Effects of cross-talk and pleiotropy on the specificity and accuracy of receptor signaling

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Abstract

Living cells and cell collectives crucially depend on the ability to sense their environment through engagement of external molecular signals with cell surface receptors. Puzzlingly, vast numbers of signaling pathways exhibit a high degree of cross-talk between different signals whereby different ligands act through the same receptor or shared components downstream. It remains unclear how a cell can accurately process information from the environment in such cross-wired pathways. We show that a feature which commonly accompanies cross-talk - receptor pleiotropy (the ability of the receptor to produce multiple outputs) - offers a solution to the cross-talk problem. In a minimal model of pleiotropic receptor cross-talk, we show that a single pleiotropic receptor is sufficient to simultaneously identify the ligand and accurately sense the concentration of a presented ligand, enabling specific and accurate signaling. We calculate the fundamental limits of the signaling specificity and accuracy of a pleiotropic receptor arising from the molecular noise both at the receptor level and downstream. We further investigate the effects of common signaling schemes such as Kinetic Proofreading (KPR) and show that KPR does not necessarily increase the signaling specificity once the effects of molecular noise are taken into account. The model serves as an elementary “building block” towards understanding more complex cross-wired receptor-ligand signaling networks, and suggests how cross-talk and pleiotropy might be inherent features of combinatorial signaling in cross-wired networks. The results of the paper suggest a novel outlook at a number of experimental systems.

Introduction

Receptor signaling via soluble ligand molecules enables living cells to communicate with each other and with their environment, and is the main mode of multi-cellular coordination in the immune, nervous, endocrine and other systems, as well as in complex populations of microorganisms. In a typical signaling pathway, binding of a ligand to a cell surface receptor activates a cascade of intracellular events that eventually lead to responses such as cellular differentiation [1,4], phenotypic change [4,7], or a change in cellular motility [8,12]. For reliable and precise communication, receptor signaling often needs to be specific, accurate, rapid, and robust to molecular noise and cellular heterogeneity [13,20]. However, fundamental physical constraints often place these different signaling goals at odds with each other [17,18,21,24], and different signaling pathways have evolved to optimize different aspects of information transmission such as specificity [18,22,25], sensitivity [5,18,26], accuracy [9,27,29], and speed [21,30,32].

Puzzlingly, receptor signaling pathways frequently exhibit a high degree of cross-talk whereby multiple ligands act through shared cell surface receptors and downstream signaling components [33,36,39,42]. Another puzzling feature that commonly accompanies cross-talk is receptor pleiotropy, the ability of a receptor to produce more than one type of output. This combination of features commonly results in “hourglass” shaped input-output networks [40,41,43,46]. This challenges the classical “one ligand/one signal” paradigm [4], while raising the question of how signaling pathways are able to effectively transmit information under such conditions [22,29,41,47,52].

The fundamental problem that cross-talk poses to effective signal transmission can be illustrated through an example of the conflict between the signaling accuracy (sensing the concentration of a given ligand) and the specificity (the ability to produce different responses to different ligands). In the presence of receptor cross-talk, these two goals are at odds as illustrated in a classical model of ligand-receptor binding, wherein the average receptor occupancy \( P \) by a ligand present at a concentration \( c \) is \( P = (c/K_d)/(1 + c/K_d) \), where \( K_d \) is the equilibrium dissociation constant [53]. The ligand concentration and the dissociation constant enter into this expression only through their ratio, \( c/K_d \), and identical receptor occupancies can be realized by a weakly binding ligand present at a high concentration, or a strongly binding one at a low concentration. Hence it is difficult to unambiguously distinguish between different ligands that act through the same receptor while accurately measuring their concentration. This example is a manifestation of a more general inference problem that arises in the presence of cross-talk - the difficulty of unambiguously inferring multiple input variables - ligand concentration (“quantity”) and affinity (“quality”), in this case - from one output variable (receptor occupancy). One prime biological example of this problem is Type I Interferon signaling [40,54].
Several recent works [29, 41, 48, 50] focused on the effects of cross-talk on the accuracy of sensing a cognate ligand in the presence of non-specific ligands. Such problems commonly arise in the context of cellular chemotaxis driven by concentration gradients of food or chemoattractants. Using an extension of the classical Berg-Purcell framework [13, 15, 55, 56], it was shown that, given sufficient separation between the affinities of the specific and non-specific ligands, detection and accurate sensing of the concentration of the high-affinity ligand is possible even if it is outnumbered by the low-affinity ligand [29, 49]. Furthermore, under certain conditions the cell is able to determine the concentration of the non-specific ligands as well [49]. In further work, it was also shown that the accuracy can in some cases be increased by a more complicated ligand-receptor network that includes two cross-wired receptors [48]. The results of these works largely rely on two important assumptions: 1) the identity of the cognate ligand, as expressed via its binding affinity to the receptor, is known and 2) the inference is based on the whole sequence of ligand-receptor binding and unbinding times.

In a related set of problems, inspired by T cell receptor (TCR) signaling, the cell needs to efficiently filter out the weak affinity “self” ligands but sensitively respond to even small numbers of the strong affinity foreign ligands. One proposed solution, relying on the “adaptive sorting” modification of the classical kinetic proofreading (KPR) scheme, enables “absolute discrimination” between different ligands based on their affinities, unconfounded by their concentrations [18, 23]. Kinetically different but conceptually similar mechanisms are involved in the ligand discrimination by dimeric receptors [22]. However, this work was focused on the sensitive detection of the ligand presence rather than on the accurate estimation of the ligand concentrations, and did not directly consider molecular noise.

In this paper we consider a general problem of accurate and specific sensing in the presence of cross-talk in signaling pathways with multiple ligands acting through a single shared receptor. This problem is motivated by the observation that in many signaling systems cells are capable of not only responding to cognate signals on the background of spurious ones, but providing substantially different responses to multiple different ligands acting through the same pathway (specificity) while maintaining dose response sensitivity for each of them (accuracy). This scenario appears in a number of signaling pathways such as cytokine and chemokine signaling, T cell response, G-protein coupled receptor (GPCR) signaling and others [40, 42, 51, 58]. These examples raise the question of how cells are able to sense signals both specifically and accurately in the presence of cross-talk [40, 41].

We show that signaling pleiotropy - the ability of the receptor to produce several different signals in response to one stimulus - provides a solution to the accuracy-specificity problem. Pleiotropy enables both discrimination between different molecular ligands based on their affinity/binding time to the receptor, and accurate measurement of their concentrations. In this paper we define a minimal, biologically motivated model of a pleotropic receptor capable of binding a large number of ligands, and show that it can unambiguously determine both the concentration and the affinity of an arbitrary number of ligands. Furthermore, we calculate the fundamental limits on the accuracy and specificity of such sensing in the presence of noise both at the receptor level and downstream. We further show how this minimal model can be extended to more complex signaling schemes, such as kinetic proofreading (KPR), serving as a “building block” for the general problem of accurate discrimination between multiple ligands in more complex multi-ligand multi-receptor signaling networks.

The paper is structured as follows. In the next section, we define the mathematical framework and formulate the problem of the accuracy-specificity tradeoff for a non-pleiotropic receptor. In the ”Pleiotropic receptor” Section we introduce the pleotropic receptor, show that it resolves the accuracy-specificity problem, and calculate the fundamental limits on the sensing accuracy and specificity. In the ”Kinetic Proofreading” Section we investigate the effects of classical specificity enhancing schemes such as KPR on the signaling accuracy and specificity in the presence of cross-talk. We conclude with a discussion and possible generalizations in Summary and Discussion.

Results

Non-pleiotropic receptor

To investigate the signaling accuracy and specificity problem, in this section we introduce a signaling receptor capable of binding a large number of ligands, as illustrated in Figure 1. In this paper we confine ourselves to the situation where the receptor is exposed to a single ligand, out of many possible ones, at concentration c (see Discussion for generalization to ligand mixtures). The identity of the ligand is defined by its binding and unbinding rates to the receptor, $k_{on}$ and $k_{off}$, respectively. In general, these rates depend in a non-trivial fashion on the molecular details of the receptor-ligand interface [36, 40, 54] and its surroundings [15, 59, 60]. For simple monomolecular binding they combine into the equilibrium dissociation constant $K_d \equiv k_{off}/k_{on} \propto e^{-c}$ where $c$ is the ligand-receptor binding energy [61]. For simplicity, we assume that the binding rate $k_{on}$ is independent of the ligand identity, which is then fully captured by its unbinding rate $k_{off}$.

While bound by a ligand, the receptor produces a single type of downstream signaling molecule at a rate $k_p$, which serves as the readout of the ligand presence outside the cell. This signaling mechanism is common to a large number of pathways, where the active form of
the output molecule is produced via phosphorylation by a receptor-bound kinase \( [3] \). Mathematically speaking, this output variable essentially measures the bound time of the receptor \([15]\). The results are trivially extended to \( N \) independent copies of the receptor.

The state of the system at time \( t \) is described by the probability \( P^n(t) \) to be in the occupancy state \( i \) (\( i = 1 \) when the receptor is occupied by the ligand, 0 otherwise) and have produced \( n \) output molecules by time \( t \). The ensemble dynamics of the system are described by the master equation for the probability \( P^n_i(t) \) \( [62-64] \):

\[
\frac{d}{dt} P^n_0 = k_{\text{off}} P^n_1 - k_{\text{on}} c P^n_0 \\
\frac{d}{dt} P^n_i = k_{\text{on}} c P^n_0 - k_{\text{off}} P^n_i + k_p P^n_{i-1} - k_p P^n_i
\]

At steady state, the probability of the receptor being occupied is \( p = x/(1+x) \) where \( x = c/K_d \). For simplicity and connection to previous work \( [29, 52] \), we currently neglect degradation of the output molecules.

The master equation \( (1) \) can be solved using the generating function \( G_i(s, t) = \sum_n s^n P^n_i(t) \). The dynamics of the vector \( \mathbf{G} = (G_0(s, t), G_1(s, t)) \) are then described by the equation \( [62-64] \):

\[
\frac{d}{dt} \mathbf{G}(s, t) = \hat{M} \mathbf{G}(s, t)
\]

with \( \hat{M} = \begin{bmatrix} -k_{\text{on}} c & k_{\text{off}} \\ k_{\text{on}} c & -k_{\text{off}} + k_p (s - 1) \end{bmatrix} \)

yielding the general solution at time \( t \) as \( \mathbf{G}(s, t) = e^{\hat{M}t} \mathbf{G}(s, 0) \). Assuming that the receptor is at steady state at the beginning of the measurement (defined as \( n = 0 \)), the initial condition is \( \mathbf{P}^0 = (1-p, p) \) for \( n = 0 \) and \( \mathbf{P}^n = (0, 0) \) otherwise, and therefore \( \mathbf{G}(1, 0) = (1-p, p) \). Similar results can be obtained if the receptor is initially unoccupied with \( \mathbf{G}(1, 0) = (1, 0) \) producing identical results in the limit of large numbers of binding events, \( k_{\text{off}} t \gg 1 \) which is the focus of this paper (see SI for details).

The mean and the variance of \( n \) are calculated as \( \langle n \rangle = \sum_i \frac{\partial G_i(s,t)}{\partial s} |_{s=1} \) and \( \langle \delta n^2 \rangle = \sum_i \frac{\partial^2 G_i(s,t)}{\partial s^2} |_{s=1} + \langle n \rangle - \langle n \rangle^2 \). For \( k_{\text{off}} t \gg 1 \), these are

\[
\langle n \rangle = k_p t \frac{x}{1+x} \\
\langle \delta n^2 \rangle = k_p t \frac{x}{1+x} + \frac{2k_p^2 t^2}{k_{\text{off}} (1+x)^3}
\]

In the long-time limit, defined as \( \min(k_{\text{on}} k_p) \gg 1/t \), the probability distribution of \( n \), \( P(n|c, k_{\text{off}}) \), tends to a Normal distribution \( N((n), \langle \delta n^2 \rangle) \), \( P(c|n, k_{\text{off}}) = (2\pi \langle \delta n^2 \rangle)^{-1/2} \exp(-\langle n - \langle n \rangle \rangle^2/(2\langle \delta n^2 \rangle)) \) (see SI). Physically, these results reflect the fact that the receptor produces molecules at rate \( k_p \) while it is occupied (on average \( x/(1+x) \) fraction of the time \( t \)). Note that the variance of \( n \) scales as \( k_p t \), in accordance with the Central Limit Theorem.

Accuracy and specificity. The classical problem of accuracy can be stated as the estimation of the concentration \( c \) from the number of signaling molecules \( n \) when \( k_{\text{off}} \) is known - a situation realized when the receptor is highly molecularly specific and can bind only one ligand type \( [13, 15] \). We formulate the problem of specificity in a similar manner - as the estimation of \( k_{\text{off}} \) given \( n \). Note that this specificity definition is different from the common measure of specificity as the ratio of the mean number of sensing molecules produced by different ligands. However, it is impossible to unambiguously estimate both \( c \) and \( k_{\text{off}} \) from a single output variable \( n \) - illustrating the fundamental accuracy-specificity trade-off - because the same number of output molecules can be produced by a weaker ligand at higher concentration as by a stronger ligand at lower concentration. This point is illustrated in Figure 1B. Within this scheme, only the combination \( x = k_{\text{on}} c/k_{\text{off}} \) can be inferred from \( n \).

In this section, we interrogate these ideas using an approximate but analytically explicit framework: exact numerical derivations are provided in the SI. Assuming \( k_{\text{off}} \) is known, the best estimate of \( c \) given \( n \), \( c^*(n) \), is provided via maximization of the likelihood \( P(n|c) \) (or equivalently, the log-likelihood \( L = \ln P \)) over \( c \); this procedure is known as maximum likelihood estimation (MLE) \( [55, 65] \). This is equivalent to the maximization of the posterior probability \( P(c|n) = P(n|c) P(c)/ \int P(n|c) P(c) dc \) for a uniform prior \( P(c) \) \( [65] \). In the long time limit, the likelihood \( P(n|c) \) is well-approximated by a Normal distribution, peaked around \( \langle n \rangle \). Neglecting the logarithmic terms in \( L(n|c) \), the MLE \( c^* \) is given by the condition \( \langle n \rangle(c^*) = n \) \( [15, 66] \), yielding

\[
\frac{k_{\text{on}} c^*}{k_{\text{off}}} = \frac{n}{k_p t (1-n/k_p t)}
\]

Interestingly, this estimate is identical to that of Endres and Wingreen \( [55] \), based on a more informative likelihood function containing the whole series of binding and unbinding events. We return to this point in the Discussion.

For a given \( n \), expanding the likelihood about \( c^*(n) \) gives, to the lowest order, a Normal distribution whose variance \( \delta c^2 = -\left( \frac{\partial^2 \ln P(n|c)}{\partial c^2} \right)_{c^*}^{-1} \) is a measure of the uncertainty for the concentration estimate \( [65] \). Repeating this over the distribution \( P(n|c) \) of possible outcomes \( n \) gives the average uncertainty \( \langle \delta c^2 \rangle = -\langle \frac{\partial^2 \ln P(n|c)}{\partial c^2} \rangle_{c^*}^{-1} \); the quantity \( -\langle \frac{\partial^2 \ln P(n|c)}{\partial c^2} \rangle_{c^*} \) is known as the Fisher Information \( [66] \). In the saddle point approximation, accurate for sharply peaked likelihoods, this simplifies to \( \langle \delta c^2 \rangle \sim -\langle \frac{\partial^2 \ln P(n|c)}{\partial c^2} \rangle_{\text{saddle}}^{-1} \approx \langle \delta n^2 \rangle/\langle \delta (n/c) \rangle^2 \) \( [15, 65, 66] \) (see SI). This expression has a simple intuitive meaning: fluctuations in \( n \) resulting
from the stochasticity of binding-unbinding and production events lead to uncertainty in the estimate \( c^* \), as illustrated in Figure 1C. The relative average error of the concentration estimate is thus

\[
\frac{\langle \delta c^2 \rangle}{c^2} = \frac{1}{k_p t} \left( 1 + \frac{1}{x} \right) \left( 1 + x \right)^2 + 2 \frac{k_p}{k_{\text{off}}}.
\]  

(5)

For \( N \) independent copies of the receptor, the above expression is multiplied by \( N^{-1/2} \) [14]. The results are summarized in Figure 1D-E, which shows the relative error of the concentration estimate \( c \), scaled by \( k_p t \), as a function of dimensionless quantities \( k_{\text{on}} c / k_p \) and \( k_{\text{off}} / k_p \).

As expected, the accuracy of the estimate of \( c \) diverges both for \( x \to 0 \) and \( x \to \infty \) because at very low concentrations the receptor does not produce enough signaling molecules for a meaningful statistical estimate, while at very high concentrations the receptor occupancy saturates independent of either affinity or concentration. In the limit \( k_p \gg k_{\text{off}} \) the expression reduces to the classical Berg-Purcell expression \( 2(1 + x) / (k_{\text{on}} c t) = 1 / (2Da c(1 - p)t) \) where \( 4Da = k_{\text{on}} \) [13, 15], as each binding event in this regime produces multiple signaling molecules, and the sensing accuracy is limited by the fluctuations in receptor occupancy. Notably, for finite \( k_p / k_{\text{off}} \), fluctuations in the production of the output molecule \( n \) play an important role, as they cause the divergence of the estimate accuracy at high \( x \) - a feature absent from the models that consider only the noise in the receptor occupancy. In particular, the concentration at which the best estimate is obtained changes significantly with \( k_p / k_{\text{off}} \), and is not necessarily close to \( x = 1 \), the point of highest response sensitivity to concentration changes.

The problem of specificity can be similarly formulated as the problem of estimation of \( k_{\text{off}} \) from \( n \) at fixed \( c \) via maximization of the likelihood \( P(n|k_{\text{off}}) \) over \( k_{\text{off}} \). Following the same approach as in Equations 4 and 5 the best estimate and its variance are again given by

\[
\frac{k_{\text{on}} c}{k_p} = \frac{m}{k_p} - \frac{1}{n/k_p}, \quad \text{and} \quad \frac{(dh^2)}{k_{\text{off}}} = \frac{(dh^2)}{k_{\text{off}}}.
\]

However, it is impossible to estimate both \( c \) and \( k_{\text{off}} \) simultaneously because the distribution \( P(n|c, k_{\text{off}}) \) does not possess a well defined peak in the \((c, k_{\text{off}})\) space but rather a ridge along
the line \( c/K_d = \frac{n}{k_p t (1 - n/k_p t)} \), as shown in Figure 1E. This could in principle be resolved by careful selection of a prior on \((c, k_{\text{off}})\), which in practice implies additional assumptions regarding the molecular inference machinery, and lies outside the scope of the present work.

### Pleiotropic receptor

In contrast to the results of the previous section, it is possible to unambiguously estimate both \( k_{\text{off}} \) and \( c \) simultaneously if the receptor is pleiotropic (i.e. produces more than one output signal upon ligand binding). In this section, we extend the model of the previous section to include a second sensing molecule, inspired by G-protein coupled receptor (GPCR) signaling \[37, 67\], shown in Figure 2A. In this scheme, the G-protein (G)–like molecule is pre-bound to the intracellular domain of the receptor and detaches once a ligand binds. For simplicity, we assume that upon ligand unbinding the receptor quickly rebinds a new GP-like molecule. Although, as defined, this type of sensing molecule has an unnatural feature that it can be produced even for infinitesimally short binding events \[48\], the model is sufficient to demonstrate the role of pleiotropy in ligand sensing; we return to this problem in Section , where we discuss other potential pleiotropic signaling schemes. Biologically, combined kinase-phosphorylation and G-protein signaling has been reported in some cytokine receptors \[35\] and other immune receptors \[68\].

We denote the total number of GP-like molecules produced by time \( t \) as \( n \), which effectively serves as a count of the number of binding events rather than the total binding time (measured by \( n \) defined in the previous section) \[29, 39, 55\]. As shown below, this model allows joint determination of the ligand identity and its quantity \((c, k_{\text{off}})\) from the two signaling outputs \((n, m)\) (see Figure 2).

The system is now described by the probability of being in a given state at time \( t \), \( P_i^{n,m}(t) \), where \( i \) denotes the receptor state \((i = 1 \text{ if bound by a ligand, } 0 \text{ if unbound})\) and \( n \) and \( m \) are the numbers of the sensing molecules accumulated by time \( t \). Similar to the previous section, the dynamics of the system are described by the following master equation:

\[
\begin{align*}
\frac{d}{dt} P_{0}^{n,m} &= k_{\text{off}} P_{1}^{n,m} - k_{\text{on}} c P_{0}^{n,m} \\
\frac{d}{dt} P_{1}^{n,m} &= k_{\text{on}} c P_{0}^{n,m} - k_{\text{off}} P_{1}^{n,m} + k_p P_{1}^{n-1,m} - k_p P_{1}^{n,m}
\end{align*}
\]

This master equation can be solved using the generating function technique, similar to Equation 2 in the previous section (see details in the SI). The mean and the variance of \( n \) remain the same as in Equation 4. The mean of \( m \), its variance \( \langle \delta m^2 \rangle \) and the covariance \( \langle \delta n \delta m \rangle \) are, in the \( k_{\text{off}} t \gg 1 \) limit,

\[
\begin{align*}
\langle m \rangle &= k_{\text{off}} t \frac{x}{1 + x} \\
\langle \delta m^2 \rangle &= k_{\text{off}} t x \frac{1 + x^2}{(1 + x)^3} \\
\langle \delta n \delta m \rangle &= k_p t x \frac{1 - x}{(1 + x)^3}.
\end{align*}
\]

These results can also be derived using renewal process theory (see SI). Note that at small \( x \), \( n \) and \( m \) are correlated because in the low concentration/weak binding limit the overall bound time is proportional to the number of events. By contrast, at large \( x \), \( n \) and \( m \) are anti-correlated because in this regime a time series with more binding-unbinding events results in lower overall bound time. However, for \( x \to \infty \), \( \langle \delta n \delta m \rangle \to 0 \) because the receptor is occupied all the time, and the number of binding events is not correlated with the total bound time.

### Accuracy and specificity of the pleiotropic receptor

The crucial feature of Equation 7 is that the variable \( m \) depends differently on the concentration \( c \) and the unbinding rate \( k_{\text{off}} \) compared to the variable \( n \) of Equation 3, which allows the estimation of both \( c \) and \( k_{\text{off}} \). As before, we assume that \( k_p \) and \( k_{\text{on}} \) are fixed constants, hardwired into the molecular machinery of the cell. In the long time limit, the likelihood \( P(n, m | c, k_{\text{off}}) \equiv P_{0}^{n,m} + P_{1}^{n,m} \) is well approximated by a multivariate Normal distribution \( \mathcal{N}(\mu, \Sigma) \) with mean and covariance

\[
\mu = \left[ \langle n \rangle \langle m \rangle \right] \quad \text{and} \quad \Sigma = \left[ \begin{array}{cc} \langle \delta n^2 \rangle & \langle \delta n \delta m \rangle \\ \langle \delta n \delta m \rangle & \langle \delta m^2 \rangle \end{array} \right].
\]

so that

\[
P(n, m | c, k_{\text{off}}) = (Z)^{-1} \exp \left( -\frac{1}{2} (n - \mu) \Sigma^{-1} (n - \mu) \right)
\]

where \( \mu = (n, m) \) and normalization factor \( Z = (2\pi)^2 \det(\Sigma)^{1/2} \).

Estimates for \( c \) and \( k_{\text{off}} \) can be found in the same manner as in the previous section, by maximizing the likelihood \( P(n, m | c, k_{\text{off}}) \) over \( c \) and \( k_{\text{off}} \), which yields:

\[
c^* = \frac{k_{\text{off}} n / (k_p t)}{k_{\text{on}} 1 - n / k_p t}, \quad \text{and} \quad k_{\text{off}}^* = k_p \frac{m}{n}.
\]

Interestingly, the same estimates are obtained using a more detailed likelihood of binding and unbinding times introduced in \[55\]. In a generalization of the one-variable procedure from the "Non-pleiotropic receptor" Section, the estimate errors are given by the width of the posterior/likelihood in the \((c, k_{\text{off}})\) space, quantified by the covariance matrix

\[
\Sigma = \left[ \begin{array}{cc} \langle \delta c^2 \rangle & \langle \delta k_{\text{off}} \delta c \rangle \\ \langle \delta k_{\text{off}} \delta c \rangle & \langle \delta k_{\text{off}}^2 \rangle \end{array} \right].
\]
Fig. 2. Accuracy and specificity of pleiotropic signaling. A. The pleiotropic receptor considered here has the same behaviour as in Figure 1 except that an additional output molecule, denoted as m, is produced for each binding event. B. The scaled relative errors of the estimates for \(c\) (blue) and \(k_{off}\) (red) as a function of \(k_{on}/k_p\), holding \(k_{off}/k_p = 1\); see Equation 10. C and D. Scaled relative estimate errors \(k_p t \langle \delta c^2 \rangle / c^2\) and \(k_p t \langle \delta k_{off}^2 \rangle / k_{off}^2\).

The lower bound on the covariance matrix is given by the inverse of the Fisher Information Matrix (FIM) \(\hat{\mathcal{I}}\) [66]:

\[
\hat{\mathcal{I}}(c, k_{off}) \equiv - \begin{bmatrix}
\langle \frac{\partial^2 \mathcal{L}}{\partial c^2} \rangle & \langle \frac{\partial^2 \mathcal{L}}{\partial c \partial k_{off}} \rangle \\
\langle \frac{\partial^2 \mathcal{L}}{\partial k_{off} \partial c} \rangle & \langle \frac{\partial^2 \mathcal{L}}{\partial k_{off}^2} \rangle
\end{bmatrix}.
\]

In the long time limit, where the likelihood is sharply peaked, the approximate relative errors of the estimates are

\[
\frac{\langle \delta c^2 \rangle}{c^2} = \frac{1 + x}{k_p t} \frac{x^2 + k_p}{k_{off}}
\]

\[
\frac{\langle \delta k_{off}^2 \rangle}{k_{off}^2} = \frac{1 + x}{k_p t} \frac{x^2 + k_p}{k_{off}}.
\]

These relative errors are plotted in Figure 2. In plain language, the cell is not capable of distinguishing ligands with affinities closer than \(\langle \delta k_{off}^2 \rangle\). An important consequence of this analysis is that signaling specificity is not determined solely by the differences between ligand affinities but also depends on the ligand concentrations, and is thus context-dependent. The relative error of the concentration estimate behaves qualitatively similar to that of the non-pleiotropic receptor, diverging in both the low and high x limits. On the other hand, the error in \(k_{off}\) inference remains low even in the high-occupancy (high x) regime because the knowledge of both \(n\) and \(m\) allows accurate determination of the average bound time [50][55].

The ratio of the concentration sensing error of the pleiotropic receptor (Equation 10) to that of the non-pleiotropic receptor (Equation 5) is \((x^2 + k_p/k_{off})/((1 + x)^2 + 2k_p/k_{off})\). Since this quantity ranges between \((k_p/k_{off})/(1 + 2k_p/k_{off})\) at low x and 1 at high x, pleiotropy always improves the concentration sensing accuracy. Likewise, the corresponding ratio for \(k_{off}\) is always less than one for \(k_p/k_{off} > 1\), indicating that pleiotropy not only enables simultaneous estimation of the concentration and the affinity but generally also increases the specificity of signalling as well.

It is instructive to estimate what these results predict with respect to the sensing accuracy and specificity in a biological context. The values of the binding affinities and physiological concentrations can vary considerably between different molecules, cell types and physiological settings. For a rough estimate, we assume as a rule of thumb that the physiological concentration of a signaling molecule is close to the receptor \(K_d\), so that \(x \approx 1\) ensuring the maximal responsiveness. For \(K_d\) in the range \(0.1 - 10 nM\), and the typical \(k_p = sec^{-1}\) and \(k_{on} \approx 10^6 M^{-1} sec^{-1}\), the time \(t\) must be on the order of \(100-1000 sec\) in order to achieve accuracy and specificity of the order \(\frac{\langle \delta c^2 \rangle}{c^2} \approx \frac{\langle \delta k_{off}^2 \rangle}{k_{off}^2} \approx 1\). This required sampling...
time is comparable to the typical measurement window in cellular signaling before the signaling is shut down by various negative feedbacks on the timescales that vary from minutes to hours.

**Kinetic proofreading**

To further understand the accuracy and the specificity of pleiotropic signaling, we examine an extension of the receptor model of the previous section to now incorporate the kinetic proofreading (KPR) - a mechanism commonly considered to improve specificity. KPR enhances the difference in affinities between different ligands by introducing additional “proofreading” steps between the ligand binding and the production of the sensing molecule (at the expense of slower sensing times) \[16,23,25,30,69,70\]. The minimal KPR scheme considered here includes a single intermediate bound state of the receptor and is depicted in Figure 3. Once the receptor reaches the final state, an m-type molecule is released, and n-type molecules are produced at rate \(k_p\) while the receptor stays in the final state. This kinetic scheme also mitigates the unnatural features of the m-type variable by introducing a delay in the formation of the final bound state so that very short binding events are unlikely to produce any signal. Other similar kinetic schemes are possible, for instance when another signal is produced while the receptor is in the intermediate state \[18,23\], as shown in the SI, with similar results.

The corresponding master equation for this KPR scheme is

\[
\begin{align*}
\frac{d}{dt}P_0^n &= k_{\text{off}} P_1^{n,m} + k_{\text{off}} P_2^{n,m} - k_{\text{on}} c P_0^{n,m} \\
\frac{d}{dt}P_1^n &= k_{\text{on}} c P_0^{n,m} - k_{\text{off}} P_1^{n,m} - k_f P_1^{n,m} \\
\frac{d}{dt}P_2^n &= k_f P_1^{n,m-1} - k_{\text{off}} P_2^{n,m} + k_p P_2^{n-1,m} - k_p P_2^{n,m}.
\end{align*}
\]

(11)

where \(P_i^{n,m}(t)\) is the probability for the receptor to be in state \(i\) (\(i = 0\) unbound, \(i = 1\) intermediate and \(i = 2\) the final state of the pathway) and have accumulated \((n,m)\) molecules. Once again using the generating function method, we find

\[
\begin{align*}
\langle n \rangle &= k_p t \frac{k_f}{k_f + k_{\text{off}}} \frac{x}{1 + x} \equiv \frac{x}{1 + g} \left( 1 + \frac{k_p t}{k_{\text{off}}} x \right) \\
\langle m \rangle &= k_{\text{off}} t \frac{k_f}{k_f + k_{\text{off}}} \frac{x}{1 + x} \equiv \frac{x}{1 + g} \left( 1 + \frac{k_{\text{off}} t}{k_f} x \right).
\end{align*}
\]

(12)

The average numbers of sensing molecules differ from the non-proofread case by the factor \(1/(1 + g)\), where \(g \equiv k_{\text{off}}/k_f\). In the limit \(g \ll 1\) these expressions reduce to the non-KPR expressions of Equations \[3\] and \(7\) as expected, because the ligand rarely unbinds before the receptor reaches the final state, where it stays for most of the time. In the other limit, \(g \gg 1\), these expressions reflect the enhancement in signal discrimination provided by KPR, as can be seen by considering the ratios \(\langle n \rangle/\langle n \rangle'\) and \(\langle m \rangle/\langle m \rangle'\) for two ligands with different affinities \(k_{\text{off}}\) and \(k_{\text{off}}'\) (at fixed concentration). With KPR, these ratios tend (at low x) to \((k_{\text{off}}'/k_{\text{off}})\) and \((k_{\text{off}}'/k_{\text{off}})\), respectively; both are enhanced by an additional Boltzmann factor \(k_{\text{off}}/k_{\text{off}}\) compared to the non-proofread case.

In the strong proofreading regime, \(g \gg 1\), the fluctuations of the variables \(n\) and \(m\) are (in the long time limit):

\[
\begin{align*}
\langle \delta n^2 \rangle &= \frac{k_p t}{k_{\text{off}}} x \left( \frac{k_p}{k_{\text{off}}} + 1 \right) \\
\langle \delta m^2 \rangle &= \frac{k_{\text{off}} t}{g} x \left( \frac{1}{1 + x} \right) \\
\langle \delta n \delta m \rangle &= \frac{k_p t}{g} x \left( \frac{1}{1 + x} \right).
\end{align*}
\]

(13)

**Accuracy and specificity with KPR.** The errors of the estimates of \(c\) and \(k_{\text{off}}\) are calculated in the same fashion as in the previous section. The results are summarized in Figure 3(C-D). The full expressions are cumbersome but for \(g \gg 1\), they simplify to (see SI for details):

\[
\begin{align*}
\langle \delta c^2 \rangle &= \frac{g}{c^2} \left( 1 + \frac{k_p}{k_{\text{off}}} \left( 2 + 2x + x^2 \right) \right) \\
\langle \delta k^2_{\text{off}} \rangle &= \frac{g}{k_p t} \left( 1 + \frac{k_p}{k_{\text{off}}} \right).
\end{align*}
\]

(14)

By comparing Equations \[14\] and \[10\] it is apparent that KPR worsens both the accuracy and specificity of signaling in the strong proofreading regime, as the relative errors are increased roughly by the factor \(g\). This feature is illustrated in Figure 3B, which shows the ratio of the determinants of the covariance matrices in the proofread and un-proofread case, as a global comparison of the covariance matrices in the two cases. Although surprising at first glance, the reason for this was already noted by McKeithan \[25\], and can be traced to the expressions for the means and the variances of the downstream signaling molecules in Equation \[13\]. Although KPR increases the ratio of the average numbers of molecules produced by two different ligands by a factor \(g\) compared to the non-proofread case, it does so at the expense of a decreased number of sensing molecules, also by a factor of \(g\). However, the standard deviation of the number of signaling molecules decreases only as \(\sqrt{g}\) compared to the non-proofread case. Therefore if the level of the non-proofread signal is \(\langle n \rangle = \delta n\), the level of the proofread signal is roughly \(\langle n \rangle + \frac{\delta n}{\sqrt{g}}\). This means that probability distributions for the number of sensing molecules, which may be well separated in the non-proofread receptor model (such as in Figure 3C), can start to overlap in the KPR scheme, impairing signal discrimination. This
Fig. 3. Specificity and accuracy of kinetic proofreading receptor. A. Schematic illustration of receptor signaling with kinetic proofreading. Upon ligand binding, the receptor transitions to the final ligand bound conformation with a rate $k_f$. Ligand unbinding can occur at either state with the rate $k_{off}$. The receptor produces both n-like and m-like signals in the final state, similar to the non-proofread receptor in Figure 2. B. The ratio of the determinant of the error covariance matrix $\Sigma$ for KPR over that of the non-proofread pleiotropic receptor for varying $k_f/k_p$ and $k_{off}/k_p$ for $k_{on}/c = 10^{-1}$; details in SI. C and D. Scaled relative estimate errors $k_f(\delta c^2)/c^2$ (C) and $k_f(\delta k_{off}^2)/k_{off}^2$ (D).

problem can potentially be rectified by stabilizing the receptor in the final state of the KPR cascade or by adaptive feedback schemes and will be analyzed elsewhere.

Summary and Discussion

Cross-talk is common in many signaling pathways, which raises the question of how cells are able to sense and thereby respond appropriately to molecularly similar signals carrying different information through these cross-wired pathways. In this paper we focused on the cross-talk at the ligand-receptor level, whereby multiple ligands can act through the same surface receptor. This situation is commonly encountered in cytokine and chemokine signaling in the immune system, developmental pathways, and other physiological and ecological systems. Ligand-receptor cross-talk entails a fundamental problem: it is impossible to discriminate between different cognate ligands based on receptor occupancy alone because the identity of the ligand (“quality”) can be confounded by its concentration (“quantity”).

In this paper we have investigated one potential solution to this problem - signaling pleiotropy - which commonly accompanies cross-talk, using biologically motivated models of receptor kinetics that account for the molecular noise at both the receptor and the downstream variables. We showed that the classical model of a receptor which binds multiple ligands but produces only one type of downstream sensing molecule is not able to simultaneously discern the ligand identity (as defined by its unbinding rate $k_{off}$), and its concentration $c$. In contrast, a pleiotropic receptor, which produces two types of downstream signaling molecules, can resolve this ambiguity. The crucial feature of the model is that the two output signaling molecules reflect physically different features of the ligand-receptor interaction - in the case studied here, one variable is proportional to the bound time of the ligand, while the other reflects the number of distinct ligand-receptor binding events. Importantly, the inference is based only on the total numbers of the produced signaling molecules and not on the whole sequence of binding-unbinding events.

In addition to providing a solution to the accuracy-specificity dilemma, our model demonstrates that the noise in the production of the downstream sensing molecules (on top of the receptor binding-unbinding fluctuations) can significantly affect the accuracy and the specificity of molecular sensing. In particular, downstream noise relieves a feature of models which only account for the randomness of the receptor binding-unbinding events that predict that the highest relative accuracy is achieved at high concentrations.
Our model is constrained by the assumptions about the nature of the output variables $n$ and $m$, which are inspired by the observed modes of signaling in cytokine and GPCR pathways. It is instructive to compare our results with those based on inference from the whole sequence of binding-unbinding events (which requires more intricate intracellular molecular networks) \[29,49,50,55\]. The likelihood of a sequence of binding/unbinding events with overall bound time $t_b$ and the overall number of binding events $m$ is given by $P(t_b,m|c,k_{off}) \sim \exp(-k_{on}c) \exp(t_b(-k_{off} + k_{on}c)) \cdot (k_{off}k_{on}c)^m$ \[55\]. Maximizing this likelihood over $(c,k_{off})$ results in the same estimates as given by Equation \[9\]. The corresponding lower bounds on the estimation errors (found by inverting the Fisher Information Matrix) in this case are $\langle \delta c^2 \rangle/c^2 = \langle \delta k_{off}^2 \rangle/k_{off}^2 = 1/\langle m \rangle$, where $\langle m \rangle = k_{off}x/(1+x)$. These expressions match Equation \[10\] in the limit $k_{on}/k_{off} \gg 1$ (at finite $x$); the deviation at finite $k_{on}/k_{off}$ is a consequence of the additional noise in the production of the signaling molecules on top of the noise of receptor-ligand binding. Notably, pleiotropy not only resolves the cross-talk problem, allowing simultaneous estimation of both the ligand concentration and identity, but also reduces estimation error of the ligand concentration, as shown in Equation \[5\] and Equation \[10\]. This is consistent with \[55\] as well as recent work suggesting that pleiotropic cross-talk can improve the accuracy of sensing in some regimes \[18\].

We also investigated the effects of pleiotropy and noise on the accuracy and specificity of a common specificity enhancing scheme - kinetic proofreading. Our results further emphasize the importance of considering not only the receptor binding noise, but also the molecular noise downstream: when the latter is taken into account, KPR does not necessarily improve signaling specificity. The reason for this is that in the strong proofreading regime, the fluctuations in the numbers of the output molecules grow faster with the proofreading factor $g$ than the corresponding means. As a result, ligands with different affinities can generate distributions that are well separated without proofreading, but overlap when proofreading is used. This has important implications for a number of suggested accuracy and specificity schemes that often rely on KPR type approaches. A number of approaches to this problem are possible and will be studied elsewhere.

The results of this paper also provide an interesting outlook on the information theory approaches to cell signaling \[28,41,75,78\] via connections between the channel capacity of a signaling pathway and the Fisher Information Matrix. These lie outside of the scope of the present work, and will be studied in the future.

Finally, in this paper we have considered only a minimal “module” of a cross-wired receptor signaling network - a receptor of one type capable of interacting with multiple types of ligands. Furthermore, we have assumed that the cell is only exposed to one type of ligand at a time. In reality, cells possess multiple receptor types and encounter combinations of various ligands in the presence of cross-talk. The ideas of this paper can be extended to the more general case of complex networks of ligands, receptors and downstream signaling molecules that may include positive and negative feedbacks mediated by such molecules \[18,30\]. The results of this paper provide a novel outlook on signaling in a number of experimental systems and can be directly tested using quantitative phospho flow cytometry and mass cytometry.

These results indicate that signaling cross-talk might not be an undesirable “noise” hampering accurate and specific ligand discrimination. Rather, combined with signaling pleiotropy, it might provide a general mechanism for accurate and specific combinatorial sensing in cross-wired networks, as long as the ligand-receptor system is capable of producing at least as many output variables as the number of ligands and their concentrations. In addition to different types of molecules produced by different receptor states in more complex signaling cascades, such additional outputs can include the time course of the signaling output \[41,79\] facilitated through feedback such as receptor internalization. Furthermore, while this paper has focused on sensing capabilities of single cells, inter-cellular interactions in multicellular environments can result in collective responses to the signaling milieu leading to collective decisions at the population level \[21,80,81\].

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