Dear Editor,

In order to address many of the great critiques and suggestions by all referees, we have done a lot of revisions to the manuscript

## Summary of changes:

Added line numbers to both main text and SI.

# Abstract

Significantly shortened the abstract

# Main text

Changed the order of the main sections of the manuscript. We agree with Referee 2 that it was not easy to follow the main text without SI, so the outline of the revised manuscript is as follows:

1. Introduction, establish relevance, previous work. lines 7-60
2. SNR: definition, intuitive behavior in 1D (Fig.1a,b) relevant for 3D models. lines 61-104
3. Numerical simulations: Fixed PDE secretion rate model. lines 105-151
   1. Fig2a,b. SNR heat map. PDE can improve SNR.
   2. Fig2c,d. PDE broadens range of cAMP detection and shifts it to higher concentrations.
   3. Fig2e,f. Chemotaxis index (could be measured) plotted to connect to experiments.
4. Analytical exact solutions: Uniform PDE model. lines 152-194
   1. Fig3a,b. SNR looks similar; only local cAMP/PDE concentrations matter.
   2. Fig3c,d,e. SNR increased by mainly increasing signal, while the noise is capped.
   3. Similar to numerical solution where the cell boundary is included (SI Section 6, SI Fig.4)
5. Spherical model in full 3D: again similar SNR. lines 195-199
6. Summary, discussion, comments. lines 200-238

# Supplementary information

Added:

* Section 1
* Section 2

Shortened first sentence in the first paragraph.

Fig.1: Added several things, tried to give intuitive idea behind the increase in SNR due to PDE secretion.

Updated Eq.1 to explicitly show that SNR definition includes sampling fold I, as used in [van Haastert, Postma, Biophys. J. 93, 1787-1796 (2007)] and defined all parameters in the same sentence.

----------------------------------------------------------------------  
Second Report of Referee A -- LT13609/Segota  
----------------------------------------------------------------------  
  
This is a substantially improved version of a previously submitted work  
concerning the role of signal degrading enzymes in chemotaxis, a quite relevant  
and interesting open problem.  
  
The idea is that in the presence of such enzymes, a characteristic degradation  
length L appears, and the steepness of the chemotactic gradient sensed by the  
cell becomes dominated by L, rather than by the distance w from the chemotactic  
source, possibly leading to an enhancement of the chemotactic signal.  
  
However, degradation also implies decreased concentration of chemotactic factors  
and stronger fluctuations.  
  
Here the Authors compute the signal-to-noise ratio (SNR) for a cell exposed to a  
chemotactic gradient, in the presence or absence of degrading enzymes, and show  
that the optimal SNR, as a function of the strength of the chemotactic source  
and of the concentration of degrading enzyme in a quasi-1D geometry, is achieved  
for non-zero concentrations of the enzyme (Fig. 1).  
  
This result is obtained by a combination of numerical computations and analytic  
estimates, making use of realistic parameter values for the main chemical  
factors involved in the chemotaxis of D. discoideum (the chemoattractant cAMP  
and PDE, a cAMP degrading enzyme).  
  
It is also shown that high chemotactic efficiency is obtained for a broader  
range of cAMP concentrations if PDE is present (Fig. 2c).  
  
The observation that degrading enzymes may systematically provide a better SNR  
and a wider sensing range is important, as chemotactic signals are often  
accompanied by the secretion of signal degrading enzymes, but their role is  
still poorly understood. The present work may also contribute to explain the  
experimental observation that mutant D. discoideum cells that do not secrete PDE  
fail to aggregate. There are however still some points that should be clarified  
(a list is included below). If such points are satisfactorily addressed, I think  
that the manuscript would satisfy the conditions of general interest and  
scientific soundness required for publication in PRL.  
  
Detailed observations  
  
- The role of the fixed secretion rate and of the fixed concentration model are  
somehow confused in the text and SI. "This analytical expression is...  plotted  
in Fig. 3, SI", so, SI Fig. 3 is a plot of Eq. (6), describing the fixed  
concentration model in the approximation of dominating receptor noise.  Then:  
"The numerical results from Fig. 1c are therefore also obtained analytically".  
But Fig. 1c refers to the fixed secretion model without any approximation,  
therefore the data in SI Fig. 3 cannot be rightly considered as the analytic  
version of those in Fig. 1c.

Thank you for pointing this out and we agree that this was not worded correctly. What we meant to say instead, is that the results from both “fixed secretion rate” and “fixed concentration” models are similar, i.e. the SNR shows a very similar response: there is some optimal range of concentration or secretion rate. We have removed this sentence and made a better distinction between the two models.

And in the SI: "Here we show the plot of... Eq. 9  
from the main text". But there is no Fig. 9 in the main text.

Thank you this part was also fixed. The exact analytical solution for shallow gradients (always the case here; main text lines: 161-164) is now shown in Eqs. 8, 9. and this is plotted in Fig.3a and SI Fig.4b to compare it to the numerical solution.  
  
- Eq. (5) is obtained under the condition of dominating receptor noise  
(sigmaR>>sigmaB), that could in principle be verified for only a subset of the  
values of cAMP and PDE concentrations shown in the corresponding plot (SI Fig.  
3). This point should be clarified.

Please see the response to the next comment, which also addresses this one.

- Why plotting in SI Fig. 3 a set of approximate values, when the exact ones are  
available? I imagine that it should be sufficient to plug in (1,2) the solutions  
of the 1D diffusion problem provided in the SI to get a plot of the exact,  
analytic solution for the fixed secret0ion model, without any approximation.

Thank you for pointing this out. As stated in the next comment, we have now replotted the results by removing this approximation altogether. There was little gained by assuming this in the first place, since at the end, the equation for SNR didn’t simplify that much nor give any additional physical interpretation.

- By rescaling the x coordinate, Fig. 1c and SI Fig 3 can apparently be  
superimposed, although Fig. 1c describes the fixed secretion model and SI Fig.  
3 the fixed concentration model. What is the reason for the similarity?  Perhaps  
only the local PDE concentration in the neighborhood of the cell matters, and  
the way it is produced (secreted or imposed from the beginning) does not really  
matter?

Exactly (these are now Fig2a and Fig3a) – this is now commented on in main text lines 165-167.

- How is the 31-fold (=8.5/0.27) SNR increase calculated? After looking  
attentively at Fig. 1d one observes that 0.27 and 8.5 are probably the zero-PDE  
and optimal-PDE SNR values for c(xleft) around 100 Kd. But what is special about  
c(xleft)=100 Kd? This point should be more clearly explained.

Thank you for this remark. The 31-fold came from somewhat arbitrarily chosen point of 100 Kd and is now removed. Instead, we show this enhancement in Fig.3f and also comment on SNR enhancement in the main text lines 179-194.

- "from SNR=0.9 for no PDE to SNR=1.6 for PDE secretion": I cannot understand to  
which points or curve of Fig. 1c or Fig. 1d these values shoud refer. The use of  
a continuous colorbar in Fig. 1d does not help: it would be much better to  
clearly indicate the c(xleft) values that correspond to the five curves in Fig.  
1d. Similarly for Figs. 1e, 2c,f,i.

Thank you for pointing this out. We agree that continuous colorbars were difficult to read and have changed it to discrete everywhere in the manuscript and SI.

- "secreting PDE can always increase the SNR": this is a confusing statement. At  
fixed cAMP concentration c(xleft), secreting more PDE leads to an increase of  
the SNR up to an optimal value, than to a decrease; what apparently is true is  
rather that secreting PDE can always increase the SNR if at the same time  
c(xleft) is suitably increased.

Thank you, this was not worded well. In lines 136-140 we instead simply described the main observation from results in Fig.2a, i.e. that there is a range of parameters for which there is an improvement in SNR, and that there is also a range c(-w/2) ≈< Kd (=c(xleft)) for which there is no improvement.

.  
- "The required PDE secretion rates range...": I don't understand the logic of  
this paragraph. Secretion rate of Bar1 is analogue to secretion rate of PDE as  
both are signal degrading enzymes (so their secretion rates can be used to  
compare with the predictions of Fig. 1c), but in Fig. 1c, the y variable is cAMP  
concentration, not cAMP secretion rate. So how the information about cAMP  
secretion rate can be used to compare to Fig. 1c? And, what is "alpha protease"?  
is it the alpha factor pheromone signal or the Bar1 protease that degrades it?

Thank you for pointing this out (and for the typo in the text about ‘alpha protease’). The idea here was to just give some rough numbers of secretion rates for different molecules, to see what is are the physiological values for some, typical, secretion rates. We rewrote this argument in a much cleaner way now; see lines 147-151.

- Which figure shows that "The average cAMP concentration on the cell at maximal  
SNR is c(xcell)=0.2Kd"? I suspect it is Fig. 3 (not referenced in this  
paragraph), but then Fig. 3 is discussed in the section about the fixed  
concentration model, not the fixed secretion rate model.

We removed the old Fig.3 for the following reason. Following the suggestions of Referee 2 to give a better picture of how SNR is improved by PDE (new Fig.3)

- "Even when we set c(xleft)=2Kd... the average cAMP concentration on the cell  
at maximal SNR is c(xcell)=0.2Kd": in this paragraph I can find no figure in  
support of this statement. It could be Fig. 3, but it is not referenced here and  
it apparently refers to the fixed concentration model. It would be more clear if  
Fig. 3 contained two curves, one for the fixed secretion rate model and one for  
the fixed concentration model, and it was referenced earlier (so it would  
probably become Fig. 2). Perhaps the two curves are indistinguishable, but then  
a comment would be in order.

Removed Fig.3.   
  
- "The analytical expression is first multiplied by a prefactor of 1.45...  (see  
SI, Sect. 7)": the prefactor is apparently computed in the SI for the case of a  
linear gradient (Fig. 4), i.e. in the absence of PDE. Is it correct to use that  
factor for all PDE values?

We now show the value of this prefactor for various combinations of cAMP and PDE concentrations (SI, Fig.4c).

- Fig. 2c apparently shows that the higher the rate of PDE secretion, the  
broader the interval of cAMP concentrations that provide a high chemotactic  
index (CI). This fact seems important, but is not discussed in relation to Fig.  
2c, neither in the figure caption, nor in the text (it is however hinted at in  
the Abstract: "respond to a broader range of cAMP concentrations"). An explicit  
discussion of this result about the CI would be quite useful.

- The values for the CI in Fig. 2c seem to saturate at CI=0.8. Why 0.8 and not  
1?

This is a consequence of the empirical relationship from ref.33 [van Haastert and Postma Biophys. J. 93, 1787 (2007)] used to relate CI and SNR (Eq.4), which is used since the authors experimentally showed that CI saturates around 0.8 for very steep gradients. This is likely due to another source of noise (not considered here) in gradient sensing.

- The paper could be improved by a short discussion about physical intuition:  
this aspect was actually more clear in the previous version of the manuscript  
and has been partially lost. It could be observed for instance that in the PDE  
case, the gradient perceived by the cell, and by consequence the best SNR, are  
approximately proportional to r / L, while in the non-PDE case, they are  
approximately proportional to r / w, with prefactors that are of the same order  
in the two cases; so, PDE somehow enhances the perceived gradient by degrading  
cAMP more rapidly in space.

The difficulty with the analysis in the previous version, as we realized,

- The main analytic result is obtained here in a quasi-1D geometry. However,  
cells live in space. It is not difficult to discuss the case of the gradient  
generated by a point source in 3D: in that case also, PDE should provide a  
better SNR. A short discussion of that case would be a quite useful addition.

3D, the gradient

- From the biological point of view, it may be interesting to observe that a  
screening of the chemotactic signal similar to the one achieved by signal  
degrading enzymes can also result from the simultaneous secretion of soluble  
attractive and repulsive factors. This seems to be the case in angiogenesis (see  
e.g. PMID 12879061, 16711846).  
  
- While the study of single-cell response is certainly the right starting point  
for understanding the role of degrading enzymes in chemotactic sensing, it is  
likely that a deeper reason for their existence lies in collective effects, such  
as the necessity to avoid saturation when a large number of cells are present  
(as suggested e.g. in Ref. 32). A comment on this point would be useful.  
  
- In Ref. 32 it is suggested that at low cAMP concentration, the role of PDE  
screening is to avoid cells to be "confused" by their own self-secreted cAMP  
signal. Again, a comment would be useful.  
  
Minor points  
  
- I was not able to find in the text either an explicit definition of w, or its  
numerical value (I imagine it is the width of the microfluidic device, w=1mm);  
also, I could not find an explicit definition of xleft, xright (I imagine it is  
xleft=-w/2, xright=w/2); also: r is implicitly defined as the diameter - not the  
radius - of the cell (10 um); it would be more clear to define it explicitly.  
Finally, the difference between c(xleft) and c(xcell) (the concentration imposed  
on the left boundary, and the concentration actually perceived by the cell)  
should be more explicitly distinguished, because it may be confusing in a first  
reading.

We appreciate these remarks and have substantially improved the readability of the entire manuscript. Fig.1c now includes the sketch of the geometry, including the symbol definitions.   
  
- A more detailed derivation of the model was provided in the previous version  
of this manuscript (for instance, the role of the quasi-stationary approximation  
was explained in more detail). I think that restoring part of it in the present  
SI would increase readability.

- In SI Sect. 2, c is actually captured by the cell receptors, so, why  
reflective boundary conditions are assigned on the cell surface? probably the  
idea is that at steady state, binding and unbinding process are in equilibrium:  
but that means that receptor internalization is neglected; is that an acceptable  
hypothesis? can estimates be provided?

Yes, precisely. We assumed the equilibrium of binding and unbinding and neglected receptor internalization. This seems reasonable since there is a separation of time scales, e.g. time scale for receptor internalization is 5 minutes [A. Serge et al. Integr. Biol., 3, 675-683, 2011] compared to the time scale of receptor dissociation of 1 second [M. Ueda et al. Science, 294, 864-867, 2001].

We moved SI section 2 to the main text, directly below the title ‘FIXED PDE SECRETION RATE MODEL’. This is now added as a reference [35] and explained, together with the boundary conditions (now in Fig.1b).

- In SI Sect. 2, it would be convenient for the reader to find the statement and  
discussion of boundary conditions in the text along with the equations, rather  
than in the figure caption.

Also in the main text in the same place: first paragraph on page 2.  
  
- In SI Sect. 2, it would be convenient for clarity to write down the explicit  
definition of K\_M.

Also in the main text in the same place: first paragraph on page 2.  
  
- In SI Sect. 3, I imagine that the constant gradient or the fixed mean  
concentrations were imposed by suitably chosing the boundary concentration  
c(xleft); it would help the reader to explain that explicitly.

- In SI Sect. 6, "where p is the PDE concentration present": is it perhaps  
"where P\_0 etc." (cf. Sect. 4)?  
  
----------------------------------------------------------------------  
Report of Referee B -- LT13609/Segota  
----------------------------------------------------------------------  
  
The paper “Extracellular amplification of chemical gradients by  
eukaryotic cells” theoretically describes how the degradation of a  
chemoattractant influences the process of chemotaxis, as can be  
measured in terms of the signal-to-noise ratio or chemotactic index.  
The main result is that the secretion of degrading enzymes increases  
the signal-to-noise ratio of the occupied receptor difference across  
the cell body.  
  
In my opinion, the presented results are of a certain interest to  
experts in the field. However, I am not sure whether PRL is the  
appropriate journal for publication. The paper lacks a clear physical  
picture and the style of presentation does not allow to understand it  
without reading the Supplementary Material in parallel. Thus, the high  
level criteria of publishing in PRL might not be satisfied; I would  
suggest to thoroughly revise the manuscript and consider resubmission  
to a biophysics-focused journal, e.g. the Physical Review E.

The main result is that the extracellular PDE does matter,

In the following, I would like to present some details, which  
contribute to my decision.  
  
- Already in the abstract the authors mention that the cAMP  
concentration for “optimal gradient sensing without PDE is at K\_d”.  
Here, it should be essential to explain the physical significance of  
the statement.

We changed the abstract but explain this at the appropriate place in the main text [REF].

The physical picture is as follows.

The mean number of occupied receptors at some concentration c is R=Nrec\*c/(c+K\_d), where Nrec is the total number of receptors (binomial distribution). When c << K\_d, R ~ c so the response is linear. When c >> K\_d, R ~ const (saturation).

For SNR:

- when c << Kd:

The signal (numerator) \Delta R ~ |\nabla c| ~ c and the noise \sigma\_{\Delta R} (denominator) scales as \sqrt{c} (this is intuitive since R ~ c and we’re just counting molecules; \sqrt{N} ~ \sqrt{c}). So, if we start at c=0 and increase c, the signal increases at a faster rate than the noise hence the SNR (their ratio) *increases*.

- when c >> Kd:

The signal \Delta R now scales as |\nabla c|/c^2 ~ 1/c. This is also expected as the signal gets saturated when most of the receptors are occupied (R ~ const. regime). The noise \sigma\_{\Delta R} ~ c^{-1/2} so both the signal and the noise now decrease, but the noise

To avoid errors due to various approximations, in our simulations SNR was directly (numerically) evaluated from R\_F – R\_B / \sqrt{ \sigma\_B^2 + \sigma\_{\Delta R}^2 }.

Furthermore, as PRL is a physical journal, it would be  
helpful to define K\_d somewhere in the main text and to give a  
physical interpretation. In addition, neither in the abstract nor in  
the paper the authors cite the reference of the statement. Comment No.  
5, where the authors address the points of Referee A, is the only hint  
that it might be taken from Ref. [9].

- All results are based on Michaelis-Menten kinetics and a reaction  
diffusion model, which is not explained in the main text. The study of  
this particular system should briefly be motivated. As a suggestion,  
one might also include Eq. (1) and the following reaction diffusion  
equations from the Supplement in the main text. Later on, the paper  
presents diffusion constants D\_p or D\_c, whose meaning is not clear at  
first sight, as not even the quantity p has been mentioned before.  
  
- The “PDE secretion rate” p\_0 is not defined in the main text. In  
addition, from my understanding, a “rate” is a quantity with unit of  
inverse time; put differently, their “rate” p\_0 seems to be a flux.  
  
- After Eq. (2), the authors introduce r without explanation.  
  
- It is not clear how the presented results depend on the model and in  
particular on the chosen values for its parameters. For example: It is  
striking that the diffusion constants D\_p and D\_c differ by almost one  
order of magnitude. Is this crucial for the results?

- The meaning of $\Delta c$ in Eq. (4) is not explained; afterwards,  
the linear scaling with the “gradient” is mentioned. After the  
subsequent Eq., the gradient $\nabla c$ is introduced for the first  
time. Is this simply a confusion between $\Delta$ and $\nabla$? In  
addition, if $CI ~ \nabla c$ were meant, it should be written as $CI ~  
|\nabla c|$. The same mixing up of vectors and scalars is in the  
discussion when writing $\nabla c \approx 3 \* 10^-2 nM µm^-1$.  
  
- Eq. (6) is multiplied by a factor of 1.45; at first glance, this  
statement seems somewhat arbitrary, so I suggest to include the  
reference from the Supplement into the main text. Next, which  
concentration profile or other input is needed in order to derive Eq.  
(6) starting from Eq. (5)? There is not even a hint of a derivation in  
the Supplementary Material.

- The authors mention that “the numerical results from Fig. 1c are  
therefore also obtained analytically”. However, I do not find any plot  
where the agreement between numerical and analytical results is  
demonstrated.  
  
- In the discussion, the authors refer to “the Peclet number argument”  
without giving any reference.  
  
- At least in the Supplement, the authors might briefly justify the  
chosen boundary condition for p=0, e.g., in the way they explain it in  
the letter to Referee A.  
  
- Is there experimental evidence whether the assumption of uniform  
secretion of PDE is indeed valid for Dictyostelium?  
  
- At a certain stage of their life cycle, Dicty cells produce cAMP  
themselves (see e.g. the model of Martiel & Goldbeter). It would be  
interesting to study the relation between the production rate of cAMP  
and the secretion rate of PDE.  
  
----------------------------------------------------------------------  
Report of Referee C -- LT13609/Segota  
----------------------------------------------------------------------  
  
This is an interesting paper which investigates the effects of PDE  
secretion during chemotaxis of an important model organism. It  
combines numerical calculations in 3D with simple analytical  
estimates. My detailed comments are as follows:  
  
1. The abstract needs improvements. In particular for PRL, the  
abstract is too detailed and long. The third sentence is out of  
bounds, the prediction that optimal cAMP concentration is below K\_a is  
rather trivial considering role of PDE, and the last sentence is  
misleading since microfluidic chambers are exactly used to wash out  
secreted cAMP and PDE to get well-defined stimuli.

We rewrote the abstract to be in-line with PRL guidelines and the referees’ suggestions.

2. The authors put a lot of effort into making experimentally  
verifiable predictions but this is sometimes at the expense of  
sounding a bit naive. For instance, to measure the chemotactic index  
precisely as predicted in Fig. 2 repetitive, tedious experiments are  
needed and any biologist would wonder how this investigation would pay  
off. Biological cells are obviously much more complicated than  
presented and modeled here. Also Dicty develops and changes during  
starvation and hence experiments, and one would expect large  
deviations from prediction due many factors. I suggest to remove last  
sentence in caption of Fig. 2, and to tone this down in general.

We appreciate very much this concern .. we are offering

To be caution against

Tone it down by “these calculations inspire new experiments”

One has to be cautious as always that the starvation response … cautios against the possibility of varying PDE secretion rate vs time and perhaps separately monitor it through, e.g. through fluorescent reporter of PDE concentration.

before planning the complete experimental program, one has to remember that the starvation response is a developing process and one has to

3. The main surprising result is that the SNR always improves with  
secretion of PDE. This finding needs to be explained intuitively. To  
do this suggest to make a plot of signal and noise separately for the  
SI, to show how improvement comes about (numerator or denominator).

Great idea, do this.

4. The title starts with "Extracellular amplification..." but the  
paper does not address this much. The authors should discuss how this  
amplification comes about in the paper, e.g. that linear gradients are  
turned into exponential gradients. A figure of the applied and actual  
cAMP concentration profiles across a cell would be helpful for the SI.  
  
5. The paper uses a round cell and suggests that chemotaxis is caused  
by difference in ligand-receptor binding between front and back  
halves. That's ok for a model but recent experiments stress the role  
of cell shape in accurate chemotaxis, e.g. Tweedy et al. Sci Rep  
(2013). I would suggest to mention this modeling limitation in the  
Discussion somewhere.

Good point, thank you for pointing this out.