# Phenomenological approach to eukaryotic chemotactic efficiency

Bo Hu,[1] Danny Fuller,[2] William F. Loomis,[2] Herbert Levine,[1] and Wouter-Jan Rappel[1]

[1]*Department of Physics and Center for Theoretical Biological Physics,*
*University of California, San Diego, La Jolla, CA 92093-0374*
[2]*Department of Biology, University of California, San Diego, La Jolla, CA 92093-0319*
(Dated: January 22, 2010)

Eukaryotic cells are capable of detecting small chemical gradients for a wide range of background concentrations. Ultimately, fluctuations place a limit on gradient sensing and recent work has focused on the role of stochastic receptor occupancy as one possible limiting factor. Here, we use a phenomenological approach to add spontaneous motility fluctuations to receptor noise and predict the directional statistics of eukaryotic chemotaxis. Specifically, an Itô diffusion equation with direction-dependent multiplicative noise is developed and analytically studied. We show that our approach can naturally accommodate recent experimental data for the chemotaxis of the social amoeba *Dictyostelium*.

## I. INTRODUCTION

During chemotaxis, cells direct their movement by sensing chemical gradients. Chemotaxis plays an important role in various biological processes including fertilization, neuronal development, wound healing and cancer metastasis [1, 2]. Unlike prokaryotes, eukaryotic cells use a spatial measurement of the asymmetric distribution of bound receptors following the external gradient. These bound receptors trigger the activation of intracellular signaling pathways, eventually leading to the generation of directional movement. Interestingly, eukaryotic cells are able to direct their motion in shallow gradients. For example, the social amoeba *Dictyostelium discoideum* is observed to crawl up a chemoattractant gradient when the front-back difference in the concentration is around 1% [3, 4]. Furthermore, the cells respond even when the average local concentration is well below the dissociation constant $K_d$. These findings has led to significant interest from the biophysics community in understanding the effect of noise on chemotaxis [5–12].

Studies to date have mainly focused on fluctuations in gradient sensing, arising for example from stochastic receptor dynamics [6, 10]. However, there are other sources of noise, as is evidenced by the fact that cells move *randomly* in the absence of directional information [13, 14]. The simplest approach to adding random motility to directional bias is to assume that the cell executes Brownian motion in a deterministic periodic potential [15]; however this approach fails to take into account uncertainties in evaluating gradient steepness and direction. Thus, what is needed is a new model of directed cell motility that takes into account both sources of fluctuations. Such a model should be able to relate the relative importance of gradient-determination noise versus motility noise to testable predictions for the behavior of chemotactic cells. Here, we propose a phenomenological, stochastic differential equation (SDE) model for this purpose and compare its predictions with the available data. As we will see, our approach naturally accounts for the
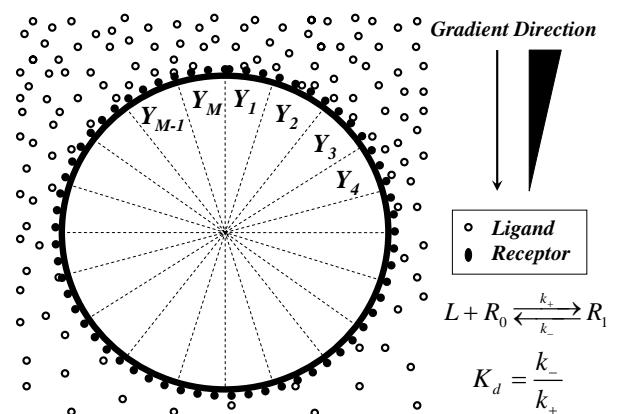


FIG. 1: (Color online). Schematic representation of our model. The forward rate $k_+$ and backward rate $k_-$ determine the transition between the unoccupied $R_0$ and occupied $R_1$ states. The dissociation constant $K_d$ is the ligand concentration at which half the receptors are bound in equilibrium.

unusual shape of the directional probability distribution for chemotaxing cells.

## II. MODEL

In our model, the cellular surface takes a circular geometry where $N$ independent receptors are uniformly distributed (Fig. 1). We divide the surface into $M$ sectors such that the local ligand concentration near each sector is constant while the number of receptors in each sector $N_m = N/M$ is large enough for the continuous approximation. For *Dictyostelium*, a typical value is $N = 60000$, and one may choose $M = 300$ and $N_m = 200$. We define the gradient steepness parameter as $p = \frac{L}{C_0}\frac{dC}{dr}$, where $C_0$ is the average local ligand concentration across the cell length $L$. Then, the local concentration at the $m_{th}$ sector with angular position $\varphi_m$ is given by $C_m = C_0[1 + \frac{p}{2}\cos(\varphi_m - \phi)]$ where $\phi$ speci-
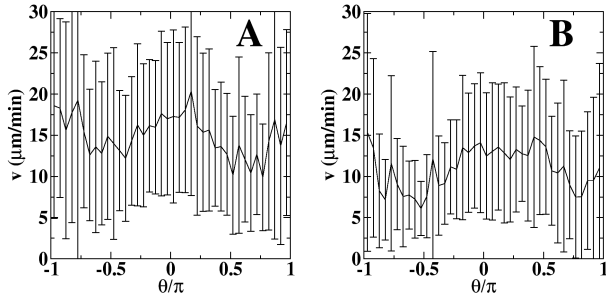
FIG. 2: The average cell speed in the experiments of Ref. [4] as a function of angle for p=5% (A) and p=10% (B). For each gradient steepness, the speed was computed by tracking the position of the centroid of 30-40 cells during 8 minutes. For more experimental details, see Ref. [4].

fies the gradient direction. The time for receptor decorrelation is dominated by the individual receptor rate $\tau_m = (k_- + k_+ C_m)^{-1}$ which is typically faster than the process by which motion can be mechanically altered [16]. This allows us to use the white-noise approximation in which the number of occupied receptors is $Y_m = \langle Y_m \rangle + \eta_m = N_m C_m/(C_m + K_d) + \eta_m$ for $m = 1, ..., M$, with $\langle \eta_m(t)\eta_n(s) \rangle \approx N_m C_m K_d/(C_m + K_d)^2 \delta(t - s)\delta_{mn}$ [7, 17]. Thus, the receptor signal is decomposed into $M$ independent Gaussian random variables.

The simplest estimate a cell can make of the gradient is obtained by the following statistic: $Z = \sum_{m=1}^{M} Y_m \cos \varphi_m + i \sum_{m=1}^{M} Y_m \sin \varphi_m \equiv z_1 + iz_2$. When $M$ is large, one can evaluate $Z$ via replacing the sum by an integral. For small gradients ($p < 10\%$), the integrand can be expanded around $p$ and we find

$$\langle Z \rangle \approx \frac{pNC_0K_de^{i\phi}}{4(C_0 + K_d)^2} + \mathcal{O}(p^3) \equiv \nu e^{i\phi} + \mathcal{O}(p^3), \quad (1)$$

$$\langle z_{1,2}^2 \rangle \approx \frac{NC_0K_d}{2(C_0 + K_d)^2} + \mathcal{O}(p^2) \equiv \sigma^2 + \mathcal{O}(p^2). \quad (2)$$

It is easy to check that $z_1$ and $z_2$ are uncorrelated Gaussian random variables, with different means but the same variance. This implies that $Z$ is a complex Gaussian variable which can be written in polar coordinates as $Z = \rho e^{i\psi}$. Here, $\rho$ measures the degree of asymmetry in the ligand-bound receptor distribution while the phase $\psi$ estimates the gradient direction $\phi$. Mathematically, $\rho$ follows the Rice distribution and $\psi$ takes a symmetric unimodal circular distribution [18]. It was found that for large signal-to-noise ratio (SNR= $\nu/\sigma \gtrsim 3$) both $\rho$ and $\psi$ are asymptotically Gaussian [18]. Thus, $\rho \approx \nu + \eta_\rho$ with $\langle \eta_\rho(t)\eta_\rho(s) \rangle = \sigma^2\delta(t - s)$, and $\psi \approx \phi + \eta_\psi$ with $\langle \eta_\psi(t)\eta_\psi(s) \rangle = (\sigma^2/\nu^2)\delta(t - s)$. As an orthogonal transformation from the Cartesian coordinates, $\eta_\rho$ is independent of $\eta_\psi$. For typical *Dictyostelium* values we find that SNR> 3, consistent with the Gaussian approximation.

Cellular motion can be decomposed into two stochastic processes: speed and direction. Recent experimental data in *Dictyostelium* [4] show that the average speed is

roughly identical for all directions. In these experiments, cells were exposed to stable exponential chemoattractant profiles using microfluidic devices. The centroid of each cell in the field of view was automatically tracked every 5s for 100 frames. Cells that moved the furthest without colliding with another cell were chosen for data analysis. Ten to twenty-five such cells were collected in each experiment, giving thousands of data points for each particular gradient. The average speed as a function of the angle is plotted in Fig. 2 for two different gradient steepnesses. From this figure, we can conclude that any possible correlation between angle and speed is smaller than can be obtained from the data. Thus, we restrict ourselves to the directional process, parametrized by the migration angle $\theta(t)$, by assuming a uniform migration speed.

Let us temporarily suppose that cells have perfect knowledge about the gradient direction $\phi$. In general, the equation of $\theta(t)$ can be written as $d\theta/dt = G(\rho, \theta-\phi)+\eta_0$ along with $\langle \eta_0(t)\eta_0(s) \rangle = \sigma_0^2\delta(t - s)$. The incorporation of the gradient-independent noise $\eta_0$ above allows the cell to perform a random walk in the absence of any gradient. Note that unlike a recent study of random motility [13], we do not include colored noise in our model. The function $G(\rho, \theta)$ must exhibit the symmetry properties [15]: (1) $2\pi$-periodicity, $G(\rho, \theta \pm 2n\pi) = G(\rho, \theta)$ for any integer $n$; (2) polar symmetry, $G(\rho, \theta \pm \pi) = -G(\rho, \theta)$, because the system's polarity will be reversed under the reversal of gradient direction; (3) reflection symmetry, $G(\rho, -\theta) = -G(\rho, \theta)$, i.e. cells cannot distinguish between left and right with respect to the direction $\phi$. The simplest form obeying these requirements is $G(\rho, \theta - \phi) = -\beta(\rho)\sin(\theta - \phi)$. Of course, cells have only imperfect knowledge of the gradient direction. The phase variable $\psi$ in our model serves as an unbiased estimator of $\phi$ for the cell. Thus, we arrive at the phenomenological Langevin equation

$$d\theta/dt = -\beta(\rho)\sin(\theta - \psi) + \eta_0. \quad (3)$$

This phenomenological equation describes a chemotactic cell trying to align its movement with the estimated gradient direction $\psi$. The first term describes a gradient-dependent restoring force which steers the cell toward the gradient direction and which contains gradient-dependent fluctuations (arising from receptor-ligand dynamics and downstream pathways) while the second term represents gradient independent noise in the random motility machinery.

Without loss of generality, we will set $\phi = 0$. In the small noise limit we can perform a Taylor expansion $d\theta/dt \approx -\beta(\nu)\sin\theta + \beta'(\nu)\sin\theta\eta_\rho + \beta(\nu)\cos\theta\eta_\psi + \eta_0 = -\beta(\nu)\sin\theta + \eta_{tot}$, where the variance of the total noise is

$$\langle \eta_{tot}^2 \rangle \equiv \sigma_{tot}^2 = [\sigma\beta'(\nu)\sin\theta]^2 + [\sigma\beta_{-1}(\nu)\cos\theta]^2 + \sigma_0^2, \quad (4)$$

with $\beta'(\nu) = \partial\beta(x)/\partial x|_{x=\nu}$ and $\beta_{-1}(\nu) = \beta(\nu)/\nu$. The first term in Eq. (4) describes the fluctuations arising from $\rho$, the amplitude of the gradient estimate, while the second term describes the fluctuations in $\psi$, the direction of the gradient estimate. Both of them vary with
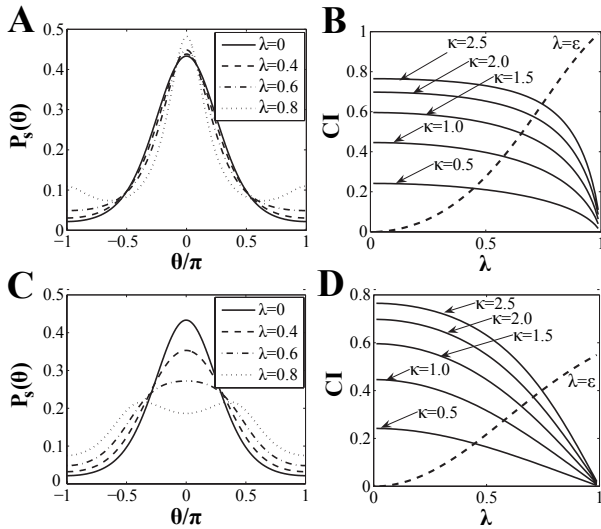
FIG. 3: The stationary distribution $P_s(\theta|\phi = 0; \kappa = 1.5, \lambda)$ for (A) $b > 1$ and (C) $b < 1$ as a function of the parameter $\lambda$ representing the ratio of direction-dependent noise to maximal total variance. The chemotaxis index (CI) vs. $\lambda$ for (B) $b > 1$ and (D) $b < 1$, with the dashed lines indicating where the bifurcation occurs.

the instantaneous direction of motion $\theta(t)$ and therefore are multiplicative fluctuations. Since the cell responds to noise at its current position, the multiplicative noise is defined with the Itô prescription. From the previous expression it is clear that the ultimate directional dependence in $\sigma_{tot}^2$ relies on the sign of $\beta'(\nu) - \beta_{-1}(\nu)$. To capture possible nonlinear input-output characteristics of downstream pathways, we assume that $\beta(\nu)$ has a power-law functional form, i.e. $\beta(\nu) = a\nu^b$. We can distinguish between two qualitatively different cases. In the first, $b > 1$, the total variance is of the "$\sin \theta$" type, $\sigma_{tot}^2 = \sigma_0^2 + (\sigma \beta_{-1})^2 + (\beta'^2 - \beta_{-1}^2)(\sigma \sin \theta)^2$ and is maximal at $\theta = \pi/2$; in the second, $b < 1$, the variance is described by a "$\cos \theta$" type and is maximal at $\theta = 0$. In the special case $b = 1$, the total variance is reduced to $\sigma_{tot}^2 = \sigma_0^2 + a^2\sigma^2$ with no directional dependence.

### III. RESULTS

We first study the case of $b > 1$ where the restoring force is ultra-sensitive to the input signal. Define the direction-independent noise $\sigma_\perp^2 = \sigma_0^2 + (\sigma\beta_{-1})^2$ and the direction-dependent noise $\sigma_\parallel^2 = (\beta'^2 - \beta_{-1}^2)\sigma^2$. Then the previous Langevin equation can be approximated as the Itô SDE

$$\frac{d\theta}{dt} = -\beta(\nu)\sin\theta + \sqrt{(\sigma_\parallel \sin\theta)^2 + \sigma_\perp^2}\,\eta(t), \qquad (5)$$

where $\eta(t)$ is white noise. One can solve the associated Fokker-Planck equation on the interval $[-\pi, \pi]$ by imposing periodic boundary conditions [19]. The stationary
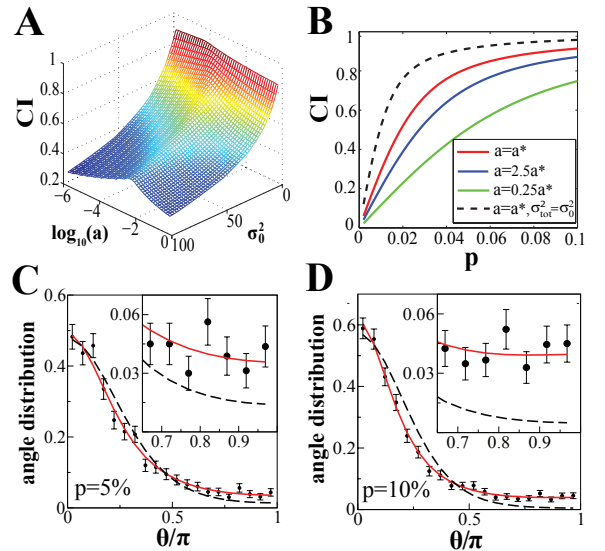


FIG. 4: (Color online). (A) CI maximized by choosing appropriate $b$ for different values of $a$ and $\sigma_0^2$, given $p = 5\%$ and $C_0 = K_d = 30nM$. (B) CI vs. $p$ for $C_0 = K_d = 30nM$, $\sigma_0^2 = 2$, $b = 1$, and $a = a^*$, $2.5a^*$, and $0.25a^*$. The solid line corresponds to a model neglecting receptor noise, i.e. $\sigma_{tot}^2 = \sigma_0^2$. (C) Experimental direction distribution (symbols) for $p = 5\%$, along with a fit using our model (solid line) and the CN distribution (dashed line). The error bars represent the standard deviation in the data set. The least square fitting parameters are $\lambda = 0.5866$, $\kappa = 1.0222$. (D) Same as (C), but now for $p = 10\%$ and with fitting parameters $\lambda = 0.7566$, $\kappa = 1.8580$. The insets show the tails of the distributions.

distribution reads

$$P_s(\theta) = \frac{\Omega \exp\left[\kappa \frac{1-\lambda^2}{\lambda}\mathrm{arctanh}(\lambda \cos\theta)\right]}{1 - (\lambda\cos\theta)^2}, \qquad (6)$$

where $\kappa = 2\beta/\sigma_\perp^2$ is a parameter that compares the direction-biased force with the direction-independent noise and where $\lambda = \sigma_\parallel/\sqrt{\sigma_\parallel^2 + \sigma_\perp^2}$ is the ratio of the direction dependent and maximal total variance. The normalization parameter $\Omega$ can be determined numerically. For a vanishing $\lambda$, we recover the Circular Normal (CN) distribution: $\lim_{\lambda \to 0} P_s(\theta; \kappa, \lambda) = \exp(\kappa \cos\theta)/(2\pi I_0(\kappa))$. For large $\lambda$, our stationary distribution will appreciably deviate from the CN distribution with a sharper peak at $\theta = 0$ and heavier tails near $\theta = \pm\pi$ (see Fig. 3A). A physical interpretation of this leptokurtic feature can be given by realizing that the first term in the Langevin equation (Eq. (3)) functions as a restoring force. Contrary to the CN case, the amplitude of this force, $\beta(\rho)$, is taken from a distribution. Thus, large values can occur and correspond to large restoring forces which lead to a sharper peak in $P_s(\theta)$. Using the same argument, one can see that the occurrence of small values in this distribution are responsible for the heavy tails in $P_s(\theta)$.

We can further explore the extrema and directionality

of $P_s(\theta)$ by recalling that $\text{arctanh}(x) = \frac{1}{2}\ln(\frac{1+x}{1-x})$ and setting $\epsilon \equiv \kappa(1-\lambda^2)/(2\lambda) = \lambda\beta(\nu)/\sigma_\parallel^2$, after which we obtain the alternative form $P_s(\theta) = \Omega(1+\lambda\cos\theta)^{\epsilon-1}/(1-\lambda\cos\theta)^{\epsilon+1}$. The solution of $\partial_\theta \ln P_s = 0$ contains five possible roots in the interval $[-\pi, \pi]$: $\theta = 0$, $\theta = \pm\pi$, and $\theta = \pm\arccos(-\epsilon/\lambda)$ which exist only if $\lambda > \epsilon$. As seen in Fig. 3A, $P_s(\theta = \pm\pi)$ correspond to global minima if $\lambda < \epsilon$, but become local maxima once $\lambda > \epsilon$, in which case the new global minima are located at $\theta = \pm\arccos(-\epsilon/\lambda)$. This change of extrema occurs at a critical point $\lambda_c = \epsilon$ (equivalent to $\sigma_\parallel^2 = \beta$) where the direction-dependent noise becomes comparable to the magnitude of the restoring force. Direct numerical simulations of the full model described by Eq. (3) indicate that our analytical expression for $P_s(\theta)$ works well when SNR$> 3$ and $\lambda < \epsilon$, justifying the Gaussian noise approximation.

As is common in the experimental literature, we can define an order parameter $\langle\cos\theta\rangle$, the Chemotaxis Index (CI), which is a measure of the directionality of the angular distribution. In Fig. 3B, we plot the CI for different values of $\kappa$ using our model. The dashed line in Fig. 3B corresponds to the CI with a value of $\kappa$ that satisfies the critical condition $\lambda = \epsilon$ or equivalently $\kappa = 2\lambda^2/(1-\lambda^2)$. To the right of this line the distribution shows a local maximum at $\pm\pi$. The slope of the CI is close to 0 over a large range of values for $\lambda$. This can be understood by realizing that the negative effect of the heavy tails on the CI is partially compensated by the positive effect of a sharpened peak.

Next we look at the case of $b < 1$. The corresponding stationary distribution is

$$P_s(\theta) = \frac{\Omega\exp\left[\kappa\frac{1-\lambda^2}{\lambda}\arctan(\lambda\cos\theta)\right]}{1+(\lambda\cos\theta)^2}. \qquad (7)$$

with $\sigma_\perp^2 = \sigma_0^2 + (\sigma\beta')^2$ and $\sigma_\parallel^2 = (\beta_{-1}^2 - \beta'^2)\sigma^2$ in the definitions for $\kappa$ and $\lambda$. The analysis proceeds as above and we only highlight the differences. The angle distribution exhibits not only heavy tails but also an obtuse peak for increasing values of $\lambda$ (Fig. 3C). This can be understood since in this case the noise in $\psi$, i.e. the second term in Eq. (4), dominates and reduces the accuracy in directionality. Due to the Gaussian approximation, the analytical probability distribution exhibits double peaks at $\theta = \pm\arccos(-\epsilon/\lambda)$ when $\lambda > \epsilon$ [20]. Also, the CI decays quickly with increasing $\lambda$ even before the bifurcation (Fig. 3D), suggesting that cells should operate in a regime where $b \geq 1$. Finally, for $b = 1$, the directional dependence in $\sigma_{tot}^2$ disappears and the stationary distribution reduces to the CN distribution with shape parameter $\tilde{\kappa} = 2a\nu/(\sigma_0^2 + a^2\sigma^2)$. Obviously, for larger values of the parameter $a$, the system is more sensitive to the gradient, and more of the receptor noise is transmitted to the directional output.

For any given $a$ and $\sigma_0^2$, there exists a unique value of $b$ that maximizes the CI (Fig. 4A). We found numerically that the combination of $a^* = \sigma_0/\sigma$ and $b^* = 1$ optimizes

the CI over the whole parameter space $(a, b)$ given the level of $\sigma_0$. The optimal pair $(a^*, b^*)$ corresponds to the ridge of the CI surface in Fig 4A. In summary, the exponent $b$ is critical for the chemotactic responses. The optimal output requires $b = 1$, while $b > 1$ is suboptimal and $b < 1$ leads to poor directionality. This can be understood by realizing that when restoring force $\beta(\nu)$ is a nonlinear function (i.e. $b \neq 1$) of the receptor signal $\nu$, the associated sensor noise is modulated to be direction-dependent, which leads to heavy tails in the angle distribution (Fig. 3). In other words, there is a significant probability that cells may move in the wrong direction and the chemotactic performance is not optimal. An important implication of our model is that the CI is always lower than that predicted by models neglecting receptor noise (see Fig. 4B). In fact, previous experimental data indicates that when the gradient steepness increases the saturating value of the CI is less than one [5, 9]. This feature is not consistent with previous CN models but may be successfully explained by our $b > 1$ model where the total noise $\sigma_{tot}^2$ becomes an increasing function of $p$.

We can compare our model to the results of recent experiments in which *Dictyostelium* cells were exposed to stable exponential chemoattractant profiles [4]. The data allow us to compute directional distributions for different gradient parameters. To eliminate the slight asymmetric bias introduced by the flow in the microfluidic chambers we collected the data into 20 bins according to their absolute deviation from the gradient direction, $|\theta-\phi|$. The resulting distribution are shown as symbols for $p = 5\%$ and $p = 10\%$ in Fig. 4C and 4D, respectively. We have also plotted as a solid line a least squares fit to the data using our model (Eq. (6)). Our fit captures well the heavy tails and the sharp peaks, features that the CN distribution, plotted as a dashed line, is unable to reproduce. A goodness of fit analysis using a Pearson's chi-square test revealed that the CN distribution is rejected at a significance level of less than 1% while our model exhibits large p-values [21]. The observed leptokurtic feature suggests that *Dictyostelium* cells are operating close to the optimal regime with the exponent $b$ estimated as slightly larger than 1.

## IV. DISCUSSION

A key assumption in our model is that the chemotaxing cell experiences a relatively stable gradient steepness ($p = const$) regardless of the cell's location or moving history. Thus, our model results can be directly compared to experiments in which the gradient is carefully quantified and controlled. If the gradient steepness is spatially varying, the chemotactic response would be heterogeneous in space, possibly obscuring the structure of the angular distribution.

Although we focus here on the role of receptor noise, it is easy to extend our model to include extra gradient-dependent fluctuations, by adding them to the magni-

tude of the restoring force, $\beta(\rho)$. Such fluctuations might arise from noise in the downstream signal transduction pathways. Future work will track cells for longer periods of time and test for the presence of long-lived cell individuality and other fluctuations sources [22]. Should the data indicate such memory effects, our model could be extended to include quenched fluctuations in cell responses.

[1] P.J.V. Haastert and P.N. Devreotes, Nat. Rev. Mol. Cell Biol. **5**, 626 (2004).

[2] C.A. Parent and P.N. Devreotes, Science. **284**, 765 (1999).

[3] L. Song, S.M. Nadkarni, H.U. Bödeker, C. Beta, A. Bae, C. Franck, W.-J. Rappel, W.F. Loomis, and E. Bodenschatz, Eur. J. Cell Biol. **85**, 981 (2006).

[4] D. Fuller, *et. al.* (submitted).

[5] P.J.V. Haastert and M. Postma, Biophys. J. **93**, 1787 (2007).

[6] K. Wang, W.-J. Rappel, R. Kerr, and H. Levine, Phys. Rev. E **75**, 061905 (2007).

[7] W.-J. Rappel and H. Levine, Phys. Rev. Lett. **100**, 228101 (2008).

[8] W.-J. Rappel and H. Levine, Proc. Natl. Acad. Sci. U.S.A. **105**, 19270 (2008).

[9] R.G. Endres and N.S. Wingreen, Proc. Natl. Acad. Sci. U.S.A. **105**, 15749 (2008).

[10] W. Bialek and S. Setayeshgar, Proc. Natl. Acad. Sci. U.S.A. **102**, 10040 (2005).

[11] T. Shibata and K. Fujimoto, Proc. Natl. Acad. Sci. U.S.A. **102**, 331 (2005).

[12] M. Ueda and T. Shibata, Biophys. J. **93**, 11 (2005).

[13] L. Li, S.F. Nørrelykke, and E.C. Cox, PLoS ONE **3**, e2093 (2008).

[14] H. Takagi, M.J. Sato, T. Yanagida, and M. Ueda, PLoS ONE, **3**, e2648 (2008).

[15] M. Schienbein, K. Franke, and H. Gruler, Phys. Rev. E **49**, 5462 (1994).

[16] Here we have used the fact that under typical conditions the contribution of ligand diffusion to the receptor correlation can be ignored [6, 7].

[17] D.A. Lauffenburger and J.J. Liderman, *Receptors: Models for Binding, Trafficking, and Signaling* (Oxford University Press, New York, 1993).

[18] H. Gudbjartsson and S. Patz, Magn. Reson. Med. **34**, 910 (1995).

[19] H. Risken, *Fokker-Planck Equation* (Springer-Verlag, Berlin, 1984).

[20] B.W. Andrews and P.A. Iglesias, PLoS Comput. Biol. **3**, e153 (2007).

[21] For $p = 5\%$, the chi-square test of our model results in $\chi^2_{our} = 14.14$, with a degree of freedom DF $= 20 - 3 = 17$ and a p-value equal to 0.657. Here, the DF is reduced by 3 since the model has two fitting parameters and the number of data points is constrained. For comparison, the CN distribution gives $\chi^2_{CN} = 81.54$ with DF $= 18$ and p-value$< 10^{-3}$. For $p = 10\%$, we find $\chi^2_{our} = 10.51$, p-value$= 0.881$, and $\chi^2_{CN} = 694.78$, p-value$< 10^{-3}$.

[22] A. Samadani, J. Mettetal, and A. van Oudenaarden, Proc. Natl. Acad. Sci. U.S.A. **103**, 11549 (2006).