High fidelity information processing in folic acid chemotaxis of *Dictyostelium* amoebae

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Living cells depend upon the detection of chemical signals for their existence. Eukaryotic cells can sense a concentration difference as low as a few per cent across their bodies. This process was previously suggested to be limited by the receptor–ligand binding fluctuations. Here, we first determine the chemotaxis response of *Dictyostelium* cells to static folic acid gradients and show that they can significantly exceed this sensitivity, responding to gradients as shallow as 0.2% across the cell body. Second, using a previously developed information theory framework, we compare the total information gained about the gradient (based on the cell response) to its upper limit: the information gained at the receptor–ligand binding step. We find that the model originally applied to cAMP sensing fails as demonstrated by the violation of the data processing inequality, i.e. the total information exceeds the information at the receptor–ligand binding step. We propose an extended model with multiple known receptor types and with cells allowed to perform several independent measurements of receptor occupancy. This does not violate the data processing inequality and implies the receptor–ligand binding noise dominates both for low- and high-chemoattractant concentrations. We also speculate that the interplay between exploration and exploitation is used as a strategy for accurate sensing of otherwise unmeasurable levels of a chemoattractant.

1. Introduction

Eukaryotic amoebae *Dictyostelium discoideum* (referred as *Dictyostelium*) in the vegetative state forage on bacteria by following gradients of folic acid (FA), a by-product of bacterial metabolism [1,2]. It is currently believed that *Dictyostelium* measure chemical gradients directly by monitoring the distribution of the occupied chemoattractant receptors. These cells can detect concentration differences as low as a few per cent across their cell bodies [3–8] and it is currently an open question what exactly limits this process. Previously, the receptor–ligand binding fluctuations were suggested as the limiting factor, which remains a possibility because a single excited receptor may amplify the signal by activating multiple G-proteins [9–11].

The chemotaxis signalling system can be described as the following Shannon communication channel [12,13]: the chemoattractant gradient direction as the input, the spatial distribution of occupied receptors as the intermediate step and the direction of cell motion as the output. Fuller *et al.* [4] recently exploited this information-theoretic framework, where a cell in a static gradient was modelled as *N* receptors arranged in a circle, each in chemical equilibrium with the local chemoattractant concentration, described by a dissociation constant $K_d$.

The joint state of all receptors $\theta_{\text{rec}}$ was assumed to depend only on the gradient direction, $\theta_{\text{grad}}$. Likewise, the probability of a cell moving in a direction $\theta_{\text{res}}$ was assumed to depend only on $\theta_{\text{rec}}$, with these three variables forming a Markov chain: $\theta_{\text{grad}} \rightarrow \theta_{\text{rec}} \rightarrow \theta_{\text{res}}$ (see the electronic supplementary material). Capital greek letters denote random variables and lowercase Greek letters their values. Fuller *et al.* [4] computed the mutual information between the gradient...
direction and the receptor distribution \( I_{\text{tot}}(\theta_{\text{grad}}, \theta_{\text{rec}}) \), ‘external mutual information’. \( I_{\text{total}} \) quantifies the information gained about the gradient through a perfect (noiseless) readout of the occupied receptors.

Furthermore, Fuller et al. [4] used Dictyostelium cAMP chemotaxis experiments to calculate the mutual information between the gradient direction and the cell response \( I_{\text{tot}}(\theta_{\text{grad}}, \theta_{\text{rec}}) \), ‘total mutual information’. \( I_{\text{total}} \) quantifies the information gained about the gradient by cells through the imperfect (noisy) readout of the occupied receptors. The data processing inequality [14, p. 34] states that in a Markov chain of variables, information can only be destroyed in each subsequent step, which here translates into \( I_{\text{total}} \leq I_{\text{ext}} \). In other words, the information gained by cells after being processed through the entire signalling pathway, cannot exceed the information gained at the receptor level. Fuller et al. [4] then argued that for low cAMP concentrations the receptor–ligand binding fluctuations dominate the entire noise (\( I_{\text{total}} \approx I_{\text{ext}} \)), because there is no further information loss downstream. Previously, Ueda & Shibata [11] also reached this conclusion using signal-to-noise ratio arguments, using stochastic receptor noise and time integration with second messengers and locomotion systems.

Here, we measure the response of a population of Dictyostelium cells to static linear FA gradients, established in an agarose gel-based microfluidic device [15]. The steady-state gradients were achieved by maintaining fixed concentrations of FA on opposite sides of a microfluidic channel (see the electronic supplementary material). A linear gradient was established by diffusion through agarose gel. Cell migration was recorded using time-lapse optical microscopy. The measured distribution of cell displacement angles \( p(\theta_{\text{grad}}|\theta_{\text{rec}}) \) was used to calculate the total mutual information \( I_{\text{ext}} \) and compared to \( I_{\text{total}} \) (using the result in [4]) to test the possibility of receptor–ligand binding fluctuations dominating the total noise.

### 2. Results and discussion

First, we use the result in Fuller et al. ([4]; electronic supplementary material, equation S56) for the external mutual information \( I_{\text{ext}} \) for shallow linear gradients

\[
I_{\text{ext}}(\theta_{\text{grad}}, \theta_{\text{rec}}) = \frac{N}{4 \ln 2 c(x)} \left( \frac{\nabla c}{1 + c(x)} \right)^2,
\]

where \( c(x) \) is the concentration measured in units of \( K_d \), \( \nabla c \) is the gradient measured in units of \( K_d R^{-1} \) (\( R \) is the radius of a hemispherical cell, taken as \( 5 \mu m \)) and the dimensionless small parameter \( \epsilon = \nabla c/(1 + c) \ll 1 \). For larger values of \( \epsilon \), one has to resort to numerical simulations. The design of our microfluidic device ensured it was applicable to use the equation (2.1) as the small parameter was in range \( 0.003 \leq \epsilon \leq 0.0065 \).

Previously, Wurster & Butz [16] and de Wit & van Haastert [17] measured the dissociation constants \( K_d \) and receptor numbers \( N \) using radioligand assays. Following Wurster & Butz [16], we used the measured \( N \) and \( K_d \) after 3 h in the buffer, which reflects the conditions in our experiments. As will be discussed below, following De Wit & van Haastert [17], we later considered multiple receptor types for which the only information available was for vegetative cells. Wurster & Butz [16] found \( K_d = 150 \text{ nM} \), \( N = 60000 \) and de Wit & van Haastert [17] found five receptor types with the following dissociation constants and receptor numbers: (i) \( K_d = 450 \text{ nM} \), \( N_1 = 80000 \), (ii) \( K_d = 70 \text{ nM} \), \( N_2 = 80000 \), (iii) \( K_d = 17 \text{ nM} \), \( N_3 = 550 \), (iv) \( K_d = 50 \text{ nM} \), \( N_4 = 50 \) and (v) \( K_d = 15 \text{ nM} \), \( N_5 = 1450 \). In both cases, Scatchard plots show that the first-order kinetics can be used with good approximation but that there is slight curvature implying either negative cooperativity or greater receptor heterogeneity. Furthermore, the binding curves for FA were measured for up to micromolar concentrations, the interesting range explored in this study.

Second, we measured the cell trajectories and the distribution of angles \( p(\theta_{\text{grad}}|\theta_{\text{rec}}) \) of total displacement vectors (figure 1a) of a population of Dictyostelium cells (see the electronic supplementary material for Methods). In each experiment, the FA gradient was uniform and the concentration varied at most three-fold across the width of a channel. Each experiment was repeated until we obtained 300–700 cell trajectories. These observations were used to calculate the total mutual information \( I_{\text{total}} \) and the chemotactic index (CI). CI is defined as \( CI = \left( \sum_i r_i \right) \cdot \bar{n} / \sum_i |r_i| \cdot \bar{n} \), where \( r_i \)
Figure 2. Comparison of the total mutual information $I_{\text{tot}}$ and external mutual information $I_{\text{ext}}$. (a) $I_{\text{tot}}$ (dashed line) and $I_{\text{ext}}$ (solid line) for the same experiments as in figure 1a, both averaged over all local concentrations (see the electronic supplementary material). Error bars for $I_{\text{tot}}$ represent s.e.m. The shaded range for $I_{\text{tot}}$ denotes its spread owing to the range of local concentrations the cells were exposed to in the microfluidic device (see text). Dotted line denotes the $I_{\text{tot}}$ for control experiments without a gradient. Annotation 1 shows the range where the data processing inequality is strongly violated, $I_{\text{tot}} > I_{\text{cAMP}}$ (b) (i) Calculated values for $I_{\text{tot}}$ (equation (2.1)); shaded area denotes the combinations of $c_0$ and $d/c d x$ inaccessible in our experiment owing to the geometry of the microfluidic device and low solubility of FA in development buffer (approx. 0.1 mM). (ii) the range of concentrations and gradients where cAMP chemotaxis has been measured, coloured by the absolute value of FA concentration and its gradient.

The instantaneous cell displacement during the time step $i$ (taken as 30 s) and $n$ is the gradient direction.

We performed 10 experiments where we varied the FA concentration in the top channel of a microfluidic device while keeping the bottom channel at concentration zero. In these experiments, both the concentration and the gradient were changed and these are plotted in figure 2a. We also performed five additional experiments (shown in figure 2c,d) where we changed the mean concentration and the gradient separately. For the range of concentrations and gradients explored here, decreasing the gradient and increasing FA concentration diminished the signal. Therefore, the FA chemotaxis can depend both on the absolute value of FA concentration and its gradient.

$I_{\text{tot}}$ was calculated by segmenting the real interval $0 \leq \theta_{\text{tot}} < 2\pi$ into $m$ bins of equal width. The bin size was $m = 14$ for all experiments, because $I_{\text{tot}}$ with that bin size correlated extremely well with CI (compare figures 1b and 2a) for which no binning was used (see the electronic supplementary material for further analysis). The fraction of total displacement angles $n_i$ ending up in the bin $\theta_{\text{tot}} / 2 \pi \leq \theta < \theta_{\text{tot}} + 1/2 \pi$ was counted and $I_{\text{tot}}$ was computed [14, pp. 247–248] as

$$I_{\text{tot}}(\Theta_{\text{grad}}, \Theta_{\text{tot}}) = \sum_{j=1}^{m} n_j \log n_j + \log m,$$

with the error due to a finite number of data points estimated as $(m-1)/2M$ [19], where $M$ is the total number of data points.

Next, we compared $I_{\text{tot}}$ and $I_{\text{ext}}$ in figure 2a. Figure 2a shows that for low concentrations and shallow gradients $I_{\text{tot}} \approx I_{\text{ext}}$, meaning the receptor–ligand binding fluctuations dominate the total noise. This possibility was previously suggested for cAMP [7, 11] using signal-to-noise ratio analysis with a biased random walk model of cell motion. The information-theoretic analysis assumes only the steady-state receptor–ligand binding fluctuations and benefits from not being tied to a particular model of cell motion, because *Dictyostelium* cells do not follow a simple random walk [20].

The most surprising result is that the response is observed for gradients as low as 0.2% across the cell body ($d/c d x = 3.2 \text{nM} \mu \text{m}^{-1}$, $c_0 = 15,000 \text{nM}$, $I_{\text{tot}} = 0.06$ bits shown in figure 2b). For these experiments, the difference in the fraction of occupied receptors front-to-back on the cell body is given by

$$\eta = \frac{c_{\text{front}}}{c_{\text{front}} + K_d} - \frac{c_{\text{back}}}{c_{\text{back}} + K_d}$$

and is shown in table 1 for different measured dissociation constants.
compared with cAMP, $K_{d}(\text{cAMP}) = 30 \text{ nM}$, whereas the number of receptors per cell is almost the same: 60 000 for FA and 70 000 for cAMP [16,18].

These simplified descriptions of FA and cAMP receptors were sufficient to explain the results in Fuller et al. [4], but do not suffice here—possibly explained by the limited range of cAMP concentrations and gradients investigated in [4] (figure 2b). The measurement with annotation 1 on fig. 2b from Varnum & Soll [5] supports this possibility. They measured CI = 0.25 for cAMP, compared to our CI = 0.13 for FA, for roughly the same mean concentration $c_0$ and gradient $dc/dx$. Therefore, the cAMP response in that range might also result in the violation of the data processing inequality. Motivated by the failure of the theory, we investigated five different modifications of the original model.

### 2.1. Effects of folic acid deaminase

First, we considered reduced FA concentrations perceived by cells as a result of FA deaminase activity, a protein that degrades FA [21]. We concluded, using both calculation and a series of control experiments (see the electronic supplementary material), that it does not significantly contribute to the observed result.

### 2.2. Effects of multiple receptor types and receptor phosphorylation

Second, we considered all different receptor types mentioned previously. This possibility was motivated by the local minimum in $I_{ext}$ shown in figure 2a indicating that perhaps there are two receptor types or states, each active in a distinct range of local ligand concentrations. We calculated $I_{ext}$ for each receptor type and added them together to investigate whether this resolves the violation of the data processing inequality. The results are shown in figure 3c and indicate that the presence of multiple receptor types reduces but does not eliminate the violation of the data processing inequality. This is because the shaded range for $I_{ext}$ in figure 3 represents the range of concentrations the cells were exposed to in our microfluidic device (and not the uncertainty), with the maximum value of $I_{ext}$ corresponding to the bottom of our device and the minimum value corresponding to the top of our device. However, the systematic uncertainty of the average $I_{ext}$ (solid line in figure 3a) is only 10% (see the electronic supplementary material), which is what is compared to the average $I_{ext}$ (we appreciate the comments of anonymous referees and Eric Siggia on this matter.) Furthermore, the double-peak feature observed in $I_{ext}$ is not exactly reproduced in $I_{ext}$ even when considering only two receptor types. This could be owing to the fact that $I_{ext}$ is only an upper limit for $I_{tot}$ and in this range, the intracellular signal processing is not negligible, so $I_{ext} < I_{tot}$. In other words, the dip could be the consequence of the extra noise somewhere downstream of the receptor–ligand binding events. It is also worth mentioning that this double-peak response prevents us from using any single receptor with fixed $K_d$ to fix the violation of the data processing inequality, unless the receptor number per cell $N$ is set to a factor 12 more than it is measured.

Therefore, this explanation could be plausible only if all the cells were concentrated near the bottom of our device. In our experiments, they were always uniformly distributed with the mean position in the centre.

Third, we hypothesized that FA receptors can be phosphorylated. Xiao et al. [22] have shown that the phosphorylation of cAMP receptors CAR1 reduced the affinity (increased $K_{d}$) of a cAMP–CAR1 process by a factor of three, from 300 to 900 nM. Here, we assume that the additional receptor types can be phosphorylated to $3 \times K_{d}$ and fit the data in the same way as for additional receptor types. The results (figure 3b) show that this only reduced the violation of the data processing inequality, but did not eliminate it.

### 2.3. Effects of cell polarization

Fourth, we considered for the possibility of cell polarization (we thank an anonymous referee for this suggestion).
previously considered in Andrews & Iglesias [13] and Hu et al. [23]. In our analysis thus far, we assumed that cells had no previous knowledge of the gradient direction, so the prior probability was \( p(\theta_{\text{grad}}) = 1/2\pi \). Now, we consider a circular normal prior distribution

\[
p(\theta_{\text{grad}}) = \frac{\exp(K \cos \theta_{\text{grad}})}{2\pi I_0(K)},
\]

where \( I_0(K) \) is the modified Bessel function of the first kind of zeroth order, and the parameter \( K \) measures the bias strength. We used the approach from Hu et al. [23] to numerically calculate \( I_{\text{tot}} \text{bias}(K) \). We also numerically calculated \( I_{\text{tot}} \text{bias}(K) \) (see the electronic supplementary material) and then compared both \( I_{\text{tot}} \text{bias} \) and \( I_{\text{tot}} \text{ext} \) to up to very biased distributions with \( K = 80 \), as larger values required significantly higher numerical precision. Figure 3c shows that the violation of the data processing inequality still persists.

2.4. Effects of multiple measurements

Finally, we investigated the effect of multiple independent measurements of the receptor occupancy [7,11], occurring if cells can choose between (i) short and imprecise gradient measurements but moving fast, and (ii) long and precise gradient measurements but moving more slowly. This is known as the trade-off between exploration and exploitation in the field of reinforcement learning [24].

Equation (2.1) is only valid for a single snapshot measurement. The information acquired from multiple independent measurements is simply the sum of the information of each contribution owing to a single measurement. Therefore, we multiply the equation (2.1) by the number of independent measurements \( N_{\text{meas}} = T_{\text{pseudo}}/T_{\text{correl}} \) [4,23], where \( T_{\text{pseudo}} \) is the time scale of pseudopod extension and \( T_{\text{correl}} \) is the receptor correlation time (this ratio gives us the maximum number of measurements that could have been performed). We note that \( T_{\text{pseudo}} \) is likely the upper bound for the integration time based on the evidence in variable gradient experiments [25] where it was observed that the cells extend their pseudopods in the gradient direction as soon as the direction of the gradient is changed. Rappel & Levine [26,27] previously noted that the correlation time depends on both receptor chemical dynamics and the diffusive process. They estimated the cAMP receptor correlation time \( T_{\text{correl}} = 5 \) s. Fuller et al. [4] concluded \( N_{\text{meas}} \). Accordingly, we estimated \( N_{\text{meas}}^{\text{FA}} \) by assuming that \( T_{\text{pseudo}} \) is inversely proportional to the mean cell speed, and the same \( T_{\text{correl}} \) for both FA and cAMP receptors (based on comparable receptor off-rates for FA and cAMP receptors [17,28])

\[
N_{\text{meas}}^{\text{FA}} = \frac{T_{\text{pseudo}}^{\text{FA}}}{T_{\text{correl}}^{\text{FA}}} \approx N_{\text{meas}}^{\text{cAMP}} \frac{v_{\text{cAMP}}}{v_{\text{FA}}},
\]

where the chemotaxis speeds are \( v_{\text{cAMP}} = 0.25 \mu m/s \) [4] and \( 0.05 \mu m/s \leq v_{\text{FA}} \leq 0.12 \mu m/s \), which gives \( 2 \leq N_{\text{meas}}^{\text{FA}} \leq 4 \). \( I_{\text{tot}} \) and \( I_{\text{ext}} \) are compared in figure 3d and show that this only reduced the violation of the data processing inequality, but again does not eliminate it. Recently developed approaches considered diffusible inhibitors in balanced inactivation model [26,27,29] and their integration time \( (T_{\text{int}} = 10 \) s) corresponds roughly to the integration times estimated here \( (T_{\text{int}} = 10–20 \) s). In addition, the models considered so far do not reproduce the double peak observed experimentally (figure 3), but this might be the consequence of a significant information loss downstream of the receptor–ligand binding events.

However, combining the effects of additional receptor types and multiple independent measurements does not result in the violation of the data processing inequality (figure 3c). \( N_{\text{meas}}^{\text{FA}} \) roughly agrees with [7] \( N_{\text{meas}}^{\text{cAMP}} \approx 2 \), which was included to explain a much greater range of concentrations and gradients than in [4] (figure 2b).

It should still be noted that the multiple independent measurements can be a consequence of integrating the information from multiple pseudopods (we thank an anonymous referee for this suggestion). During the 30 s time interval, cells extend a number of small protrusions (sometimes simultaneously), some of which are retracted quickly (see fig. 6 in [30]). Taking this into account would lead to a different definition of the total mutual information than that used here where the centroid of each cell is used to specify its position. One direction for future studies is then to perform experiments with higher resolution to quantify the information acquired about the gradient using this alternative measure.

2.5. Other effects

Figure 3e implies that the total noise is indeed dominated by the receptor–ligand binding fluctuations at both low and high gradients and concentrations. This seems plausible because in that range, the receptors are either mostly unoccupied or occupied. In the intermediate range where \( I_{\text{tot}} \gg I_{\text{int}} \), the internal noise dominates. We note that it has been shown [31] that there is always a fraction of cell population which does not respond to gradients and polarizes in random directions, independent of the external cAMP gradient. Since in our experiments we only have static gradients, we could not separately identify these cells and they had to be included in the data analysis. Exclusion of this subpopulation from our analysis would increase the total mutual information \( I_{\text{tot}} \) even further and the violation of the data processing inequality would be even larger.

The possibility of receptor interactions was ruled out owing to uniform receptor distributions for both FA [32] and cAMP receptors [33,34]. Unlike in the cAMP case [4], here the non-circularity of cell shapes is not an issue because the cells are circular when sensing FA. However, there is still a possibility of a more complicated mechanism if FA receptors also transport FA into the cell [9], serving as a different communication channel, or if a FA transporter is a separate protein, as a separate communication channel.

The possibility that FA and cAMP receptors share the majority of the internal signalling pathway [35] implies equal FA and cAMP responses, if rescaled by their respective parameters \( K_d \) and \( N \). This remains to be investigated with more cAMP and FA chemotaxis measurements in the same concentration and gradient range. The results here and in [4,11] confirmed that the external noise dominates for both chemotactants in low concentration range. This is in contrast to the conclusion reached in the Supplementary Information of Samadani & Mettetal [31]. SI possibly caused by using single-pulse temporal gradients, as opposed to defined static gradients used here and in [4].

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