Juxtacrine signaling is inherently noisy

Supplementary information

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Supplementary Figures

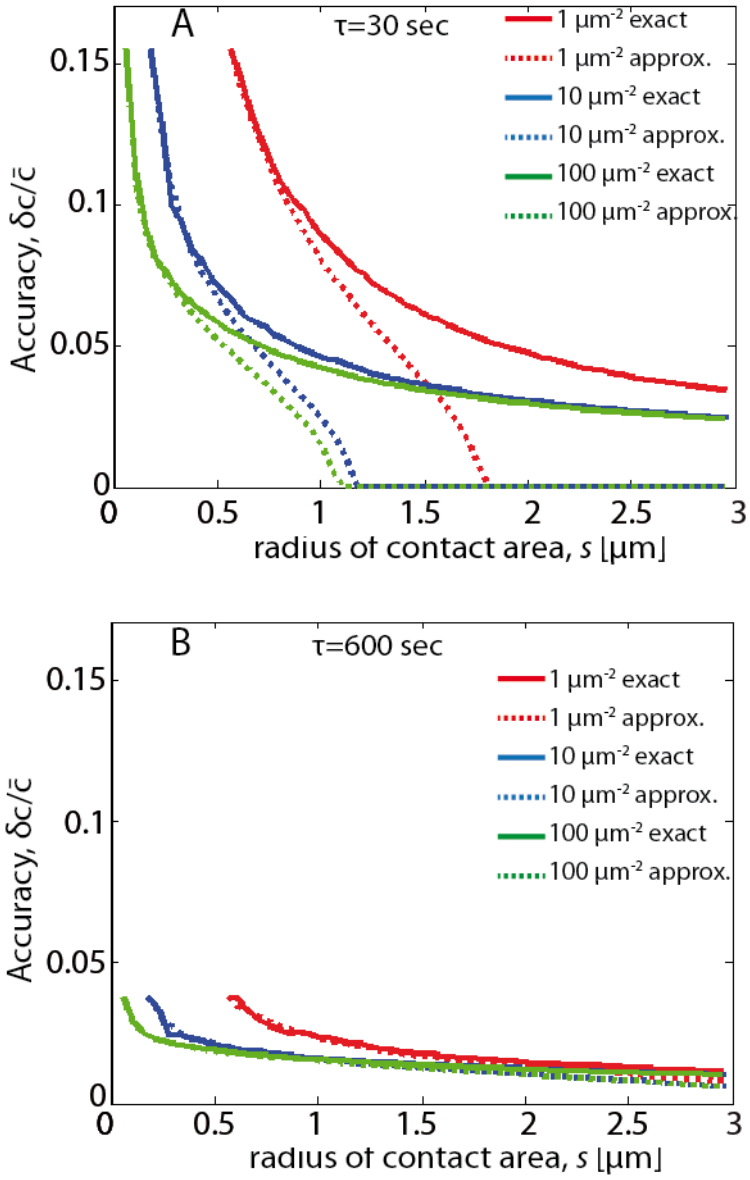

Figure S1: Comparison of exact and approximate solutions for the accuracy of measurement. (A,B) Dependence of measurement accuracy of ligand concentration, $\frac{\delta c}{\bar{c}}$, on the radius of the contact area, s, for relatively short integration time (A, τ=30 sec) and for relatively long integration time (B, τ=600 sec) calculated either with *Eq.* 12 (solid lines) or *Eq.* 13 (dashed lines). The plot shows that the approximate solution works well for long integration times (B) but breaks down for short integration times and large contact radii (A). This is since the condition $s < \lambda, \lambda^*$ no longer holds in this parameter regime. For these plots the same parameters as Fig. 2C,D were used.

Supplementary methods

The purpose of this supplementary information is to describe in detail, the calculation of the accuracy of measuring ligand concentration by one receptor and its extension to several receptors. The supplementary information is divided into four sections. In the first section we calculate the accuracy of ligand concentration measurement by one receptor acting as a perfect instrument ('perfect monitoring disk' approximation), assuming fluctuations induced by the diffusion of ligands. In the second section we extend the calculation of accuracy of ligand concentration to the case of several receptors. In the third section we present a detailed computation of the accuracy of ligand sensing by a perfect absorbing receptor. In the fourth section we present an alternative computation of the accuracy in measuring ligand concentration based on the fluctuation dissipation theorem (FDT) (1,2) and assuming intrinsic noise in the receptor-ligand system.

Accuracy of ligand concentration measurement by one receptor

The ligand concentration on the membrane of the ligand cell is described by $c(\boldsymbol{x},t)$. We assume that ligands are continuously recycled in and out of the membrane (e.g. through endocytosis and exocytosis with rates k_{endo} and k_{exo}, respectively. The dynamics of receptor-ligand interactions is dictated by

$$\frac{\partial c(\boldsymbol{x},t)}{\partial t} + \nabla \cdot j = -k_{endo} c(\boldsymbol{x},t) + k_{exo} c_{cyto} \text{ , (S1)}$$

where j is the ligands diffusion current on the cell membrane and to first order can be written as $j = -D_2 \nabla c$.

D_2 is the ligand diffusion coefficient. External noise is included by adding a current $j_d = -\gamma c \nabla \mu$, where γ is the ligand mobility and μ is the chemical potential. In thermodynamic equilibrium, the ligand concentration satisfies the Boltzman relation $\mu = k_B T ln c$, where k_B is the Boltzman constant and T represents the temperature. Therefore $\nabla \cdot (\beta D_2 c \nabla \mu + \gamma c \nabla \mu)$=0, where $\beta = \frac{1}{KT}$, leads to $\beta D_2 = \gamma$ (3).

Hence, in the presence of an external noise, Eq. S1 is replaced by

$$\frac{\partial c(\boldsymbol{x},t)}{\partial t} - D_2 \nabla^2 c = \beta D_2 \nabla (c \nabla \mu) - k_{endo} c(\boldsymbol{x},t) + k_{exo} c_{cyto} \text{ . (S2)}$$

Next we introduce a random noise in the chemical potential μ that satisfies

$$\langle \delta\mu(t,\boldsymbol{k}) \delta\mu(t',-\boldsymbol{k}) \rangle = A(k)\delta(t-t'), \text{ (S3)}$$

where $k = |\boldsymbol{k}|$. The notation $\langle \dots \rangle$ represents an ensemble average. The fluctuation in the chemical potential is defined by $\delta\mu = \mu - \bar{\mu}$, where $\bar{\mu}$ is the chemical

potential average value, and $\mu(t, \boldsymbol{k})$ is the spatial Fourier transform of the chemical potential. We define the Fourier transform in spatial and temporal variables of a function $f(\boldsymbol{x}, t)$ according to the following standard definition

$f(\omega, \boldsymbol{k})$=$\int dt \int d^2x\, e^{i(-\boldsymbol{k}\cdot\boldsymbol{x}+\omega t)} f(\boldsymbol{x}, t)$. (S4)

Similarly we define the fluctuations in the ligand concentration by δc^*, $c = \bar{c} + \delta c^*$, where $\bar{c}$ represents the ligand average concentration. Eq. S2 may be rewritten in terms of the fluctuations δc^* and $\delta\mu$ as

$\frac{\partial \delta c^*(\boldsymbol{x},t)}{\partial t} - D_2\nabla^2\delta c^* = \beta D_2 \bar{c}\, \nabla^2(\delta\mu) - k_{endo}\delta c^*(\boldsymbol{x}, t)$. (S5)

The Fourier transform of Eq. S5 in spatial and temporal variables leads to

$\delta c^*(\omega, \boldsymbol{k}) = \frac{-\beta \bar{c} D_2 k^2}{-i\omega + D_2 k^2 + k_{endo}} \delta\mu(\omega, \boldsymbol{k})$. (S6)

The next step is to compute the power spectrum of the fluctuation δc^*: $S_c(\omega, \boldsymbol{k}) = \langle \delta c^*(w, \boldsymbol{k}) \delta c^*(-\omega, -\boldsymbol{k}) \rangle$ (4). We calculate the power spectrum $S_c(\omega, \boldsymbol{k})$ using Eq. S3 and Eq. S6 obtaining

$S_c(\omega, \boldsymbol{k}) = \mathrm{A}\beta^2 \bar{c}^2 \frac{(D_2 k^2)^2}{\omega^2 + (D_2 k^2 + k_{endo})^2}$. (S7)

In the case of a solution at low concentration (ideal gas approximation) it is known that $\mu = \beta^{-1} ln\, c$, then applying the equipartition theorem (5) to the variable c and its conjugate $\mu = \frac{\partial E}{\partial c}$,we get $\langle \delta c^*(t) \delta\mu(t) \rangle = \beta^{-1} = \beta^{-1}\bar{c}^{-1}\langle \delta c^*(t)^2 \rangle$. Therefore (4)

$\langle \delta c^*(t)^2 \rangle$ =$\bar{c}$. (S8)

It is well known that the power spectrum satisfies (2)

$\langle \delta c^*(t)^2 \rangle = \int \frac{d\omega}{2\pi} S_c(\omega, \boldsymbol{k})$. (S9)

Substituting Eq. S7, into Eq. S9 we find

$\langle \delta c^*(t)^2 \rangle = \frac{A\beta^2 \bar{c}^2 (D_2 k^2)^2}{2(D_2 k^2 + k_{endo})}$.(S10)

Replacing Eq. S8 into Eq. S10 we obtain

$A = \frac{2(D_2 k^2 + k_{endo})}{\bar{c}\beta^2\, (D_2 k^2)^2}$, (S11)

and hence the power spectrum $S_c(\omega, \boldsymbol{k})$ becomes

$S_c(\omega, \boldsymbol{k}) = \frac{2\bar{c}(D_2 k^2 + k_{endo})}{\omega^2 + (D_2 k^2 + k_{endo})^2}$. (S12)

This expression is a generalization of the correlation function of the fluctuations of the number of solute particles in a weak solution where particles are continuously recycled (6). Next, we consider a receptor that can measure ligand concentration in a radius roughly equivalent to its size, a, over an integration time τ. The average ligand concentration measured by a receptor will be given by

$$\tilde{c}(t,x) = \int d^2x'dt'\, w_r(\boldsymbol{x}-\boldsymbol{x}')k_r(t-t')\, c(t',\boldsymbol{x}'). \text{ (S13)}$$

where the function $w_r(\boldsymbol{x}-\boldsymbol{x}')$ defines the receptor spatial distribution and $k_r(t-t')$ describes the receptor temporal response. We take the limit of a 'perfect' receptor which can count all the ligands that arrive at its close vicinity.

We choose Gaussian distributions $w_r(\boldsymbol{x}-\boldsymbol{x}') = \frac{e^{-(x-x')^2/(2a^2)}}{2\pi a^2}$ and

$k_r(t-t') = \frac{e^{-(t-t')^2/(2\tau^2)}}{\sqrt{2\pi}\tau}$.

Both Gaussian distributions are normalized: $\int d^2\, x\, w_r(\boldsymbol{x}-\boldsymbol{x}') = 1$ and $\int dt\ k_r(t-t') = 1$. As a matter of comparison, note that a slightly different kernel function $w_j(t)$ was defined by Berg and Purcell (7), being equal to 1 if the ligand molecule is at time t inside a small sphere representing a receptor and 0 otherwise.

Using Eq. S13, and writing $\delta c^*(t',\boldsymbol{x}')$ in terms of its Fourier transform, we find that $\langle\delta\tilde{c}(t,\boldsymbol{x})^2\rangle$ is given by

$$\langle\delta\tilde{c}(t,\boldsymbol{x})\delta\tilde{c}(t,\boldsymbol{x})\rangle = \int d^2x'dt'd\omega\,\frac{d^2k}{(2\pi)^3}\,d^2x''dt''\,d\omega'\,\frac{d^2k'}{(2\pi)^3}$$

$$w_r\left(\boldsymbol{x}-\boldsymbol{x}'\right)k_r\left(t-t'\right)e^{i\left(\boldsymbol{k}\cdot\boldsymbol{x}'-\omega t'\right)}w_r\left(\boldsymbol{x}-\boldsymbol{x}''\right)k_r\left(t-t''\right)e^{i\left(\boldsymbol{k}'\cdot\boldsymbol{x}''-\omega' t''\right)}\langle\delta c^*(\omega,\boldsymbol{k})\delta c^*\left(\omega',\boldsymbol{k}'\right)\rangle$$

$$= \int d^2x'dt'd\omega\,\frac{d^2k}{(2\pi)^3}\,\mathrm{d}^2x''dt''$$

$$d\omega'\,\frac{d^2k'}{(2\pi)^3}\,\frac{e^{-(t-t')^2/(2\tau^2)}}{\sqrt{2\pi}\tau}\,\frac{e^{-(x-x')^2/(2a^2)}}{2\pi a^2}\,e^{i(\boldsymbol{k}\cdot\boldsymbol{x}'-\omega t')}\,\frac{e^{-(t-t'')^2/(2\tau^2)}}{\sqrt{2\pi}\tau}\,\frac{e^{-(x-x'')^2/(2a^2)}}{2\pi a^2}\,e^{i(\boldsymbol{k}'\cdot\boldsymbol{x}''-\omega' t'')}$$

$\langle\delta c^*(\omega,\boldsymbol{k})\delta c^*(\omega',\boldsymbol{k}')\rangle$. (S14)

It is easy to see that

$$\int dt'\,\frac{e^{-(t-t')^2/(2\tau^2)}}{\sqrt{2\pi}\tau}\,e^{-i\omega t'} = e^{-i\omega t}e^{-\tau^2\omega^2/2}\text{ , (S15)}$$

and

$$\int d^2x'\,\frac{e^{-(x-x')^2/(2a^2)}}{2\pi a^2}\,e^{i\boldsymbol{k}\cdot\boldsymbol{x}'} = e^{i\boldsymbol{k}\cdot\boldsymbol{x}}e^{-a^2k^2/2}\text{. (S16)}$$

Then taking into account that $(\langle \delta c^*(\omega, \boldsymbol{k}) \delta c^*(\omega', \boldsymbol{k}') \rangle = (2\pi)^3 \delta(\omega + \omega') \delta(\boldsymbol{k} + \boldsymbol{k}') S_{c^*}(\omega, \boldsymbol{k}))$ (4), we obtain

$$\langle \delta \tilde{c}^2 \rangle = \int \frac{d\omega d^2k}{(2\pi)^3} \frac{2\bar{c}(D_2 k^2 + k_{endo})}{\omega^2 + (D_2 k^2 + k_{endo})^2} e^{-\tau^2 \omega^2} e^{-a^2 k^2} \quad \text{(S17)}$$

where $\langle \delta \tilde{c}^2 \rangle = \langle \delta \tilde{c}(t, \boldsymbol{x}) \delta \tilde{c}(t, \boldsymbol{x}) \rangle$.

Due to the radial symmetry of the function appearing in Eq. S17 it is natural to introduce polar coordinates $(k = |\boldsymbol{k}|, \theta)$. After performing the integration in the azimuthal angle, Eq. S17 can be rewritten as

$$\langle \delta \tilde{c}^2 \rangle = \int_{-\infty}^{\infty} d\omega \, e^{-\tau^2 \omega^2} \int_0^{\infty} \frac{kdk}{(2\pi)^2} e^{-a^2 k^2} \frac{2\bar{c}(D_2 k^2 + k_{endo})}{\omega^2 + (D_2 k^2 + k_{endo})^2}. \text{(S18)}$$

In order to compute Eq. S18 we introduce the following dimensionless parameters

$$\alpha = \frac{a^2}{D_2 \tau}, \; v = ka, u = \omega\tau.$$

Then $\langle \delta \tilde{c}(t, x)^2 \rangle$ becomes

$$\langle \delta \tilde{c}^2 \rangle = \frac{\bar{c}\alpha}{\pi^2 a^2} \int_0^{\infty} du \, e^{-u^2} \int_0^{\infty} dv \, e^{-v^2} \frac{v(v^2 + \alpha k_{endo} \tau)}{\alpha^2 u^2 + (v^2 + \alpha k_{endo} \tau)^2} =$$

$$\frac{\bar{c}\alpha}{\pi^2 a^2} \int_0^{\infty} du \, e^{-u^2} \int_0^{\infty} dv \, e^{-v^2} \frac{v\left(v^2 + \left(\frac{a}{\lambda}\right)^2\right)}{\alpha^2 u^2 + (v^2 + \left(\frac{a}{\lambda}\right)^2)^2}, \text{(S19)}$$

where we define the diffusive length scale $\lambda = \sqrt{\frac{D_2}{k_{endo}}}$.

The parameter *a* for typical receptors is about 1-10 *nm*, D_2 is in the range 0.01-0.1 μm^2/sec (8,9), and k_{endo} varies in the range 0.001-0.01 1/sec (10-13).

Hence we have $\left(\frac{a}{\lambda}\right)^2 \sim 10^{-8} - 10^{-5} \ll 1$. The parameter α^2 for large integration times is expected to be in the range $[10^{-16}, 10^{-10}]$.

The u –integrand, due to the term e^{-u^2}, tends to zero for $u \sim 2$. Then, introducing a sharp cut-off $u_{max} = \pi$, or $(\omega_{max} = \frac{\pi}{\tau})$, we may approximate the integral from 0 to ∞ of the Gaussian by an integral with a sharp cutoff and the Gaussian substituted by 1. Hence we proceed by evaluating the *u* integral,

$$\langle \delta \tilde{c}^2 \rangle = \frac{\bar{c}\alpha}{\pi^2 a^2} \int_0^{\pi} du \int_0^{\infty} dv \, e^{-v^2} \frac{v\left(v^2 + \left(\frac{a}{\lambda}\right)^2\right)}{\alpha^2 u^2 + (v^2 + \left(\frac{a}{\lambda}\right)^2)^2} = \frac{\bar{c}\alpha}{\pi^2 a^2} \int_0^{\infty} dv \, e^{-v^2} v \left(v^2 + \left(\frac{a}{\lambda}\right)^2\right) \frac{\arctan\left(\frac{\alpha\pi}{v^2 + \left(\frac{a}{\lambda}\right)^2}\right)}{\alpha(v^2 + \left(\frac{a}{\lambda}\right)^2)} = \frac{\bar{c}}{\pi^2 a^2} \int_0^{\infty} dv \, e^{-v^2} v \arctan\left(\frac{\alpha\pi}{v^2 + \left(\frac{a}{\lambda}\right)^2}\right). \text{(S20)}$$

The v −integrand of Eq. S20, drops to almost 0 for $v\sim 2$, due to the exponential function e^{-v^2} and that the function $\frac{v\left(v^2+\left(\frac{a}{\lambda}\right)^2\right)}{\alpha^2u^2+(v^2+\left(\frac{a}{\lambda}\right)^2)^2}$ is bounded. Therefore most of the contribution to the integral (Eq. S20) comes from values of $v<2$. We simplify the calculation by approximating the v-integral appearing in Eq. S20 by a new integral with a sharp cut-off $v_{max}=1$ (i.e. $k_{max}=\frac{1}{a}$) where the function e^{-v^2} is replaced by 1 (2,14) (see below, Eq. S21).

Defining $\delta c=\sqrt{\langle\delta\tilde{c}^2\rangle}$, and recalling that the argument of the $arctan$ is small for large enough integration times ($\alpha\ll\left(\frac{a}{\lambda}\right)^2$, or equivalently $\tau\gg\frac{1}{k_{endo}}$), we finally obtain

$$\left(\frac{\delta c}{\bar{c}}\right)^2=\frac{\alpha}{\pi^2a^2}\int_0^1 dv\ \frac{\pi v}{v^2+\left(\frac{a}{\lambda}\right)^2}=\frac{1}{\pi D_2\tau\bar{c}}\ln\left(\frac{\lambda}{a}\right),\ \text{(S21)}$$

where we have assumed that $\frac{\lambda}{a}\gg 1$.

For the case of short integration times ($\tau\ll\frac{1}{k_{endo}}$) we proceed by first evaluating the v −integral (here we also assumed a sharp cutoff to take care of the integrands containing the Gaussians)

$$\langle\delta\tilde{c}^2\rangle=\frac{\bar{c}\alpha}{\pi^2a^2}\int_0^\pi du\int_0^1 dv\ \frac{v\left(v^2+\left(\frac{a}{\lambda}\right)^2\right)}{\alpha^2u^2+(v^2+\left(\frac{a}{\lambda}\right)^2)^2}.\ \text{(S22)}$$

The v integral can be evaluated analytically using the expression $\int dv\frac{v(v^2+l)}{b^2+(v^2+a)^2}=\frac{1}{4}\ln[b^2+(v^2+l)^2]$. Hence Eq.S22 is equivalent to

$$\langle\delta\tilde{c}^2\rangle=\frac{\bar{c}\alpha}{\pi^2a^2}\int_0^\pi du\frac{1}{4}\ln(1+\frac{(2\left(\frac{a}{\lambda}\right)^2+1)}{\left(\frac{a}{\lambda}\right)^4+\alpha^2u^2}).\ \text{(S23)}$$

This integral can be computed analytically using

$$\int dx ln(1+\frac{a}{b+cx^2})=xln\left(\frac{a}{b+cx^2}+1\right)+\frac{2\sqrt{a+b}\,tan^{-1}(\frac{\sqrt{c}\,x}{\sqrt{a+b}})}{\sqrt{c}}-2\sqrt{\frac{b}{c}}tan^{-1}(\sqrt{\frac{c}{b}}x),\quad\text{then}$$

we obtain

$$\langle \delta\tilde{c}^2 \rangle =$$

$$\frac{\bar{c}\alpha}{\pi^2 a^2}\frac{1}{4}\left[\ln\left(1+\frac{\left(2\left(\frac{a}{\lambda}\right)^2+1\right)}{\left(\frac{a}{\lambda}\right)^4+\alpha^2}\right)+\frac{2}{\alpha}\sqrt{\left(2\left(\frac{a}{\lambda}\right)^2+1\right)+\left(\frac{a}{\lambda}\right)^4}\,tan^{-1}\left(\frac{\alpha}{\sqrt{\left(2\left(\frac{a}{\lambda}\right)^2+1\right)+\left(\frac{a}{\lambda}\right)^4}}\right)-\right.$$

$$\left.\frac{2k_{endo}\tau}{\alpha}\,tan^{-1}\left(\frac{\alpha}{k_{endo}\tau}\right)\right]. \text{ (S24)}$$

Note that the expression shown in Eq.S24 is valid for any integration time τ.

In the limit $\left(\frac{a}{\lambda}\right)^2 \ll 1$, and assuming $\left(\frac{a}{\lambda}\right)^4 \ll \alpha^2$ (i.e. $\tau \ll \frac{1}{k_{endo}}$, for short integration times), we find that

$$\langle \delta\tilde{c}^2 \rangle = \frac{\bar{c}\alpha}{\pi a^2}\frac{1}{4}\left[\ln\left(1+\frac{1}{\alpha^2}\right)+\frac{2}{\alpha}tan^{-1}\alpha-\frac{2k_{endo}\tau}{\alpha}\,tan^{-1}\left(\frac{\alpha}{k_{endo}\tau}\right)\right]. \text{ (S25)}$$

The parameter α is expected to be in the range $(10^{-4}-10^{-1})$. The term $\ln\left(\frac{1}{\alpha^2}+1\right)$ may be approximated by $\ln\left(\frac{1}{\alpha^2}\right)$. The difference between the terms $\frac{2}{\alpha}tan^{-1}\alpha$, and $\frac{2k_{endo}\tau}{\alpha}\,tan^{-1}\left(\frac{\alpha}{k_{endo}\tau}\right)$, is small compared to the $\ln\left(\frac{1}{\alpha^2}\right)$ term . Hence we get that, for short integration times,

$\langle \delta\tilde{c}^2 \rangle = \frac{\bar{c}}{\pi D_2 \tau}\ln\left(\sqrt{\frac{D_2\tau}{a^2}}\right) = \frac{\bar{c}}{\pi D_2 \tau}\ln\left(\frac{\lambda^*}{a}\right)$, where $\lambda^* \equiv \sqrt{D_2\tau}$ is the corresponding natural length scale. Then the relative uncertainty for short integration times becomes

$$\left(\frac{\delta c}{\bar{c}}\right)^2 = \frac{1}{\pi D_2 \tau \bar{c}}\ln\left(\frac{\lambda^*}{a}\right). \text{ (S26)}$$

By plotting the function defined by Eq.S24 it is easy to verify that the limits given by Eqs. S21 and S26 are good approximations for the accuracy at short and large integration times.

Note that in the very extreme case where α gets so large (i.e. integration time very close to 0, or very small diffusion coefficient) that the dominant term in Eq. S24 is the second term, $\frac{2}{\alpha}tan^{-1}(\alpha)\sim\frac{\pi}{\alpha}$, then we get $\langle \delta\tilde{c}^2 \rangle = \frac{\bar{c}\alpha}{\pi^2 a^2}\frac{1}{4}\frac{\pi}{\alpha}$ and $\left(\frac{\delta c}{\bar{c}}\right)^2 = \frac{1}{4\pi\bar{c}a^2}$. Time average does not appear, but just the size of the receptor. In this limit the ligands can hardy diffuse over distances larger than the receptor size, and then it makes sense that there will not be any diffusion noise. Without diffusion the only

source of noise would be Poisson counting noise $\frac{1}{\sqrt{N}}$, where N is the number of ligands placed in the contact area (namely, $\left(\frac{\delta c}{\bar{c}}\right)^2 \sim \frac{1}{a^2\bar{c}}$).

Accuracy of ligand concentration measurement by multiple receptors

In order to take into account the presence of *m* multiple receptors, we assume

$\delta\tilde{c}(t,\boldsymbol{x}) = \sum_{\mu=1}^{m} \int d^2x dt'\, w_r(\boldsymbol{x_\mu} - \boldsymbol{x'}) k_r(t-t')\, \delta c^*(t', \boldsymbol{x'})$, (S27)

where $w_r(\boldsymbol{x_\mu} - \boldsymbol{x'}) = \frac{e^{-(x_\mu - x\prime)^2/(2a^2)}}{2\pi a^2 m}$. (S28)

Proceeding in a similar way to the case of one receptor, we compute $\langle\delta\tilde{c}^2\rangle$:

$\langle\delta\tilde{c}^2\rangle = \sum_{\mu,\nu} \int d^2x' dt' d\omega \frac{d^2k}{(2\pi)^3} d^2x'' \mathrm{d}t''$

$d\omega' \frac{d^2k'}{(2\pi)^3} \frac{e^{-(x_\mu - x\prime)^2/(2a^2)}}{2\pi a^2 m} \frac{e^{-(t-t')^2/(2\tau^2)}}{\sqrt{2\pi}\tau} \frac{e^{-(x'' - x_\nu)^2/(2a^2)}}{2\pi a^2 m} \frac{e^{-(t-t'')^2/(2\tau^2)}}{\sqrt{2\pi}\tau} e^{i(\boldsymbol{k}\cdot\boldsymbol{x'} - \omega t')}$

$e^{i(\boldsymbol{k'}\cdot\boldsymbol{x''} - \omega' t')} \langle \delta c^*(\omega, \boldsymbol{k}) \delta c^*(\omega', \boldsymbol{k'}) \rangle$. (S29)

As in the one receptor case, we set $\omega' = -\omega$ and $\boldsymbol{k'} = -\boldsymbol{k}$ (4), and obtain

$\langle\delta\tilde{c}^2\rangle = \sum_{\mu,\nu} \int d\omega \frac{d^2k}{(2\pi)^3}\, e^{-\tau^2\omega^2} e^{-a^2k^2} (\frac{1}{m})^2 e^{i\boldsymbol{k}\cdot\boldsymbol{x_\mu}} e^{-i\boldsymbol{k}\cdot\boldsymbol{x_\nu}} \frac{2\bar{c}(D_2k^2 + k_{endo})}{\omega^2 + (D_2k^2 + k_{endo})^2}$. (S30)

We first consider low frequencies ($\omega \approx 0$) (2). Then we replace the infinite integral in ω (Eq.S30) by an integral with sharp cutoff $\omega_{max} \sim \frac{\pi}{\tau}$ and substitute the Gaussian kernel by the constant value 1 (similarly to the case of one receptor). Then Eq.S30 can be rewritten as

$\langle\delta\tilde{c}^2\rangle = \sum_{\mu,\nu} \int_0^\infty d\omega \int \frac{d^2k}{(2\pi)^3} e^{-\tau^2\omega^2} e^{-a^2k^2} e^{i\boldsymbol{k}\cdot(\boldsymbol{x_\mu} - \boldsymbol{x_\nu})} (\frac{1}{m})^2 \frac{4\bar{c}(D_2k^2 + k_{endo})}{\omega^2 + (D_2k^2 + k_{endo})^2}$ (S31).

The integral in ω is just $\frac{\pi}{\tau}$, therefore Eq.S31 becomes

$\langle\delta\tilde{c}^2\rangle \sim \sum_{\mu,\nu} \int \frac{d^2k}{(2\pi)^3}\, e^{-a^2k^2} e^{i\boldsymbol{k}\cdot(\boldsymbol{x_\mu} - \boldsymbol{x_\nu})} (\frac{1}{m})^2 \frac{4\pi\bar{c}/(D_2\tau)}{k^2 + k_{endo}/D_2}$ (S32).

The dominant terms in Eq.S32 come from the terms with small ka, due to the drop of the Gaussian kernel. Therefore, we may approximate the Gaussian by the constant 1 and limit the integral in k from 0 to $\frac{1}{a}$. Note that the integral (in Eq. S32) is in addition highly oscillatory for large values of k. In particular, the Fourier integral is highly oscillatory in the exponent $\boldsymbol{k}\cdot(\boldsymbol{x_\mu} - \boldsymbol{x_\nu})$. The maximal value of k contributing to the integral is $k_{max} \sim \frac{1}{|\boldsymbol{x_\mu} - \boldsymbol{x_\nu}|_{min}} \sim 1/a$. Therefore we may consider

the integral again over all possible values of k, because large values of k do not contribute to the Fourier integral. Hence Eq. S32 is replaced by

$$\langle \delta\tilde{c}^2 \rangle \approx \sum_{\mu,\nu} \left(\frac{1}{m^2}\right) \frac{4\pi\bar{c}}{D_2\tau} \int \frac{d^2k}{(2\pi)^3} e^{i\boldsymbol{k}\cdot(\boldsymbol{x_\mu}-\boldsymbol{x_\nu})} \frac{1}{k^2+\left(\frac{1}{\lambda}\right)^2}. \quad \text{(S33)}$$

Now the integral term appearing in Eq. S33 is the 2D inverse Fourier transform of the generalized function $\frac{1}{k^2+\left(\frac{1}{\lambda}\right)^2}$. This corresponds to one of the well-known radial inverse Fourier transform, and it can be written in terms of the modified Bessel function K_0 (15-18).

$$\int d^2k \, \frac{e^{i\boldsymbol{k}\cdot\boldsymbol{r}}}{k^2+\frac{1}{\Lambda^2}} = 2\pi K_0\left(\frac{|\boldsymbol{r}|}{\Lambda}\right). \quad \text{(S34)}$$

Therefore, Eq. S33 can be expressed as

$$\langle \delta\tilde{c}^2 \rangle \approx \sum_{\mu\neq\nu} \left(\frac{1}{m^2}\right) \frac{\bar{c}}{\tau D_2 \pi} K_0\left(|\boldsymbol{x_\mu} - \boldsymbol{x_\nu}|/\lambda\right) + \frac{\bar{c}}{\pi m \tau D_2} \ln\left(\frac{\lambda}{a}\right). \quad \text{(S35)}$$

The only limitations of Eq.S35 are $\frac{\lambda}{a} \gg 1$ and $\tau \gg \frac{1}{k_{endo}}$.

For the sake of simplicity, we will assume a cluster of m receptors of size *a* distributed equidistantly along a ring of radius *s* (2). This assumption allows us to simplify Eq. S35.

Let's define the following variables

$$\theta_i = i\frac{2\pi}{m}, \; x_1 = 0, y_1 = s, x_i = s\, sin\theta_i, y_i = s\, cos\theta_i. \quad \text{(S36)}$$

It is easy to see that

$$|(x_1 - x_i)| = \sqrt{2}s\sqrt{1 - cos\theta_i} = 2s\, \sin(\frac{\pi i}{m}). \quad \text{(S37)}$$

Therefore, defining $\delta c = \sqrt{\langle \delta\tilde{c}^2 \rangle}$ we obtain for large integration times

$$\left(\frac{\delta c}{\bar{c}}\right)^2 \approx \left[\frac{\ln(\frac{\lambda}{a})}{\pi m D_2 \bar{c}\tau} + \frac{\sum_{i=1}^{m-1} K_0(\frac{2s}{\lambda_1}\sin(\frac{\pi i}{m}))}{\pi m D_2 \bar{c}\tau}\right], \quad \text{(S38)}$$

or

$$\left(\frac{\delta c}{\bar{c}}\right) \approx \frac{1}{\sqrt{\pi D_2 \bar{c}\tau m}} \sqrt{\ln\left(\frac{\lambda}{a}\right) + \sum_{i=1}^{m-1} K_0\left(\frac{2s}{\lambda}\sin\left(\frac{\pi i}{m}\right)\right)}. \quad \text{(S39)}$$

As a limiting case we consider $\lambda \gg s$. In this case we can use the limiting form for the modified Bessel function K_0 for $x \ll 1$ (18):

$$K_0(x \ll 1) \approx -\left(\ln\left(\frac{x}{2}\right) + C\right), \qquad \text{(S40)}$$

where C=0.5772 (Euler-Mascheroni constant).

Using the identity (18):

$$\sin(\pi m\varphi) = 2^{m-1}\prod_{i=0}^{m-1} sin\pi(\frac{i}{m} + \varphi), \text{ (S41)}$$

it is easy to show that

$$\prod_{i=1}^{m-1} sin\left(\frac{\pi i}{m}\right) = 2^{1-m}m, \text{ for m≥2 . (S42)}$$

Therefore, the sum of modified Bessel functions can be approximated as follows

$$\sum_{i=1}^{m-1} K_0\left(\frac{2s}{\lambda}\sin\left(\frac{\pi i}{m}\right)\right) = -\sum_{i=1}^{m-1} ln\left(\frac{s}{\lambda}\sin\left(\frac{\pi i}{m}\right)\right) + C = -\sum_{i=1}^{m-1} ln\left(\frac{se^C}{\lambda}\sin\left(\frac{\pi i}{m}\right)\right) =$$

$$\prod_{i=1}^{m-1} ln\left(\frac{se^C}{\lambda}\sin\left(\frac{\pi i}{m}\right)\right) = -\ln[\left(\frac{se^C}{\lambda}\right)^{m-1} 2^{1-m}m] = -\Bigg((m-1)\ln\left(\frac{se^C}{2\lambda}\right) + \ln(m)\Bigg) = (m-1)\ln\left(\frac{2\lambda}{se^C}\right) - \ln(m). \qquad \text{(S43)}$$

Substituting Eq. S43 into Eq. S39 we obtain the final result

$$\frac{\delta c}{\bar{c}} \approx \frac{1}{\sqrt{\pi D_2 \bar{c}\tau}}\sqrt{\frac{\ln\left(\frac{\lambda}{ma}\right)}{m} + \left(\frac{m-1}{m}\right)\ln\left(1.1228\frac{\lambda}{s}\right)}. \qquad \text{(S44)}$$

It should be mentioned that this same result was obtained by introducing internal noise to the receptor-ligand system and computing the accuracy using the fluctuation dissipation theorem (see below).

Calculation of accuracy in the absence of endocytosis

We start with Eq.S30 and consider the limit case $k_{endo} = 0$. Then

$$\left\langle\left(\frac{\delta c}{\bar{c}}\right)^2\right\rangle = \sum_{\mu,\nu}\int_0^\infty d\omega\,\frac{d^2k}{(2\pi)^3}\, e^{-\tau^2\omega^2}e^{-a^2k^2}\left(\frac{1}{m}\right)^2 e^{i\boldsymbol{k}\cdot\boldsymbol{x_\mu}}e^{-i\boldsymbol{k}\cdot\boldsymbol{x_\nu}}\frac{4D_2k^2}{\omega^2+(D_2k^2)^2}. \text{ (S45)}$$

In order to perform the ω-integral we assume a cutoff at $\omega_{max}\sim\frac{\pi}{\tau}$, and approximate the Gaussian by the constant 1 in the low frequency limit $\omega \approx 0$ (similarly to the way we computed Eq.S30). Then the integral in ω is just $\frac{\pi}{\tau}$ and we obtain

$$\left\langle\left(\frac{\delta c}{\bar{c}}\right)^2\right\rangle = \frac{4\pi}{\bar{c}\tau}\sum_{\mu,\nu}\int\frac{d^2k}{(2\pi)^3}\, e^{-a^2k^2}\left(\frac{1}{m}\right)^2 e^{i\boldsymbol{k}\cdot\boldsymbol{x_\mu}}e^{-i\boldsymbol{k}\cdot\boldsymbol{x_\nu}}\,\frac{1}{D_2k^2}. \text{ (S46)}$$

In order to compute the integral in Eq.S46 we will follow also an approach similar to the one we used to compute Eq. S32. We note that the major contributions come from small values of , again this is due to the Gaussian kernel $e^{-a^2k^2}$ that drops for $k \gg \frac{1}{a}$ and also because the Fourier integral is highly oscillatory in the exponent $\boldsymbol{k}\cdot(\boldsymbol{x_\mu}-\boldsymbol{x_\nu})$. We can see that the maximal value of k contributing to the integral is $k_{max}\sim\frac{1}{|\boldsymbol{x_\mu}-\boldsymbol{x_\nu}|_{min}}\sim 1/a$. Therefore the procedure is to approximate the Gaussian by 1, and then compute Eq.S46 using Fourier transform of radial functions (15). Hence we obtain

$$\left(\frac{\delta c}{\bar{c}}\right)^2 \approx \sum_{\mu,\nu}\left(\frac{1}{m^2}\right)\frac{4\pi}{D_2\tau\bar{c}}\int\frac{d^2k}{(2\pi)^3}\, e^{i\boldsymbol{k}\cdot(\boldsymbol{x_\mu}-\boldsymbol{x_\nu})}\frac{1}{k^2} = -\sum_{\mu\neq\nu}\frac{1}{\pi m D_2\tau\bar{c}}\left(\ln\left[\frac{|\boldsymbol{x_\mu}-\boldsymbol{x_\nu}|}{\lambda^*}\right]+C\right)+ \frac{1}{m\pi D_2\tau\bar{c}}\ln\left(\frac{\sqrt{D_2\tau}}{a}\right). \text{(S47)}$$

where C~0.5772, and λ^* is a typical length scale. We adopt $\lambda^* \sim\sqrt{D_2\tau}$. The length scale λ^* (in the absence of endocytosis) plays the role of the length scale $\lambda = \sqrt{\frac{D_2}{k_{endo}}}$ present when endocytosis is involved. An additional justification for the typical length scale $\lambda^* \equiv \sqrt{D_2\tau}$ in the absence of endocytosis is shown using the FDT (see below section dedicated to the FDT approach).

Hence the accuracy will be given by

$$\left(\frac{\delta c}{\bar{c}}\right)^2 = \frac{1}{m\pi D_2\tau\bar{c}}\ln\left(\frac{\sqrt{D_2\tau}}{a}\right) - \frac{1}{\pi m D_2\tau\bar{c}}\left(\sum_{i=1}^{m-1} ln\left(\frac{s}{\sqrt{D_2\tau}}\sin\left(\frac{\pi i}{m}\right)\right)+C\right). \text{(S48)}$$

The second term involving $\sum_{i=1}^{m-1} ln\left(\frac{s}{\sqrt{D_2\tau}}\sin\left(\frac{\pi i}{m}\right)\right)$ can be simplified. Then we may use the result of Eq.S43 and obtain

$$\left(\frac{\delta c}{\bar{c}}\right)^2 = \frac{1}{\pi D_2\tau\bar{c}}\left[\frac{\ln\left(\frac{\sqrt{D_2\tau}}{ma}\right)}{m}+\left(\frac{m-1}{m}\right)\ln\left(1.1228\frac{\sqrt{D_2\tau}}{s}\right)\right] \text{(S49)}$$

For large number of receptors we may just use the approximate expression $\left(\frac{\delta c}{\bar{c}}\right)^2 = \frac{1}{\pi D_2\tau\bar{c}}\ln\left(1.1228\frac{\sqrt{D_2\tau}}{s}\right)$. (S50).

Note that this expression has a physical meaning only for $\lambda^* > s$.

Note that in the absent of endocytosis and assuming $\frac{s}{\sqrt{D_2\tau}} \ll 1$, we obtained an expression for the accuracy (Eq.S49) similar to the one obtained when endocytosis is present and $\lambda \gg s$ (Eq.S44). We infer that for more general cases, i.e. where the contact radio is larger than the typical length scale $\lambda^* \equiv \sqrt{D_2\tau}$, the accuracy for short integration times will be given by the expression Eq. S39 but with λ replaced by λ^*.

One way of seeing this is by noticing that for short integration times, endocytosis role is negligible, being λ^* the only relevant length scale affecting the accuracy.

Improvement of accuracy by averaging over neighboring cells

We assume that each cell is surrounded by *N* neighboring cells and receives a signal from all its neighbors. The total signal received by the cell, *S(t)*, is the sum of the individual signals from all its neighbors. Assuming that the signal from each neighbor is proportional to its ligand concentration we can write

$$S(t) = \sum_{i=1}^{N} c_i\,(t) \quad \text{(S51)}$$

(for simplicity we assumed that the proportionality constant is 1). The average value of the signal measured by all neighbor cells is given by $\bar{S_1}(t) = \sum_{i=1}^{N} \bar{c}_i\,(t)$.

The accuracy of the total signal is therefore defined as

$$\delta S = S - \bar{S} = \sum_{i=1}^{N}(c_i\text{-}\bar{c}_i)=\sum_{i=1}^{N} \delta c_i. \quad \text{(S52)}$$

Squaring both terms we obtain

$$(\delta S)^2 = \sum_{i=1}^{N}(\delta c_i)^2 + \sum_{i,i\neq j}\sum_{j}(\delta c_i)\left(\delta c_j\right). \quad \text{(S53)}$$

We take ensemble average of Eq. S53. Assuming that the measurements performed by each neighbor cell are statistically independent, and since $\langle(\delta c_i)\rangle = 0$, we see that $\langle(\delta c_i)\left(\delta c_j\right)\rangle = \langle(\delta c_i)\rangle\,\langle\left(\delta c_j\right)\rangle = 0$, and hence $\langle(\delta S)^2\rangle = \sum_{i=1}^{N}\langle(\delta c_i)^2\rangle$. (S54)

The relative accuracy of measuring ligand concentration $\frac{\langle(\delta S_1)^2\rangle}{\bar{S_1}^2}$ turns out to be

$$\frac{\langle(\delta S)^2\rangle}{\bar{S}^2} = \frac{\sum_{i=1}^{N}\langle(\delta c_i)^2\rangle}{(\sum_{i=1}^{N}\bar{c}_i)^2} \quad \text{(S55)}$$

There are several interesting limiting cases.

First case: If ligand concentration in all neighbors is approximately the same, namely $c_i \approx c_0$, where c_0 is the average concentration in a tissue. We then get

$$\frac{(\delta S)^2}{\bar{S}^2} = \frac{(\delta c_0)^2}{N c_0^2}\,. \quad \text{(S56)}$$

We note that averaging by *N* neighbor cells the accuracy gets improved by a factor of $\frac{1}{\sqrt{N}}$, as expected from averaging N independent random variables.

Second case: The ligand concentration in one of the cells is much larger than in the other cells, $c_i \gg c_{j\neq i}$. Then, defining $c_i = c_0$,

$$\frac{(\delta S)^2}{\bar{S}^2} = \frac{(\delta c_0)^2}{c_0^2}\,. \qquad \text{(S57)}$$

In this case the noise is dominated by the cell with the higher ligand concentration.

Third case: There is a gradient of ligand concentration (as in the vein boundary case discussed in the text). Assuming a two dimensional array of hexagonal cells (*N*=6 neighbors) and a linear gradient in ligand concentration we can write the concentration in each of the cells as:

$$c_{i-1,j-1} = c_0(1+\frac{a}{2});\ c_{i+1,j-1} = c_0(1-\frac{a}{2});\ \ c_{i-1,j} = c_0(1+a);\ c_{i+1,j} = c_0(1-a);$$
$$c_{i-1,j+1} = c_0(1+\frac{a}{2});\ c_{i+1,j+1} = c_0\left(1-\frac{a}{2}\right). \qquad \text{(S58)}$$

where $c_{i,j}$ denotes the ligand concentration measured by the cell located at row *i* and column *j*.

In this case, since $\sum(\delta c_{i,j})^2$=6$(\delta c_0)^2$ we get the same result as with a uniform ligand concentration with $c_i = c_0$.

Processing of receptor-ligand pair improves accuracy by a factor of up to $\sqrt{2}$

We consider a receptor with radius *a.* Every ligand molecule reaching the receptor is immediately absorbed, hence we may assume that the ligand concentration is zero at the border of the receptor *r=a*. We also assume that there is a constant ligand concentration far away. The ligand concentration satisfies the diffusion equation

$$\frac{\partial c(\boldsymbol{x},t)}{\partial t} = D_2\nabla^2 c - k_{endo}c(x,t) + k_{exo}c_{cyto} \quad \text{(S59)}$$

with the boundary condition $c(r=a)=0$, meaning the ligand molecules are trapped as soon as they reach the receptor. The second condition corresponds to a reflective boundary $\frac{\partial c}{\partial r}(r=b)=0$. This means that same number of ligands is crossing back and forth the external boundary $r=b$ (20).
Due to the symmetry of the problem, we introduce polar coordinates. Then the steady state solution will satisfy the following ODE:
$D_2\frac{1}{r}\frac{d}{dr}\left(r\frac{dc}{dr}\right) - k_{endo}c(r,t) + \beta = 0$ (S60),
where $\beta = k_{exo}c_{cyto}$.
In order to solve this equation, we define $c = c^* + \frac{\beta}{k_{endo}}$. Therefore c^* satisfies the equation
$\frac{1}{r}\left[D\frac{\partial c^*}{\partial r} + Dr\frac{\partial^2 c^*}{\partial r^2}\right] - r^2 k_{endo}c^* = 0$(S61).
This corresponds to a Modified Bessel's equation.
The most general solution of this equation can be written in terms of the modified Bessel functions as
$c^*(r) = AI_0\left(\frac{r}{\lambda}\right) + BK_0\left(\frac{r}{\lambda}\right)$ (S62),

where $= \sqrt{\frac{D_2}{k_{endo}}}$, and I_0, K_0 are the modified Bessel functions of order 0 (18).
Then it is very easy to show that the solution to this boundary value problem (perfect absorbing receptor) is
$c(r) = \frac{\beta}{k_{endo}}\left[1 - \frac{I_1\left(\frac{b}{\lambda}\right)K_0\left(\frac{r}{\lambda}\right)+K_1\left(\frac{b}{\lambda}\right)I_0\left(\frac{r}{\lambda}\right)}{I_1\left(\frac{b}{\lambda}\right)K_0\left(\frac{a}{\lambda}\right)+K_1\left(\frac{b}{\lambda}\right)I_0\left(\frac{a}{\lambda}\right)}\right]$ (S63),
I_0, K_0 and K_1 are the modified Bessel functions of order 0 and 1 (18).

In order to find the flux at the receptor's border we need to compute the current $J_r = D_2\frac{\partial c}{\partial r}$. The number of ligand molecules impinging on the receptor per unit time is given by $J_r 2\pi a$. Then, the rate of particles absorbed by the receptor during an integration time τ is given by $N = J_r 2\pi a\tau$.
The ligands behave independently; therefore we assume they are distributed according to Poisson distribution, $\langle(\delta N)^2\rangle = \langle N\rangle$. Hence, for a perfectly absorbing receptor the uncertainty in measuring ligands concentration is given by (21)
$\left(\frac{\delta c}{\bar{c}}\right)^2 = \frac{\langle(\delta N)^2\rangle}{\langle N\rangle^2} = \frac{1}{\langle N\rangle} = \frac{1}{J_r 2\pi a\tau}$ (S64).
Let's compute the flux:

$J_r = D_2\frac{\partial c}{\partial r} = -\frac{D_2\beta}{k_{endo}}\left[\frac{I_1\left(\frac{b}{\lambda}\right)\frac{d}{dr}K_0\left(\frac{r}{\lambda}\right)+K_1\left(\frac{b}{\lambda}\right)\frac{d}{dr}I_0\left(\frac{r}{\lambda}\right)}{I_1\left(\frac{b}{\lambda}\right)K_0\left(\frac{a}{\lambda}\right)+K_1\left(\frac{b}{\lambda}\right)I_0\left(\frac{a}{\lambda}\right)}\right]$. (S65)
It is known that $\frac{d}{dz}I_0(z) = I_1(z)$ and $\frac{d}{dz}K_0(z) = -K_1(z)$
Therefore the current of ligands at $r = a$ is
$J_r = -\frac{D_2\beta}{k_{endo}}\left[\frac{-I_1\left(\frac{b}{\lambda}\right)K_1\left(\frac{a}{\lambda}\right)+K_1\left(\frac{b}{\lambda}\right)I_1\left(\frac{a}{\lambda}\right)}{I_1\left(\frac{b}{\lambda}\right)K_0\left(\frac{a}{\lambda}\right)+K_1\left(\frac{b}{\lambda}\right)I_0\left(\frac{a}{\lambda}\right)}\right.$ (S66).

For the case $\frac{a}{\lambda} \ll 1$, we may exploit the asymptotic expansions corresponding to the modified Bessel functions for small arguments (18):
$K_0\left(\frac{a}{\lambda}\right) \sim -ln\left(\frac{a}{\lambda}\right)$ (S67),
$\frac{d}{dr}I_0\left(\frac{r}{\lambda}\right)(r = a) \sim 0$ (S68).
Therefore,
$J_r = -\frac{D_2\beta}{k_{endo}}\frac{I_1\left(\frac{b}{\lambda}\right)\left(\frac{1}{a}\right)}{I_1\left(\frac{b}{\lambda}\right)\left(\ln\left(\frac{a}{\lambda}\right)\right)} = -\frac{D_2\beta}{k_{endo}}\frac{1}{a\ln\left(\frac{a}{\lambda}\right)} = \frac{D_2\beta}{k_{endo}}\frac{1}{a\ln\left(\frac{\lambda}{a}\right)}$ (S69).
Hence

$N = \frac{D_2\beta}{k_{endo}}\frac{2\pi a\tau}{a\ln\left(\frac{\lambda}{a}\right)} = \frac{D_2\beta}{k_{endo}}\frac{2\pi\tau}{\ln\left(\frac{\lambda}{a}\right)}$ (S70).

The uncertainty is given by

$\left(\frac{\delta c}{\bar{c}}\right)^2 = \frac{1}{\langle N\rangle} = \frac{k_{endo}}{2\pi\tau D_2\beta}\ln\left(\frac{\lambda}{a}\right)$ (S71).

Since at steady state far away from the absorber $\bar{c} = \frac{\beta}{k_{endo}}$, we finally get:

$\left(\frac{\delta c}{\bar{c}}\right)^2 = \frac{1}{\langle N \rangle} = \frac{1}{2\pi\tau D_2 \bar{c}} \ln\left(\frac{\lambda}{a}\right)$ (S72),
which is a factor of 2 smaller than our result with 'perfect monitoring disk' approximation (Eq. S21).

Accuracy of ligand concentration measurement by one receptor using the FDT

We conclude the supplementary methods with an alternative computation of the accuracy in measuring ligand concentration. We analyze the effects of intrinsic fluctuations of the receptor-ligand system with the help of the fluctuation dissipation theorem (1,2)**.** First we calculate the accuracy in ligand concentration measurement due to binding to a single fixed receptor. Afterwards we extend the calculation to include the possibility of binding to several fixed receptors. We arrived to the same accuracy due to diffusion noise we obtained when we considered a perfect receptor with extrinsic noise in the receptor-ligand system.

We define *n(t)* as the occupation probability of one receptor bound to a ligand at position x_0 on the membrane of the receptor cell. The ligand concentration on the membrane of the ligand cell is described by $c(\boldsymbol{x}, t)$. We also assume that ligands are continuously recycled in and out of the membrane (e.g. through endocytosis and exocytosis with rates k_{endo} and k_{exo}, respectively. The dynamics of ligand-receptor is governed by

$$\frac{dn(t)}{dt} = k_+ c(\boldsymbol{x}, t)\big(1 - n(t)\big) - k_- n(t), \qquad \text{(S73)}$$

$$\frac{\partial c(\boldsymbol{x},t)}{\partial t} = D_2 \nabla^2 c(\boldsymbol{x}, t) - \delta(\boldsymbol{x} - \boldsymbol{x_0}) \frac{dn(t)}{dt} - k_{endo} c(\boldsymbol{x}, t) + k_{exo} c_{cyto}\,. \qquad \text{(S74)}$$

where D_2 is the 2D diffusion coefficient for the ligands on the cell membrane, k_+ and $k_-$are the binding and unbinding rates of the ligand-receptor complex, $\delta(\boldsymbol{x})$ is the Dirac delta function, and c_{cyto} is the concentration of a cytoplasmic pool of the Delta ligand (assumed to be constant in this study).

The rate constants obey the detailed balance equation:

$$\frac{k_+ \bar{c}}{k_-} = \exp\left(\frac{F}{kT}\right), \qquad \text{(S75)}$$

where F is the difference in the free energies between unbound and bound states of the receptor. We introduce small perturbations around the stationary solutions. The perturbations are defined according to the following

$$k_\pm = \overline{k_\pm} + \delta k_\pm,\ n = \bar{n} + \delta n, c = \bar{c} + \delta c,\ F = \bar{F} + \delta F. \qquad \text{(S76)}$$

The bar over the variables denotes steady state equilibrium values.

Substituting Eq. S76 into Eq. S75 leads to

$$\frac{\delta k_+}{k_+} - \frac{\delta k_-}{k_-} = \frac{\delta F}{k_B T}\,. \qquad \text{(S77)}$$

By substituting Eq. S77 into Eq. S73 we obtain that the perturbation δn satisfies

$$\frac{d\delta n(t)}{dt} = -\left(\bar{k}_+\bar{c} + \bar{k}_-\right)\delta n(t) + \bar{c}(1-\bar{n})\delta k_+ + \bar{k}_+\delta c(1-\bar{n}) - \bar{n}\delta k_-\,. \qquad \text{(S78)}$$

The perturbations of the rate constants $\delta k_\pm$ are connected by the Eq. S77, hence Eq. S78 becomes

$$\frac{kT}{\bar{k}_+\bar{c}(1-\bar{n})}\frac{d\delta n}{dt} + \frac{kT(\bar{k}_+\bar{c}+\bar{k}_-)}{\bar{k}_+\bar{c}(1-\bar{n})}\,\delta n(t) - kT\frac{\delta c}{\bar{c}} = \delta F\,. \qquad \text{(S79)}$$

In a similar way, Eq. S74 may be rewritten in terms of the perturbations δc and δn as follows

$$\frac{\partial \delta c(\boldsymbol{x},t)}{\partial t} = D_2\nabla^2\delta c(\boldsymbol{x},t) - \delta(\boldsymbol{x}-\boldsymbol{x_0})\frac{d\delta n(t)}{dt} - k_{endo}\delta c(\boldsymbol{x},t). \qquad \text{(S80)}$$

Fourier transform in spatial and temporal variables are defined as in Eq. S4.

The Fourier transform of Eq. S79 in the temporal variable is

$$\delta n(\omega)\left[-\frac{kT(i\omega)}{\bar{k}_+\bar{c}(1-\bar{n})} + \frac{kT(\bar{k}_+\bar{c}+\bar{k}_-)}{\bar{k}_+\bar{c}(1-\bar{n})}\right] - \frac{kT}{\bar{c}}\delta c = \delta F\,. \qquad \text{(S81)}$$

The Fourier transform in spatial and temporal variables of Eq. S80 becomes:

$$\delta c(\boldsymbol{k},\omega) = i\omega\frac{e^{-i\boldsymbol{k}\cdot x_0}\delta n(\omega)}{[-i\omega + D_2k^2 + k_{endo}]}\,. \qquad \text{(S82)}$$

The inverse Fourier transform in 2D is defined by

$$\delta c(\boldsymbol{x},\omega) = \frac{1}{(2\pi)^2}\int d^2k\; e^{i\boldsymbol{k}\cdot\boldsymbol{x}}\delta c(\boldsymbol{k},\omega). \qquad \text{(S83)}$$

Applying the inverse Fourier transform to Eq. S82 we obtain:

$$\delta c(\boldsymbol{x_0},\omega) = \frac{i\omega}{(2\pi)^2}\int d^2k\;\frac{\delta n(\omega)}{[-i\omega + D_2k^2 + k_{endo}]}. \qquad \text{(S84)}$$

Substituting Eq. S84 into the Eq. S81 we get:

$$\delta n(\omega)\left[-\frac{kT(i\omega)}{\bar{k}_+\bar{c}(1-\bar{n})} + \frac{kT(\bar{k}_+\bar{c}+\bar{k}_-)}{\bar{k}_+\bar{c}(1-\bar{n})} - \frac{kT}{\bar{c}}\frac{i\omega}{(2\pi)^2}\int d^2k\;\frac{1}{[-i\omega + D_2k^2 + k_{endo}]}\right] = \delta F. \qquad \text{(S85)}$$

We define the linear response function or the generalized susceptibility α by (2)

$$\alpha = \frac{\delta n(\omega)}{\delta F(\omega)}. \qquad \text{(S86)}$$

The generalized susceptibility in our particular case turns out to be

$$\alpha = \frac{\bar{k}_+\bar{c}(1-\bar{n})}{kT}\frac{1}{-i\omega\left[1+\bar{k}_+(1-\bar{n})\int\frac{d^2k}{(2\pi)^2}\frac{1}{[-i\omega+D_2k^2+k_{endo}]}\right]+(\bar{k}_+\bar{c}+\bar{k}_-)}. \quad \text{(S87)}$$

Defining

$$\sum(\omega) = \bar{k}_+(1-\bar{n})\int\frac{d^2k}{(2\pi)^2}\frac{1}{[-i\omega+D_2k^2+k_{endo}]}, \quad \text{(S88)}$$

we rewrite Eq. S87 as

$$\alpha = \frac{\bar{k}_+\bar{c}(1-\bar{n})}{kT}\frac{1}{-i\omega[1+\sum(\omega)]+(\bar{k}_+\bar{c}+\bar{k}_-)}. \quad \text{(S89)}$$

Since we are averaging over a time τ large compared to the noise correlation time $\tau_c = \left(\bar{k}_+\bar{c}+\bar{k}_-\right)^{-1}$, we need to take into consideration only the low frequency limit of the noise spectrum.

Eq. S88 diverges for large k. In order to regularize Eq. S88, we introduce a cut off for large k. This is equivalent to assume that the receptor has a finite size (2). Introducing polar coordinates $d^2k = kd\theta dk$, Eq. S88 can be rewritten as:

$$\sum(\omega\sim 0) = \bar{k}_+(1-\bar{n})\int\frac{d^2k}{(2\pi)^2}\frac{1}{D_2k^2+k_{endo}} =$$
$$\frac{\bar{k}_+(1-\bar{n})2\pi}{2(2\pi)^2D_2}[\ln(D_2k^2+k_{endo})]_0^{\frac{1}{a}} = \frac{\bar{k}_+(1-\bar{n})}{2\pi D_2}\ln\left(\frac{D_2}{a^2k_{endo}}+1\right)^{1/2} \approx \frac{\bar{k}_+(1-\bar{n})}{2\pi D_2}\ln\left(\frac{\lambda}{a}\right), \quad \text{(S90)}$$

with

$$\lambda \equiv \sqrt{\frac{D_2}{k_{endo}}}, \quad \text{(S91)}$$

as previously defined. For the derivation of Eq. S90 it was assumed $\lambda \gg a$, where a is the radius of the receptor.

The power spectrum or spectral density of a random variable $y(t)$ is defined as

$$S_y(\omega) = \lim_{T\to\infty}\frac{2}{T}\left|\int_{-T/2}^{T/2}[y(t)-\bar{y}]e^{i\omega t}\right|^2, \quad \text{(S92)}$$

and it satisfies

$$\int\frac{d\omega}{2\pi}S_y(\omega) = \lim_{T\to\infty}\frac{1}{T}\int_{-T/2}^{T/2}[y(t)-\bar{y}]^2 = \langle(\delta y)^2\rangle. \quad \text{(S93)}$$

In particular, the power spectrum $S_n(\omega)$ in occupancy may be defined by

$$\langle\delta n(\omega)\delta n(\omega')\rangle = 2\pi\delta(\omega+\omega')S_n(\omega). \quad \text{(S94)}$$

The Fluctuation Dissipation theorem connects the generalized susceptibility $\alpha(\omega)$ with the power spectrum $S_n(\omega)$ (2) by the relation $S_n(\omega) = \frac{2kT}{\omega} Im$ (α(ω)). Here we compute $S_n(\omega)$ and obtain

$$S_n(\omega) = \frac{2\bar{k}_+\bar{c}(1-\bar{n})[1+\sum(0)]}{\omega^2[1+\sum(0)]^2+\left((\bar{k}_+\bar{c}+\bar{k}_-)\right)^2}. \qquad \text{(S95)}$$

Using Eq. S90, the power spectrum $S_n(\omega)$ in occupancy can be rewritten as

$$S_n(\omega\sim 0) = \frac{2\bar{k}_+\bar{c}(1-\bar{n})[1+\sum(0)]}{(\bar{k}_+\bar{c}+\bar{k}_-)^2} = \frac{2\bar{n}(1-\bar{n})}{(\bar{k}_+\bar{c}+\bar{k}_-)} + \frac{\bar{n}^2(1-\bar{n})^2}{\pi D_2\bar{c}} \ln\left(\frac{\lambda}{a}\right). \qquad \text{(S96)}$$

Averaging over a time τ, the accuracy δn will take into account only low frequencies $|\omega| < \frac{1}{\tau_{int}}$:

$$\langle(\delta n)^2\rangle \sim \int_0^{|\omega|<\frac{1}{\tau}} \frac{d\omega}{2\pi} S_n(\omega) \sim \int_0^{|\omega|<\frac{1}{\tau}} \frac{d\omega}{2\pi} S_n(\omega\sim 0) = S_n(\omega\sim 0)\frac{\omega}{2\pi} = \frac{S_n(\omega\sim 0)}{\tau}. \text{ (S97)}$$

Therefore,

$$\delta n = \sqrt{S_n(0)/\tau}. \qquad \text{(S98)}$$

Finally using Eq. S96 we obtain that

$$\delta n > \frac{\bar{n}(1-\bar{n})}{\sqrt{\pi D_2\bar{c}\tau}} \sqrt{\ln\left(\frac{\lambda}{a}\right)}. \qquad \text{(S99)}$$

We may relate δc with δn using spectral densities of fluctuations. The power spectrum $S_c(\omega)$ satisfies,

$$\langle\delta c(t)\delta c(t')\rangle = \int \frac{d\omega}{2\pi} S_c(\omega)\, e^{-i\omega(t-t')}. \qquad \text{(S100)}$$

A total variation in the concentration *c* is equivalent to a variation in the chemical potential μ. Since $\frac{\Delta c}{\bar{c}} = \frac{\Delta F}{k_B T}$, we will have

$$S_c(\omega) = \left(\frac{\bar{c}}{k_B T}\right)^2 S_F(\omega). \qquad \text{(S101)}$$

The spectral density for F satisfies

$$S_n(\omega) = \alpha^2 S_F(\omega); \qquad \text{(S102)}$$

therefore

$$S_F(\omega) = \frac{2k_B T}{\omega\left|\frac{\delta\hat{n}}{\delta\hat{F}}\right|^2} Im\left[\frac{\delta\hat{n}}{\delta\hat{F}}\right], \qquad \text{(S103)}$$

which is equivalent to

$$S_F(\omega) = \frac{-2k_BT}{\omega} Im[\frac{\delta \hat{F}}{\delta \hat{n}}] \,. \qquad \text{(S104)}$$

Similarly to δn, the accuracy δc satisfies:

$$\delta c = \sqrt{S_c(\omega \sim 0)/\tau}. \qquad \text{(S105)}$$

Combining Eq. S105, Eq. S104, Eq. S101 and Eq. S99 we obtain

$$\frac{\delta c}{\bar{c}} > \frac{1}{\sqrt{\pi D_2 \bar{c} \tau}} \sqrt{\ln\left(\frac{\lambda}{a}\right)} \,. \qquad \text{(S106)}$$

Length scale in the absence of endocytosis

Now we proceed to show that endocytosis is not required in order to deal with the IR divergence in the zero frequency limit. If we neglect endocytosis, the diffusion length scale is replaced by $\sqrt{D_2\tau}$. In order to prove that, we rewrite Eq. S88 as

$$\sum(\omega) = \bar{k}_+(1-\bar{n}) \int \frac{d^2k}{(2\pi)^2} \frac{1}{[-i\omega + D_2k^2]} = \frac{\bar{k}_+(1-\bar{n})}{2\pi(2D_2)} [\ln(D_2k^2 - i\omega)]_0^{\frac{1}{a}} =$$

$$\frac{\bar{k}_+(1-\bar{n})}{2\pi(2D_2)} \left[\ln\left(\frac{D_2}{a^2} - i\omega\right) - \ln(-i\omega)\right]. \qquad \text{(S107)}$$

The log of a complex number $z = re^{i\theta}$ is defined by

$$\ln(z) = \ln(r) + i\theta. \qquad \text{(S108)}$$

Therefore

$$\sum(\omega) = \frac{\bar{k}_+(1-\bar{n})}{2\pi(2D_2)} [\frac{1}{2}\ln(\left(\frac{D_2}{a^2}\right)^2 + \omega^2) + i\theta_1 - \ln(\omega) - i\theta_2] = \sum_{real} + i\sum_{imaginary} \,, \qquad \text{(S109)}$$

θ_1 is the argument of the complex number $\frac{D_2}{a^2} - i\omega$ and $\theta_2 = \frac{3\pi}{2}$.

The generalized susceptibility is given by

$$\alpha = \frac{\bar{k}_+\bar{c}(1-\bar{n})}{kT} \frac{1}{-i\omega[1+\sum(\omega)] + (\bar{k}_+\bar{c} + \bar{k}_-)} \,. \qquad \text{(S110)}$$

The power spectrum $S_n(\omega)$ is

$$S_n(\omega) = \frac{2kT}{\omega} Im\,(\alpha(\omega)) = \frac{2\bar{k}_+\bar{c}(1-\bar{n})[1+\sum(\omega)_{real}]}{\omega^2[1+\sum(\omega)_{real}]^2 + \left((\bar{k}_+\bar{c} + \bar{k}_-) + \omega\sum_{imaginary}\right)^2} \,. \qquad \text{(S111)}$$

The IR divergence is contained solely in the term $\ln(\omega)$ in the zero frequency limit. The other terms containing ω are well behaved for small ω and can be set to 0 in the evaluation of $\langle(\delta n)^2\rangle \sim \int_0^{|\omega|<\frac{1}{\tau}} \frac{d\omega}{2\pi} S_n(\omega)$. The imaginary part $\omega\sum_{imaginary}$ disappears in the zero frequency limit.

We set $\omega \sim 0$ in all the terms that do not have IR divergence (i.e. except in the term involving $\ln(\omega)$).

Averaging over a time τ, for frequencies satisfying $|\omega| < \frac{1}{\tau_{int}}$ we obtain

$$\langle(\delta n)^2\rangle \sim \int_0^{|\omega|<\frac{1}{\tau}} \frac{d\omega}{2\pi} S_n(\omega) \sim \frac{2\bar{k}_+\bar{c}(1-\bar{n})}{(\bar{k}_+\bar{c}+\bar{k}_-)^2} \int_0^{|\omega|<\frac{1}{\tau}} \frac{d\omega}{2\pi} \frac{\bar{k}_+(1-\bar{n})}{2\pi(2D_2)} [(1 + \ln(\frac{D_2}{a^2})^2 - \ln\left(\frac{1}{\tau}\right)]$$

$$= \frac{2\bar{k}_+\bar{c}(1-\bar{n})}{(\bar{k}_+\bar{c}+\bar{k}_-)^2} \frac{\bar{k}_+(1-\bar{n})}{2\pi(2D_2)} \{[1 + 2\ln\left(\frac{D_2}{a^2}\right) + \ln(\tau)]\left(\frac{1}{\tau}\right) = \frac{2\bar{k}_+\bar{c}(1-\bar{n})}{(\bar{k}_+\bar{c}+\bar{k}_-)^2} \frac{\bar{k}_+(1-\bar{n})}{2\pi(2D_2)} \frac{2}{\tau}[\ln\left(\frac{D_2\tau}{a^2}\right)] = \frac{(\bar{n}(1-\bar{n}))^2}{\pi D_2 \bar{c}\tau} \ln\left(\frac{\sqrt{D_2\tau}}{a}\right).$$

(S112)

Here, we note we may define a new length scale

$$\lambda = \sqrt{D_2\tau}\,. \qquad \text{(S113)}$$

From here following the same computation we did for k_{endo} (see Eqs. S100-S106) we may obtain a similar expression to Eq. S26: $\frac{\delta c}{\bar{c}} > \frac{1}{\sqrt{\pi D_2 \bar{c}\tau}} \sqrt{\ln\left(\frac{\lambda^*}{a}\right)}$ with the length scale $\lambda^* = \sqrt{D_2\tau}$.

Accuracy of ligand concentration measurement by multiple receptors using the FDT

The extended equations for ligand and multiple molecule receptors become

$$\frac{dn_\mu(t)}{dt} = k_+ c(\boldsymbol{x_\mu}, \mathrm{t})\left(1 - n_\mu(t)\right) - k_- n_\mu(t), \qquad \text{(S114)}$$

$$\frac{\partial c(\boldsymbol{x},t)}{\partial t} = D_2\nabla^2 c(\boldsymbol{x},t) - \sum_{\mu=1}^{m} \delta(\boldsymbol{x} - \boldsymbol{x_\mu}) \frac{dn_\mu(t)}{dt} - k_{endo} c(\boldsymbol{x},t) + k_{exo} c_{cyto}. \qquad \text{(S115)}$$

As in the case of a single receptor, detailed balance requires Eq. S75.

Similarly to the case of one receptor, we introduce small perturbations around the stationary solutions:

$$k_\pm = \overline{k_\pm} + \delta k_\pm,\ n_\mu = \overline{n_\mu} + \delta n_\mu,\ c = \bar{c} + \delta c,\ F = \bar{F} + \delta F. \qquad \text{(S116)}$$

The rate constants $\delta k_\pm$ obey Eq. S77.

Substituting Eq. S116 into Eq. S114 we obtain

$$\frac{d\delta n_\mu}{dt} = -\left(\bar{k}_+\bar{c} + \bar{k}_-\right)\delta n_\mu(t) + \bar{c}\left(1-\overline{n_\mu}\right)\delta k_+ - \overline{n_\mu}\delta k_- + \bar{k}_+\delta c\left(1-\overline{n_\mu}\right). \quad \text{(S117)}$$

With the help of Eq. S77 we rewrite Eq. S117 as

$$\frac{kT}{\bar{k}_+\bar{c}\left(1-\overline{n_\mu}\right)}\frac{d\delta n_\mu}{dt} + \frac{kT(\bar{k}_+\bar{c}+\bar{k}_-)}{\bar{k}_+\bar{c}\left(1-\overline{n_\mu}\right)}\,\delta n_\mu(t) - k_BT\frac{\delta c}{\bar{c}} = \delta F. \qquad \text{(S118)}$$

We proceed to rewrite the ligand equation for the perturbation δc in terms of the corresponding Fourier transforms of δc and δn_μ (similar to the computation that lead to Eq. S82)

$$-i\omega\delta c(\boldsymbol{k},\omega) = \\ (-D_2k^2 - k_{endo})\delta c(\boldsymbol{k},\omega) + \iint d^2x\, dt \sum_{\mu=1}^{m}\delta\left(\boldsymbol{x}-\boldsymbol{x_\mu}\right)(i\omega)\delta n_\mu(\mathrm{t})e^{i(-\boldsymbol{k}\cdot\boldsymbol{x}+w\mathrm{t})}.$$

(S119)

Therefore,

$$\delta c\,(\boldsymbol{x},\omega) = \frac{1}{(2\pi)^2}(i\omega)\sum_{\mu=1}^{m}\int\frac{d^2k\;\delta n_\mu(\omega)}{[-i\omega+D_2k^2+k_{endo}]}e^{ik(-\boldsymbol{x_\mu}+\boldsymbol{x_\nu})}. \qquad \text{(S120)}$$

The integral appearing in Eq. S120 can be split into two cases (similar to the treatment of the 3D case by (2), namely μ=ν and μ≠ν.

The term corresponding to μ=ν is equal to

$$\delta c\,(\boldsymbol{x_\nu},\omega) = \delta n_\nu(\omega)(i\omega)\int\frac{1}{(2\pi)^2}\frac{d^2k}{[-i\omega+D_2k^2+k_{endo}]}, \qquad \text{(S121)}$$

which can be evaluated explicitly (the same integral was evaluated in Eq. S100)

$$\delta c\,(\boldsymbol{x_\nu},\omega) = \delta n_\nu(\omega)(i\omega)\frac{\ln\sqrt{\left(\frac{D_2}{a^2k_{endo}}+1\right)}}{2\pi D_2}. \qquad \text{(S122)}$$

The term corresponding to μ≠ν:

$$\delta c\,(\boldsymbol{x_\nu},\omega{\sim}0,\mu\neq\nu) = \frac{1}{(2\pi)^2D_2}(i\omega)\sum_{(\mu\neq\nu)=1}^{m}\int\frac{d^2k\;\delta n_\mu(\omega)}{\left[-\frac{i\omega}{D_2}+k^2+\frac{k_{endo}}{D_2}\right]}e^{i\boldsymbol{k}\cdot(-\boldsymbol{x}_\mu+\boldsymbol{x_\nu})}. \quad \text{(S123)}$$

The integral term appearing in Eq. S116 (for $\omega{\sim}0$) is the 2D inverse Fourier transform of the generalized function $\frac{1}{\left[k^2+\frac{k_{endo}}{D_2}\right]}$. This was computed already (Eqs.S30-S34).

Therefore, Eq. S116 can be rewritten as

$$\delta c\,(\boldsymbol{x_\nu}, \omega{\sim}0, \mu \neq \nu) = \frac{1}{(2\pi)^2 D_2}(i\omega)\sum_{(\mu\neq\nu)=1}^{m}\delta n_\mu(\omega)(2\pi)K_0\left(\sqrt{\frac{k_d}{D_2}}\left|\boldsymbol{x_\mu}-\boldsymbol{x_\nu}\right|\right).\ (S124)$$

This can be expressed in terms of the diffusion length λ (Eq. S84)

$$\delta c\,(\boldsymbol{x_\nu}, \omega{\sim}0, \mu \neq \nu) = \frac{1}{(2\pi)^2 D_2}(i\omega)\sum_{(\mu\neq\nu)=1}^{m}\delta n_\mu(\omega)(2\pi)K_0\left(\frac{\left|(x_\mu-\boldsymbol{x_\nu}\right|}{\lambda}\right). \quad (S125)$$

Now, the Fourier transform of Eq. S118 is

$$\text{-}i\omega\delta n_\mu = k_+\bar{c_\mu}\left(1-\overline{n_\mu}\right)\frac{\delta F(\omega)}{kT} + k_+\left(1-\overline{n_\mu}\right)\delta c\left(x_\mu,\omega\right) - \left(\overline{k_+}\bar{c_\mu} + \overline{k_-}\right)\delta n_\mu. \quad (S126)$$

Substituting Eq. S122 and Eq. S123 into Eq. S126 and summing over all receptors, we obtain

$$-i\omega\delta N(\omega) = -\left[(k_+\bar{c}+k_-) - (i\omega)k_+(1-\bar{n})\frac{\ln\sqrt{\left(\frac{D_2}{a^2k_{endo}}+1\right)}}{2\pi D_2}\right]\delta N + k_+(1-$$

$$\bar{n})\frac{(i\omega)}{(2\pi)^2 D_2}\sum_{\nu=1}^{m}\sum_{\mu\neq\nu}\delta n_\mu(\omega)\ K_0(\left|(\boldsymbol{x_\mu}-\boldsymbol{x_\nu})\right|/\lambda)(2\pi) + mk_+(1-\bar{n})\bar{c}\frac{\delta F(\omega)}{kT}.\ (S127)$$

Then,

$$-i\omega\delta N(\omega) =$$

$$-\left[\left(\overline{k_+}\,\bar{c}+\overline{k_-}\right) - (i\omega)\overline{k_+}(1-\bar{n})\frac{\ln\left(\frac{\lambda}{a}\right)}{2\pi D_2}\right]\delta N +$$

$$\overline{k_+}(1-\bar{n})\frac{(i\omega)}{2\pi D_2}\sum_{\nu=1}^{m}\sum_{\mu\neq\nu}\delta n_\mu(\omega)\ K_0(\left|(\boldsymbol{x_\mu}-\boldsymbol{x_\nu})\right|/\lambda) + m\overline{k_+}(1-\bar{n})\bar{c}\frac{\delta F(\omega)}{kT}.\ (S128)$$

Here we added over all receptors and defined the total occupancy of the receptor cluster as $\delta N(\omega) = \sum_{\mu=1}^{m}\delta n_\mu$.

In cases where the inner sum is independent of x_ν (like in the symmetries contemplated in (2)) we rewrite the sum as

$$\sum_{\nu=1}^{m}\sum_{\mu\neq\nu}\delta n_\mu(\omega)\ K_0\left(\frac{\left|(\boldsymbol{x_\mu}-\boldsymbol{x_\nu}\right|}{\lambda}\right) = \delta N(\omega)\sum_{\mu=2}K_0\left(\frac{\left|(x_\mu-x_1\right|}{\lambda}\right). \quad (S129)$$

For the sake of simplicity, we will assume a cluster of m receptors of size *a* distributed equidistantly along a ring of radius *s* (Eqs. S36-S37). This assumption allows us to simplify Eq. S129.

Then following a similar procedure to the one used to calculate the accuracy of ligand concentration measurement by one receptor, we obtain

$$S_F(\omega{\sim}0) = -2k_BT[-\frac{\ln\left(\frac{\lambda}{a}\right)}{2\pi\bar{c}mD_2} - \frac{1}{\overline{k_+}(1-\bar{n})m\bar{c}} - \frac{\sum_{i=1}^{m-1}K_0\left(\frac{2s}{\lambda}\sin\left(\frac{\pi i}{m}\right)\right)}{2\pi\bar{c}mD_2}]. \quad (S130)$$

So, using Eq. S111 and Eq. S129 we obtain

$$S_c(\omega\sim 0) = 2\bar{c}\left[\frac{\ln(\frac{\lambda}{a})}{2\pi m D_2} + \frac{1}{k_+(1-\bar{n})m} + \frac{\sum_{i=1}^{m-1} K_0(\frac{2s}{\lambda}\sin(\frac{\pi i}{m}))}{2\pi m D_2}\right]. \qquad \text{(S131)}$$

And therefore

$$\left(\frac{\delta c}{\bar{c}}\right)^2 = \left[\frac{\ln(\frac{\lambda}{a})}{\pi m D_2 \bar{c}\tau} + \frac{2}{k_+(1-\bar{n})\tau m\bar{c}} + \frac{\sum_{i=1}^{m-1} K_0(\frac{2s}{\lambda}\sin(\frac{\pi i}{m}))}{\pi m D_2 \bar{c}\tau}\right], \qquad \text{(S132)}$$

Hence

$$\left(\frac{\delta c}{\bar{c}}\right) > \frac{1}{\sqrt{\pi D_2 \bar{c}\tau m}}\sqrt{\ln(\frac{\lambda}{a}) + \sum_{i=1}^{m-1} K_0\left(\frac{2s}{\lambda}\sin\left(\frac{\pi i}{m}\right)\right)}. \qquad \text{(S133)}$$

Eq. S133 is similar to Eq. S39. Then again for the limiting case $\frac{s}{\lambda} \ll 1$

we obtain the final expression

$$\frac{\delta c}{\bar{c}} > \frac{1}{\sqrt{\pi D_2 \bar{c}\tau}}\sqrt{\frac{\ln\left(\frac{\lambda}{ma}\right)}{m} + \left(\frac{m-1}{m}\right)\ln\left(1.1228\frac{\lambda}{s}\right)}, \qquad \text{(S134)}$$

(see Eq. S44).

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