Supporting Information: The Berg-Purcell limit revisited

Kazunari Kaizu,[1] Wiet de Ronde,[2] Joris Paijmans,[2] Koichi Takahashi,[1] Filipe Tostevin,[2] and Pieter Rein ten Wolde[2]
[1]*RIKEN Quantitative Biology Center, Osaka 5650874, Japan*
[2]*FOM Institute AMOLF, Science Park 104, 1098 XG Amsterdam, The Netherlands*

RELATIONS BETWEEN THE VARIANCE OF A TIME-ESTIMATE, THE CORRELATION FUNCTION AND THE POWER SPECTRUM

In this section we briefly describe how the variance of a time estimate of a specific quantity is related to the correlation function. We describe the state of a receptor with the variable n, where $n = 0, 1$ depending on whether the receptor is free or bound, respectively. The estimate n_T of the receptor occupancy $\overline{n}$, obtained by integrating $n(t)$ over an integration time T, is

$$n_T = \frac{1}{T}\int_0^T n(t)dt. \tag{S1}$$

The variance in n_T, $\sigma^2_{n_T}$, is given by

$$\sigma^2_{n_T} = \langle n_T^2\rangle - \langle n_T\rangle^2 \tag{S2}$$
$$= \frac{1}{T^2}\int_0^T\int_0^T dtdt' \langle n(t)\, n(t')\rangle - \langle n_T\rangle^2 \tag{S3}$$
$$= \frac{1}{T^2}\int_0^T\int_{-t}^{T-t} dtd\tau \langle n(0)\, n(\tau)\rangle - \langle n\rangle^2, \tag{S4}$$

where in Eq. S4 we have defined $\tau = t' - t$ and assumed the process to be stationary. The angular brackets denote an ensemble average over a large number of independent measurements. The correlation function for the observable $n(t)$ is defined as

$$C_n(\tau) \equiv \langle n(0)\, n(\tau)\rangle - \langle n\rangle^2. \tag{S5}$$

Substitution of Eq. S5 into Eq. S4 yields

$$\sigma^2_{n_T} = \frac{1}{T^2}\int_0^T dt\int_{-t}^{T-t} d\tau C(\tau), \tag{S6}$$

which in the limit of large T becomes

$$\sigma^2_{n_T} \underset{T\gg\tau_n}{=} \frac{1}{T}\int_{-\infty}^{\infty} d\tau C_n(\tau)$$
$$= \frac{2\sigma_n^2\tau_n}{T}. \tag{S7}$$

Here we have used the fact that $\lim_{\tau\gg\tau_n} C_n(\tau) = 0$ and introduced the correlation time τ_n:

$$\tau_n \equiv \frac{1}{\sigma_n^2}\int_0^\infty C_n(\tau)\, d\tau. \tag{S8}$$

The correlation function and the power spectrum $P_n(\omega)$ are related through the Fourier Transform

$$C_n(\tau) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} d\omega P_n(\omega)\, e^{i\omega\tau}, \tag{S9}$$
$$P_n(\omega) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} d\tau C_n(\tau)\, e^{-i\omega\tau}, \tag{S10}$$

such that

$$C_n(0) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} d\omega P_n(\omega) = \sigma_n^2, \tag{S11}$$
$$P_n(0) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} d\tau C_n(\tau) = 2\sigma_n^2\tau_n = T\sigma^2_{n_T}. \tag{S12}$$

Because the correlation function is real and even in time, we have

$$P_n(\omega) = \hat{C}_n(s = i\omega) + \hat{C}_n(s = -i\omega), \tag{S13}$$

where $\hat{C}_n(s) = \int_0^\infty C_n(t)e^{-st}$ is the Laplce transform of the correlation function. The correlation time is therefore related to the Laplace transform of the correlation function by

$$2\sigma_n^2\tau_n = P_n(0) \tag{S14}$$
$$= 2\mathrm{Re}\left[\hat{C}(s = i\omega)\right]_{\omega=0}. \tag{S15}$$

TIME-DEPENDENT RATE CONSTANTS

Following Agmon and Szabo [1], we consider a single static receptor at the origin and a single ligand molecule that moves with diffusion constant D. The probability that the ligand molecule is at distance r at time t given that it was initially at a distance r_0 is given by the Green's function $p(r, t|r_0)$. The evolution of the Green's function is given by the diffusion equation

$$\frac{\partial p(r,t|r_0)}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}Dr^2e^{-\beta U(r)}\frac{\partial}{\partial r}e^{\beta U(r)}p(r,t|r_0), \tag{S16}$$

where β is the inverse temperature and $U(r)$ is the interaction potential. The reaction between receptor and ligand is modeled as a boundary condition to the solution of this equation. If receptor and ligand can associate with the intrinsic association rate k_a when they are at the contact distance $r = \sigma$, then the boundary condition is

$$4\pi\sigma^2 D\left.\frac{\partial p(r,t|r_0)}{\partial r}\right|_{r=\sigma} = k_a p(\sigma, t|r_0). \tag{S17}$$

If k_a is finite, then the boundary condition is called a radiation boundary condition, while if $k_a \to \infty$, the boundary condition is an absorbing coundition. The latter must be used to obtain the rate constant of diffision-limited reactions, where receptor and ligand associate upon the first collision.

The survival probability $S_\alpha(t|r_0)$ is the probability that a particle, which starts at a position r_0, has not yet reacted at a later time t. It is given by

$$S_\alpha(t|r_0) = 4\pi \int_\sigma^\infty dr r^2 p(r,t|r_0). \tag{S18}$$

The subscript α is either "rad" or "abs", corresponding to k_a being finite or infinite, respectively.

The propensity function $R_\alpha(t|r_0)$ is the probabiltiy that a ligand particle, which starts at $r = r_0$, reacts for the first time at a later time t:

$$R_\alpha(t|r_0) = -\frac{\partial S_\alpha(t|r_0)}{\partial t}. \tag{S19}$$

The time-dependent rate constant $k_\alpha(t)$ is

$$k_\alpha(t) = 4\pi \int_\sigma^\infty dr_0 r_0^2 R_\alpha(t|r_0) p_{\rm eq}(r_0). \tag{S20}$$

The distribution $p_{\rm eq}(r_0)$ is the equilibrium radial distribution function, $p_{\rm eq}(r) = e^{-\beta U(r)}$. If ligand and receptor only interact at contact as assumed in this study, then $U(r) = 0$ for $r \geq \sigma$ and $p_{\rm eq} = 1$, meaning that the equilibrium distribution corresponds to a spatially uniform distribution. The time-dependent rate constant $k_\alpha(t)$ divided by the volume V is the probability per unit amount of time that receptor and ligand associate for the first time at a later time t, averaged over all initial positions r_0 drawn from the equilibrium distribution $p_{\rm eq}(r_0)$.

For N non-interacting ligand molecules in a volume V at concentration $c = N/V$, which initially have an equilibrium distribution, the probability that no ligand molecules have reacted at a later time t is $\mathscr{S}_\alpha(t|{\rm eq})$. In the limit that $V \to \infty$ and $N \to \infty$, the probability that a ligand molecule reacts for the first time at a later time t is

$$-\frac{\partial \mathscr{S}_\alpha(t|{\rm eq})}{\partial t} = k_{\rm rad}(t) c \mathscr{S}_\alpha(t|{\rm eq}). \tag{S21}$$

This can be integrated to yield

$$\mathscr{S}_\alpha(t|{\rm eq}) = e^{-c\int_0^t dt' k_\alpha(t')}. \tag{S22}$$

The expressions Eq. S18 - Eq. S22 hold for both radiating and absorbing boundary conditions, corresponding to k_a being finite and infinite, respectively. When k_a is finite, $R_{\rm rad}(t|r_0)$ is also given by

$$R_{\rm rad}(t|r_0) = k_a p(\sigma, t|r_0) \tag{S23}$$

and the time-dependent rate constant $k_{\rm rad}(t)$ is then also given by

$$k_{\rm rad}(t) = 4\pi k_a \int_\sigma^\infty dr_0 r_0^2 p(\sigma, t|r_0) p_{\rm eq}(r_0) \tag{S24}$$

To relate $k_{\rm rad}(t)$ to $k_{\rm abs}(t)$ in what follows below it will be useful to exploit the detailed-balance condition

$$p_{\rm eq}(r_0) p(r, t|r_0) = p_{\rm eq}(r) p(r_0, t|r). \tag{S25}$$

We can integrate this equation over r_0 to find

$$4\pi \int dr_0 r_0^2 p(r,t|r_0) p_{\rm eq}(r_0) = p_{\rm eq}(r) S_\alpha(t|r). \tag{S26}$$

Combining this equation with Eq. S24 we find that

$$k_{\rm rad}(t) = p_{\rm eq}(\sigma) k_a S_{\rm rad}(t|\sigma), \tag{S27}$$

which for $V(r) = 0$, as assumed here, reduces to

$$k_{\rm rad}(t) = k_a S_{\rm rad}(t|\sigma). \tag{S28}$$

The time-dependent rate constant $k_{\rm rad}(t)$ can be related to the time-dependent rate constant $k_{\rm abs}(t)$ via

$$k_{\rm rad}(t) = \int_0^t dt' R_{\rm rad}(t - t'|\sigma) k_{\rm abs}(t'). \tag{S29}$$

This can be understood by noting that $k_{\rm abs}(t')/V$ is the probability per unit amount of time that receptor and ligand come in contact for the first time at time t', while $R_{\rm rad}(t-t'|\sigma)$ is the probability that receptor and ligand which start at contact $r = \sigma$ at time t' associate a time $t - t'$ later. In Laplace space, the above expression reads

$$\hat{k}_{\rm rad}(s) = \hat{R}_{\rm rad}(s|\sigma) \hat{k}_{\rm abs}(s). \tag{S30}$$

Since $R_{\rm rad}(t|\sigma) = -\partial S_{\rm rad}(t|\sigma)/\partial t$, $\hat{R}_{\rm rad}(s|\sigma)$ is also given by

$$\hat{R}_{\rm rad}(s|\sigma) = 1 - s\hat{S}_{\rm rad}(s|\sigma). \tag{S31}$$

The Laplace transform of Eq. S28 yields $\hat{k}_{\rm rad}(s) = k_a \hat{S}_{\rm rad}(s|\sigma)$. Combining this with Eq. S30 and Eq. S31 yields

$$\hat{k}_{\rm rad}(s) = \frac{k_a \hat{k}_{\rm abs}(s)}{k_a + s\hat{k}_{\rm abs}(s)}. \tag{S32}$$

DERIVATION OF EQ. 13 OF MAIN TEXT

In this section we derive the correlation function and the correlation time for a receptor which switches between a ligand-bound state $n = 1$ and a ligand-unbound state $n = 0$. The correlation function for the receptor state n is

$$C_n(\tau) = p_*^0 \left(p_{*|*}(\tau) - p_*^0 \right), \tag{S33}$$

where $p_*^0 = \langle n \rangle = \overline{n}$ is the equilibrium probability that the receptor is bound $(*)$ and $p_{*|*}(\tau)$ is the probability that the receptor is bound at time $t = \tau$, given that it was bound initially. For every two-state process we can write

$$p_{*|*}(\tau) = 1 - p_{0|*}(\tau) \tag{S34}$$
$$= 1 - \mathscr{S}_{\rm rev}(\tau|*), \tag{S35}$$

where $\mathscr{S}_{\rm rev}(\tau|*)$ is the probability that the receptor is free at time τ given that it was bound initially; note that in between the receptor may have switched between the bound and unbound state many times. In Laplace space

$$\hat{p}_{*|*}(s) = s^{-1} - \hat{\mathscr{S}}_{\rm rev}(s|*), \tag{S36}$$

such that we have

$$\hat{C}_n(s) = p_*^0 \left(s^{-1} - \hat{\mathscr{S}}_{\rm rev}(s|*) - \frac{p_*^0}{s} \right). \tag{S37}$$

The initial value theorem states that the $s \to \infty$ limit of $s\hat{C}_n(s)$ in the Laplace domain is equal to the $t \to 0$ limit of $C(t)$ in the time domain, thus

$$\lim_{s\to\infty} s\hat{C}_n(s) = \lim_{t\to 0} C_n(t) = \sigma_n^2. \tag{S38}$$

For a binary process the variance is

$$\sigma_n^2 = p_*^0\left(1 - p_*^0\right) = \overline{n}(1-\overline{n}). \tag{S39}$$

The Laplace transform of Eq. 8 of the main text yields

$$\mathcal{L}\left[\mathscr{S}_{\rm rev}(t|*) = k_d \int_0^t \left[1 - \mathscr{S}_{\rm rev}(t'|*)\right] \mathscr{S}_{\rm rev}(t-t'|\sigma)\, dt'\right] \tag{S40}$$

$$\implies s\hat{\mathscr{S}}_{\rm rev}(s|*) = \frac{k_d \hat{\mathscr{S}}_{\rm rad}(s|\sigma)}{1 + k_d \hat{\mathscr{S}}_{\rm rad}(s|\sigma)}. \tag{S41}$$

We assume the ligand particles are non-interacting and, following Agmon and Szabo [1], we approximate the survival probability as

$$\mathscr{S}_{\rm rad}(t|\sigma) = \mathscr{S}_{\rm rad}(t|{\rm eq})\, S_{\rm rad}(t|\sigma). \tag{S42}$$

This is Eq. 9 of the main text. Here $S_{\rm rad}(t|\sigma)$ is the survival probability for the geminate receptor-ligand pair at contact; it is the probability that a receptor which initially is surrounded by *only one* ligand molecule at contact, is still free at a later time t. The quantity $\mathscr{S}_{\rm rad}(t|{\rm eq})$ is the survival probability for the receptor in a sea of equilibrated ligand molecules. In the main text we elaborate on the assumptions underlying this approximation.

We now relate the survival probability $\mathscr{S}_{\rm rad}(t|{\rm eq})$ for a receptor surrounded by an equilibrium distribution of ligand molecules to the survival probability $S_{\rm rad}(t|\sigma)$ for a receptor with only ligand molecule at contact. To this end, we exploit that both survival probabilities can be related to the time-dependent rate constant $k_{\rm rad}(t)$. As shown in the previous section (Eq. S28), detailed balance yields [1]

$$k_{\rm rad}(t) = k_a S_{\rm rad}(t|\sigma). \tag{S43}$$

On the other hand, we know that $\mathscr{S}_{\rm rad}(t|{\rm eq})$ is given by (see Eq. S22 in previous section)

$$\mathscr{S}_{\rm rad}(t|{\rm eq}) = e^{-c\int_0^t k_{\rm rad}(t')dt'}. \tag{S44}$$

Combining these two equations yields the following expression for the time derivative of $\mathscr{S}_{\rm rad}(t|{\rm eq})$:

$$\frac{d\mathscr{S}_{\rm rad}(t|{\rm eq})}{dt} = -ck_{\rm rad}(t)\,\mathscr{S}_{\rm rad}(t|{\rm eq}) \tag{S45}$$
$$= -ck_a S_{\rm rad}(t|\sigma)\,\mathscr{S}_{\rm rad}(t|{\rm eq}) \tag{S46}$$
$$= -ck_a \mathscr{S}_{\rm rad}(t|\sigma). \tag{S47}$$

The Laplace transform of Eq. S47 is

$$s\hat{\mathscr{S}}_{\rm rad}(s|{\rm eq}) - 1 = -ck_a \hat{\mathscr{S}}_{\rm rad}(s|\sigma). \tag{S48}$$

Combining this equation with Eq. S41 gives

$$1 - s\hat{\mathscr{S}}_{\rm rev}(s|*) = \frac{cK_{\rm eq}}{1 + cK_{\rm eq} - s\hat{\mathscr{S}}_{\rm rad}(s|{\rm eq})}, \tag{S49}$$

where $K_{\rm eq} = k_a/k_d$ is the equilibrium constant. Substituting this result in Eq. S37, we find

$$\hat{C}_n(s) = \frac{\overline{n}}{s}\left(1 - s\hat{\mathscr{S}}_{\rm rev}(s|*) - \overline{n}\right)$$
$$= \frac{n}{s}\left(\frac{cK_{\rm eq}(1-\overline{n}) + \overline{n}s\hat{\mathscr{S}}_{\rm rad}(s|{\rm eq}) - \overline{n}}{1 + cK_{\rm eq} - s\hat{\mathscr{S}}_{\rm rad}(s|{\rm eq})}\right)$$
$$= \sigma_n^2 \frac{\overline{n}\hat{\mathscr{S}}_{\rm rad}(s|{\rm eq})}{1 - (1-\overline{n})\, s\hat{\mathscr{S}}_{\rm rad}(s|{\rm eq})}, \tag{S50}$$

where in deriving the last line we have used that $cK_{\rm eq} = \overline{n}/(1-\overline{n})$ and $\sigma_n^2 = \overline{n}(1-\overline{n})$. Noting that $\overline{n} = k_a c \tau_c$ and $1 - \overline{n} = k_d \tau_c$, with $\tau_c = (k_a c + k_d)^{-1}$ the intrinsic correlation time, Eq. 11 of the main text is obtained.

To continue, an expression for $\hat{\mathscr{S}}_{\rm rad}(s|{\rm eq})$ is required. A general expression for the Laplace transform of Eq. S44 is not available. We can, however, expand $\mathscr{S}_{\rm rad}(t|{\rm eq})$,

$$\mathscr{S}_{\rm rad}(t|{\rm eq}) = e^{-c\int_0^t k_{\rm rad}(t')dt'} \tag{S51}$$
$$\approx 1 - c\int_0^t k_{\rm rad}(t')\,dt' + \ldots, \tag{S52}$$

and now take the Laplace transform [1]:

$$\hat{\mathscr{S}}_{\rm rad}(s|{\rm eq}) = s^{-1} - s^{-1}c\hat{k}_{\rm rad}(s) + \ldots \tag{S53}$$
$$\approx s^{-1}\left(1 + c\hat{k}_{\rm rad}(s)\right)^{-1}. \tag{S54}$$

For small times and low concentrations, the above approximation is accurate because the higher order terms in

the expansions of Eq. S52 and Eq. S53 can be neglected. For long times, $k_{\rm rad}(t)$ becomes constant, $k_{\rm rad}(t\to\infty) = k_{\rm on}$, and the Laplace transform of $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)$ is exactly given by Eq. S54, with $\hat{k}_{\rm rad}\left(s\right) = k_{\rm on}/s$.

The Laplace transform $\hat{k}_{\rm rad}(s)$ of the time-dependent rate constant $k_{\rm rad}(t)$ can be related to the Laplace transform $\hat{k}_{\rm abs}(s)$ of the time-dependent rate constant $k_{\rm abst}(t)$ of a diffusion-limited reaction via (see previous section) [1]

$$\hat{k}_{\rm rad}(s) = \frac{k_a k_{\rm abs}(s)}{k_a + s k_{\rm abs}(s)}. \tag{S55}$$

The time-dependent rate constant $k_{\rm abs}(t)$ of a diffusion-limited reaction is [2]

$$k_{\rm abs}(t) = 4\pi\sigma D\left(1 + \sigma/\sqrt{\pi D t}\right), \tag{S56}$$

which in the Laplace domain becomes

$$s\hat{k}_{\rm abs}(s) = k_D\left(1 + \sigma\sqrt{s/D}\right), \tag{S57}$$

$$= k_D\left(1 + \tau(s)\right), \tag{S58}$$

where $\tau\left(s\right) \equiv \sigma\sqrt{s/D} = \sqrt{s\tau_m}$ with the molecular time scale $\tau_m = \sigma^2/D$ and $k_D \equiv k_{\rm abs}(t\to\infty) = 4\pi\sigma D$ is the diffusion-limited reaction rate. Substituting this in Eq. S55 gives

$$\hat{k}_{\rm rad}(s) = \frac{k_a k_D}{s}\frac{1+\tau\left(s\right)}{k_a + k_D\left(1+\tau\left(s\right)\right)}, \tag{S59}$$

which can be plugged into Eq. S54 to yield

$$\hat{\mathscr{S}}_{\rm rad}\left(s|{\rm eq}\right) \approx \frac{k_a + k_D\left(1+\tau\left(s\right)\right)}{s\left(k_a + k_D\left(1+\tau\left(s\right)\right)\right) + c k_a k_D\left(1+\tau\left(s\right)\right)}. \tag{S60}$$

We now insert the above expression into Eq. S50, which gives

$$\begin{aligned}\hat{C}_n\left(s\right) &= \sigma_n^2\frac{\overline{n}\left(k_a + k_D(1+\tau(s))\right)}{c k_a k_D(1+\tau(s)) + \overline{n}s\left(k_a + k_D(1+\tau(s))\right)} \\ &= \sigma_n^2\frac{\tau_c\left(k_a + k_D(1+\tau(s))\right)}{k_D(1+\tau(s)) + \tau_c s\left(k_a + k_D(1+\tau(s))\right)},\end{aligned} \tag{S61}$$

where we have used that $\overline{n} = k_a c\tau_c$, with $\tau_c = (k_a c + k_d)^{-1}$ the intrinsic correlation time of the receptor. We define

$$\Sigma(s) \equiv \frac{k_a}{k_D(1+\tau(s))} \tag{S62}$$

and the receptor correlation time $\tau_c'(s)$ renormalized by concentration fluctuations:

$$\tau_c'(s) \equiv \tau_c\left(1 + \Sigma(s)\right), \tag{S63}$$

$$= \frac{k_a + k_D(1+\tau(s))}{k_D(1+\tau(s))}. \tag{S64}$$

We now substitute the above expression in Eq. S61 to arrive at Eq. 13 of the main text:

$$\hat{C}_n\left(s\right) = \sigma_n^2\frac{\tau_c'(s)}{s\tau_c'(s)+1}. \tag{S65}$$

To obtain the correlation time, we take the limit $s = 0$ since ${\rm Re}\left[\hat{C}_n\left(s=0\right)\right] = \sigma_n^2\tau_n$ (see Eq. S14):

$$\hat{C}_n\left(s=0\right) = \frac{P_n\left(\omega=0\right)}{2} \tag{S66}$$

$$= \sigma_n^2\frac{k_a + k_D}{\left(k_a c + k_d\right)k_D}. \tag{S67}$$

Hence, the receptor correlation time, normalized by concentration fluctuations, is

$$\tau_n = \tau_c'(s\to 0), \tag{S68}$$

$$= \frac{k_a + k_D}{\left(k_a c + k_d\right)k_D}. \tag{S69}$$

VALIDITY OF ASSUMPTIONS UNDER BIOLOGICALLY RELEVANT CONDITIONS

Our theory makes two assumptions: **I** After receptor dissociation, the unbound receptor-ligand pair is surrounded by a uniform distribution of ligand molecules; this is described by Eq. 9 of the main text and Eq. S42 of the SI; **II** the Laplace transform of $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)$ is given by Eq. 12 of the main text and Eq. S54 of the SI. Below, we address the validity of these two assumptions. But before doing so, we give a qualitative argument for why both assumptions are satisfied.

Timescale separation

The validity of both assumptions is rooted in a timescale separation: the time a molecule spends near the receptor is very short compared to the timescale on which molecules bind the receptor from the bulk, *i.e.* starting from a uniform distribution. This is illustrated in Fig. S1. It is seen that $S_{\rm rad}\left(t|\sigma\right)$ (blue dashed line) decays much more rapidly than $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)$ (red solid line). Indeed, a ligand molecule near the receptor effectively either instantly (re)binds the receptor or diffuses into the bulk. Consequently, the probability of rebinding interference is very small, meaning that assumption **I** is satisfied.

The second assumption **II** follows from the observation that the range over which $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right) = e^{-c\int_0^t dt' k_{\rm rad}(t')}$ deviates from its exponential decay at long times, $\lim_{t\to\infty}\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right) \approx e^{-k_{\rm on}ct}$, is given by $S_{\rm rad}\left(t|\sigma\right)$—the rate at which $k_{\rm rad}(t)$ reaches its limiting value $k_{\rm on}$ is given by $S_{\rm rad}\left(t|\sigma\right)$, see Eq. S43. Because $S_{\rm rad}\left(t|\sigma\right)$ decays much faster than $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)$ (see Fig. S1), $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)$ can be

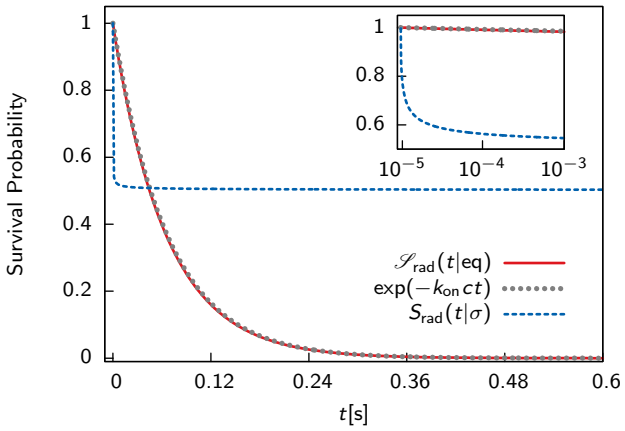

FIG. S1. Illustration of timescale separation, which underlies the validity of our theory and provides the motivation for the simple-coarse-grained model. The survival probability of a free receptor surrounded by only one ligand molecule which initially is at contact, $S_{\rm rad}(t|\sigma)$ (blue dashed line), decays much more rapidly than the survival probability for a free receptor that is initially surrounded by an equilibrium, uniform distribution of ligand molecules, $\mathscr{S}_{\rm rad}(t|{\rm eq}) = e^{-c\int_0^t k_{\rm rad}(t')dt'}$ (solid red line). This shows that the time a molecule spends near the receptor (given by the decay of $S_{\rm rad}(t|\sigma)$) is very small compared to the average time on which molecules arrive from the bulk (given by $\tau_{\rm off} = \int_0^\infty dt' \mathscr{S}_{\rm rad}(t'|{\rm eq})$). Indeed, on this timescale, a ligand molecule near the receptor effectively either instantly (re)binds the receptor or diffuses into the bulk. This separation of timescales means that rebinding interference occurs only rarely (see also Fig. S3) and that assumption **I** of our theory, Eq. S42, is satisfied; it also makes it possible to integrate out the rebindings. The figure also shows that $\mathscr{S}_{\rm rad}(t|{\rm eq})$ is well approximated by its long-time behavior $\lim_{t\to\infty}\mathscr{S}_{\rm rad}(t|{\rm eq}) \approx e^{-k_{\rm on}ct}$ (dotted grey line). This means that bulk molecules arrive at the receptor in a memoryless fashion at a constant rate $k_{\rm on}c$ and that assumption **II** is satisfied. Parameters: $\sigma = 10\,{\rm nm}$, $c = 0.4\mu{\rm M}$, $D = 1\,\mu{\rm m}^2{\rm s}^{-1}$, $k_a = 75\,\mu{\rm M}^{-1}{\rm s}^{-1}$, $k_D = 75\,\mu{\rm M}^{-1}{\rm s}^{-1}$.

well approximated by its long-time limit $\mathscr{S}_{\rm rad}(t|{\rm eq}) \approx e^{-k_{\rm on}ct}$. This means that also assumption II is satisfied.

We now address both assumptions quantitatively.

Assumption I: the equilibrium assumption

As discussed in the main text, the equilibrium assumption, Eq. 9 of the main text (Eq. S42), holds when there is no rebinding interference. To address the probability of rebinding interference, we first study the propensity for receptor binding given that a ligand molecule has just dissociated and now is at contact with the receptor; the other molecules have the equilibrium, uniform distribution. From Eq. 9 of the main text (Eq. S42) we have

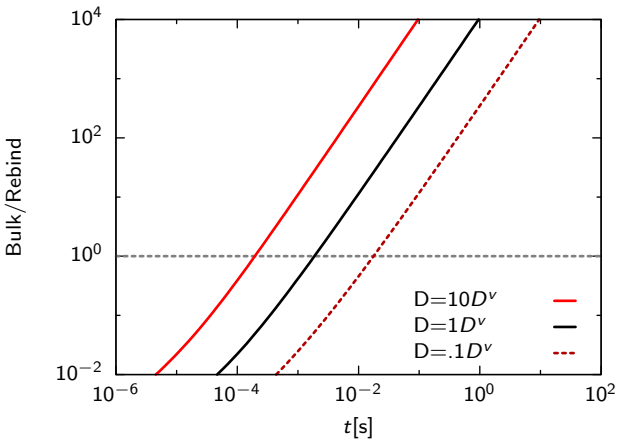

FIG. S2. The ratio of the change in $\mathscr{S}_{\rm rad}$ due to a bulk binding and a rebinding of a dissociated ligand particle; this is the ratio of the two terms on the right-hand-side of Eq. S70. The bulk dominates the propensity function already at very small t. Parameters: $\sigma = 10\,{\rm nm}$, $c = 0.4\,\mu{\rm M}$, $D^v = 1\,\mu{\rm m}^2\,{\rm s}^{-1}$, $k_a = 75\,\mu{\rm M}^{-1}\,{\rm s}^{-1}$.

$$\frac{d\mathscr{S}_{\rm rad}(t|\sigma)}{dt} = \overbrace{S_{\rm rad}(t|\sigma)\frac{d\mathscr{S}_{\rm rad}(t|{\rm eq})}{dt}}^{\rm bulk-binding} + \overbrace{\mathscr{S}_{\rm rad}(t|{\rm eq})\frac{dS_{\rm rad}(t|\sigma)}{dt}}^{\rm rebinding}. \quad ({\rm S70})$$

This is the propensity function for receptor binding, *i.e.* the probability that a receptor with a ligand molecule at contact and surrounded by a uniform distribution of ligand molecules, binds a ligand molecule for the first time at a later time t. The first term is the probability that this ligand molecule is a molecule from the bulk, while the second gives the probability that this is the ligand molecule that was in contact. Fig. S2 shows the ratio of these two terms. It is seen that only at very short times, rebindings dominate the bulk bindings. For long times, receptor binding is completely dominated by the binding of molecules from the bulk, which in our theory, as well as in the simulations, bind the receptor in a memoryless fashion (see below for further details).

Eq. S70 allows us to derive an expression for the probability $p_{\rm int}$ that an interference occurs. Here, interference is defined as an event in which the binding of a molecule from the bulk pre-empts, and thereby prevents, the receptor rebinding of a molecule that has just dissociated from the receptor. If the unbound receptor-ligand pair at contact is surrounded by an equilibrium distribution of ligand molecules, then the probability that a ligand molecule from the bulk does not interfere with the recep-

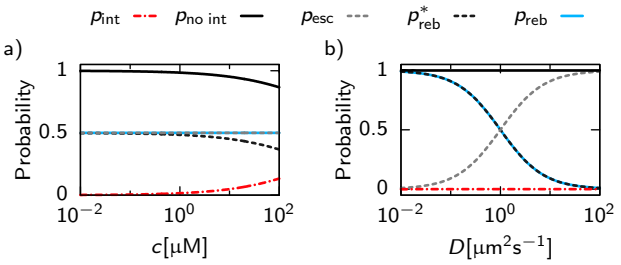

FIG. S3. Probability $p_{\rm int}$ (red dash-dotted line) that a ligand molecule from the bulk interferes with the receptor rebinding of a dissociated ligand molecule as a function of the concentration c (a) and diffusion constant D (b). The probability $1-p_{\rm int}$ of no interference (black line) consists of two terms (see Eq. S71): the probability $p^*_{\rm reb}$ that the dissociated ligand molecule rebinds the receptor before a molecule from the bulk does (dashed black line) and the probability $p_{\rm esc}=S_{\rm rad}(\infty|\sigma)=k_D/(k_a+k_D)=1-p_{\rm reb}$ that it escapes into the bulk (dashed gray line; second term). It is seen that for concentrations $c\lesssim\mu$M bulk molecules hardly interfere with the receptor rebinding of dissociated ligand molecules. This is the motivation for the simplified model of Fig. 3 of the main text. In the simplified coarse-grained model, the probability of rebinding is approximated as $p_{\rm reb}=1-p_{\rm esc}\approx S_{\rm rad}(t\to\infty|\sigma)=k_a/(k_a+k_D)$ (solid blue line); this corresponds to assuming that in the expression for $p_{\rm reb}$, the first term on the right-hand side of Eq. S71, $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)\approx 1$. See also Fig. S1. Parameters: $k_a=75\,\mu{\rm M}^{-1}{\rm s}^{-1}$, $\sigma=10\,{\rm nm}$; in panel a) $D=1\,\mu{\rm m}^2{\rm s}^{-1}$ and $k_D=75\,\mu{\rm M}^{-1}{\rm s}^{-1}$; because $k_a=k_D$, $p_{\rm reb}=p_{\rm esc}=0.5$; in panel b) $c=0.4\mu$M.

tor rebinding of the ligand molecule at contact, is

$$1-p_{\rm int}=-\int_0^\infty\frac{dS_{\rm rad}\left(t|\sigma\right)}{dt}\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)dt+S_{\rm rad}\left(\infty|\sigma\right). \tag{S71}$$

Here the first term on the right-hand-side is the integral of the second term on the right-hand-side of Eq. S70 — it is the probability that the ligand molecule which has just dissociated from the receptor rebinds the receptor before a ligand molecule from the bulk does; the second term $S_{\rm rad}(\infty|\sigma)$ is the probability that the ligand molecule at contact (with no other ligand molecules present) escapes into the bulk — when the ligand at contact escapes into the bulk, then, by definition, it does not rebind and rebindig interference therefore does not arise. Combining the above expression with Eq. S70 shows that the probability of rebinding interference is

$$p_{\rm int}=-\int_0^\infty dt\left[S_{\rm rad}\left(t|\sigma\right)-S_{\rm rad}\left(\infty|\sigma\right)\right]\frac{d\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)}{dt}, \tag{S72}$$

where $\left[S_{\rm rad}\left(t|\sigma\right)-S_{\rm rad}\left(\infty|\sigma\right)\right]d\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)/dt$ is the probability that a molecule from the bulk binds the receptor before the ligand molecule which started at contact and that would have rebound the receptor if there were no other ligand molecules, does. Fig. S3 shows that for biologically relevant concentrations and diffusion constants, the probability of rebinding interference, $p_{\rm int}$, is very small, which means that the central assumption holds.

Assumption II: Survival probablity of a receptor surrounded by an equilibrium distribution of particles

The second assumption is that the Laplace transform of $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)$ is given by Eq. 12 of the main text and Eq. S54 of the *SI*. As mentioned above, this expression captures both the short- and long-time limit of the survival probabiliy. Fig. S1 illustrates, however, that biologically relevant concentrations are so low that, to a very good approximation, the survival probability is given by its long-time limit: $\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)=e^{-c\int_0^t k_{\rm rad}(t')dt'}\approx e^{-k_{\rm on}ct}$. This means that the molecules other than the one which has dissociated last bind, to an excellent approximation, the receptor in a memoryless fashion with a constant rate $k_{\rm on}c$.

We can quantify this approximation further by investigating the mean unbound time $\tau_{\rm off}=\int_0^\infty\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)dt=\hat{\mathscr{S}}_{\rm rad}\left(s=0|{\rm eq}\right)$; this is the mean waiting time for binding. Eq. S50 shows that the central approximation of our theory, Eq. S42 (Eq. 9 of the main text), predicts that the zero-frequency limit of the power spectrum and hence the correlation time τ_n and the precision of the concentration estimate, are determined by $\overline{n}$ and $\tau_{\rm off}$:

$$\hat{C}_n(s=0)=\sigma_n^2\overline{n}\tau_{\rm off}. \tag{S73}$$

Indeed, because $\hat{C}_n(s=0)=\sigma_n^2\tau_n$ (see Eq. S15), $\tau_n=\overline{n}\tau_{\rm off}$. The exact mean unbound time for a free receptor surrounded by ligand molecules obeying the equilibrium distribution is $\tau_{\rm off}=\int_0^\infty\mathscr{S}_{\rm rad}\left(t|{\rm eq}\right)dt=\int_0^\infty e^{-c\int_0^t k_{\rm rad}(t')dt'}dt$. In contrast, the approximation of Eq. S54 predicts an average unbound time of $1/(k_{\rm on}c)$. Note that while this is the mean waiting time for Markovian binding with rate $k_{\rm on}c$, the approximation of Eq. S54 does *not* assume that binding is Markovian for all times—only in the long-time limit does binding occur with a constant rate. Fig. S4 shows the relative error in the mean unbound time as a function of the concentration. It is seen that for biologically relevant concentrations $c\lesssim\mu$M the relative error is indeed small, less than 10%.

COMMENTS ON COMPARISON BETWEEN THEORY AND SIMULATIONS

Our theory accurately predicts the power spectrum over the full frequency range, even though our simulation box is finite and the theory is for an unbounded

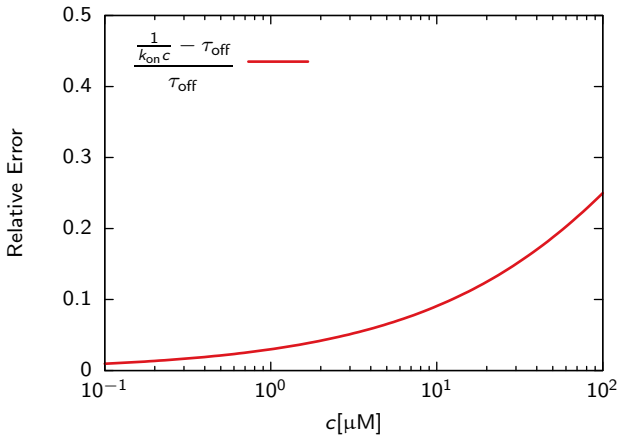

FIG. S4. The relative error in the average off-time, *i.e.* the average waiting time for a new molecule to associate, assuming that the receptor is initially surrounded by a uniform distribution of ligand molecules. The exact unbound time $\tau_{\rm off} \equiv \int_0^\infty \mathscr{S}_{\rm rad}\,(t|{\rm eq})\,dt = \int_0^\infty e^{-c\int_0^t k_{\rm rad}(t')dt'}dt$ while Eq. S54 assumes that it is $1/(k_{\rm on}c)$. Eq. S54 does not assume that binding is Markovian for all times; yet its average waiting time is equal to that of a Markovian binding process. The simple, coarse-grained model assumes binding is Markovian for all times. Note that for $c \lesssim \mu$M the theory and hence the coarse-grained model gives a reasonable prediction for the off time. Parameters: $D = 1\,\mu{\rm m}^2{\rm s}^{-1}$, $k_a = 55\,\mu{\rm M}^{-1}{\rm s}^{-1}$, $\sigma = 10\,{\rm nm}$, $k_D = 75\,\mu{\rm M}^{-1}{\rm s}^{-1}$.

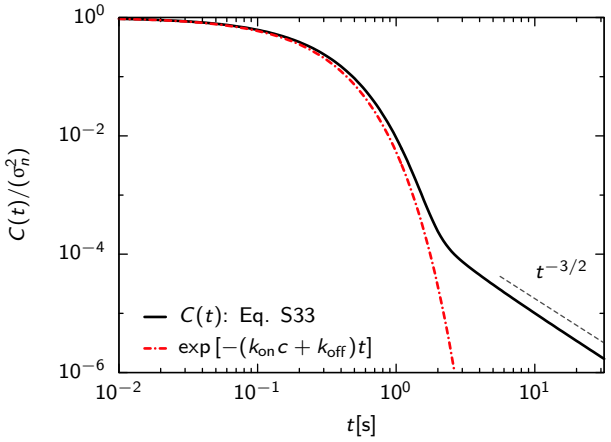

FIG. S5. The correlation function of our theory (black solid line) compared with that of a random telegraph process (red dotted curve). At long times, our theory exhibits a small algebraic tail, which is the remnant of $S_{\rm rad}(t|\sigma)$ in Eq. S42. $D = 1\,\mu{\rm m}^2{\rm s}^{-1}$, $k_a = 75\,\mu{\rm M}^{-1}{\rm s}^{-1}$, $\sigma = 10\,{\rm nm}$, $k_D = 75\,\mu{\rm M}^{-1}{\rm s}^{-1}$, $c = 0.4\,\mu$M.

domain. It is well known that in an unbounded system, the correlation function exhibits an algebraic tail [3, 4]. This is because the relaxation of a fluctuation of the receptor state involves not only the binding and unbinding of ligand, but also the diffusive transport of ligand to and from the receptor. While the binding and unbinding of ligand continually perturbs the ligand concentration profile, diffusion counteracts these perturbations, leading to the relaxation of the concentration profile back to its equilibrium shape. In an unbounded system, the relaxation of the concentration profile involves diffusion-mediated transport of ligand on infinitely long length and time scales, thereby dominating the relaxation of the receptor state at long times. This is the origin of the algebraic tail of the receptor correlation function for diffusion-influenced reactions in an unbounded system.

In contrast, the simulation box of our system is finite and the collisions of the ligand molecules with the cell boundaries randomize their trajectories on time scales longer than L^2/D. Consequently, in the simulations the bulk molecules thus bind the receptor in a Markovian fashion at a constant rate at long times, leading to exponential relaxation of fluctuations of the receptor state at long times.

The correlation function of our theory does exhibit a small algebraic tail (Fig. S5), which is the remnant of the factor $S_{\rm rad}(t|\sigma)$ in Eq. S42—the probability that a free receptor with a single molecule at contact is still free at a later time t *in an unbounded system*. However, in Eq. S42 we assume that the other ligand molecules, forming the "bulk" with survival probability $\mathscr{S}_{\rm rad}\,(t|{\rm eq})$, do have a uniform distribution. At long times $t \gg \tau_m$ these molecules will associate with the receptor in a memoryless fashion with a constant rate $k_{\rm on}c$, as described above (Fig. S1). Moreover, in this limit they dominate receptor binding (Fig. S2), as a result of which the algebraic tail of our theory is very small—smaller than observed for diffusion-influenced reactions in an unbounded domain [3, 4]. Both in our theory and our simulations, at long times the receptor dynamics is thus to a good approximation a random telegraph process with an on rate $k_{\rm on}c$ and an off rate $k_{\rm off}$.

TEST FOR HIGHER CONCENTRATIONS

Fig. S6 shows the zero-frequency limit of the power spectrum $P_n(\omega \to 0)$ for two higher concentrations, $c = 4\mu$M and $c = 36\mu$M, respectively. While for higher concentrations, it becomes increasingly difficult to obtain good statistics, our results suggest that for concentrations up to at least $c = 36\mu$M, our theory accurately predicts the precision by which chemical concentrations can be measured.

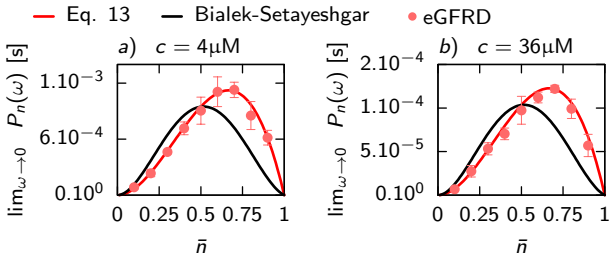

FIG. S6. The zero-frequency limit of the power spectrum as a function of the receptor occupancy $\overline{n}$ for a concentration and box size of $c = 4\mu$M and $L = 0.25\,\mu$m (panel a) and $c = 36\mu$M and $L = 0.80\,\mu$m (panel b), respectively. The red dots show the simulation results, while the red line shows the prediction of our theory. It is seen that the agreement is good, even for the highest concentration. In contrast, the result of Bialek and Setaygeshar deviates markedly from our results. Parameters: $k_a = 552\,\mu\mathrm{M}^{-1}\mathrm{s}^{-1}$; $\sigma = 10$nm and $D = 1\,\mu\mathrm{m}^2\mathrm{s}^{-1}$, such that $k_D = 75\,\mu\mathrm{M}^{-1}\mathrm{s}^{-1}$. The occupancy $\overline{n}$ is varied by changing k_d.

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