Supplementary Material: Information content in stochastic pulse sequences of intracellular messengers

1 SUPPLEMENTARY CALCULATIONS

1.1 Properties of the model in the Poisson and the GNF limits

The model presented in the paper is characterized, on one hand, by the following probability density that, given that a pulse occurred at time 0, the first subsequent pulse occurs at time, $t + T_{cell}$:

$$p(t|\lambda) = \lambda(1 - e^{-\rho t}) \exp(-\int_0^t \lambda(1 - e^{-\rho t'})dt'). \tag{S1}$$

In the model of Skupin and Falcke (2007) and here, the inter-spike time is the sum, $t + T_{cell}$, where T_{cell} is a (fixed) determinisitc component and t a stochastic one. The other component of our model is the relation between $T \equiv \langle t\rangle|_{t|\lambda}$ and the external ligand concentration, C. To this end we use the relationship introduced in Thurley et al. (2014) to explain various experimental observations:

$$T \equiv \langle t\rangle|_{t|\lambda} = A\exp(-BC), \tag{S2}$$

where $\langle\cdot\rangle|_{t|\lambda}$ represents the mean over the distribution, $p(t|\lambda)$. We here derive some properties of the model in the two limits that we are interested in: the Global Negative Feedback (GNF) one which is defined by $x \equiv \lambda/\rho \gg 1$ and the Poisson limit which is defined by $x \ll 1$. As we show in what follows, even if we consider a range of values for λ, if A and B are fixed, ρ can always be chosen so that each of these limits hold over all the range of λ values.

1.1.1 Relationship between the “firing” rate, $\boldsymbol{\lambda}$, and the external ligand concentration, $\boldsymbol{C}$

We show here that Eq. (S2) implies that:

$$\lambda = \alpha\exp(\beta C), \tag{S3}$$

in the GNF and Poisson limits, with α and β fixed parameters that can be functions of ρ.

As it is clear from Eq. (S1), for a given value of λ and in the $x \ll 1$ limit, the model reduces to a Poisson process for which the distribution, $p(t|\lambda)$, is exponential with $T = 1/\lambda$. Introducing this last relation in Eq. (S2) we obtain:

$$\lambda = \frac{1}{A}e^{BC}, \quad \text{for } x \ll 1. \tag{S4}$$

To relate λ and C in the $x \gg 1$ (GNF) limit we use the result of Skupin and Falcke (2007) which shows that the mean, T, for the model of Eq. (S1) is given by:

$$T = \frac{e^x x^{1-x}}{\lambda} \left(\Gamma(x) - \Gamma(x,x) \right), \tag{S5}$$

where $\Gamma(x)$ is the Gamma function, $\Gamma(s,t)$ is the upper incomplete Γ function and $x = \lambda/\rho$. The difference in the r.h.s of Eq. (S5) can be rewritten as:

$$\Gamma(x) - \Gamma(x,x) = \gamma(x,x), \tag{S6}$$

with $\gamma(s,t)$ the lower incomplete Γ function. We now prove that:

$$\lim_{x\to\infty} \frac{e^x}{x^x}\gamma(x+1,x) - \sqrt{\frac{\pi}{2}x} = 0. \tag{S7}$$

In fact, using the generalized Laplace method we obtain:

$$\gamma(x+1,x) = \int_0^x z^x e^{-z} dz \sim x^x e^{-x} \int_0^\infty e^{-\frac{1}{2x}(z-x)^2} dz = \frac{1}{2} x^x e^{-x} \sqrt{2\pi x}, \tag{S8}$$

for $x = \lambda/\rho$ large enough from which Eq. (S7) immediately follows. This in turn implies that:

$$\gamma(x,x) = x^{x-1}e^{-x} + x^{-1}\gamma(x+1,x) = x^{x-1}e^{-x} + x^{x-1}e^{-x}\sqrt{\frac{\pi x}{2}}, \quad x \gg 1. \tag{S9}$$

Combining Eqs. (S9), (S5) and (S6) we obtain:

$$T = \frac{1}{\lambda}\left(1 + \sqrt{\frac{\pi x}{2}}\right) \approx \sqrt{\frac{\pi x}{2\lambda^2}} = \sqrt{\frac{\pi}{2\lambda\rho}}, \quad x \gg 1. \tag{S10}$$

Combining Eqs. (S2) and (S10) we obtain:

$$\lambda = \frac{\pi}{2\rho A^2} e^{2BC}, \quad \text{for } x \gg 1. \tag{S11}$$

Eqs. (S4) and (S11) imply that, in both limits, it is:

$$\lambda = \alpha e^{\beta C}, \tag{S12}$$

with $\alpha = \pi/(2\rho A^2)$ and $\beta = 2B$ for the GNF limit and $\alpha = 1/A$ and $\beta = B$ for the Poisson one. Eqs. (S4) and (S11) also imply that, for fixed values of A, B and ρ, the value of the external ligand, C, univocally determines the value of the "firing" rate, λ. If, as done later, we consider that C can take on any value over the interval, $[0, C_M]$, Eq. (S12) then implies that λ can take on any value in the range $\alpha \le \lambda \le \alpha \exp(\beta C_M)$. By choosing ρ so that $\alpha/\rho = \pi/(2\rho^2 A^2) \gg 1$ we guarantee that the GNF limit, $x = \lambda/\rho \gg 1$, holds $\forall \lambda \in [\alpha, \alpha \exp(\beta C_M)]$ with $\alpha = \pi/(2\rho A^2)$ and $\beta = 2B$. Likewise, by choosing ρ so that $\alpha \exp(\beta C_M)/\rho = \exp(BC_M)/(A\rho) \ll 1$, we guarantee that the

Poisson limit, $x \ll 1$, holds $\forall\lambda \in [\alpha, \alpha\exp(\beta C_M)]$ with $\alpha = 1/A$ and $\beta = B$. Thus, ρ can always be chosen so that each of the two limits of interest hold over all the corresponding range of λ values.

1.1.2 Relationship between the mean and standard deviation of the stochastic component of the inter-pulse time in the GNF and the Poisson limits

As shown in Skupin and Falcke (2007), the mean of the stochastic part of the inter-pulse time, T, is given by Eq. (S5), while its variance is given by:

$$\sigma^2 = \langle t^2 \rangle - T^2 = 2e^x {}_2F_2 \begin{pmatrix} x & x \\ 1+x & 1+x \end{pmatrix}; -x \Big) - T^2, \tag{S13}$$

where ${}_2F_2$ is the (2,2) generalized hypergeometric function. For both limits of the model, the mean of the stochastic part, T, and the standard deviation, σ, are related by:

$$\sigma = kT. \tag{S14}$$

This relationship is trivial in the Poisson limit ($x \ll 1$) for which $T = \sigma = 1/\lambda$ and $k = 1$. Outside this limit, the relationship was obtained numerically in Skupin and Falcke (2007). As we show now, it can be derived analytically for $x \gg 1$ as well yielding $k = \sqrt{\frac{4}{\pi} - 1}$.

In order to determine $k = \sigma/T$ for the $x \gg 1$ limit we will calculate:

$$1 + \frac{\sigma^2}{T^2} = \lim_{x\to\infty} \frac{\langle t^2 \rangle}{T^2} = \lim_{x\to\infty} \frac{2e^x {}_2F_2 \left(\begin{matrix} x & x \\ 1+x & 1+x \end{matrix}; -x \right)}{\left(e^x x^{1-x} \left(\Gamma(x) - \Gamma(x,x)\right)\right)^2}. \tag{S15}$$

We have already proved that $T = e^x x^{1-x}(\Gamma(x) - \Gamma(x,x)) \approx \sqrt{\pi x/2} + 1$ for $x \gg 1$ (see Eq. (S10)). We now compute the numerator of Eq. (S15). To this end we use the integral transformation of Euler (see *e.g.*Slater (1966)):

$${}_{A+1}F_{B+1} \left(\begin{matrix} a_1 & \ldots & a_A & c \\ b_1 & \ldots & b_B & d \end{matrix}; z \right) = \frac{\Gamma(d)}{\Gamma(c)\Gamma(d-c)} \int_0^1 t^{c-1}(1-t)^{d-c-1} {}_AF_B \left(\begin{matrix} a_1 & \ldots & a_A \\ b_1 & \ldots & b_B \end{matrix}; tz \right) dt. \tag{S16}$$

Taking into account that $\frac{\Gamma(x+1)}{\Gamma(x)\Gamma(1)} = x$, applying this formula twice to go from ${}_0F_0$ to ${}_2F_2$ we obtain:

$${}_2F_2 \left(\begin{matrix} x & x \\ 1+x & 1+x \end{matrix}; -x \right) = x^2 \int_0^1 \int_0^1 (tt')^{x-1} {}_0F_0\left(; -tt'x\right) dt dt'. \tag{S17}$$

Given that ${}_0F_0\left(; -tt'x\right) = e^{-tt'x}$, Eq. (S17) becomes:

$${}_2F_2 \left(\begin{matrix} x & x \\ 1+x & 1+x \end{matrix}; -x \right) = x^2 \int_0^1 \int_0^1 (tt')^{x-1} e^{-tt'x} dt dt'. \tag{S18}$$

This equation can be solved via the substitution:

$$u = \ln\left(\sqrt{\frac{t}{t'}}\right), \tag{S19a}$$

$$v = \sqrt{tt'}, \tag{S19b}$$

which Jacobian is $2|v|$. The limits of integration, on the other hand, get transformed into $0 < v < 1$, $\ln(v) < u < -\ln(v)$. Thus, the integral in the r.h.s of Eq. (S18) can be rewritten as:

$$\int_0^1 \int_0^1 (tt')^{x-1} e^{-tt'x} dt dt' = \int_0^1 \int_{\ln(v)}^{-\ln(v)} (v^2)^{x-1} e^{-v^2 x} 2v du dv = \int_0^1 (v^2)^{x-1} e^{-v^2 x} 2v(-2\ln(v)) dv. \tag{S20}$$

Rewriting $2\ln(v) = \ln(v^2)$ and introducing the substitution $w = v^2$, we can rewrite Eq. (S20) as:

$$\int_0^1 \int_0^1 (tt')^{x-1} e^{-tt'x} dt dt' = \int_0^1 (w)^{x-1} e^{-wx} (-\ln(w)) dw \tag{S21}$$

Thus, the limit of Eq. (S15) is equivalent to:

$$\lim_{x\to\infty} \frac{2e^x x^2 \int_0^1 (w)^{x-1} e^{-wx} (-\ln(w)) dw}{\frac{\pi}{2}x + \sqrt{2\pi x} + 1} = \lim_{x\to\infty} \frac{4x \int_0^1 (w)^{x-1} e^{(1-w)x} (-\ln(w)) dw}{\pi}. \tag{S22}$$

Thus, proving that

$$\lim_{x\to\infty} x \int_0^1 (w)^{x-1} e^{(1-w)x} (-\ln(w)) dw = 1, \tag{S23}$$

is enough to demonstrate that $1 + \sigma^2/T^2 \to 4/\pi$ for $x \gg 1$ and, therefore, that $k = \sigma/T = \sqrt{\frac{4}{\pi} - 1} \sim 0.523$. In order to prove Eq. (S23) we use the modified Laplace method. With this in mind we can observe that the integrand in this equation tends to 0 for every value of w outside a neighborhood of $w = 1$. Thus, we can use the approximation $-\frac{\ln(w)}{w} \sim 1 - w + \mathcal{O}((1-w)^2)$. Inserting in into Eq. (S23) we obtain:

$$x \int_0^1 w^{x-1} e^{(1-w)x} (-\ln(w)) dw \sim \int_0^1 w^x e^{(1-w)x} x(1-w) dw = \int_0^1 e^{x(\ln(w)+(1-w))} x(1-w) dw. \tag{S24}$$

Approximating $\ln(w) + (1-w) \sim -\frac{1}{2}(w-1)^2$ we arrive at:

$$x \int_0^1 (w)^{x-1} e^{(1-w)x} (-\ln(w)) dw \sim \int_0^1 e^{-\frac{1}{2}x(w-1)^2} x(1-w) dw, \tag{S25}$$

which can be solved intoducing the change of variables $y = \frac{1}{2}x(w-1)^2$. In this way we obtain

$$x \int_0^1 (w)^{x-1} e^{(\ln(w)+(1-w))x} (-\ln(w)) dw \approx \int_0^{\frac{1}{2}x} e^{-y} dy \approx \int_0^\infty e^{-y} dy = 1, \tag{S26}$$

and so we prove Eq. (S23).

In summary we have proved here that in the GNF and the Poisson limits T and σ are related by Eq. (S14) with $k = 1$ in the Poisson case and $k = \sqrt{\frac{4}{\pi} - 1}$ in the GNF one.

1.2 Quantifying the information contained in a pulse.

Here we show the calculation of the information contained in the time separation between two successive pulses. More specifically, we compute the mutual information between t and C which is the same as the mutual information between t and λ:

$$\begin{aligned} I(C,t) = I(\lambda,t) &= \iint p_\lambda(\lambda) p(t|\lambda) \log_2 \left(p(t|\lambda)/p(t)\right) dt d\lambda \\ &= \int p_\lambda(\lambda) \int p(t|\lambda) \log_2 \left(p(t|\lambda)\right) dt d\lambda - \int p_t(t) \log_2 \left(p_t(t)\right) dt \\ &\equiv -H(t|\lambda) + H(t), \end{aligned} \tag{S27}$$

where

$$p_t(t) = \int p_{\{t,\lambda\}}(t,\lambda) d\lambda = \int p(t|\lambda) p_\lambda(\lambda) d\lambda, \tag{S28}$$

with $p_{\{t,\lambda\}}$ the joint probability density of t and λ. Now, given that λ is a function of the ligand concentration, which typically changes with time, λ will change as well. The calculations we are going to perform are good either for constant λ or if λ varies slowly enough with respect to $\langle t \rangle|_{t|\lambda}$. In this way, we use Eq. (S3) to relate the λ probability density, p_λ, and that of the external ligand concentration, p_C, by:

$$p_\lambda(\lambda) = \frac{1}{\alpha\beta} e^{-\beta C} p_C(C) = \frac{1}{\lambda\beta}\, p_C\left(\frac{1}{\beta}\ln\left(\frac{\lambda}{\alpha}\right)\right). \tag{S29}$$

We first compute $H(t)$. Assuming that λ is constant, we write:

$$\int_0^t -\lambda\left(1 - \exp(-\rho t')\right) dt' = -\lambda t - \frac{\lambda}{\rho}\left(\exp(-\rho t) - 1\right), \tag{S30}$$

and

$$p(t|\lambda) = -\frac{\partial e^{\lambda\left(-t - \frac{1}{\rho}(e^{-\rho t} - 1)\right)}}{\partial t}. \tag{S31}$$

Defining $\tau = (-t - \frac{1}{\rho}(e^{-\rho t} - 1))$ we rewrite

$$p_t(t) = \int -\frac{\partial e^{\lambda\tau(t)}}{\partial t} p_\lambda(\lambda) d\lambda = -\frac{\partial M_\lambda(\tau(t))}{\partial t} \equiv -M'_\lambda, \tag{S32}$$

where we have introduced the definition:

$$M_\lambda(\tau) = \int e^{\lambda\tau} p_\lambda(\lambda) d\lambda, \tag{S33}$$

which is the moment-generating function. We then rewrite H in terms of M_λ as:

$$H(t) = \frac{1}{\ln(2)}\left(-\int -M'_\lambda(\tau)\ln(-M'_\lambda(\tau))dt\right), \tag{S34}$$

where the prime indicates the derivative with respect to t (see Eq. (S32)). Given that $-M'_\lambda(\tau) = -\frac{\partial M_\lambda(\tau)}{\partial\tau}\tau'(t) = \frac{\partial M_\lambda(\tau)}{\partial\tau}(-\tau'(t))$ we rewrite Eq. (S34) as:

$$H(t) = -\frac{1}{\ln(2)}\left(\int -M'_\lambda(\tau)\ln\left(\frac{\partial M_\lambda(\tau)}{\partial\tau}\right)dt + \int -M'_\lambda(x)\ln(-\tau'(t))dt\right). \tag{S35}$$

The last term in this equation can be written as:

$$\frac{1}{\ln(2)}\int M'_\lambda(\tau)\ln(-\tau'(t))dt = -\frac{1}{\ln(2)}\int p_t(t)\ln(1-e^{-\rho t})dt \equiv -\frac{1}{\ln(2)}\langle\ln(1-e^{-\rho t})\rangle|_t. \tag{S36}$$

Further changing the variable of integration from t to $\tau = -t - 1/\rho(\exp(-\rho t) - 1)$, we obtain:

$$H(t) = \frac{1}{\ln(2)}\left(-\int_{-\infty}^{0}\frac{\partial M_\lambda(\tau)}{\partial\tau}\ln\left(\frac{\partial M_\lambda(\tau)}{\partial\tau}\right)d\tau - \langle\ln(1-e^{-\rho t})\rangle|_t\right), \tag{S37}$$

where we have used $\tau(t=0) = 0$ and $\tau(t\to\infty)\to-\infty$.

We now compute $H(t|\lambda)$. Taking into account Eqs. (S1) and (S30) we write:

$$\int p(t|\lambda)\log_2(p(t|\lambda))\,dt = \frac{1}{\ln(2)}\left(\langle\ln(\lambda(1-e^{-\rho t}))\rangle|_{t|\lambda} - \lambda\langle t\rangle|_{t|\lambda} - \frac{\lambda}{\rho}\left(\langle e^{-\rho t}\rangle|_{t|\lambda} - 1\right)\right). \tag{S38}$$

The term, $\langle e^{-\rho t}\rangle|_{t|\lambda}$, can be rewritten as:

$$\int_0^\infty e^{-\rho t}\lambda(1-e^{-\rho t})e^{-\lambda t-\frac{\lambda}{\rho}\left(e^{-\rho t}-1\right)}dt = \int_0^1\frac{\lambda}{\rho}(1-u)e^{\frac{\lambda}{\rho}\ln(u)-\frac{\lambda}{\rho}(u-1)}du = \int_0^1\frac{\lambda}{\rho}(1-u)u^{\frac{\lambda}{\rho}}e^{-\frac{\lambda}{\rho}(u-1)}du, \tag{S39}$$

where we have introduced $u = \exp(-\rho t)$. Further replacing u by $v = \lambda u/\rho$, we obtain:

$$\begin{aligned}\langle\exp(-\rho t)\rangle|_{t|\lambda} &= \int_0^1\frac{\lambda}{\rho}(1-u)u^{\frac{\lambda}{\rho}}e^{-\frac{\lambda}{\rho}(u-1)}du = e^{\frac{\lambda}{\rho}}\left(\int_0^{\frac{\lambda}{\rho}}\left(\frac{v\rho}{\lambda}\right)^{\frac{\lambda}{\rho}}e^{-v}dv - \int_0^{\frac{\lambda}{\rho}}\left(\frac{v\rho}{\lambda}\right)^{\frac{\lambda}{\rho}+1}e^{-v}dv\right)\\ &= \frac{e^{\frac{\lambda}{\rho}}}{(\frac{\lambda}{\rho})^{\frac{\lambda}{\rho}}}\gamma\left(\frac{\lambda}{\rho}+1,\frac{\lambda}{\rho}\right) - \frac{e^{\frac{\lambda}{\rho}}}{(\frac{\lambda}{\rho})^{\frac{\lambda}{\rho}+1}}\gamma\left(\frac{\lambda}{\rho}+2,\frac{\lambda}{\rho}\right),\end{aligned} \tag{S40}$$

where $\gamma(x,y)$ is the lower incomplete Gamma function. We rewrite $\gamma(\frac{\lambda}{\rho}+2,\frac{\lambda}{\rho})=(\frac{\lambda}{\rho}+1)\gamma(\frac{\lambda}{\rho}+1,\frac{\lambda}{\rho})-(\frac{\lambda}{\rho})^{\frac{\lambda}{\rho}+1}e^{-\frac{\lambda}{\rho}}$ and Eq. (S40) becomes:

$$\langle\exp(-\rho t)\rangle|_{t|\lambda}=-\frac{e^{\frac{\lambda}{\rho}}}{\left(\frac{\lambda}{\rho}\right)^{\frac{\lambda}{\rho}+1}}\left(\gamma\left(\frac{\lambda}{\rho}+1,\frac{\lambda}{\rho}\right)-\left(\frac{\lambda}{\rho}\right)^{\frac{\lambda}{\rho}+1}e^{-\frac{\lambda}{\rho}}\right). \tag{S41}$$

1.2.1 The $\boldsymbol{\lambda/\rho\gg 1}$ limit

We now advance with the computation assuming that $x=\lambda/\rho\gg 1$. Combining Eqs. (S7) (which holds for $x\gg 1$) and (S41) we obtain

$$\frac{\lambda}{\rho}\langle\exp(-\rho t)\rangle|_{t|\lambda}\sim-\sqrt{\frac{\pi}{2}\frac{\lambda}{\rho}}+\frac{\lambda}{\rho}, \tag{S42}$$

for $\lambda/\rho\gg 1$. Inserting Eqs. (S10) and (S42) in Eq. (S38) we obtain:

$$\begin{aligned}\int p(t|\lambda)\log_2\left(p(t|\lambda)\right)dt &\approx \frac{1}{\ln(2)}\left(\langle\ln(\lambda(1-e^{-\rho t}))\rangle|_{t|\lambda}-\sqrt{\frac{\pi\lambda}{2\rho}}-1+\sqrt{\frac{\pi}{2}\frac{\lambda}{\rho}}-\frac{\lambda}{\rho}+\frac{\lambda}{\rho}\right)\\ &= \frac{1}{\ln(2)}\left(\ln(\lambda)-1+\langle\ln(1-e^{-\rho t})\rangle|_{t|\lambda}\right). \end{aligned}\tag{S43}$$

The first term in Eq. (S27) is then given by:

$$\begin{aligned}-H(t|\lambda) &\equiv \int p_\lambda(\lambda)\int p(t|\lambda)\log_2\left(p(t|\lambda)\right)dtd\lambda\\ &= \int p_\lambda(\lambda)\frac{1}{\ln(2)}\left(\ln(\lambda)-1+\langle\ln(1-e^{-\rho t})\rangle|_{t|\lambda}\right)d\lambda\\ &= \frac{1}{\ln(2)}\left(\langle\ln(\lambda)\rangle|_\lambda-1+\langle\ln(1-e^{-\rho t})\rangle|_t\right), \end{aligned}\tag{S44}$$

where the mean $\langle\cdot\rangle|_\lambda$ is computed using the distribution, p_λ, and $\langle\cdot\rangle|_t$ using the distribution, p_t, defined in Eq. (S28). The last term in this equation cancels out with the similar one coming from $H(t)$ when computing I (see Eqs. (S27), (S37) and (S44)) and we have:

$$I(\lambda,t)=\frac{1}{\ln(2)}\left(-\int_{-\infty}^{0}\frac{\partial M_\lambda(\tau)}{\partial\tau}\ln\left(\frac{\partial M_\lambda(\tau)}{\partial\tau}\right)d\tau-1+\langle\ln(\lambda)\rangle|_\lambda\right), \tag{S45}$$

with M given by Eq. (S33).

1.2.2 The $\lambda/\rho \ll 1$ limit.

We now compute Eq. (S27) in the limit, $\lambda/\rho \ll 1$, for which the model can be approximated by a Poisson process with the probability :

$$p(t|\lambda) = \lambda e^{-\lambda t} \tag{S46a}$$

$$\lambda = \alpha e^{\beta C} \tag{S46b}$$

In this case, Eq. (S38) becomes:

$$\int p(t|\lambda)\log_2\left(p(t|\lambda)\right)dt = \frac{1}{\ln(2)}\left(\ln(\lambda)-1\right) \tag{S47}$$

Therefore:

$$H(t|\lambda) = \frac{1}{\ln(2)}\left(1-\langle\ln(\lambda)\rangle|_\lambda\right). \tag{S48}$$

Eq. (S37), on the other hand, becomes

$$H(t) = \frac{1}{\ln(2)}\left(-\int_{-\infty}^{0}\frac{\partial M_\lambda(\tau)}{\partial\tau}\ln\left(\frac{\partial M_\lambda(\tau)}{\partial\tau}\right)d\tau\right), \tag{S49}$$

with M given by Eq. (S33) as before. Thus, the information in this limit can also be written as in Eq. (S45), *i.e.*, as in the $\lambda/\rho \gg 1$ limit. Summarizing, the information has the same expression (Eq. (S45)) in both limits. We can rewrite it using some constant, μ, which we will either take equal to $\langle\lambda\rangle$ or to α to make all time variables dimensionless. Namely, we define $\tilde{\tau} = \mu\tau$ and $\tilde{\lambda} = \lambda/\mu$. In this way, Eq. (S33) can be rewritten as:

$$M_\lambda(\tilde{\tau}) = \int e^{\tilde{\lambda}\tilde{\tau}}p_{\tilde{\lambda}}(\tilde{\lambda})d\tilde{\lambda}, \quad \text{with} \quad p_{\tilde{\lambda}}(\tilde{\lambda}) = \frac{1}{\tilde{\lambda}\beta}\,p_C\left(\frac{1}{\beta}\ln\left(\frac{\tilde{\lambda}\mu}{\alpha}\right)\right), \tag{S50}$$

and Eq. (S45) becomes:

$$I(\lambda,t) = \frac{1}{\ln(2)}\left(-\int_{-\infty}^{0}\frac{\partial M_\lambda}{\partial\tilde{\tau}}\ln\left(\frac{\partial M_\lambda}{\partial\tilde{\tau}}\right)d\tilde{\tau} - 1 + \langle\ln\left(\tilde{\lambda}\right)\rangle|_{\tilde{\lambda}}\right). \tag{S51}$$

1.2.3 Dependence of the information on the parameters of the problem

If we choose $\mu = \alpha$ to define the dimensionless time variables, Eq. (S12) implies that $\tilde{\alpha} = \exp(\beta C)$. We then deduce from Eqs. (S50)–(S51) that, at least in the two limits that we are studiying, I does not depend on α. Similarly, if we choose $\mu = \alpha\exp(\beta\langle C\rangle$, it is $\tilde{\lambda} = \exp(\beta(C-\langle C\rangle)$ and we then conclude that I does not depend on $\langle C\rangle$. The dependence on ρ is more subtle and we discuss it in the main paper. In order to study the dependence on β, we now make an assumption for the distribution of C. Namely, we assume a uniform distribution over the interval $[0, C_M]$. In such a case it is:

$$p_C(C) = \begin{cases} \frac{1}{C_M}, & \text{if } 0 \le C \le C_M, \\ 0, & \text{otherwise,} \end{cases} \tag{S52}$$

so that

$$p_\lambda(\lambda) = \begin{cases} \frac{1}{C_M \beta \lambda}, & \text{if } \alpha \le \lambda \le \alpha \exp(\beta C_M), \\ 0, & \text{otherwise.} \end{cases} \tag{S53}$$

Then, $\partial M_\lambda / \partial \tilde{\tau}$ (Eq. (S33) with $\tilde{\tau} = \mu\tau$, $\mu = \alpha$) is given by:

$$\frac{\partial M_\lambda}{\partial \tilde{\tau}} = \int_1^{\exp(\beta C_M)} e^{\tilde{\lambda}\tilde{\tau}} \frac{1}{C_M \beta} d\tilde{\lambda} = \frac{1}{C_M \beta \tilde{\tau}} \left(\exp\left(\tilde{\tau} e^{\beta C_M} \right) - \exp\left(\tilde{\tau}\right) \right) \tag{S54}$$

On the other hand:

$$\langle \ln(\tilde{\lambda}) \rangle = \beta \langle C \rangle = \beta \frac{C_M}{2} \tag{S55}$$

Then, the only thing left to calculate I in Eq. (S51) is:

$$HM'_\lambda \equiv - \int_{-\infty}^{0} \frac{\partial M_\lambda}{\partial \tilde{\tau}} \ln\left(\frac{\partial M_\lambda}{\partial \tilde{\tau}} \right) d\tilde{\tau}. \tag{S56}$$

We define

$$g(\tilde{\tau}) \equiv \beta C_M \frac{\partial M_\lambda}{\partial \tilde{\tau}} = \frac{e^{\tilde{\tau} e^{C_M \beta}} - e^{\tilde{\tau}}}{\tilde{\tau}}, \tag{S57}$$

which can be rewritten as:

$$g(\tilde{\tau}) = \frac{2}{\tilde{\tau}} e^{\tilde{\tau} \frac{e^{\beta C_M}+1}{2}} \sinh\left(\tilde{\tau} \frac{e^{\beta C_M} - 1}{2} \right). \tag{S58}$$

Thus:

$$\ln(g(\tilde{\tau})) = \ln\left(\frac{\partial M_\lambda}{\partial \tilde{\tau}} \right) + \ln(\beta C_M) = \ln(2) - \ln(\tilde{\tau}) + \tilde{\tau} \frac{e^{\beta C_M} + 1}{2} + \ln(\sinh(\tilde{\tau} \frac{e^{\beta C_M} - 1}{2})). \tag{S59}$$

Given Eqs. (S32))-(S33) and that $\tilde{\tau}(t=0) = 0$ and $\tilde{\tau}(t \to \infty) \to -\infty$, then

$$- \int_0^\infty f(t) \frac{\partial M_\lambda}{\partial t} dt = \int_{-\infty}^{0} f(t(\tilde{\tau})) \frac{\partial M_\lambda}{\partial \tilde{\tau}} d\tilde{\tau} = \int_0^\infty f(t) p_t(t) dt = \langle f \rangle|_t, \tag{S60}$$

for any function $f(t)$ (or of $\tilde{\tau}$ through the relationship $\tilde{\tau} = \mu(-t - \frac{1}{\rho}(e^{-\rho t} - 1))$). Thus, I can be written as:

$$I(\lambda,t) = \frac{1}{\ln(2)}\left(-\int_{-\infty}^{0} \frac{\partial M_\lambda}{\partial \tilde{\tau}} \ln(g(\tilde{\tau}))d\tilde{\tau} + \ln(\beta C_M) - 1 + \beta\frac{C_M}{2}\right) =$$
$$\frac{1}{\ln(2)}\left(-\ln(2) + \langle \ln(\tilde{\tau})\rangle|_t - \langle\tilde{\tau}\rangle|_t \frac{e^{\beta C_M}+1}{2} - \langle \ln(\sinh(\tilde{\tau}\frac{e^{\beta C_M}-1}{2}))\rangle|_t + \ln(\beta C_M) - 1 + \beta\frac{C_M}{2}\right). \tag{S61}$$

Computing each term in this equation, taking special care of the $\tilde{\tau} = 0$ limit of integration, we arrive at the following expression

$$\begin{aligned} I(\lambda,t) = {} & \frac{1}{\ln(2)}\left(\frac{\sinh(\beta C_M)}{\beta C_M} + \ln\left(\beta\frac{C_M}{2}\right) - \ln\left(\frac{e^{\beta C_M}-1}{2}\right) - 1 + \beta\frac{C_M}{2}\right) \\ & - \frac{1}{\beta C_M \ln(2)}\int_{-\infty}^{0} \frac{e^{\zeta}}{\zeta}\ln\left(\frac{e^{\beta C_M}\sinh(\zeta e^{-\beta C_M}\frac{e^{\beta C_M}-1}{2})}{\sinh(\zeta\frac{e^{\beta C_M}-1}{2})}\right)d\zeta, \end{aligned} \tag{S62}$$

the last term of which we computed numerically using Mathematica. The result of this numerical computation is shown in the main body of the paper where it may be observed that it is an increasing function of βC_M. Given that, in the GNF limit, β is twice as large as in the Poisson limit, $I(C,t)$ is always smaller in the latter than in the former limit. Given that $I(C,t)$ does not depend on $\langle C\rangle$ (see discussion at the beginning of Sec. 1.2.3), the dependence on βC_M comes from the dependence of I on the standard deviation of C, which, for a uniform distribution as the one we have considered, is $C_M/\sqrt{12}$. As we have already discussed, $I(C,t)$ does not depend on the parameter, α. Eqs. (S3) and (S27) then imply that it depends on the standard deviation of C through its product with the parameter β. If instead of using the least informative distribution for C (uniform over an interval) we consider that C can take on only one value (*i.e.*, it is a δ function with zero variance) we obtain $I(t,C) = 0$ in the two limits. We then expect that $I(C,t)$ will increase with the product between β and the standard deviation of C regardless of the distribution that we might consider for C. This argument and the results we obtain for the information between the number of pulses and C in the following sections lead us to think that $I(C,t)$ is always larger in the GNF than in the Poisson limit.

1.3 Information contained in $N \gg 1$ pulses.

Now we look at the problem when there are many (N) subsequent pulses. In order to perform analytic calculations we will consider the limit $N \gg 1$. Basically, we are assuming that we observe the system for a fixed time, t_{tot}, such that $t_{tot} \gg (T_{cell} + T)$. We then want to compute the mutual information between N and the external ligand concentration, C, assuming fixed values of ρ, α and β (or of A and B) as we have done before. To this end, we need to know the conditional probability that N pulses occur during an observation time, t_{tot} for a given value of C, *i.e.*, $p(N|C, t_{tot})$. The problem at hand is an example of a *renewal process* Ross (2014). According to the Central Limit

Theorem for this type of processes, for large enough N, it is:

$$p(N|C,t_{tot}) \approx \left(2\pi \frac{t_{tot}}{T_{cell}+T}\frac{\sigma^2}{(T_{cell}+T)^2}\right)^{-1/2} \exp\left(-\frac{(N-\frac{t_{tot}}{T_{cell}+T})^2}{2\frac{t_{tot}}{T_{cell}+T}\frac{\sigma^2}{(T_{cell}+T)^2}}\right), \tag{S63}$$

where both the mean, $T_{cell}+T$, and the standard deviation, σ, of the inter-pulse time, t, depend on the external ligand concentration, C. In order to simplify the calculations, from now on we will assume that $T_{cell} \ll T$. All the calculations can be performed in the same way as described here if this assumption is not taken into account. Considering the simplification and given Eq. (S14) (which holds for the Poisson and GNF limits), Eq. (S63) can be rewritten as:

$$p(N|C,t_{tot}) = p(N|T,t_{tot}) \approx \left(\frac{2\pi k^2 t_{tot}}{T}\right)^{-1/2} \exp\left(-\frac{(N-\frac{t_{tot}}{T})^2}{2k^2 t_{tot}/T}\right). \tag{S64}$$

For fixed values of ρ, α and β, the mean, T, is uniquely determined by C. In such a case, the mutual information between N and C is the same as the information between N and T. We will then compute the latter treating N as a continuous variable, namely:

$$\begin{aligned} I(C,N) = I(N,T) = -\int p(N|t_{tot}) \log_2\left(p(N|t_{tot})\right) dN + \\ \iint p(N|T,t_{tot}) p_T(T) \log_2\left(p(N|T,t_{tot})\right) dT dN, \end{aligned} \tag{S65}$$

where $p_T(T)$ is the probability density of the mean, T, which is to be derived from that of the ligand concentration, $p_C(C)$, and

$$p(N|t_{tot}) = \int p(N|T,t_{tot}) p_T(T) dT. \tag{S66}$$

It is convenient to introduce the change of variables $\bar{N} = \frac{N}{t_{tot}}$, so that:

$$\begin{aligned} I(N,T) = -\int p(\bar{N}|t_{tot}) \log_2\left(p(\bar{N}|t_{tot})\right) d\bar{N} + \\ \iint p(\bar{N}|T,t_{tot}) p_T(T) \log_2\left(p(\bar{N}|T,t_{tot})\right) dT d\bar{N}, \end{aligned} \tag{S67a}$$

$$p(\bar{N}|T,t_{tot}) = \sqrt{\frac{t_{tot}T}{2\pi k^2}} \exp\left(-\frac{(\bar{N}-\frac{1}{T})^2}{\frac{2k^2}{t_{tot}T}}\right). \tag{S67b}$$

In order to compute the first term in Eq. (S67a) we assume that t_{tot} is large enough so that we can approximate:

$$
\begin{aligned}
p(\bar{N}|t_{tot}) = \int \frac{1}{\sqrt{\frac{2\pi k^2}{t_{tot}T}}} e^{-\frac{(\bar{N}-\frac{1}{T})^2}{\frac{2k^2}{t_{tot}T}}} p_T(T)dT &\approx \int \delta(\bar{N} - \frac{1}{T}) p_T(T)dT + O\left(\frac{1}{t_{tot}}\right) . \\
&= \frac{1}{\bar{N}^2} p_T\left(\frac{1}{\bar{N}}\right) + O\left(\frac{1}{t_{tot}}\right)
\end{aligned}
\tag{S68}
$$

The last equality is demonstrated in 1.3.1. In this way, the first term in Eq. (S67a) becomes:

$$
\begin{aligned}
&-\int p(\bar{N}|t_{tot}) \log_2 \left(p(\bar{N}|t_{tot})\right) d\bar{N} \\
&\approx -\int \left(\frac{1}{\bar{N}^2} p_T\left(\frac{1}{\bar{N}}\right) + O\left(\frac{1}{t_{tot}}\right)\right) \log_2 \left(\frac{1}{\bar{N}^2} p_T\left(\frac{1}{\bar{N}}\right) + O\left(\frac{1}{t_{tot}}\right)\right) d\bar{N} \\
&= -\int p_T(T) \left(\log_2 \left(p_T(T)\right) + 2\log_2(T)\right) dT + O\left(\frac{1}{t_{tot}}\right) \\
&= H(T) - 2\langle \log_2(T)\rangle_T,
\end{aligned}
\tag{S69}
$$

with the entropy, H, as defined in Eq. (S27) and $\langle\cdot\rangle_T$ the mean computed using the probability density, p_T. Then, remembering that the entropy of a normal distribution of standard deviation σ is $\frac{1}{2}\log_2(2\pi e\sigma^2)$, the second term in Eq. (S67a) can be written as:

$$
\begin{aligned}
&\iint p(\bar{N}|T, t_{tot}) p_T(T) \log_2 \left(p(\bar{N}|T, t_{tot})\right) dT d\bar{N} = \\
&\int p_T(T) \int \frac{1}{\sqrt{\frac{2\pi k^2}{t_{tot}T}}} e^{-\frac{(\bar{N}-\frac{1}{T})^2}{\frac{2k^2}{t_{tot}T}}} \log_2 \left(\frac{1}{\sqrt{\frac{2\pi k^2}{t_{tot}T}}} e^{-\frac{(\bar{N}-\frac{1}{T})^2}{\frac{2k^2}{t_{tot}T}}}\right) d\bar{N} dT = \\
&-\frac{1}{2}\int p_T(T) \log_2 \left(\frac{2\pi e}{t_{tot}T} k^2\right) dT = -\frac{1}{2}\log_2\left(\frac{2\pi e k^2}{t_{tot}}\right) - \frac{1}{2}\int p_T(T) \log_2 \left(\frac{1}{T}\right) dT.
\end{aligned}
\tag{S70}
$$

Inserting Eqs. (S69) and (S70) into Eq. (S67a) we finally obtain:

$$
I(T, N) = H(T) - \frac{3}{2}\langle \log_2(T)\rangle_T - \frac{1}{2}\log_2\left(\frac{2\pi e k^2}{t_{tot}}\right). \tag{S71}
$$

Under the assumption that the mean, T, is a function of the external ligand concentration, C, (see Eq. (S2)), Eq. (S71) implies that, for any given distribution, $p_C(C)$ or, equivalently, $p_T(T)$, the only difference in $I(T, N)$ between the Poisson and the GNF limits of the model is given by k. We recall that k, the constant of proportionality between standard deviation, σ, and the mean, T, of (the stochastic part of) the interpulse time (see Eq. (S14), is 1 in the Poisson limit and $\sqrt{4/\pi - 1} \approx 0.523$ in the GNF one. Thus, the difference between the information for the GNF and the Poisson limits is 0.936 regardless of the distribution that we might consider for C. There is an extra term in $I(T,N)$ of $\mathcal{O}(\frac{1}{\sqrt[3]{t_{tot}}})$ which is related to the residual skewness of the probability distribution $p(\bar{N}|T,t_{tot})$, that is explored in section 1.3.2. Adding this term Eq. (S71) becomes:

$$I(T,N) = H(T) - \frac{3}{2}\langle \log_2(T)\rangle_T - \frac{1}{2}\log_2\left(\frac{2\pi e k^2}{t_{tot}}\right) + \mathcal{O}\left(\frac{1}{\sqrt[3]{t_{tot}}}\right). \tag{S72}$$

Assuming that C is uniformly distributed as before (Eq. (S52)) and that T and C are related by: $A\exp(-BC)$ (see Eq. (S2)) we obtain:

$$p_T(T) = \begin{cases} \frac{1}{C_M B T}, & \text{if} \quad A\exp(-BC_M) \le T \le A, \\ 0, & \text{otherwise,} \end{cases} \tag{S73}$$

so that $1/T = C_M B p_T(T)$ for the values of T for which p_T is not zero. This implies that

$$H(T) = -\int p_T(T)\log_2(p_T)dT = \int p_T(T)\log_2(C_M B T)dT = \log_2(C_M B) + \langle \log_2(T)\rangle_T, \tag{S74}$$

so that Eq. (S71) becomes:

$$I(T,N) = \log_2(C_M B) - \frac{1}{2}\langle \log_2(T)\rangle_T - \frac{1}{2}\log_2\left(\frac{2\pi e k^2}{t_{tot}}\right). \tag{S75}$$

The mean, $\langle \log_2(T)\rangle_T$, can readily be computed using Eq. (S73). We obtain:

$$\langle \log_2(T)\rangle_T = \log_2(A) - \frac{BC_M}{2\ln(2)}. \tag{S76}$$

Inserting Eq. (S76) into Eq. (S75) we obtain:

$$I(T,N) = \log_2(AC_M B) - \frac{BC_M}{2\ln(2)} - \frac{1}{2}\log_2\left(\frac{2\pi e k^2}{t_{tot}}\right). \tag{S77}$$

Including the $\mathcal{O}(\frac{1}{\sqrt[3]{t_{tot}}})$ term, the final result can be written as:

$$I(T,N) = f(A, BC_M) + \frac{1}{2}\log_2\left(\frac{t_{tot}}{k^2}\right) + \mathcal{O}\left(\frac{1}{\sqrt[3]{t_{tot}}}\right). \tag{S78}$$

1.3.1 Approximate calculation of $p(\bar{N}|t_{tot})$

We want to calculate approximately the function, $p(\bar{N}|T, t_{tot})$, given by Eq. (S67b) for large enough t_{tot}. Performing a Fourier transform, $\mathcal{F}$, (with respect to $\bar{N}$) on it we obtain:

$$\mathcal{F}\left(\frac{1}{\sqrt{2\pi\frac{1}{t_{tot}T}k^2}}e^{-\frac{(\bar{N}-\frac{1}{T})^2}{\frac{1}{t_{tot}T}k^2}}\right) = \frac{1}{\sqrt{2\pi}}e^{i[\frac{1}{t_{tot}T}k^2]\omega}e^{-\frac{1}{2t_{tot}T}k^2\omega^2} \sim$$
$$\frac{1}{\sqrt{4\pi}}e^{i[\frac{1}{T}]\omega}e^{-\frac{1}{2t_{tot}T}k^2\omega^2} \sim \frac{1}{\sqrt{4\pi}}e^{i(\frac{1}{T})\omega}\left(1-\frac{k^2\omega^2}{2t_{tot}T}+\mathcal{O}\left(\frac{1}{t_{tot}^2})\right)\right). \tag{S79}$$

Antitransforming the last expression we obtain $\delta(\bar{N}-\frac{1}{T})+\mathcal{O}(\frac{1}{t_{tot}})$.

1.3.2 Going beyond the Central Limit Theorem

In Eq. (S64) (or, equivalenty, Eq. (S67b) we assumed that the probability density function of the number of pulses, N, for given values of C and t_{tot} could be approximated by a normal distribution. This result is derived from the Central Limit Theorem. In this subsection we explore what happens if we deform slightly the normal distribution to include some skewness. Without loss of generality we will introduce a change of variables so that $\langle\bar{N}\rangle = 0$. Namely, we consider that the probability density for the rescaled variable, $\bar{N} = N/t_{tot}$ is given by:

$$p(\bar{N}|T, t_{tot}) \sim \frac{1}{\sqrt{2\pi\bar{\sigma}_N^2}}e^{-\frac{\bar{N}^2}{2\bar{\sigma}_N^2}}f\left(\varphi\frac{\bar{N}}{\bar{\sigma}_N}\right), \tag{S80}$$

instead of Eq. (S67b), where $\varphi \to 0$, $f(0) = 1$, $\int_{-\infty}^{\infty}\frac{1}{\sqrt{2\pi\bar{\sigma}_N^2}}e^{-\frac{\bar{N}^2}{\bar{\sigma}_N^2}}f(\varphi\frac{\bar{N}}{\sigma}) = 1$. A possible specific $f(\zeta)$ could be $2\Phi(\zeta)$ with Φ the cumulative distribution function of the normal distribution. In this way we would have the skew-normal distribution. Now we remind the readers that we are working in the limit of large t_{tot} so that that $\bar{\sigma}_N \sim \mathcal{O}(\frac{1}{\sqrt{t_{tot}}})$. The actual probability density function for the variable N, $p(N|T, t_{tot})$, in the Poisson limit of the model is the Poisson distribution which can be approximated by a normal distribution of the same variance. The skewness of the Poisson distribution with variance, $\sigma_N^2 \sim t_{tot}$, as in the normal approximation of Eq. (S64)) satisfies:

$$S = \frac{\langle(N-\langle N\rangle)^3\rangle}{\sigma_N^3} = \frac{1}{\sigma_N} \sim \frac{1}{\sqrt{t_{tot}}}. \tag{S81}$$

Given that $\sigma_N^2 \sim t_{tot}$, Eq. (S81) implies that $\langle(N-\langle N\rangle)^3\rangle \sim t_{tot}$ so that:

$$\langle(\bar{N}-\langle\bar{N}\rangle)^3\rangle \sim \mathcal{O}\left(\frac{1}{t_{tot}^2}\right). \tag{S82}$$

We now approximate the function f in Eq. (S80) by its Taylor expansion, $f(x) \approx 1 + a_1 x + a_2 x^2 + a_3 x^3$, and compute:

$$\begin{aligned}\langle \bar{N} \rangle \sim \int_{-\infty}^{\infty} \frac{1}{\sqrt{2\pi\bar{\sigma}_N^2}} e^{-\frac{\bar{N}^2}{\bar{\sigma}_N^2}} f(\frac{\varphi}{\bar{\sigma}_N}\bar{N})\bar{N}d\bar{N} = \\ (\frac{\bar{\sigma}_N}{\varphi}) \int_{-\infty}^{\infty} \frac{1}{\sqrt{2\pi\varphi^2}} e^{-\frac{u^2}{\varphi^2}} (1 + a_1 u + a_2 u^2 + a_3 u^3) u du = (\frac{\bar{\sigma}_N}{\varphi})[a_1\varphi^2 + 3a_3\varphi^4],\end{aligned} \tag{S83}$$

$$\begin{aligned}\langle \bar{N}^2 \rangle \sim \int_{-\infty}^{\infty} \frac{1}{\sqrt{2\pi\bar{\sigma}_N^2}} e^{-\frac{\bar{N}^2}{\bar{\sigma}_N^2}} f(\frac{\varphi}{\bar{\sigma}_N}\bar{N})\bar{N}d\bar{N} = \\ (\frac{\bar{\sigma}_N}{\varphi})^2 \int_{-\infty}^{\infty} \frac{1}{\sqrt{2\pi\varphi^2}} e^{-\frac{u^2}{\varphi^2}} (1 + a_1 u + a_2 u^2 + a_3 u^3) u^2 du = (\frac{\bar{\sigma}_N}{\varphi})^2[\varphi^2 + 3a_2\varphi^4],\end{aligned} \tag{S84}$$

$$\begin{aligned}\int_{-\infty}^{\infty} \frac{1}{\sqrt{2\pi\bar{\sigma}_N^2}} e^{-\frac{\bar{N}^2}{\bar{\sigma}_N^2}} f(\frac{\varphi}{\bar{\sigma}_N}\bar{N})\bar{N}^3 d\bar{N} = \\ (\frac{\bar{\sigma}_N}{\varphi})^3 \int_{-\infty}^{\infty} \frac{1}{\sqrt{2\pi\varphi^2}} e^{-\frac{u^2}{\varphi^2}} (1 + a_1 u + a_2 u^2 + a_3 u^3) u^3 du = (\frac{\bar{\sigma}_N}{\varphi})^3[3a_1\varphi^4 + 15a_3\varphi^6].\end{aligned} \tag{S85}$$

The latter gives:

$$\begin{aligned}\langle (\bar{N} - \langle \bar{N} \rangle)^3 \rangle = \langle \bar{N}^3 \rangle - 3\langle \bar{N} \rangle \langle \bar{N}^2 \rangle + 2\langle \bar{N} \rangle^3 \sim \\ (\frac{\bar{\sigma}_N}{\varphi})^3[(3a_1\varphi^4 + 15a_3\varphi^6) - 3(a_1\varphi^2 + 3a_3\varphi^4)(\varphi^2 + 3a_2\varphi^4) + 2(a_1\varphi^2 + 3a_3\varphi^4)^3] \sim \\ (\bar{\sigma}_N\varphi)^3[15a_3 - 9a_1a_3 + 9a_1a_2 + 2a_1^3] \sim \mathcal{O}((\bar{\sigma}_N\varphi)^3).\end{aligned} \tag{S86}$$

Considering that $\bar{\sigma}_N \sim \mathcal{O}(\frac{1}{\sqrt{t_{tot}}})$, Eqs. (S82) and (S86) imply that $\varphi \sim \mathcal{O}(\frac{1}{\sqrt[6]{t_{tot}}})$. Thus, when computing the second term in the r.h.s. of Eq. (S67a) we would have to perform a calculation of the form:

$$\begin{aligned}\int \frac{1}{\sqrt{2\pi\bar{\sigma}_N^2}} e^{-\frac{\bar{N}^2}{2\bar{\sigma}_N^2}} f\left(\frac{\varphi}{\bar{\sigma}_N}\bar{N}\right) \log_2\left(f\left(\frac{\varphi}{\bar{\sigma}_N}\bar{N}\right)\right) d\bar{N} = \\ \int \frac{1}{\sqrt{2\pi\varphi^2}} e^{-\frac{u^2}{2\varphi^2}} (1 + a_1 u + a_2 u^2 + a_3 u^3) \log_2\left(1 + a_1 u + a_2 u^2 + a_3 u^3\right) du \sim \\ \frac{1}{\ln(2)} \int \frac{1}{\sqrt{2\pi\varphi^2}} e^{-\frac{u^2}{2\varphi^2}} \left(a_1 u + \frac{a_1^2 + 2a_2}{2} u^2\right) du \sim \\ \frac{1}{\ln(2)} \left(\frac{a_1^2 + 2a_2}{2}\varphi^2\right) \sim \mathcal{O}(\varphi^2) \sim \mathcal{O}(\frac{1}{\sqrt[3]{t_{tot}}}).\end{aligned} \tag{S87}$$

We then conclude that the error that is made when calculating the mutual information, $I(C,N)$, using the normal approximation of the probability density function, $p(N|T,t_{tot})$, is $\sim \mathcal{O}(\frac{1}{\sqrt[3]{t_{tot}}})$. The simulations presented in the main body of the paper confirm this conclusion.

REFERENCES

Skupin A, Falcke M. Statistical properties and information content of calcium oscillations. *Genome Inform Ser* **18** (2007) 44–53.

Thurley K, Tovey SC, Moenke G, Prince VL, Meena A, Thomas AP, et al. Reliable encoding of stimulus intensities within random sequences of intracellular ca2+ spikes. *Science Signaling* **7** (2014) ra59–ra59. doi:10.1126/scisignal.2005237.

Slater LJ. *Generalized Hypergeometric Functions* (Cambridge: Cambridge University Press) (1966).

Ross SM. *Introduction to Probability Models, 11th ed.* (Waltham, MA: Academic Press) (2014).