde{\Delta}/\tau_\ell}}{\tau_\ell} d\tilde{\Delta} \quad (45)$$

From this expression we can identify $p(\tilde{\Delta}) = \frac{e^{-\tilde{\Delta}/\tau_\ell}}{\tau_\ell}$ as the distribution of times between two randomly chosen (not necessarily consecutive) samples, when molecules can decay. Simulations confirm this distribution.

Completing the integral and using it in the expression for the sensing error gives the first term in Eq. 6 in the main text for the effective spacing $\Delta = 2\tau_\ell/N$ (here, $R_T = 1$). We have made the simplifying assumptions that $N \gg 1$ (it does not make sense to talk of the spacing between just one sample) and $\tau_\ell \gg \tau_c$, a standard assumption. The effective spacing is not the mean nearest-neighbor spacing, but it is qualitatively similar and serves to summarize the fact that samples taken further apart in time are more independent. Clearly, from Eq. 45, the error depends on the distribution of all-pairs spacings, not necessarily nearest-neighbor spacings, and it depends on the full distribution, not just the mean.

Finally, we iterate that we can perform an independent check on the derivation in this section by computing the sensing error using the linear-noise approximation, which is exact for this linear network. As mentioned, this gives exactly the same result.

No trade-offs among resources

In Fig. 3D of the main text, we show how the sensing error depends on the pair of resources (readout copy number X_T , energy w). These results were obtained via numerical minimization of Eq. 6 subject to constraints on X_T and w .

In Fig. 3E of the main text, we show how the sensing error depends the pair of resources (time/receptor copy number, energy). The plot for (time/receptor copy number, readout copy number) is the same. In this section, we describe the derivation of the results shown in this figure. In order to consider τ_r/τ_c not necessarily large, we need to use a form of the Berg-Purcell bound that is valid for short integration times (10):

$$\left(\frac{\delta c}{c}\right)_{\min}^2 > \frac{1}{p(1-p)} \frac{1}{R_T \left(1 + \frac{\tau_r}{\tau_c}\right)} \quad (46)$$

which identifies $R_T \left(1 + \frac{\tau_r}{\tau_c}\right)$ as a limiting resource, rather than the result of the main text, $R_T \frac{\tau_r}{\tau_c}$, which only holds in the limit $\tau_r \gg \tau_c$.

To elucidate how the sensing error depends on (time/receptor copy number, energy) and (time/receptor copy number, readout copy number), we calculate the

minimum sensing error by optimizing over all parameters while fixing $R_T(1 + \tau_r/\tau_c)$ and either w or X_T , respectively. For a fixed $R_T(1 + \frac{\tau_r}{\tau_c})$ and a fixed work w , the minimum sensing error is:

$$\begin{aligned} \left(\frac{\delta c}{c}\right)_{\min}^2 &= \frac{w}{32} \left(-\frac{1}{\left(R_T \left(1 + \frac{\tau_r}{\tau_c}\right)\right)^2} \right. \\ &\quad + \sqrt{\frac{1}{R_T \left(1 + \frac{\tau_r}{\tau_c}\right)}} \left(\frac{1}{R_T \left(1 + \frac{\tau_r}{\tau_c}\right)} + \frac{32}{w} \right)^{3/2} \\ &\quad \left. + \frac{128}{w^2} + \frac{80}{w \left(R_T \left(1 + \frac{\tau_r}{\tau_c}\right)\right)} \right) \end{aligned} \quad (47)$$

The equation for the dependence of the sensing error on (time/receptor copy number, readout copy number) is the same, with w replaced by X_T . The minimum is plotted in Fig. 3E. The minimum tracks the worst bound, again showing that the resources do not compensate each other.

Additional constraints on the values of rate constants will generally prevent the network from achieving these bounds. In particular, it is common to consider that the binding of ligand to receptor is diffusion-limited, so that the bound $4/\left(R_T \left(1 + \frac{\tau_r}{\tau_c}\right)\right)$ is never achieved. Of course, additional constraints cannot improve the performance of the network beyond the bounds required here, nor can they alter the fact that all the resources are needed for sensing.

Additional networks

Networks are often more complicated than a simple one-level push-pull cascade. We investigate some common motifs to understand whether they relax the trade-offs faced by sensory networks.

Multi-level cascades: Often the signaling molecule activated by the receptor is not taken as the final read-out; rather that molecule catalyzes the activation of another molecule, and so on in a signaling cascade. All of the molecules are reversibly degraded. Using the same approach as for the one-level cascade, we find that the sensing error is bounded by the work done driving just the last step of the cascade: $\bar{N} \leq \frac{\dot{w}_i \tau_r}{4p}$, where $\dot{w}_i = \dot{n}_i \Delta \mu_i$ is the product of the flux of the last molecule through its cycle and the free-energy drop across that cycle, and τ_r is the slowest relaxation time in the cascade (i.e. the reciprocal of the largest eigenvalue of the relaxation matrix.) Even more work is done at other levels of the cascade. The results suggest that cascades do not enable more energy efficient sensing. Additionally, each sample of an active state (bound receptor or active molecule upstream) still requires a molecule to store it.

Positive and negative feedback: A simple model of positive feedback is autocatalysis, in which the receptor-catalyzed activation of the read-out is enhanced by the activated form of the read-out, x^* : $x + x^* + RL \rightleftharpoons 2x^* + RL$. A simple model of negative feedback can be implemented by requiring inactive x for the activation: $2x + RL \rightleftharpoons x + x^* + RL$. In both cases, x^* degrades according to $x^* \rightleftharpoons x$. Neither positive feedback nor negative feedback changes the energetic requirements for sensing: $\bar{N} = \frac{\dot{n}\tau}{p} \frac{(e^{\Delta\mu_1}-1)(e^{\Delta\mu_2}-1)}{e^{\Delta\mu}-1}$. As before, the free-energy drops across the reactions were calculated as the ratio of mass-action propensities.

Cooperative activation of the read-out: If the catalytic activation of the read-out is mediated cooperatively by the receptors (i.e. $x + nRL \rightleftharpoons x^* + nRL$), then the error is reduced by a factor n^2 for the same amount of energy. One way to interpret the result is that each sample requires the same amount of energy as before, but the samples are individually more informative because they reflect n ligand bindings, instead of one — indeed, the instantaneous error is lower.

Trade-offs between equilibrium and non-equilibrium sensing

To understand how energy shapes the design of a network, we modify the push-pull network so that the read-out actually binds the ligand-bound receptor, which can boot the read-out off in a modified state: $RL + x \rightleftharpoons RLx$, $RLx \rightleftharpoons RL + x^*$. The active read-out decays, as before: $x^* \rightleftharpoons x$. The reaction $RLx \rightleftharpoons RL + x^*$ coarse-grains the reactions $RLx \rightleftharpoons RLx^*$ and $RLx^* \rightleftharpoons RL + x^*$; explicitly adding these reaction gives the same results because they essentially can always be integrated out. This network interpolates between the equilibrium and non-equilibrium networks considered in the main text. Choosing the rate constants of the booting and decay reactions to be 0, the network reduces to the sequestration network studied in the equilibrium section. Choosing the rate constants so that the read-out is rarely bound to the receptor, the network reduces to the push-pull network studied in the non-equilibrium section. No resources are coarse-grained in these reductions, though the latter breaks the retroactivity of receptor-read-out binding: energy is required to break reversibility, not retroactivity.

We focus on the relationship between the number of receptors (the equilibrium resource) and the work (a non-equilibrium resource), as the network shifts from binding to catalysis. The work is defined as $w = \dot{w}\tau_r$, as in the main text, where the relaxation time τ_r is chosen as the negative reciprocal of the smallest eigenvalue of the regression matrix of the network. From a scaling argument and dimensional analysis, the relationship between these

resources must take the form:

$$\frac{1}{R_T} \left(\frac{\delta c}{c} \right)^2 \geq f \left(\frac{\dot{w}\tau_r}{R_T} \right) \quad (48)$$

for some function f independent of any parameters.

We probe this function numerically (Fig. 4). The figure shows results from 2.5 million explicit parameter evaluations and from about 25,000 numerical minimization trials. Minimization trials were constrained steepest descent minimizations, randomly initialized for logarithmically distributed rate constants between $\exp(-15)$ and $\exp(15)$. To promote uniform sampling of the space, we minimized estimation error subject to constraints on the work; we minimized work subject to constraints on the estimation error; and we minimized the product of the work and the estimation error subject to constraints on either. We also continued the best solutions over variations in the constraints to probe the global minima.

As seen in the figure, when the work per receptor is less than about $1 \text{ k}_B\text{T}$, the equilibrium scheme of binding is optimal, recovering the equilibrium bound for the sensing error, $(\delta c/c)^2 \geq 4/R_T$ (Eq. 2 in the main text with $p = 1/2$). When the work per receptor is greater than about $4 \text{ k}_B\text{T}$, the non-equilibrium scheme of catalysis is optimal, recovering the bound from the main text, $(\delta c/c)^2 \geq 4/(\dot{w}\tau_r)$. Roughly, it only makes sense to use the nonequilibrium catalysis scheme if the energy budget is sufficient to take more than one sample per receptor ($4 \text{ k}_B\text{T}$ per sample of the bound receptor), since the equilibrium scheme can take one sample of the bound receptor without any energy. Around $1 \text{ k}_B\text{T}$ there is an intermediate regime in which the network outperforms both these regimes by partially utilizing the bound receptor-read-out state.

Assessing the limiting resource in biochemical networks

In the main text, we argue that the TNF network could transmit much more than one bit if it were time/receptor limited. Here, we describe how we arrived at that conclusion.

Even if the integration time of the network were zero and the network did not integrate the receptor state, it would still be able to transmit the information in the instantaneous receptor state. The information about the ligand concentration, c , in the instantaneous receptor occupancy, $RL(T)$, is given by:

$$I(RL(T), c) = 1/2 \log_2 (\pi R_T / (2e)) \quad (49)$$

To arrive at this result, we calculated the information transfer of a biochemical system that takes the receptor occupancy, and not a downstream readout, as the final output. We assumed simple ligand-binding kinetics, $R + L \rightleftharpoons RL$, and assumed that ligand binding is

not affected by any downstream processes. More complicated kinetics (e.g. cooperativity) would likely increase the instantaneous information transfer. The result assumes that the ligand-binding kinetics are optimized with respect to the distribution of input concentrations of the ligand; i.e. the information transfer calculated is the channel capacity of the network. The channel capacity is the appropriate quantity to consider, because it is the experimentally reported quantity in the paper by (36). We followed the method in [49] to calculate the channel capacity.

TNF signaling utilizes $R_T = 2000$ receptors on the cell surface (50), corresponding to $1/2 \log_2 (\pi R_T / (2e)) = 5$ bits of information. If the network integrates the receptor state, the information could be even higher. The fact that the actual information transfer is instead much less than 5 bits suggests that receptors/time do not limit the accuracy of sensing, but rather another resource, such as copy numbers of signaling components or energy.

The following paragraphs address various nuances to the above argument. First, note that restrictions on the probability distribution of inputs can prevent the system from achieving the channel capacity. This is true both for our bound and for the calculated information transmission through the entire network in the paper by Cheong *et al.* One biologically relevant restriction on the probability distribution of inputs is the support of the distribution, particularly the maximum biologically relevant concentration of the ligand; if achieving the channel capacity requires input distributions with large probability for concentrations that are much higher than those biologically observed, then the channel capacity is not really a relevant measure for the capacity of the network. Important in this context is that the dissociation constant K_D for TNF binding is 0.323 ng/mL (51), about the same as the half-saturation for the TNF response as measured by Cheong *et al.*. So achieving the channel capacity at the level of the receptors does not require higher concentrations than achieving the channel capacity of the whole network. This means that, while restrictions on the maximum input concentration would prevent the system from achieving the channel capacity of 5.5 bits at the level of the receptors, they would also prevent the system from achieving the channel capacity of 1 bit at the level of the output, maintaining the discrepancy.

The above arguments assume that a) the principal role of the signaling network is to time integrate the receptor, and b) that this improves information transmission if energy and the copy numbers of the signaling molecules are not limiting, and the network is hence not too noisy. However, signaling systems with enough fuel and signaling molecules that time-integrate the receptor, do not necessarily increase information transmission. They can also reduce information transmission by collapsing many input states onto the same output state. This can happen when the input-output relation is (strongly) non-

linear. However, the experimental data in the paper by Cheong *et al.* suggest that this is not the case for the TNF network, as the response increases mono-modally and gradually with the input. In fact, the output is also noisy. Indeed, the authors attribute the loss of information transmission to biochemical noise, which, according to our analysis, could be due to limiting amounts of read-out molecules or energy.

A final note is that while the above arguments show that the number of receptors and time are not limiting and suggest that downstream molecules or energy are limiting, it is cannot be ruled out that other sources of noise, which we have not modeled, are instead limiting. For example, the sensing precision could be limited by cell-to-cell variability in the copy numbers of signaling molecules (expression or capacity noise (52)). These could even involve variations in the number of receptors themselves. However, back-of-the-envelope calculations suggest that such variations are not enough to explain the discrepancy above. Moreover, many biological systems, including some two-component systems, are insulated against fluctuations in protein expression (53), supporting the idea that in these cases energy or protein copy numbers are indeed limiting the accuracy of sensing.

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