Stochastic branching-diffusion models for gene expression

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A challenge to both understanding and modeling biochemical networks is integrating the effects of diffusion and stochasticity. Here, we use the theory of branching processes to give exact analytical expressions for the mean and variance of protein numbers as a function of time and position in a spatial version of an established model of gene expression. We show that both the mean and the magnitude of fluctuations are determined by the protein's Kuramoto length—the typical distance a protein diffuses over its lifetime—and find that the covariance between local concentrations of proteins often increases if there are substantial bursts of synthesis during translation. Using high-throughput data, we estimate that the Kuramoto length of cytoplasmic proteins in budding yeast to be an order of magnitude larger than the cell diameter, implying that many such proteins should have an approximately uniform concentration. For constitutively expressed proteins that live substantially longer than their mRNA, we give an exact expression for the deviation of their local fluctuations from Poisson fluctuations. If the Kuramoto length of mRNA is sufficiently small, we predict that such local fluctuations become approximately Poisson in bacteria in much of the cell, unless translational bursting is exceptionally strong. Our results therefore demonstrate that diffusion can act to both increase and decrease the complexity of fluctuations in biochemical networks.

A challenge in systems biology is to understand how the spatial structure of cells influences signal transduction and information processing (1). Diffusion is not only fundamental to many developmental processes (2), but also to responses in differentiated cells: it is, for example, necessary for nanoclusters of signaling proteins to form temporarily at the cell membrane (3) and to allow some cells to polarize (4). Yet, most modelers assume that a cell is "well stirred"—that the effects of diffusion are negligible and that any location in the cell is identical to any other. Modeling space and diffusion in biochemical networks is particularly challenging because these networks are now recognized to often be substantially stochastic (5–7). Consequently, the standard approach is to use numerical simulation (8), but many simulations are required to build intuition on a system of interest. Further, multiple different methodologies for such simulations exist because of the difficulties of the underlying theory of stochastic reaction-diffusion systems (9).

Here, we present analytical solutions to a reaction-diffusion version of a well-known model of gene expression (10). We give expressions for the spatial correlations in the system and determine the limits under which the well-stirred approximation holds and when diffusion dominates, generating fluctuations that, unlike the well-stirred case, are locally approximately Poissonian. As well as building intuition, our analytical results should provide useful tests to validate algorithms for stochastic spatial simulations.

The key to our approach is to consider gene expression as a stochastic branching process. When solving stochastic systems, the master equation is usually mapped to a partial differential equation describing the evolution of the generating function of the system. With space and diffusion, however, this equation becomes a partial differential equation for a functional (11), which is difficult to solve. In our approach, spatial diffusion generates a system of partial differential equations for the evolution of a set of generating functions. These equations are considerably more tractable.

To begin, we illustrate our method by first considering gene expression without diffusion.

Gene Expression Without Diffusion

We consider a model of constitutive gene expression: Transcription of the gene can always occur. Fig. 1*A* shows the processes involved. The probability of having N_2 mRNAs and N_3 proteins obeys a master equation:

$$\begin{aligned}\frac{\partial P_{N_2,N_3}}{\partial t} &= v_2(P_{N_2-1,N_3} - P_{N_2,N_3}) + v_3 N_2(P_{N_2,N_3-1} - P_{N_2,N_3}) \\ &\quad + d_2[(N_2+1)P_{N_2+1,N_3} - N_2 P_{N_2,N_3}] \\ &\quad + d_3[(N_3+1)P_{N_2,N_3+1} - N_3 P_{N_2,N_3}]. \end{aligned} \quad [1]$$

This model of gene expression is a branching process because each molecule behaves independently and does not interact with any other molecules: there are no binary reactions. This independence means that a system with initially N molecules either of one or of several different species is statistically identical to the sum of N independent systems, each of which has a single initial molecule. We can consequently describe the evolution of the system by three different generating functions, all having one initial molecule, which can be either a DNA, an mRNA, or a protein.

We will first consider the standard generating function and its evolution (12). Letting N_i be the number of molecules of species i, where $i = 1$ for DNA, $i = 2$ for mRNA, and $i = 3$ for protein, then the generating function, defined as $\sum_n s^n P_n$ for a one dimensional system, can be written as

$$g(t, s_1, s_2, s_3) = \mathbb{E}\left[\prod_{i=1}^{3} s_i^{N_i(t)}\right], \quad [2]$$

where the s_i are auxiliary variables with $0 \le s_i \le 1$. We do not explicitly write the dependence on the initial condition. The expectation is taken over $P_{N_1,N_2,N_3}(t)$ and is shown as a time dependence in the exponent of Eq. **2**. Rescaling time by the degradation rate of protein, d_3, so that $\tau = d_3 t$ and substituting Eq. **2** into Eq. **1** gives

$$\frac{\partial g}{\partial \tau} = \left[a(s_2 - 1) + \gamma(b s_2(s_3 - 1) - (s_2 - 1))\frac{\partial}{\partial s_2} - (s_3 - 1)\frac{\partial}{\partial s_3}\right] g, \quad [3]$$

where $a = v_2/d_3$ is the number of mRNAs transcribed during a typical lifetime of a protein and $b = v_3/d_2$ is the burst size or the

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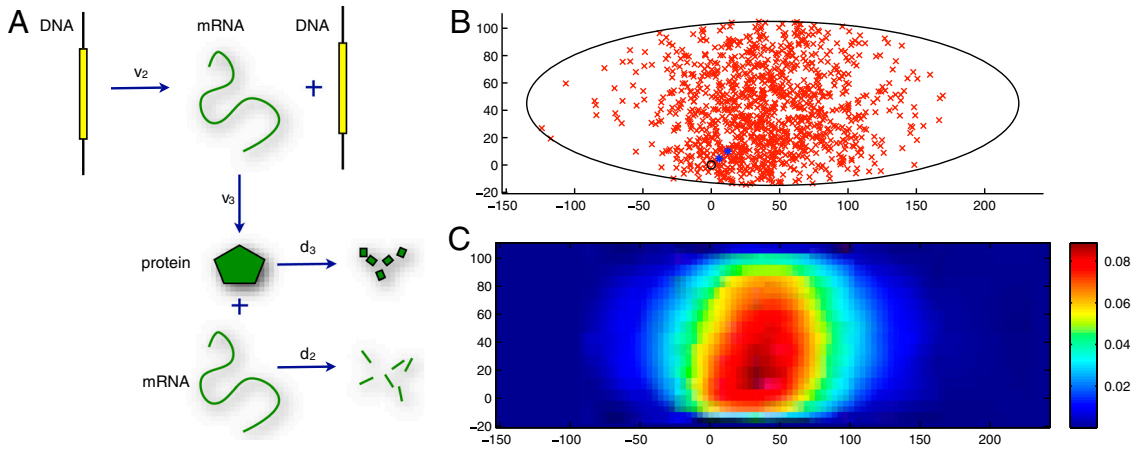

Fig. 1. (*A*) A model of constitutive gene expression (10). DNA is transcribed into mRNA with a probability per unit time of v_2; mRNA is translated into protein with a probability per unit time of v_3. Both mRNA and protein are degraded: mRNA with a probability per unit time of d_2 and protein with a probability per unit time of d_3. (*B*) Protein usually diffuses further from the DNA than mRNA because mRNA has both a lower diffusion coefficient and degrades faster. A snapshot of simulation results for an elliptical cell. Red crosses denote protein, blue asterisks denote mRNA, and the black circle at the origin denotes the DNA. We set $d_2 = 0.006\ \text{s}^{-1}$ (a half-life of 2 min), $d_3 = 0.0002\ \text{s}^{-1}$ (a half-life of 1 h), $v_2 = 0.01\ \text{s}^{-1}$, and $v_3 = 0.12\ \text{s}^{-1}$ (10). Consequently, $a \simeq 52$, $b = 20$, and $\gamma = 30$. The diffusion coefficients are $D_2 = 0.5\ \mu\text{m}^2\ \text{s}^{-1}$ and $D_3 = 5\ \mu\text{m}^2\ \text{s}^{-1}$ giving Kuramoto lengths of $\kappa_2 = 9.3\ \mu\text{m}$ and $\kappa_3 = 160\ \mu\text{m}$. Axis labels are in micrometers. (*C*) A corresponding heat map of the steady-state number density of molecules of protein.

typical number of proteins synthesized from a single mRNA during the mRNA's lifetime (13). The parameter γ is the ratio of the lifetime of the protein to the mRNA—$\gamma = d_2/d_3$—and is typically larger than one (5). Eq. **3** has the initial condition $g(0, s_1, s_2, s_3) = \prod_{i=1}^{3} s_i^{N_i(0)}$ when there is initially $N_i(0)$ molecules of species i. It can be solved approximately for $\gamma \gg 1$ giving a negative binomial distribution for protein numbers at steady state (5).

We consider an alternative description of this system using techniques from the theory of branching processes (14, 15). The number of molecules of species i at time t is a sum of the number of species of type i generated by each of the initial molecules. Writing $N_{i|j}^{(k)}(t)$ to be the number of molecules of species i at time t that are generated from the kth initial molecule of species j, then the number of molecules of species i is $N_i(t) = \sum_{j=1}^{3} \sum_{k=1}^{N_j(0)} N_{i|j}^{(k)}(t)$. The $N_{i|j}^{(k)}$ for $k = 1, \ldots, N_j(0)$ are identically distributed and independent. From Eq. **2**, we can then write the generating function as

$$g = \mathbb{E}\left[\prod_{i=1}^{3}\prod_{j=1}^{3}\prod_{k=1}^{N_j(0)} s_i^{N_{i|j}^{(k)}(t)}\right] = \prod_{j=1}^{3}\prod_{k=1}^{N_j(0)} \mathbb{E}\left[\prod_{i=1}^{3} s_i^{N_{i|j}^{(k)}(t)}\right], \qquad [4]$$

but, again from Eq. **2**, this last expectation is the generating function for a gene expression process that starts with a single molecule of species j. We will define u_j to be such a generating function:

$$u_j(t, s_1, s_2, s_3) = g(t, s_1, s_2, s_3 | 1 \text{ molecule of species } j). \qquad [5]$$

Hence,

$$g = \prod_{j=1}^{3}\prod_{k=1}^{N_j(0)} u_j = \prod_{j=1}^{3} u_j^{N_j(0)}, \qquad [6]$$

and so the three u_j collectively contain the same information as the generating function g.

From the chemical reactions occurring during gene expression, we can derive how the u_j evolve over time. For example, consider the generating function corresponding to a single initial DNA molecule, u_1, at time $t + dt$ for a small time interval dt. From Eq. **2**, this generating function obeys

$$u_1(t + dt) = \mathbb{E}\left[\prod_{i=1}^{3} s_i^{N_i(t+dt)} \middle| \mathbf{N}(0) = (1, 0, 0)\right] \qquad [7]$$

by definition. If we consider the time interval dt to be at the start of the dynamics of the system when only a single DNA molecule is present and to be small enough that only one reaction can possibly have occurred then this reaction can only be the synthesis of an mRNA. Consequently, remembering that v_2 is the probability of synthesis of an mRNA per unit time, we can write

$$\begin{aligned} u_1(t + dt) &= v_2 dt \mathbb{E}\left[\prod_{i=1}^{3} s_i^{N_i(t+dt)} \middle| \mathbf{N}(dt) = (1, 1, 0)\right] \\ &+ (1 - v_2 dt)\mathbb{E}\left[\prod_{i=1}^{3} s_i^{N_i(t+dt)} \middle| \mathbf{N}(dt) = (1, 0, 0)\right] \\ &= v_2 dt u_1 u_2 + (1 - v_2 dt)u_1, \end{aligned} \qquad [8]$$

and so that

$$\frac{\partial}{\partial t} u_1 = v_2 u_1 (u_2 - 1). \qquad [9]$$

Similarly, and if we rescale time to $\tau = d_3 t$, the three generating functions satisfy

$$\frac{\partial}{\partial \tau}\begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix} = \begin{bmatrix} a u_1 (u_2 - 1) \\ \gamma(b u_2 (u_3 - 1) - (u_2 - 1)) \\ -(u_3 - 1) \end{bmatrix}, \qquad [10]$$

with the initial condition $u_j(0, s_1, s_2, s_3) = s_j$ because at $t = 0$ only one molecule is present.

Gene Expression with Diffusion

We now include diffusion within Γ, a region of $\mathbb{R}^d$ (16, 17) (Fig. 1 *B* and *C*). Γ may either be all of $\mathbb{R}^d$ or a subregion with a boundary reflecting diffusing molecules. Let mRNA molecules have a diffusion coefficient of D_2 and protein molecules have a diffusion coefficient of D_3. We will consider DNA molecules that neither diffuse nor decay but are fixed at a point ξ_0 in Γ. If space has d dimensions then ξ_0 is a d-dimensional vector. We will let $x_i^{(k)}$ be the spatial location of the k'th molecule of species i. The generating function now depends on s_i that are functions of space, $s_i = s_i(\xi)$, with ξ in Γ, but has a similar form to Eq. **2**:

$$g(t, s_1, s_2, s_3) = \mathbb{E}\left[\prod_{i=1}^{3}\prod_{k=1}^{N_i(t)} s_i(x_i^{(k)}(t))\right]. \qquad [11]$$

The moments of the distribution for the number of molecules are derived analogously to the well-stirred model but now by functional differentiation of $g(t, s_1, s_2, s_3)$ by the variables $s_i(\xi)$ and then evaluating at $s_i = 1$. As before, we define generating functions for systems that initially have only one molecule of species j at location ξ_0

$$u_j(t, s_1, s_2, s_3|\xi_0) = g(t, s_1, s_2, s_3|1 \text{ molecule of species } j \text{ at } \xi_0), \quad [12]$$

and then the generating function can be expressed in terms of these new u_j

$$g(t, s_1, s_2, s_3) = \prod_{j=1}^{3} \prod_{k=1}^{N_j(0)} u_j(t, s_1, s_2, s_3|x_j^{(k)}(0)). \quad [13]$$

The evolution equations for the u_i are also similar although they contain the Laplacian operator

$$\left(\frac{\partial}{\partial \tau} - \mathbf{D}\nabla^2_{\xi_0}\right)\begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix} = \begin{bmatrix} au_1(u_2 - 1) \\ \gamma(bu_2(u_3 - 1) - (u_2 - 1)) \\ -(u_3 - 1) \end{bmatrix}, \quad [14]$$

with the initial conditions $u_i(0, s_1, s_2, s_3|\xi_0) = s_i(\xi_0)$ and where the matrix $\mathbf{D}$ is the diffusion matrix: $\mathbf{D} = d_3^{-1}\text{diag}(0, D_2, D_3)$. Eq. **14** holds for all ξ_0 in Γ, with the normal derivatives of the u_i being zero on the boundary of Γ, if applicable. By using branching processes, we have a Laplacian evaluated at the coordinates of the initial condition. Consequently, the evolution equations for the moments of the process are simpler than those derived directly from g.

Eq. **10** can be recovered from Eq. **14** if the initial conditions are constant in space: $u_i(0, s_1, s_2, s_3|\xi_0) = \text{constant}$ because then the term $\nabla^2_{\xi_0}\mathbf{u}$ vanishes.

First- and Second-Order Statistics

Including space, we must now consider fields for the molecular species rather than numbers of molecules. Let the field for species i be $X_i(t, \xi)$. It is defined as a distribution determined by the locations of the molecules of species i: $X_i(t, \xi) = \sum_{k=1}^{N_i(t)} \delta(x_i^{(k)}(t) - \xi)$. The field for DNA is, however, deterministic and constant with respect to time because we have one DNA molecule fixed at ξ_0.

We can obtain the moments of the process at a single point in time by differentiating the u_i with respect to $s_i(\xi)$. The first-order statistics are mean density fields; the second-order statistics are distributions on $\mathbb{R}^d \times \mathbb{R}^d$. Let $M_{i|j}$ be the mean density of species i given one initial molecule of species j at ξ_0. Then

$$M_{i|j}(\tau, \xi|\xi_0) = \mathbb{E}[X_i(\tau, \xi)|X_k(0, \xi) = \delta_{jk}\delta(\xi - \xi_0)] = \left.\frac{\delta u_j}{\delta s_i(\xi)}\right|_{s=1}. \quad [15]$$

For example, with this definition, the mean protein field is

$$\left.\frac{\delta u_j}{\delta s_3(\xi)}\right|_{s=1} = \left.\mathbb{E}\left[\frac{\delta}{\delta s_3(\xi)}\prod_{i=1}^{3}\prod_{k=1}^{N_i(t)} s_i(x_i^{(k)}(t))\right]\right|_{s=1}$$
$$= \mathbb{E}\left[\sum_{k=1}^{N_i(t)} \delta(\xi - x_3^{(k)}(t))\right] = \mathbb{E}[X_3(t, \xi)], \quad [16]$$

where we have not explicitly written the dependence on one initial molecule of species j at ξ_0. Integrating Eq. **15** over a volume gives the expected number of molecules of species j in the volume. Similarly, let $C_{ii'|j}$ denote the covariance densities between molecules of species i in one spatial location and molecules of species i' at another, given one initial molecule of species j at ξ_0, then

$$C_{ii'|j}(\tau, \xi, \xi'|\xi_0) = \mathbb{E}[X_i(\tau, \xi)X_{i'}(\tau, \xi')] - M_{i|j}(\tau, \xi|\xi_0)M_{i'|j}(\tau, \xi'|\xi_0)$$
$$= \left.\frac{\delta^2 u_j}{\delta s_i(\xi)\delta s_{i'}(\xi')}\right|_{s=1} + M_{i|j}(\tau, \xi|\xi_0)\delta_{ii'}\delta(\xi - \xi') - M_{i|j}(\tau, \xi|\xi_0)M_{i'|j}(\tau, \xi'|\xi_0), \quad [17]$$

where again we have not written explicitly the dependence of the expectation on one initial molecule of species j at ξ_0. Integrating Eq. **17** with respect to ξ over one volume and with respect to ξ' over another volume gives the expected covariance between the number of molecules of species i in the first volume with the number of molecules of species i' in the second volume.

Analytical Results

Reaction-diffusion systems are often characterized by the Kuramoto length (18): the distance a molecule typically diffuses over its lifetime. We will define a Kuramoto length for both mRNA and protein:

$$\kappa_2 = \sqrt{\frac{D_2}{d_2}}; \qquad \kappa_3 = \sqrt{\frac{D_3}{d_3}}. \quad [18]$$

We will consider either a d-dimensional space or specialize to three dimensions.

In principle, our main results hold for arbitrary geometries and are presented in terms of a general probability density describing diffusion of single molecules. Usually, and for simplicity, we will give results for free space, assuming that gene expression occurs sufficiently far from a confining membrane. Then the diffusion density, $f(t, \xi|\xi_0)$, is a Gaussian function: $f(t, \xi|\xi_0) = (4\pi t)^{-\frac{d}{2}}\exp[-\frac{|\xi - \xi_0|^2}{4t}]$. Alternatively, for Brownian diffusion in a region Γ with molecules reflecting off the boundary, we must solve the diffusion equation for $f(t, \xi|\xi_0)$ with the boundary condition that the spatial derivative of f normal to the boundary is zero. For general domains Γ, this system does not have a closed-form solution, but a series solution exists in terms of the eigenvalues and eigenmodes of the Laplacian operator on the region (19).

Mean and Covariance. To begin, we calculate the first and second moments of the distribution as integrals over the diffusion density. We functionally differentiate Eq. **14** with respect to $s_i(\xi)$ and evaluate at $s_i = 1$ to obtain a system of inhomogeneous linear reaction-diffusion equations. These equations can be solved using Green's function techniques (*SI Text*).

The mean density of mRNA is then

$$M_{2|1}(\tau, \xi|\xi_0) = a\int_0^\tau e^{-\gamma\tau_1} f(\kappa_2^2\gamma\tau_1, \xi|\xi_0)\mathrm{d}\tau_1, \quad [19]$$

and the mean density of protein is

$$M_{3|1}(\tau, \xi|\xi_0) = ab\gamma\int_0^\tau\int_0^{\tau_1} e^{-\gamma(\tau_1 - \tau_2) - \tau_2} \times f(\kappa_2^2\gamma(\tau_1 - \tau_2) + \kappa_3^2\tau_2, \xi|\xi_0)\mathrm{d}\tau_2\mathrm{d}\tau_1. \quad [20]$$

The single-time covariance matrix, $\mathbf{C}_{|1}$, has components $C_{ii'|1}$ and obeys

$$\mathbf{C}_{|1} = \delta(\xi-\xi')\begin{bmatrix}0&0&0\\0&M_{2|1}&0\\0&0&M_{3|1}\end{bmatrix} + ab\gamma\int_0^\tau\int_0^{\tau_1} e^{-\gamma(\tau_1-\tau_2)} \times \int_{\mathbb{R}^d} f(\kappa_2^2\gamma(\tau_1-\tau_2),\zeta|\xi_0)\mathbf{F}(\tau_2,\xi,\xi'|\zeta)\mathrm{d}\zeta\mathrm{d}\tau_2\mathrm{d}\tau_1, \quad [21]$$

where the matrix $\mathbf{F}$ is

$$\mathbf{F}(\tau,\xi,\xi'|\xi_0) = \begin{bmatrix}0&0&0\\0&0&M_{2|2}M'_{3|3}\\0&M_{3|3}M'_{2|2}&M_{3|3}M'_{3|2}+M_{3|2}M'_{3|3}\end{bmatrix}. \quad [22]$$

We use a prime to denote evaluation at ξ' so that $M'_{j|i} = M_{j|i}(\tau,\xi'|\xi_0)$ and $M_{j|i} = M_{j|i}(\tau,\xi|\xi_0)$. The mean densities with different initial conditions are

$$\begin{aligned} M_{2|2}(\tau,\xi|\xi_0) &= e^{-\gamma\tau}f(\kappa_2^2\gamma\tau,\xi|\xi_0)\\ M_{3|2}(\tau,\xi|\xi_0) &= b\gamma\int_0^\tau e^{-\gamma(\tau-\tau_1)-\tau_1} \times f(\kappa_2^2\gamma(\tau-\tau_1)+\kappa_3^2\tau_1,\xi|\xi_0)\mathrm{d}\tau_1\\ M_{3|3}(\tau,\xi|\xi_0) &= e^{-\tau}f(\kappa_3^2\tau,\xi|\xi_0). \end{aligned} \quad [23]$$

We will use these statistics to find radial correlation functions, which can be calculated explicitly.

Specializing to free space, our results when integrated over all space agree, as expected, with the nonspatial case. Setting $\xi_0 = 0$ and letting the overline denote integration over all spatial variables, the average total number of mRNAs in all of $\mathbb{R}^d$ is

$$\overline{M_{2|1}}(\tau) = \int_{\mathbb{R}^d} M_{2|1}(\tau,\xi|0)\mathrm{d}\xi = a\int_0^\tau e^{-\gamma\tau_1}\int_{\mathbb{R}^d} f(\kappa_2^2\gamma\tau_1,\xi|0)\mathrm{d}\xi\mathrm{d}\tau_1 = \frac{a}{\gamma}(1-e^{-\gamma\tau}), \quad [24]$$

agreeing with earlier work (5). Similarly, the average total number of protein molecules is

$$\overline{M_{3|1}}(\tau) = ab\left(1-\frac{\gamma}{\gamma-1}e^{-\tau}+e^{-\gamma\tau}\right), \quad [25]$$

as expected (5, 10).

For the covariance functions, we need to integrate over ξ and ξ' to compare with the nonspatial system. We find that the protein-protein covariance in the limit of $\tau\to\infty$ is

$$\overline{C_{33|1}}(\tau)\overset{\tau\to\infty}{\to} ab\left(1+\frac{b\gamma}{1+\gamma}\right), \quad [26]$$

again as expected (5, 10). For small τ, we find $\overline{C_{33|1}}(\tau) = ab(1+\frac{1}{3}b\gamma^2\tau^3)+o(\tau^4)$, a behavior similar to Poisson fluctuations.

The Steady-State Limit: Means. We use Laplace transforms to calculate the steady-state limits (*SI Text*). Assuming free space and that the DNA is fixed at the origin ($\xi_0 = 0$), the mean mRNA and protein densities at steady-state are

$$M_{2|1}(\xi) = \frac{a}{\gamma}\frac{e^{-|\xi|/\kappa_2}}{4\pi|\xi|\kappa_2^2}; \qquad M_{3|1}(\xi) = ab\frac{e^{-|\xi|/\kappa_3}-e^{-|\xi|/\kappa_2}}{4\pi|\xi|(\kappa_3^2-\kappa_2^2)}. \quad [27]$$

We can derive the steady-state mean squared distance of mRNA and protein from the origin from these equations, by integrating the product of $|\xi|^2$ and either $M_{2|1}$ or $M_{3|1}$ over all space:

$$\langle|\xi_2|^2\rangle = \frac{6a\kappa_2^2}{\gamma}; \qquad \langle|\xi_3|^2\rangle = 6ab(\kappa_2^2+\kappa_3^2),$$

and we see that the mean squared distance of protein is given by the sum of the squares of the Kuramoto lengths of mRNA and protein because protein is synthesized from a diffusing source (of mRNA).

The Steady-State Limit: Radial Correlation Functions. Even assuming free space, the covariance densities do not appear to yield simple analytic forms in the limit as $\tau\to\infty$. Instead, we can calculate explicit expression for the radial correlation functions where the radius r is the distance between the two fields whose covariation we are studying. For species i and j with fields X_i and X_j at time τ, we define $\Gamma_{ij}(\tau,r)$ to be the radial correlation function. It satisfies

$$r^{d-1}A_d\Gamma_{ij}(\tau,r) = \int_{\mathbb{R}^d}\int_{\mathbb{R}^d}\delta(r-|\xi'|)C_{ij|1}(\tau,\xi,\xi+\xi')\mathrm{d}\xi\mathrm{d}\xi', \quad [28]$$

where A_d is the area of the unit sphere in d dimensions, and ξ and ξ' are vectors measured from ξ_0, the location of the DNA molecule. The radial correlation function is still a density and determines how on average the covariance between a molecule of species i and a molecule of species j depends on the distance between these two molecules. It is calculated by averaging over all possible positions of these molecules relative to the DNA molecule. With this definition, we find that the radial correlation functions can be written as integrals (over time), but become explicit functions when $\tau\to\infty$. Considering only $d = 3$, free space, and writing $\kappa^2 = \frac{\gamma\kappa_2^2+\kappa_3^2}{\gamma+1}$, then

$$\Gamma_{22}(r) = \frac{\delta(r)}{4\pi r^2}\frac{a}{\gamma}; \qquad \Gamma_{23}(r) = \frac{ab}{\gamma\kappa_2^2+\kappa_3^2}\frac{e^{-r/\kappa}}{4\pi r} \quad [29]$$

and

$$\Gamma_{33}(r) = \frac{\delta(r)}{4\pi r^2}ab + \frac{ab^2}{\kappa_3^2-\kappa_2^2}\frac{e^{-r/\kappa_3}-e^{-r/\kappa}}{4\pi r}. \quad [30]$$

The $\delta(r)$ is interpreted in the right-handed sense: $\int_0^\epsilon\delta(z)g(z)\mathrm{d}z = g(0)$ for all $\epsilon > 0$. Eqs. **29** and **30** should be integrated over r to be interpreted as covariances of numbers of molecules. Integrating Eq. **29** over any region that does not include $r = 0$ shows that there is no covariance between spatially separated mRNAs, and integrating Eq. **30** over all r (multiplying by $4\pi r^2$ because we are using spherical coordinates) recovers Eq. **26**.

We further verified Eqs. **27**, **29**, and **30** by comparing integrals of these densities with Monte Carlo simulations (*SI Text*).

Limiting Cases

To more easily interpret the expressions for the mean of the protein field, Eq. **20**, and its covariance, Eq. **17**, we consider two limiting cases: the limit of rapid degradation of mRNA compared to degradation of protein ($\gamma\to\infty$) and the limit of fast diffusion of proteins ($\kappa_3\to\infty$).

The Limit of Fast Degradation of mRNA Compared to Protein. Many proteins have substantially longer lifetimes than their corresponding mRNAs (5). In the limit of $\gamma\gg 1$, with a and b remaining finite, mRNA diffuses relatively little over its lifetime compared to protein, and it appears that all the protein is synthesized at the DNA at the length scale associated with the diffusion of the protein, κ_3. Taking $\gamma\to\infty$ and $\xi_0 = 0$, we find at steady-state and in free space that

$$M_{3|1}(\xi) = \frac{ab}{4\pi|\xi|\kappa_3^2}\exp(-|\xi|/\kappa_3)$$

$$C_{33|1}(\xi,\xi') = \delta(\xi-\xi')M_{3|1}(\xi) + \frac{ab^2}{2\pi^3\kappa_3^6}\frac{K_2(\sqrt{2\theta})}{\theta}, \quad [31]$$

where $\theta = (|\xi|^2 + |\xi'|^2)/\kappa_3^2$. The function K_2 is the order 2 modified Bessel function of the second kind. In this $\gamma \gg 1$ limit, Eq. **31** implies that the correlation between the protein field at two different points depends only on the distance of each of the points to the DNA, and not on the position of the points relative to each other. These correlations arise because of the "burstiness" of the synthesis of protein at the origin: A single fixed Poisson source of protein would lead to a protein density field that is uncorrelated at distinct points.

One application of Eq. **31** is to estimate how far the distribution of proteins differs from being Poisson. For a reaction-diffusion system at equilibrium, detailed balance holds and the distribution of numbers of each chemical species is Poisson (11). Most biological systems are, however, far from equilibrium, but fluctuations averaged over a small volume should be dominated by diffusion and can be approximately Poisson (11). For a small region of volume, we can find the mean and variance of the number of molecules in that volume by integrating $M_{3|1}$ and $C_{33|1}$ over the region. At steady-state, we would obtain $C_{33|1}(\xi,\xi') = \delta(\xi-\xi')M_{3|1}(\xi)$ for a Poisson field, and the mean and variance would be equal. We can therefore measure the deviation from being Poisson in a small region by computing the Fano factor (the variance divided by the mean). At steady-state, when $\gamma \to \infty$, and for a small volume $\Delta\xi^3$ at a distance ξ from the origin, the result is (*SI Text*)

$$\text{Fano factor} = 1 + b\left(\frac{\Delta\xi}{\kappa_3}\right)^3 \mathcal{G}(\xi/\kappa_3), \quad [32]$$

where b is the burst size or the typical number of proteins synthesized per mRNA and $\mathcal{G}(z) = K_2(2z)\exp(z)/(2\pi^2 z)$ (*SI Text*).

We see from Eq. **32** that for large γ the deviation from being Poisson becomes small from either the burst size b being small, the volume of the region measured in units of the protein's Kuramoto length being small, or else the region being far from the DNA source (Fig. 2). This requirement for $\gamma \gg 1$ (the ratio of protein to mRNA lifetimes being large) implies short mRNA lifetimes and so the Kuramoto length of mRNA is negligible. (The effective lifetime of a protein cannot be increased arbitrarily because proteins are lost at cell division as well as being degraded.) Nevertheless, Eq. **32** confirms the observations of Saunders and Howard (20).

Limit of Fast Protein Diffusion. Spatially extended chemical systems are often assumed to be spatially homogeneous, obviating the need for considering the location of molecules. One justification for this assumption is that if diffusion rates of relevant species are fast compared to reaction rates, the species behave as if they are uniformly distributed over the region in question. For example, Grima and Schnell argue that diffusion effects become important when the average intermolecular distance and the Kuramoto length are of the same order (8). In our model, we can examine the limit of large κ_3. In free space, this limit implies that the steady-state protein density will converge to zero everywhere because proteins will rapidly leave any neighborhood of the DNA. To obtain a nontrivial limit, we therefore include diffusion in confined geometries. Let Γ be a reference region of dimensionless unit diameter (such as a sphere or a cube). We consider our system confined to the region $L\Gamma$ where L has units of length, so that our region has volume $L^d\text{Vol}(\Gamma)$ with d being the dimension of space, and use a series solution for $f(t,\xi|\xi_0)$ (*SI Text*).

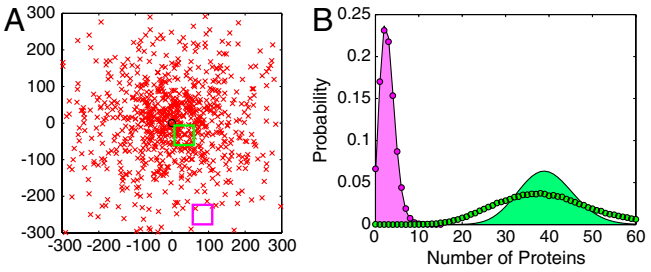

Fig. 2. Local fluctuations in protein numbers become approximately Poisson sufficiently far from the DNA. (*A*) A snapshot of a simulation of gene expression with diffusion in free space. Protein is denoted by red crosses and the DNA by the black circle. In this snapshot, there is by chance no mRNA. Parameter values are given in Fig. 1. Axis labels are in micrometers. (*B*) Histograms of counts of proteins sampled from the purple and green boxes in *A*. A total of 10^6 samples were taken at 10 s intervals. Each box has $\Delta\xi/\kappa_3 = 0.31$. The colored dots show data collected from the simulation. The filled curves show the best Poisson fits to the data. For the green box $|\xi|/\kappa_3 = 0.28$ and Eq. **32** predicts a deviation from being Poisson of about 0.8; for the purple box $|\xi|/\kappa_3 = 1.55$ with a predicted deviation from Poisson of about 0.005.

We find a uniform protein concentration approximately holds when the diameter of the region of interest is small compared to the protein's Kuramoto length. To be well mixed, the mean protein density needs to be constant over the region. We computed a series expansion of $M_{3|1}$ in powers of L/κ_3 at steady-state. To first-order, we find

$$M_{3|1}(\xi) = \frac{ab}{L^d\text{Vol}(\Gamma)}\left[1 + \left(\frac{L}{\kappa_3}\right)^2 \eta(\xi/L) + \cdots\right], \quad [33]$$

where η is a dimensionless function of position depending on the geometry of Γ. We see that $M_{3|1}$ approaches the well-stirred result of a concentration of $ab/L^d\text{Vol}(\Gamma)$ when $L \ll \kappa_3$.

Estimating the Kuramoto Lengths of Cytoplasmic Proteins

Our results show that the Kuramoto length determines both the size and the nature of local fluctuations, and we therefore estimated the Kuramoto length for cytoplasmic proteins in budding yeast. Although there are high-throughput measurements of protein lifetimes (21), there have been almost no measurements of diffusion coefficients in budding yeast. To estimate diffusion coefficients, we therefore used the measured diffusion coefficient for the kinase Fus3p (22) and rescaled this diffusion coefficient by the cubed root of the ratio of the molecular mass of Fus3p to the molecular mass of the protein of interest (*SI Text*). Although this approach assumes that proteins are uniformly dense spheres and so our results are only approximate, we believe that they are still informative.

All of the proteins we considered had Kuramoto lengths larger than a typical cell diameter (*SI Text*): Both the mean and the median Kuramoto lengths are well over 100 μm (160 and 128 μm, respectively), but the diameter of a cell is only approximately 4 μm (23). We expect this difference to also hold for some proteins in bacteria. For example, Green Fluorescent Protein (GFP) has a diffusion coefficient of 7.7 $\mu m^2\,s^{-1}$ in *Escherichia coli* (24) and typically decays only through dilution. With a cell-cycle time of 40 min, GFP then has a Kuramoto length of approximately 160 μm. An *E. coli* cell, however, has a length of around 2.5 μm (25).

Our results therefore indicate that cytoplasmic proteins are, perhaps typically, approximately uniformly distributed in *E. coli* and in budding yeast, at least assuming spherical proteins with constant diffusion coefficients and Brownian diffusion. Although we have not explicitly included the nucleus in our calculations, Eq. **33** is valid for the volume between two spheres providing this volume is not small compared to the volume of the outer sphere. We have also assumed constitutive expression, and Eq. **33** may

change with sufficiently large bursts of transcription. In contrast, for mRNAs, even neglecting nuclear export, we predict the Kuramoto length to be an order of magnitude smaller than the Kuramoto length of a protein and so of similar size to the cell diameter (assuming diffusion coefficients that are an order of magnitude less than those of protein and degradation rates that are an order of magnitude greater).

Discussion

To include spatial effects into models of biochemical networks is challenging. Cells have complex internal geometries, are intracellularly heterogeneous, and are packed with molecules, potentially generating substantial volume exclusion (26). Furthermore, combining spatial and stochastic effects even in simple geometries and homogeneous environments is mathematically challenging (11). Here, we have shown how techniques from the theory of branching processes can be used to derive analytical expressions for both the local mean and variance of proteins in an established model of gene expression, at least for point molecules and homogeneous, Brownian diffusion. Such Brownian diffusion is appropriate for proteins, at least when measured in *E. coli* (27), but anomalous diffusion (28) and active transport (29) has been reported for mRNA.

Our approach is extensible to other first-order biochemical networks in arbitrary geometries and with Markovian diffusion. For example, we need not only consider constitutively expressed genes but can also include regulated gene expression. Such expression is often modeled using a promoter with two states: one "off," with no expression, and the other "on," with a constant probability of expression per unit time (30). This model has been applied widely from bacteria (31) to human cells (32) and fits within our framework of branching processes (*SI Text*). To include the nucleus in our model, we should use a diffusion density for a confined region (such as that between two spheres) to describe the cytoplasm and consider the source of transcription in Fig. 1*A* not as a DNA molecule but as a nuclear pore complex stochastically exporting mRNA. Indeed, we can describe the export of mRNA from multiple nuclear pores diffusing on the outer membrane of the nucleus as a branching process.

Despite the complexity of the intracellular environment, our results indicate the local fluctuations of some proteins in bacteria can be well approximated by Poisson fluctuations, at least for those constitutively expressed and with small Kuramoto lengths for their mRNA. For example, assuming $\Delta\xi$ in Eq. **32** is 1/8 of the length of the cell, a conservative estimate of the Kuramoto length of 10 μm, and that the limit of large γ holds (and so negligible Kuramoto lengths for mRNA), then to have a correction to Poisson behavior of at least 10% in Eq. **32**, the burst size must be greater than 50 if the local volume is a distance of 0.75 μm from the DNA and greater than 600 if the local volume is at distance 1.75 μm. From measurements of around 1,000 genes in *E. coli*, however, over 92% of genes have proteins expressed with a b value less than 50 and over 99% have a b value of less than 600 (33). Almost all genes, though, even if the Kuramoto length for their mRNA is sufficiently small, would still have non-Poissonian fluctuations in protein numbers if the local volume is only 0.25 μm away from the DNA.

Our results imply that cytoplasmic proteins are often uniformly distributed in budding yeast and *E. coli* providing the levels of these proteins have had time to reach steady-state and the approximations we have made, particularly of spherical proteins and constitutive expression, are valid. Further, we have shown that diffusion can cause local fluctuations to be close to Poisson sufficiently far from, but not close to, the DNA. We expect non-Poisson behavior in cellular compartments that have diameters smaller than the Kuramoto length of protein, however, because of the reflecting boundary conditions imposed by the walls of the compartment (12). We also predict non-Poissonian behavior if translational bursting is sufficiently strong. Although our results are most applicable to bacteria because we have not explicitly included the nucleus, we expect that the intuition gained holds more generally (11, 12). With our analytical calculations, we have thus demonstrated that although space and diffusion are often a source of increased complexity when studying intracellular dynamics as a whole, they have, in contrast, the potential to simplify local behavior.

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Supporting Information

Cottrell et al. 10.1073/pnas.1201103109

SI Text

Analytical results: Mean and Covariance. Here, we explain how to derive and solve the equations for the moments of the particle fields. The generating functions for the gene expression system in the main text satisfy

$$\left(\frac{\partial}{\partial\tau}-\mathbf{D}\Delta_{\xi_0}\right)\mathbf{u}=\begin{bmatrix} au_1(u_2-1)\\ \gamma(bu_2(u_3-1)-(u_2-1))\\ -(u_3-1)\end{bmatrix}=\underbrace{\begin{bmatrix}0 & a & 0\\ 0 & -\gamma & b\gamma\\ 0 & 0 & -1\end{bmatrix}}_{\mathbf{A}}(\mathbf{u}-\mathbf{1})+\begin{bmatrix} a(u_1-1)(u_2-1)\\ b\gamma(u_2-1)(u_3-1)\\ 0\end{bmatrix}, \quad [S1]$$

where $\mathbf{D}=d_3^{-1}\mathrm{diag}(0,D_2,D_3)$ and $\mathbf{1}=(1,1,1)^T$.

We can obtain equations for the first and second moments by taking functional derivatives of Eq. **S1** with respect to $s_i(\xi), i=1,2,3$ and evaluating at $s_i(\xi)=1, i=1,2,3, \xi\in\mathbb{R}^d$. Using the notation described in the main text, we introduce the matrix of means, $[\mathbf{M}]_{ij}=M_{j|i}$, and the matrix of second moments

$$[\Theta_i]_{jk}=\Theta_{jk|i}=\left(\frac{\delta^2u_i}{\delta s_j(\xi)\delta s_k(\xi')}+\delta_{jk}\delta(\xi-\xi')\frac{\delta u_i}{\delta s_j(\xi)}\right)\Big|_{s=1}, \quad [S2]$$

$$\Theta=\begin{pmatrix}\Theta_1\\ \Theta_2\\ \Theta_3\end{pmatrix}.$$

After differentiating and evaluating Eq. **S1** as mentioned above, we obtain

$$\left(\frac{\partial}{\partial\tau}-\mathbf{D}\Delta_{\xi_0}\right)\mathbf{M}=\mathbf{A}\mathbf{M}, \quad [S3]$$

$$\left(\frac{\partial}{\partial\tau}-(\mathbf{D}\otimes\mathbf{I})\Delta_{\xi_0}\right)\Theta=(\mathbf{A}\otimes\mathbf{I})\Theta+\begin{pmatrix}\mathbf{F}_1\\ \mathbf{F}_2\\ 0\end{pmatrix}, \quad [S4]$$

where $\mathbf{A}$ is defined in Eq. **S1**, $\otimes$ is the matrix tensor product, $\mathbf{F}_2=\mathbf{F}(\tau,\xi,\xi'|\xi_0)$ is given in the main text, and

$$\mathbf{F}_1=\mathbf{F}_1(\tau,\xi,\xi'|\xi_0)=\begin{bmatrix}0 & M_{1|1}M'_{2|2} & M_{1|1}M'_{3|2}\\ M_{2|2}M'_{1|1} & M_{2|2}M'_{2|1}+M_{2|1}M'_{2|2} & M_{2|2}M'_{3|1}+M_{2|1}M'_{3|2}\\ M_{3|2}M'_{1|1} & M_{3|2}M'_{2|1}+M_{3|1}M'_{2|2} & M_{3|2}M'_{3|1}+M_{3|1}M'_{3|2}\end{bmatrix}. \quad [S5]$$

The moments satisfy the initial conditions $M_{j|i}(0,\xi|\xi_0)=\delta_{ij}\delta(\xi-\xi_0)$ and $\Theta_{jk|i}(0,\xi,\xi'|\xi_0)=\delta_{ij}\delta_{jk}\delta(\xi-\xi_0)\delta(\xi'-\xi_0)$.

Analytical solution of these equations is accomplished by splitting $\mathbf{A}=\mathbf{A}_d+\mathbf{A}_u$ into diagonal and strictly upper diagonal parts, introducing an integrating factor to absorb the term involving $\mathbf{A}_d$ and solving the remaining equations iteratively using standard Green's function or transform methods for the inhomogeneous heat equation. The solutions for the $\mathbf{M}$ are given in the main text. The second moments are

$$\Theta_1(t,\xi,\xi'|\xi_0)=\delta(\xi-\xi')\begin{bmatrix}M_{1|1} & 0 & 0\\ 0 & M_{2|1} & 0\\ 0 & 0 & M_{3|1}\end{bmatrix}(\tau,\xi|\xi_0)+a\int_0^\tau\mathbf{F}_1(\tau_1,\xi,\xi'|\xi_0)\mathrm{d}\tau_1+ab\gamma\int_0^\tau\int_0^{\tau_1}e^{-\gamma(\tau_1-\tau_2)}\times\int_{\mathbb{R}^d}f(\kappa_2^2\gamma(\tau_1-\tau_2),\zeta|\xi_0)\mathbf{F}_2(\tau_2,\xi,\xi'|\zeta)\mathrm{d}\zeta\mathrm{d}\tau_2\mathrm{d}\tau_1 \quad [S6]$$

$$\Theta_2(t,\xi,\xi'|\xi_0)=\delta(\xi-\xi')\begin{bmatrix}0 & 0 & 0\\ 0 & M_{2|2} & 0\\ 0 & 0 & M_{3|2}\end{bmatrix}(\tau,\xi|\xi_0)+b\gamma\int_0^\tau e^{-\gamma(\tau-\tau_1)}\int_{\mathbb{R}^d}f(\kappa_2^2\gamma(\tau-\tau_1),\zeta|\xi_0)\times\mathbf{F}_2(\tau_1,\xi,\xi'|\zeta)\mathrm{d}\zeta\mathrm{d}\tau_1 \quad [S7]$$

$$\Theta_3(\tau,\xi,\xi'|\xi_0)=\delta(\xi-\xi')\begin{bmatrix}0 & 0 & 0\\ 0 & 0 & 0\\ 0 & 0 & M_{3|3}\end{bmatrix}(\tau,\xi|\xi_0). \quad [S8]$$

We are primarily interested in obtaining expressions for the covariance

$$\mathbf{C}_1(t,\xi,\xi'|\xi_0)=\Theta_\mathbf{1}-\mathbf{M}_{1:}^T(t,\xi|\xi_0)\mathbf{M}_{1:}(t,\xi'|\xi_0). \quad [S9]$$

It turns out that the second term in the solution for $\Theta_\mathbf{1}$ is exactly

$$a\int_0^\tau\mathbf{F}_1(\tau_1,\xi,\xi'|\xi_0)\mathrm{d}\tau_1=\mathbf{M}_{1:}^T(t,\xi|\xi_0)\mathbf{M}_{1:}(t,\xi'|\xi_0)-\delta(\xi-\xi')\begin{bmatrix}M_{1|1}(\tau,\xi|\xi_0) & 0 & 0\\ 0 & 0 & 0\\ 0 & 0 & 0\end{bmatrix}, \quad [S10]$$

so it cancels with the matrix of the products of means and part of the first term. The first and third terms remain and yield the expressions listed in the main text. From these solutions of the moment equations, we can derive expressions for other statistical quantities such as variance densities and radial distribution functions.

The Steady-State Limit: Means. We compute the $\tau\to\infty$ limit of the means and radial correlation functions in a number of ways in order to verify our results. Recall that the Laplace transform of a function $g=g(\tau)$ is given by

$$(\mathscr{L}g)(s)=\int_{0^-}^\infty e^{-s\tau}g(\tau)\mathrm{d}\tau. \quad [S11]$$

The Laplace transform is particularly useful for calculating large time limits (1) via the identity

$$\lim_{\tau\to\infty}\int_0^\tau g(\tau_1)\mathrm{d}\tau_1 = \lim_{s\downarrow 0}(\mathscr{L}g)(s). \qquad \text{[S12]}$$

Using this identity, the long-term mRNA density can be calculated:

$$\begin{aligned}\lim_{\tau\to\infty} M_{2|1}(\tau,\xi|\xi_0) &= a\lim_{s\downarrow 0}\mathscr{L}[e^{-\gamma\tau}f(\kappa_2^2\gamma\tau,\xi|\xi_0)](s)\\ &= a\lim_{s\downarrow 0}\mathscr{L}[f(\kappa_2^2\gamma\tau,\xi|\xi_0)](s+\gamma)\\ &= \frac{a}{\kappa_2^2\gamma}\lim_{s\downarrow 0}\mathscr{L}[f(\tau,\xi|\xi_0)]\left(\frac{s+\gamma}{\kappa_2^2\gamma}\right)\\ &= \frac{a}{\kappa_2^2\gamma}L_d\left(\frac{1}{\kappa_2^2},|\xi-\xi_0|\right), \qquad \text{[S13]}\end{aligned}$$

where L_d is given by Eq. **S78**. Using the identity from Eq. **S80**, we can verify that $\int_{\mathbb{R}^d} M_{2|1}(\infty,\xi|\xi_0) = a/\gamma = v_0/d_0 = m_2$. Employing the same technique and a judicious change of variables, we can compute the $\tau\to\infty$ limit of $M_{3|1}$ when $\kappa_2\neq\kappa_3$:

$$\begin{aligned}\lim_{\tau\to\infty} M_{3|1}(\tau,\xi|\xi_0) = \frac{ab}{\kappa_3^2-\kappa_2^2}\Bigg(&L_d\left(\frac{1}{\kappa_3^2},|\xi-\xi_0|\right)\\ &- L_d\left(\frac{1}{\kappa_2^2},|\xi-\xi_0|\right)\Bigg). \qquad \text{[S14]}\end{aligned}$$

The case $\kappa_2=\kappa_3$ is given by the limit

$$\lim_{\kappa_2\to\kappa_3}\lim_{\tau\to\infty} M_{3|1}(\tau,\xi|\xi_0) = \frac{ab}{4\pi\kappa_3^4}L_{d-2}\left(\frac{1}{\kappa_3^2},|\xi-\xi_0|\right). \qquad \text{[S15]}$$

Typically $\kappa_3\gg\kappa_2$, so for regions a moderate distance $|\xi|\gg\kappa_2$ from the DNA source, we have

$$M_{3|1}(\infty,\xi|\xi_0)\approx\frac{ab}{\kappa_3^2}L_d\left(\frac{1}{\kappa_3^2},|\xi-\xi_0|\right). \qquad \text{[S16]}$$

With the aid of the identity in Eq. **S80**, we can integrate $M_{3|1}(\infty,\xi|\xi_0)$ over $\mathbb{R}^d$ to see that the mean total number of particles agrees with the nonspatial result:

$$\int_{\mathbb{R}^d} M_{3|1}(\infty,\xi|\xi_0)\mathrm{d}\xi = ab = v_0v_1/(d_0d_1) = m_3. \qquad \text{[S17]}$$

The Steady-State Limit: Radial Correlation Functions. Due to the symmetry of the covariance densities and the Gaussian structure of the diffusion mechanism, it is possible to compute tractable analytical expressions for Γ_{ij}. In the main text, we define the radial correlation functions using Dirac delta functions. A more rigorous definition is given by

$$\begin{aligned}\int_0^{\tilde r} z^{d-1}\Gamma_{ij}(\tau,z)\mathrm{d}z = \frac{1}{A_d}\int_{\mathbb{R}^d}\int_{\mathbb{R}^d} &H(\tilde r-|\zeta-\zeta'|)\\ &\times C_{ij|1}(\tau,\zeta,\zeta')\mathrm{d}\zeta\mathrm{d}\zeta', \qquad \text{[S18]}\end{aligned}$$

where H is the Heaviside function $H(r)=0, r\le 0, H(r)=1, r>0$ and A_d is the surface area of the d-dimensional sphere. Formally, we can think of Eq. **S18** as

$$\begin{aligned}\tilde r^{d-1}\Gamma_{ij}(\tau,\tilde r) &= \frac{1}{A_d}\int_{\mathbb{R}^d}\int_{\mathbb{R}^d}\delta_{\tilde r}(|\zeta-\zeta'|)C_{ij|1}(\tau,\zeta,\zeta')\mathrm{d}\zeta\mathrm{d}\zeta'\\ &\equiv \int_{\substack{(\zeta,\zeta')\in\mathbb{R}^{2d}\\ |\zeta-\zeta'|=\tilde r}} C_{ij|1}(\tau,\zeta,\zeta')\mathrm{d}\zeta\mathrm{d}\zeta', \qquad \text{[S19]}\end{aligned}$$

where $\delta_{\tilde r}(x)=\delta(x-\tilde r)$. Note that we are using an "unnormalized" version of the delta function in the sense that, $\iint\delta(|\zeta-\zeta'|-\tilde r)\mathrm{d}\zeta\mathrm{d}\zeta' = A_d\tilde r^{d-1}$. Also note that if the integrand $C_{ij|1}$ possesses integrable singularities [in (ζ,ζ')], the definition in the main text should be used rather than the formal delta function definition.

In both the mRNA-Pr $(i=2,j=3)$ and the Pr-Pr $(i=3,j=3)$ cases, the expressions involve terms of the form

$$\iint\delta(|\zeta-\zeta'|-\tilde r)\int f(T_3,\eta)f(T_1,\zeta-\eta)f(T_2,\zeta'-\eta)\mathrm{d}\eta\mathrm{d}\zeta\mathrm{d}\zeta'. \qquad \text{[S20]}$$

We can change the order of integration in Eq. **S20** and make a change of variables $\zeta\mapsto\zeta-\zeta'+\eta, \zeta'\mapsto\zeta'+\eta$ to simplify:

$$\begin{aligned}\text{Eq. S20} &= \int f(T_3,\eta)\mathrm{d}\eta\int\delta(|\zeta|-\tilde r)\int f(T_2,\zeta')f(T_1,\zeta-\zeta')\mathrm{d}\zeta'\mathrm{d}\zeta\\ &= \int\delta(|\zeta|-\tilde r)f(T_1+T_2,\zeta)\mathrm{d}\zeta\\ &= A_d\int_0^\infty r^{d-1}\delta(r-\tilde r)f(T_1+T_2,r)\mathrm{d}r\\ &= A_d\tilde r^{d-1}f(T_1+T_2,\tilde r), \qquad \text{[S21]}\end{aligned}$$

recalling that $f(\tau,\zeta)$ depends only on τ and $|\zeta|$. $\delta(|\zeta-\zeta'|-\tilde r)$ is the Dirac delta distribution on the sphere $|\zeta|=\tilde r$ in $\mathbb{R}^d$. With this identity we can obtain tractable analytic expressions for the radial correlation function Γ.

To facilitate comparison of these analytical expression to Monte Carlo estimates, we introduce the radial distribution functions (rdfs) $\rho_{ij}(r)$ and $\tilde\rho_{ij}(r)$. The rdfs are defined by

$$\begin{aligned}\int_0^{\tilde r} z^{d-1}\rho_{ij}(\tau,z)\mathrm{d}z = \frac{1}{A_d}\int_{\mathbb{R}^d}\int_{\mathbb{R}^d} &H(\tilde r-|\zeta-\zeta'|)\\ &\times\mathbb{E}(X_\tau^i(\zeta)X_\tau^j(\zeta'))\mathrm{d}\zeta\mathrm{d}\zeta' \qquad \text{[S22]}\end{aligned}$$

and

$$\begin{aligned}\int_0^{\tilde r} z^{d-1}\tilde\rho_{ij}(\tau,z)\mathrm{d}z = \frac{1}{A_d}\int_{\mathbb{R}^d}\int_{\mathbb{R}^d} &H(\tilde r-|\zeta-\zeta'|)\\ &\times M_{i|1}(\tau,\zeta)M_{j|1}(\tau,\zeta')\mathrm{d}\zeta'\mathrm{d}\zeta. \qquad \text{[S23]}\end{aligned}$$

If the spatial dimension $d=3$, the background term $\tilde\rho_{ij}\equiv\rho_{ij}-\Gamma_{ij}$ can also be computed analytically. The computation of this integral serves two purposes. Firstly, it allows us to analytically compute the effective radial distribution function $\rho_{ij}=\Gamma_{ij}+\tilde\rho_{ij}$. Secondly, since it is costly to estimate $\tilde\rho_{ij}$ by straightforward Monte Carlo methods, having an analytic expression for $\tilde\rho_{ij}$ is essential for comparing the theory to simulation. Fig. S1 shows ρ computed from analytics and simulation. The subfigures with error bars give the relative differences plus/minus one standard deviation of the sample estimates.

The following three subsections detail the calculation of the rcf Γ and the background term $\tilde\rho$ for the mRNA-Protein and Protein-Protein fields.

mRNA-protein (Γ_{23}). The unconvolved form of the mRNA-protein covariance is

$$C_{23|1}(\tau,\zeta,\zeta') = \mathbf{II}_{23}, \quad \text{[S24]}$$

where $\mathbf{II}_{23} =$

$$ab\gamma\int_0^\tau\int_0^{\tau_1}\int e^{-\gamma\tau_1-\tau_2}f(\kappa_2^2\gamma(\tau_1-\tau_2),\eta) \times f(\kappa_2^2\gamma\tau_2,\zeta-\eta)f(\kappa_3^2\tau_2,\zeta'-\eta)\mathrm{d}\eta\mathrm{d}\tau_2\mathrm{d}\tau_1. \quad \text{[S25]}$$

Applying the above manipulations,

$$\begin{aligned}A_d\tilde{r}^{d-1}\Gamma_{23} &= \iint\delta(|\zeta-\zeta'|-\tilde{r})\mathbf{II}_{23}(\tau,\zeta,\zeta')\mathrm{d}\zeta\mathrm{d}\zeta'\\ &= ab\gamma\iint\delta(|\zeta-\zeta'|-\tilde{r})\int_0^\tau\int_0^{\tau_1}e^{-\gamma\tau_1-\tau_2}\\ &\quad\times\int f(\kappa_2^2\gamma(\tau_1-\tau_2),\eta)f(\kappa_2^2\gamma\tau_2,\eta-\zeta')\\ &\quad\times f(\kappa_3^2\tau_2,\eta-\zeta)\mathrm{d}\eta\mathrm{d}\tau_2\mathrm{d}\tau_1\mathrm{d}\zeta\mathrm{d}\zeta'\\ &= ab\gamma A_d\tilde{r}^{d-1}\int_0^\tau\int_0^{\tau_1}e^{-\gamma\tau_1-\tau_2}f((\kappa_2^2\gamma+\kappa_3^2)\tau_2,\tilde{r})\mathrm{d}\tau_2\mathrm{d}\tau_1.\end{aligned} \quad \text{[S26]}$$

In the limit as $\tau\to\infty$, we can exchange the order of integration to obtain

$$\begin{aligned}\Gamma_{23}(\tilde{r}) &= ab\gamma\int_0^\infty\int_0^{\tau_1}e^{-\gamma\tau_1-\tau_2}f((\kappa_2^2\gamma+\kappa_3^2)\tau_2,\tilde{r})\mathrm{d}\tau_2\mathrm{d}\tau_1\\ &= ab\gamma\int_0^\infty\int_{\tau_2}^\infty e^{-\gamma\tau_1-\tau_2}f((\kappa_2^2\gamma+\kappa_3^2)\tau_2,\tilde{r})\mathrm{d}\tau_1\mathrm{d}\tau_2\\ &= ab\gamma\frac{1}{\gamma}\int_0^\infty e^{-(1+\gamma)\tau_2}f((\kappa_2^2\gamma+\kappa_3^2)\tau_2,\tilde{r})\mathrm{d}\tau_2\\ &= ab\int_0^\infty e^{-(1+\gamma)\tau_2}(4\pi\hat{\kappa}^2\tau_2)^{-d/2}e^{-\tilde{r}^2/(4\hat{\kappa}^2\tau_2)}\mathrm{d}\tau_2,\end{aligned} \quad \text{[S27]}$$

where $\hat{\kappa}^2=\kappa_2^2\gamma+\kappa_3^2$. In the $\tau\to\infty$ limit, we can obtain an explicit expression for Γ_{23}:

$$\Gamma_{23}(\infty,\tilde{r}) = \frac{ab}{\hat{\kappa}^2}L_d\left(\frac{1+\gamma}{\hat{\kappa}^2},\tilde{r}\right). \quad \text{[S28]}$$

Protein-protein (Γ_{33}). The unconvolved form of the protein-protein covariance is

$$C_{33|1}(\tau,\zeta,\zeta'|\zeta_0) = \delta(\zeta-\zeta')M_{3|1}(\tau,\zeta|\zeta_0)+\mathbf{II}_{33}, \quad \text{[S29]}$$

where

$$\begin{aligned}\mathbf{II}_{33} &= ab\gamma\int_0^\tau\int_0^{\tau_1}e^{-\gamma(\tau_1-\tau_2)}\int f(\kappa_2^2\gamma(\tau_1-\tau_2),\eta)\\ &\quad\times\mathbf{F}_{33}(\tau_2,\zeta,\zeta'|\eta)\mathrm{d}\eta\mathrm{d}\tau_2\mathrm{d}\tau_1\\ &= a(b\gamma)^2\int_0^\tau\int_0^{\tau_1}\int_0^{\tau_2}e^{-\gamma(\tau_1-\tau_2)}e^{-\gamma(\tau_2-\tau_3)-\tau_2-\tau_3}\\ &\quad\times\int f(\kappa_2^2\gamma(\tau_1-\tau_2),\eta)\\ &\quad\times[f(\kappa_2^2\gamma(\tau_2-\tau_3)+\kappa_3^2\tau_3,\zeta-\eta)f(\kappa_3^2\tau_2,\zeta'-\eta)\\ &\quad+f(\kappa_2^2\gamma(\tau_2-\tau_3)+\kappa_3^2\tau_3,\zeta'-\eta)f(\kappa_3^2\tau_2,\zeta-\eta)]\mathrm{d}\eta\mathrm{d}\tau_3\mathrm{d}\tau_2\mathrm{d}\tau_1.\end{aligned} \quad \text{[S30]}$$

Plugging $C_{33|1}$ into the definition of Γ we see that the first term is

$$\begin{aligned}&\int_{\mathbb{R}^d}\int_{\mathbb{R}^d}H(\tilde{r}-|\zeta-\zeta'|)\delta(\zeta-\zeta')M_{3|1}(\tau,\zeta)\mathrm{d}\zeta\mathrm{d}\zeta'\\ &\quad=\int_{\mathbb{R}^d}M_{3|1}(\tau,\zeta)\mathrm{d}\zeta = ab\end{aligned} \quad \text{[S31]}$$

for all $\tilde{r}>0$. This means that Γ_{33} is a distribution that can formally be written as

$$A_d\tilde{r}^{d-1}\Gamma_{33}(\tau,\tilde{r}) = \delta(\tilde{r})ab+\text{“}\mathbf{II}_{33}\text{term”}. \quad \text{[S32]}$$

The second term, after some manipulation as discussed above, gives

$$\begin{aligned}\text{“}\mathbf{II}_{33}\text{term”} &= \iint\delta(\tilde{r}-|\zeta-\zeta'|)\mathbf{II}_{33}\mathrm{d}\zeta\mathrm{d}\zeta'\\ &= 2a(b\gamma)^2A_d\tilde{r}^{d-1}\int_0^\tau\int_0^{\tau_1}\int_0^{\tau_2}\Phi\mathrm{d}\tau_3\mathrm{d}\tau_2\mathrm{d}\tau_1,\end{aligned} \quad \text{[S33]}$$

where

$$\Phi = e^{-\gamma(\tau_1-\tau_2)-\gamma(\tau_2-\tau_3)-\tau_2-\tau_3}f(\kappa_2^2\gamma(\tau_2-\tau_3)+\kappa_3^2(\tau_2+\tau_3),\tilde{r}). \quad \text{[S34]}$$

We take the limit as $\tau\to\infty$ and rearrange the order of integration. Letting $\kappa^2=(\gamma\kappa_2^2+\kappa_3^2)/(\gamma+1)$ and changing variables gives

$$\Phi = e^{-\gamma S_3}e^{-(1-\kappa^2/\kappa_3^2)S_2}e^{-S_1/\kappa_3^2}f(S_1,\tilde{r}), \quad \text{[S35]}$$

where $S_1=(\kappa_2^2\gamma+\kappa_3^2)(\tau_2-\tau_3)+2\kappa_3^2\tau_3$, $S_2=(1+\gamma)(\tau_2-\tau_3)$, $S_3=(\tau_1-\tau_2)$. In the limit $\tau\to\infty$, the integral in Eq. **S33** becomes

$$\text{“}\mathbf{II}_{33}\text{ term”} = \frac{a(b\gamma)^2}{(1+\gamma)\kappa_3^2}A_d\tilde{r}^{d-1}\int_0^\infty\int_0^{S_1/\kappa^2}\int_0^\infty\Phi dS_3dS_2dS_1. \quad \text{[S36]}$$

Performing the integrals for S_3 and S_2 and simplifying gives

$$\begin{aligned}\text{“}\mathbf{II}_{33}\text{ term”} &= \frac{ab^2}{\kappa_3^2-\kappa_2^2}A_d\tilde{r}^{d-1}\int_0^\infty[1\\ &\quad-e^{-(1/\kappa_3^2-1/\kappa^2)S_1}]e^{-S_1/\kappa_3^2}f(S_1,\tilde{r})dS_1.\end{aligned} \quad \text{[S37]}$$

An explicit form can be obtained using Eq. **S77**:

$$\begin{aligned}A_d\tilde{r}^{d-1}\Gamma_{33}(\infty,\tilde{r}) &= \delta(\tilde{r})ab\\ &\quad+A_d\tilde{r}^{d-1}\frac{ab^2}{\kappa_3^2-\kappa_2^2}\left(L_d(\frac{1}{\kappa_3^2},\tilde{r})\right.\\ &\quad\left.-L_d\left(\frac{1+\gamma}{\gamma\kappa_2^2+\kappa_3^2},\tilde{r}\right)\right).\end{aligned} \quad \text{[S38]}$$

The term $\tilde{\rho}$. As mentioned above, the background term $\tilde{\rho}$ (Eq. **S23**) relating the rdf ρ and the rcf Γ is a useful quantity that is slightly more difficult to estimate directly using Monte Carlo techniques than ρ in that it requires the generation of independent samples of the fields. We can calculate this quantity either analytically or by using quadrature.

We restrict ourselves to the case $d=3$ and change to radial coordinates and use the fact that the mean densities are radially symmetric. We note also that the integral over the angular coor-

dinates depends only on the radial lengths r, r' and $\tilde{r}$. Furthermore, this quantity either vanishes or is equal to the solid angle of the spherical cap of a cone with angle $\tilde{\theta}(r, r', \tilde{r})$. The key result is that

$$B_3(r, r', \tilde{r}) \equiv \int_0^{2\pi}\int_0^{\pi}\int_0^{2\pi}\int_0^{\pi} H(\tilde{r} - |\zeta - \zeta'|)\mathrm{d}\phi'\mathrm{d}\theta'\mathrm{d}\phi\mathrm{d}\theta$$
$$= \begin{cases} 8\pi^2(1-\cos\tilde{\theta}(r,r',\tilde{r})), & |\tilde{r}-r| \le r' \le r+\tilde{r} \\ 16\pi^2, & r \le \tilde{r}, r' \le \tilde{r}-r \\ 0, & \text{otherwise} \end{cases}, \quad \textbf{[S39]}$$

where $\tilde{\theta}$ is given by

$$\cos\tilde{\theta}(r, r', \tilde{r}) = \frac{1}{2}\frac{r^2 + (r')^2 - \tilde{r}^2}{rr'}. \quad \textbf{[S40]}$$

We can then write the integral from Eq. **S23** as

$$[\textbf{S23}] = 4\pi^2 \int_0^{\infty}\int_{|r-\tilde{r}|}^{r+\tilde{r}} rr'(\tilde{r}^2 - (r-r')^2) \times M_{i|1}(\tau, r)M_{j|1}(\tau, r')\mathrm{d}r'\mathrm{d}r + 16\pi^4 \int_0^{R}\int_0^{\tilde{r}-r} r^2(r')^2 M_{i|1}(\tau, r)M_{j|1}(\tau, r')\mathrm{d}r'\mathrm{d}r. \quad \textbf{[S41]}$$

These integrals can be computed in closed form. For example, in the limit as $\tau \to \infty$, the mean densities happen to be linear combinations of functions L_3 (Eq. **S78**), and Eq. **S23** can be written in terms of integrals of the form

$$I(s, s', \tilde{r}) \equiv \int_0^{\infty}\int_0^{\infty} B_3(r, r', \tilde{r})L_3(s, r)L_3(s', r')\mathrm{d}r\mathrm{d}r'. \quad \textbf{[S42]}$$

The quantity in Eq. **S42** can be calculated explicitly using symbolic math software such as Maple. The resulting expression is extremely long, so we omit its presentation here.

We can now write the rdfs (for dimension $d = 3$) in terms of the function in Eq. **S42**. We can then analytically compute $\rho_{ij}(\infty, \tilde{r}) = \Gamma_{ij}(\infty, \tilde{r}) + \tilde{\rho}_{ij}(\infty, \tilde{r})$, though we omit the details here.

Comparison to simulation. We verified Eqs. **23**, **29**, and **30** in the main text by comparing integrals of these densities over regions with exact Monte Carlo simulations. For our simulations, we used an event-driven algorithm that is a slight modification of the Gillespie algorithm (2): Over a given time interval of length Δt, each molecule of type i moves by a random amount in a random direction so that its displacement is a Gaussian random variable with a mean of zero and covariance of $D_i\Delta t$. Since diffusion is occurring in free space, there are no boundary conditions to implement.

As an example, to check the validity of our expression for $M_{2|1}(\infty, \xi|0)$ in Eq. **23**, we use simulations to estimate the mean number of steady-state mRNAs between two spheres of radius r_i and $r_i + \Delta r_i$. This number should equal the integral of $M_{2|1}$ over the volume:

$$\int_{r_i}^{r_i+\Delta r_i} 4\pi r^2 \frac{a}{\gamma}\frac{e^{-r/\kappa_2}}{4\pi r\kappa_2^2}\mathrm{d}r = -\frac{a}{\gamma}e^{-r/\kappa_2}(1 + r/\kappa_2)\Big|_{r=r_i}^{r=r_i+\Delta r_i}, \quad \textbf{[S43]}$$

as we show in Fig. S2.

Limiting Cases. ***The limit of fast degradation of mRNA compared to protein.*** For the following calculation, we assume that we are in $\mathbb{R}^3$ with no bounding walls and that the DNA molecule is at $\xi_0 = 0$. Accordingly, we suppress the dependence on ξ_0 in our notation. The limit $\gamma \to \infty$ with a and b fixed can be achieved by fixing v_2, d_3 and letting both $d_2, v_3 \to \infty$. Since we also fix D_2 and D_3, we have that $\kappa_2 \to 0$. From Eqs. **S13** and **S14**, we get, for $\gamma \to \infty$,

$$\lim_{\gamma\to\infty} M_{2|1}(\infty, \xi) = 0, \qquad \lim_{\gamma\to\infty} M_{3|1}(\infty, \xi) = ab\frac{e^{-|\xi|/\kappa_3}}{4\pi|\xi|\kappa_3^2}. \quad \textbf{[S44]}$$

In the following, we will use the identity

$$\lim_{\gamma\to\infty}\gamma\int_0^{\tau} e^{-\gamma t}g(t)dt = g(0) \quad \textbf{[S45]}$$

for $\tau > 0$, which can be checked using integration by parts.

To get $C_{33|1}$ in the limit $\gamma \to \infty$, we start with Eqs. **21** and **22** in the main text. We need to find the expressions for $M_{3|3}$ and $M_{3|2}$ in the $\gamma \to \infty$ limit. For the calculation of $M_{3|2}$, we use the change of variables $t = \tau - \tau_1$ to get

$$\lim_{\gamma\to\infty} M_{3|2}(\tau, \xi) = \lim_{\gamma\to\infty} b\gamma\int_0^{\tau} e^{-\gamma(\tau-\tau_1)-\tau_1}f(\kappa_2^2\gamma(\tau-\tau_1) + \kappa_3^2\tau_1, \xi)d\tau_1$$
$$= \lim_{\gamma\to\infty} b\gamma\int_0^{\tau} e^{-\gamma t}e^{-(\tau-t)}f(\kappa_2^2\gamma t + \kappa_3^2(\tau-t), \xi)dt$$
$$= be^{-\tau}f(\kappa_3^2\tau, \xi) \quad \textbf{[S46]}$$

and from Eq. **23**

$$M_{3|3}(\tau, \xi|\xi_0) = e^{-\tau}f(\kappa_3^2\tau, \xi), \quad \textbf{[S47]}$$

which does not depend on γ.

Using the change of variables

$$\tau_1 = x + y, \qquad \tau_2 = y, \qquad d\tau_2 = dy, \qquad d\tau_1 = dx, \quad \textbf{[S48]}$$

we obtain

$$\lim_{\gamma\to\infty} C_{33|1}(\xi, \xi') = \delta(\xi - \xi')M_{3|1} + \lim_{\gamma\to\infty} ab\gamma\int_0^{\infty}\int_0^{\tau_1} e^{-\gamma(\tau_1-\tau_2)} \times \int_{\mathbf{R}^d} f(\kappa_2^2\gamma(\tau_1-\tau_2), \zeta)F_{33}(\tau_2, \xi, \xi'|\zeta)d\zeta d\tau_1 d\tau_2$$
$$= \delta(\xi - \xi')M_{3|1} + ab\int_0^{\infty}\lim_{\gamma\to\infty}\gamma\int_0^{\infty} e^{-\gamma x} \times \int_{\mathbf{R}^d} f(\kappa_2^2\gamma x, \zeta)F_{33}(y, \xi, \xi'|\zeta)d\zeta dx dy$$
$$= \delta(\xi - \xi')M_{3|1} + ab\int_0^{\infty}\int_{\mathbf{R}^d} f(0, \zeta)F_{33}(y, \xi, \xi'|\zeta)d\zeta dy$$
$$= \delta(\xi - \xi')M_{3|1} + ab\int_0^{\infty} F_{33}(y, \xi, \xi')dy. \quad \textbf{[S49]}$$

Recall that

$$F_{33}(y, \xi, \xi') = M_{3|3}M'_{3|2} + M_{3|2}M'_{3|3} = 2be^{-2y}f(\kappa_3^2 y, \xi)f(\kappa_3^2 y, \xi'). \quad \textbf{[S50]}$$

The integral of F_{33} can be evaluated:

$$\int_0^\infty F_{33}(y,\xi,\xi')dy = 2b\frac{K_2(\sqrt{2}\sqrt{|\xi|^2+|\xi'|^2}/\kappa_3^2)}{4\pi^3\kappa_3^4(|\xi|^2+|\xi'|^2)}, \quad \text{[S51]}$$

where K_2 is the order 2 modified Bessel function of the second kind (3). Defining

$$\theta = (|\xi|^2+|\xi'|^2)/\kappa_3^2, \quad \text{[S52]}$$

we obtain

$$\begin{aligned}\lim_{\gamma\to\infty} C_{33|1}(\infty,\xi,\xi') &= \delta(\xi-\xi')M_{3|1}(\infty,\xi) \\ &\quad + 2ab^2\frac{K_2(\sqrt{2}\sqrt{|\xi|^2+|\xi'|^2}/\kappa_3)}{4\pi^3\kappa_3^4(|\xi|^2+|\xi'|^2)} \\ &= \delta(\xi-\xi')M_{3|1}(\infty,\xi) + \frac{ab^2}{2\pi^3\kappa_3^6}\frac{K_2(\sqrt{2\theta})}{\theta}.\end{aligned} \quad \text{[S53]}$$

Now, we consider the deviation of protein distribution from Poisson. Consider a cubic volume of length $\Delta\xi$ on each side. We imagine measuring the number of protein molecules contained in the volume at a given point in time. To determine how far the distribution of this variable differs from being Poissonian, we compute the variance divided by the mean which is the Fano factor. Assuming that $\Delta\xi$ is small, so that $M_{3|1}(\infty,\xi)$ and $C_{33|1}(\infty,\xi,\xi')$ are effectively constant over the volume, the mean number of particles is approximately

$$\Delta\xi^3 M_{3|1}(\infty,\xi) \quad \text{[S54]}$$

and the variance is approximately

$$\Delta\xi^3 M_{3|1}(\infty,\xi) + \Delta\xi^6\frac{ab^2}{2\pi^3\kappa_3^4}\frac{K_2(2|\xi|/\kappa_3)}{|\xi|^2}. \quad \text{[S55]}$$

So we have

$$\begin{aligned}\text{Fano factor} &= 1+\frac{\Delta\xi^6\frac{ab^2}{2\pi^3\kappa_3^4}\frac{K_2(2|\xi|/\kappa_3)}{|\xi|^2}}{\Delta\xi^3 M_{3|1}(\infty,\xi)} = 1+\frac{\Delta\xi^3\frac{ab^2}{2\pi^3\kappa_3^4}\frac{K_2(2|\xi|/\kappa_3)}{|\xi|^2}}{ab\frac{e^{-|\xi|/\kappa_3}}{4\pi|\xi|\kappa_3^2}} \\ &= 1+\Delta\xi^3\frac{b}{2\pi^2\kappa_3^2|\xi|}K_2(2|\xi|/\kappa_2)\exp(|\xi|/\kappa_3) \\ &= b\left(\frac{\Delta\xi}{\kappa_3}\right)^3\frac{K_2(2|\xi|/\kappa_2)\exp(|\xi|/\kappa_3)}{2\pi^2|\xi|/\kappa_3} \\ &= 1+b\left(\frac{\Delta\xi}{\kappa_3}\right)^3\mathcal{G}(|\xi|/\kappa_3),\end{aligned} \quad \text{[S56]}$$

where

$$\mathcal{G}(z) = \frac{K_2(2z)\exp(z)}{2\pi^2 z}. \quad \text{[S57]}$$

Limit of fast protein diffusion. Here, we obtain expressions for $M_{3|1}$ in a closed region with reflecting walls, in the limit of $\gamma\to\infty$ and of $L/\kappa_3\to 0$. For the purposes of this calculation, we may assume that protein molecules originate at a single fixed DNA molecule at ξ_0 at a constant rate. (These assumptions would not give the correct result for $C_{33|1}$.) Recall that we have rescaled time so that the rate of protein decay is 1.

Let our domain be $L\Gamma$, where Γ is a reference region with length 1 and L has units of length. We obtain for the mean density of protein at ξ

$$M_{3|1}(\xi|\xi_0) = ab\int_0^\infty e^{-\tau}f_L(\kappa_3^2\tau,\xi|\xi_0)dy. \quad \text{[S58]}$$

Here, f_L is the diffusion kernel of a particle diffusing at rate 1 within $L\Gamma$ with reflecting boundary conditions. A scaling argument yields that

$$f_L(y,\zeta|\zeta_0) = L^{-d}f(y/L^2,\zeta/L|\zeta_0/L), \quad \text{[S59]}$$

where f is the corresponding diffusion kernel for $L=1$.

We use the eigenfunction expansion of $f(t,\zeta|\zeta_0)$, dropping the dependence on ζ_0. Using separation of variables in the diffusion equation gives

$$f(t,\zeta) = \sum_{j=0}^\infty e^{-\lambda_j t}c_j\phi_j(\zeta), \quad \text{[S60]}$$

where c_j are constants, λ_j are the eigenvalues of the Laplacian on the domain, and ϕ_j are the corresponding orthogonal eigenfunctions. We use the normalization $\int_\Gamma\phi_j(\zeta)^2d\zeta = 1$. Now,

$$\delta(\zeta-\zeta_0) = f(0,\zeta) = \sum_{j=0}^\infty c_j\phi_j(\zeta). \quad \text{[S61]}$$

Integrating both sides against $\phi_j(\zeta)$ gives

$$\phi_j(\zeta_0) = \int_\Gamma\delta(\zeta-\zeta_0)\phi_j(\zeta)d\zeta = c_j. \quad \text{[S62]}$$

So,

$$f(t,\zeta|\zeta_0) = \sum_{j=0}^\infty e^{-\lambda_j t}\phi_j(\zeta_0)\phi_j(\zeta) \quad \text{[S63]}$$

and

$$\begin{aligned}f_L(y,\zeta|\zeta_0) &= L^{-d}f(y/L^2,\zeta/L|\zeta_0/L) \\ &= L^{-d}\sum_{j=0}^\infty e^{-\lambda_j y/L^2}\phi_j(\zeta_0/L)\phi_j(\zeta/L).\end{aligned} \quad \text{[S64]}$$

The reflecting boundary conditions tell us that ϕ_0 is constant and $\lambda_0 = 0$. All the other λ_j are positive. Since

$$\int_\Gamma\{\phi_0(\zeta|\zeta_0)\}^2d\zeta = 1, \quad \text{[S65]}$$

we have $\phi_0(\zeta|\zeta_0) = \text{vol}(\Gamma)^{-1/2}$ for all ζ.

Looking at mean protein concentration gives

$$\begin{aligned}
M_{3|1}(\xi|\xi_0) &= ab\int_0^\infty e^{-y} f_L(\kappa_3^2 y, \xi|\xi_0)dy \\
&= \frac{ab}{L^d}\int_0^\infty e^{-y}\sum_{j=0}^\infty e^{-y\lambda_j\kappa_3^2/L^2} c_j\phi_j(\xi/L)dy \\
&= \frac{ab}{L^d}\sum_{j=0}^\infty\left[\int_0^\infty e^{-y-y\lambda_j\kappa_3^2/L^2}dy\right]c_j\phi_j(\xi/L) \\
&= \frac{ab}{L^d}\sum_{j=0}^\infty \frac{1}{1+\lambda_j\kappa_3^2/L^2}c_j\phi_j(\xi/L) \\
&= \frac{ab}{L^d}c_0\phi_0(\xi/L) \\
&\quad + \frac{ab}{L^d}\sum_{j=1}^\infty \frac{L^2}{\lambda_j\kappa_3^2}\frac{1}{L^2/(\kappa_3^2\lambda_j)+1}c_j\phi_j(\xi/L) \\
&= \frac{ab}{L^d\mathrm{vol}(\Gamma)} \\
&\quad + \frac{ab}{L^d}\sum_{j=1}^\infty \frac{L^2}{\kappa_3^2}\frac{1}{\lambda_j}c_j\phi_j(\xi/L)\sum_{i=0}^\infty\left(\frac{L^2}{\kappa_3^2}\right)^i(-\lambda_j)^{-i} \\
&= \frac{ab}{L^d\mathrm{vol}(\Gamma)} \\
&\quad + \frac{ab}{L^d\mathrm{vol}(\Gamma)}\sum_{i=0}^\infty\left(\frac{L^2}{\kappa_3^2}\right)^{i+1}\mathrm{vol}(\Gamma)\sum_{j=1}^\infty c_j\phi_j(\xi/L)(-\lambda_j)^{-i} \\
&= \frac{ab}{L^d\mathrm{vol}(\Gamma)} + \frac{ab}{L^d\mathrm{vol}(\Gamma)}\sum_{i=1}^\infty\left(\frac{L^2}{\kappa_3^2}\right)^i\eta_i(\xi/L).
\end{aligned}$$

[S66]

Here, $\eta_i(\xi/L)$ is a dimensionless quantity depending on ξ, ξ_0 and the geometry of Γ.

Kuramoto Lengths for Proteins in Budding Yeast. There have been few direct measurements of the diffusion coefficient of proteins in budding yeast, and we therefore estimated diffusion coefficients using the molecular mass of the protein (obtained from www.uniprot.org) and the measured value of the diffusion coefficient of the kinase Fus3p (4). For a spherical protein, Stokes' law relates the diffusion coefficient, D, to the radius, R, of the protein (5):

$$D = \frac{kT}{6\pi\eta R}, \qquad \textbf{[S67]}$$

where k is Boltzmann's constant and T is temperature. The coefficient of viscosity is denoted η. Assuming each protein is also uniformly dense, then the mass, M, of a protein is proportional to the volume of a sphere or R^3. Consequently, the ratio of two diffusion coefficients obeys

$$\frac{D}{D'} = \frac{R'}{R} = \left(\frac{M'}{M}\right)^{\frac{1}{3}} \qquad \textbf{[S68]}$$

for uniformly dense, spherical proteins. We used Eq. **S68** to estimate diffusion coefficients with D' being the diffusion coefficent and M' being the molecular mass of Fus3p. To find the Kuramoto lengths, we took the square root of the diffusion coefficient multipled by the lifetime of the protein using the data of Belle et al. (6). We emphasize that our results are only approximate: They rely on the accuracy of the measurement of the diffusion coefficient of Fus3p and assume that cytoplasmic proteins are uniformly dense spheres.

Using gene ontologies (www.geneontology.org), we asked if particular classes of proteins had on average relatively small or large Kuramoto lengths. Given the large absolute Kuramoto lengths of all the proteins we investigated, there may, however, be little selection on exact values, but rather only to maintain values larger than the cell diameter. Proceeding nevertheless, we found that proteins involved in responses to chemical stimuli and stress, in sporulation, transporters, signal transduction, and in the generation of energy all had relatively low Kuramoto lengths despite having relatively high diffusion coefficients. These proteins may then be rapidly degraded to maintain higher local concentrations, perhaps for both specificity (7) and efficiency.

Regulated Expression of Genes. The model presented in the main text considers constituitively expressed genes, wherein the DNA is always active. We can extend our framework to genes that have regulated expression.

To do this, we consider four species of molecule: Active DNA, Inactive DNA, mRNA, and protein. Active DNA produces mRNA exactly as DNA does in the model in the main text. Inactive DNA does not produce mRNA. With rate k_0 inactive DNA switches into active DNA, and with rate k_1 active DNA switches into inactive DNA. This system also satisfies the criterion for a branching process: Once a particle is produced, its subsequent evolution and that of its offspring are independent of all other particles in the system. As in the main text, we rescale time by the rate of protein decay d_3 and use parameters $\kappa_0 = k_0/d_3$, $\kappa_1 = k_1/d_3$.

We first deal with the spatially homogeneous case. Let N_i be the number of molecules of species i, where $i = 0$ for inactive DNA, $i = 1$ for active DNA, and $i = 2, 3$ are for mRNA and protein, respectively. We define the generating functions as

$$g(t, s_0, s_1, s_2, s_3) = \mathbb{E}\left[\prod_{i=0}^3 s_i^{N_i(t)}\right] \qquad \textbf{[S69]}$$

and

$$u_j(t, s_0, s_1, s_2, s_3) = g(t, s_0, s_1, s_2, s_3|1 \text{ molecule of species } j). \qquad \textbf{[S70]}$$

Hence,

$$g = \prod_{j=0}^3\prod_{k=1}^{N_j(0)} u_j = \prod_{j=0}^3 u_j^{N_j(0)}. \qquad \textbf{[S71]}$$

Following a similar derivation as in the main text, we obtain the following equations for the u_i:

$$\frac{\partial}{\partial\tau}\begin{bmatrix}u_0\\u_1\\u_2\\u_3\end{bmatrix} = \begin{bmatrix}\kappa_0(u_1-u_0)\\ \kappa_1(u_0-u_1)+au_1(u_2-1)\\ \gamma[bu_2(u_3-1)-(u_2-1)]\\ -(u_3-1)\end{bmatrix}.$$

To include the effects of diffusion we allow mRNA and protein to diffuse through a region Γ as in the main text, but fix the position of the DNA, regardless of whether it is active or not. The generating functions depend on functions $s_i = s_i(\xi)$ for ξ in Γ and $i = 0, \ldots, 3$. We let

$$g(t, s_0, s_1, s_2, s_3) = \mathbb{E}\left[\prod_{i=0}^3\prod_{k=1}^{N_i(t)} s_i(x_i^{(k)}(t))\right] \qquad \textbf{[S72]}$$

and

$$u_j(t, s_0, s_1, s_2, s_3|\xi_0) = g(t, s_0, s_1, s_2, s_3|1 \text{ molecule of species } j \text{ at } \xi_0), \quad \text{[S73]}$$

which gives

$$g(t, s_0, s_1, s_2, s_3) = \prod_{j=0}^{3} \prod_{k=1}^{N_j(0)} u_j(t, s_0, s_1, s_2, s_3|x_j^{(k)}(0)). \quad \text{[S74]}$$

Similarly, the equations for the u_i are

$$\left(\frac{\partial}{\partial \tau} - \mathbf{D}\Delta_{\xi_0}\right)\mathbf{u} = \begin{bmatrix} \kappa_0(u_1 - u_0) \\ \kappa_1(u_0 - u_1) + au_1(u_2 - 1) \\ \gamma(bu_2(u_3 - 1) - (u_2 - 1)) \\ -(u_3 - 1) \end{bmatrix}, \quad \text{[S75]}$$

where $\mathbf{D} = d_3^{-1}\text{diag}(0, 0, D_2, D_3)$.

Appendix. Recall that $f(\tau, \xi|\xi_0)$ is the Gaussian density given by

$$f(\tau, \xi|\xi_0) = f(\tau, |\xi - \xi_0|) \equiv (4\pi\tau)^{-\frac{d}{2}} e^{-\frac{|\xi - \xi_0|^2}{4\tau}}. \quad \text{[S76]}$$

The Laplace transform of f (in τ) is given by

$$\mathscr{L}[f(t, \zeta)](s) = L_d(s, \zeta), \quad \text{[S77]}$$

for $\zeta > 0$, where

$$L_d(s, \zeta) \equiv \frac{1}{(2\pi)^{\frac{d}{2}}} \left(\frac{\zeta}{\sqrt{s}}\right)^{1-\frac{d}{2}} K_{\frac{d}{2}-1}(\sqrt{s}\zeta) = \begin{cases} \frac{1}{2\sqrt{s}} e^{-\sqrt{s}\zeta}, & d = 1 \\ \frac{1}{2\pi} K_0(\sqrt{s}\zeta), & d = 2 \\ \frac{1}{4\pi\zeta} e^{-\sqrt{s}\zeta}, & d = 3 \end{cases} \quad \text{[S78]}$$

and K_n is a modified Bessel function of the second kind with degree n (8). L_d satisfies the scaling relations

$$L_d(s, \sqrt{a}\zeta) = a^{1-\frac{d}{2}} L_d(as, \zeta) \quad \text{[S79]}$$

for $d = 1, 2, 3$. There are two useful identities involving integrals of L_d over $\mathbb{R}^d$. They can be computed using symbolic software such as Maple:

$$\int_{\mathbb{R}^d} L_d(s, \zeta)\mathrm{d}\zeta = A_d \int_0^\infty r^{d-1} L_d(s, r)\mathrm{d}r = \frac{1}{s},$$
$$\int_{\mathbb{R}^d} L_{d-2}(s, \zeta)\mathrm{d}\zeta = A_d \int_0^\infty r^{d-1} L_{d-2}(s, r)\mathrm{d}r = \frac{2\pi}{s^2}. \quad \text{[S80]}$$

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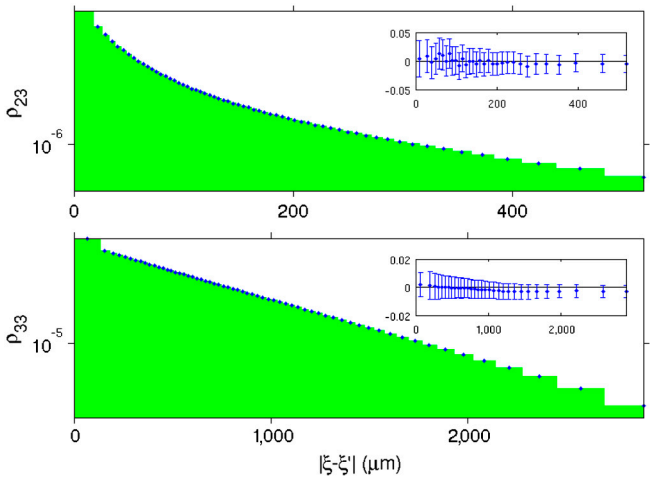

Fig. S1. Comparison of Monte Carlo estimates (blue points) and analytic expression (green solid areas) for rdfs ρ. The subplots show the relative difference of the MC estimates and theory with error bars corresponding to the standard deviation of the estimates. The parameters used are the same as in the main text, but with $D_3 = 50\ \mu\text{m}^2\ \text{s}^{-1}$.

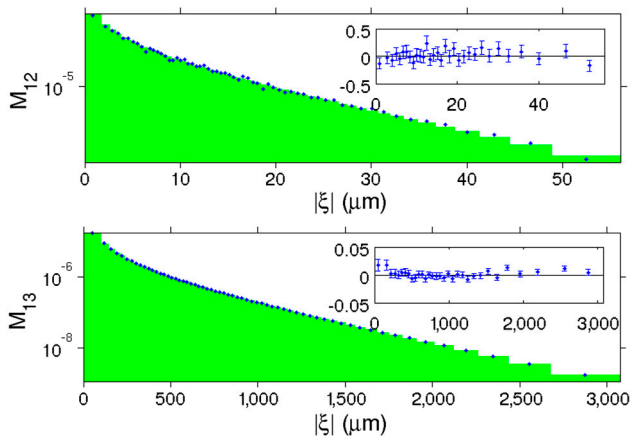

Fig. S2. Comparison of Monte Carlo estimates (blue points) and analytic expression (green solid areas) for means $M_{2|1} = M_{12}$ and $M_{3|1} = M_{13}$. The subplots show the relative difference of the MC estimates and theory with error bars corresponding to the standard deviation of the estimates. The parameters used are the same as in the main text, but with $D_3 = 50\ \mu m^2\ s^{-1}$.

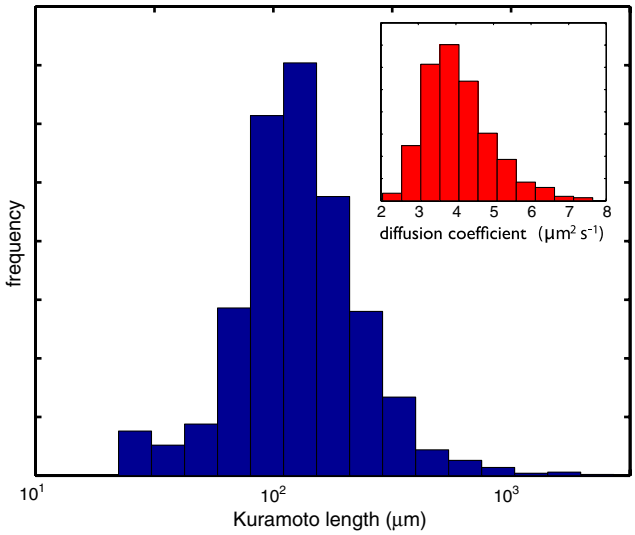

Fig. S3. The Kuramoto length for cytoplasmic proteins in budding yeast is expected to be larger than then the cell diameter (approximately 4 μm). Using a diffusion coefficient of 4.2 $\mu m^2\ s^{-1}$ for Fus3p (4), measurements of protein half-lives (6) and molecular masses (www.uniprot.org), we estimated the diffusion coefficient (*Inset*) and the Kuramoto length for approximately 1,400 cytoplasmic proteins in budding yeast.