Positional information readout in Ca\textsuperscript{2+} signaling

Vaibhav H. Wasnik,\textsuperscript{1} Peter Lipp,\textsuperscript{2} and Karsten Kruse\textsuperscript{1}

\textsuperscript{1}NCCR Chemical Biology, Departments of Biochemistry and Theoretical Physics, University of Geneva, 1211 Geneva, Switzerland

\textsuperscript{2}Institute for Molecular Cell Biology, Research Centre for Molecular Imaging and Screening, Center for Molecular Signaling (PZMS), Medical Faculty, Saarland University, Homburg/Saar, Germany

(Dated: June 26, 2017)

Living cells respond to spatially confined signals. Intracellular signal transmission often involves the release of second messengers like Ca\textsuperscript{2+}. They will eventually trigger a physiological response, for example, by activating kinases that in turn activate target proteins through phosphorylation. Here, we investigate theoretically how positional information can be accurately read out by protein phosphorylation in spite of rapid second messenger diffusion. We find that accuracy is increased by binding of the kinases to the cell membrane prior to phosphorylation and by increasing the rate of Ca\textsuperscript{2+} loss from the cell interior. These findings could explain some salient features of conventional protein kinases.

Living cells respond to external chemical and physical stimuli. In many cases, external factors result in global cellular responses with substrate-stiffness dependent cell differentiation being a particularly dramatic example \cite{1}. In other cases, signals carry spatial information on a subcellular scale \cite{2}. In this way, localized uptake of extracellular material through endocytosis can be initiated \cite{3}, as well as targeted release through exocytosis \cite{4}. Amoeba migrate along chemical gradients \cite{5}, neurons reinforce or weaken synapses \cite{6,7}, and T cells of the immune system polarize in response to infection with pathogens \cite{8}.

There is another reason why the localization of signals is important. Typically an external stimulus is translated into the release of a second messenger \cite{9}. Examples of such molecules are cyclic Adenosine-Monophosphate, Ca\textsuperscript{2+} ions, and diacylglycerol (DAG). These then activate further downstream responses. Notably, this involves the Ca\textsuperscript{2+}-binding protein calmodulin (CaM), as well as the family of conventional protein kinases C (cPKCs). For activation, the latter require simultaneous binding to DAG in the cell membrane \cite{10}. The signal is further relayed, in the case of cPKCs, by direct phosphorylation of a target protein or, in the case of CaM, by binding to and thus activating a kinase, which in turn phosphorylates a target protein. Remarkably, binding of Ca\textsuperscript{2+} to CaM and cPKCs is nonspecific and yet these proteins can trigger specific responses \cite{10}. It has been suggested that specificity results from localized signalling domains \cite{11,12}. They could result, for example, from Ca\textsuperscript{2+} puffs or sparks, which originate from a spatially restricted and transient opening of Ca\textsuperscript{2+} channels \cite{13}.

In this work, we address the question how cells can optimally detect the position of a transient and spatially localized signal. Work on physical limits of detecting spatial information contained in cellular signals has so far focused on gradient sensing \cite{14,15} and on extracting positional information from chemical gradients \cite{17}. A prominent example is the transfer of positional information from the bicoid gradient in developing drosophila flies \cite{18,19}. In addition, the efficient detection of shallow gradients through cell-cell communication has been investigated \cite{20,21}. Motivated by the dynamics of CaM and cPKCs, we consider the spatial distribution of phosphorylation events in response to localized Ca\textsuperscript{2+} release. Through numerical and analytical work, we find that kinases binding to the membrane detect the position of the incoming signal better than cytosolic kinases. For both types of kinases, spatial accuracy increases with the rate at which Ca\textsuperscript{2+} unbinds and is lost from the system. Furthermore, the accuracy increase more slowly than the square root of the number of Ca\textsuperscript{2+} ions in a puff.

We start with the case of a diffusible kinase. Consider a single Ca\textsuperscript{2+} released into the cell interior at \(x = 0\), Fig. 1. We will neglect possible intermediate steps and assume that it can associate directly with a kinase. After binding Ca\textsuperscript{2+}, the kinase is active and can phosphorylate target proteins at rate \(\nu_k\). The Ca\textsuperscript{2+} can dissociate from the kinase at rate \(\nu_d\). Free Ca\textsuperscript{2+} is lost from the system at rate \(\nu_l\). The diffusion constants of Ca\textsuperscript{2+} and the kinase are \(D_C\) and \(D_K\), respectively. For simplicity, we will assume that the kinases form a reservoir, such that there is a constant association rate \(\nu_a\) of Ca\textsuperscript{2+} with a kinase. Finally, we specify the geometry: the membrane is located at \(y = 0\) and extends infinitely into the \(x\)-direction. We neglect the dynamics in the \(z\)-direction and the intracellular space is the half-space with \(y < 0\). By abuse of terminology, we will call this the calmodulin scenario and refer to the kinase as calmodulin (CaM).

The readout signal or estimated position of Ca\textsuperscript{2+} release, \(\hat{x}\), is the average position of the phosphorylation events along the \(x\)-axis. In Figure 1, we present the probability distribution \(P\) of the estimated positions of the Ca\textsuperscript{2+} release obtained from numerical simulations of \(10^6\) Ca\textsuperscript{2+} release events. In our numerical simulations, we draw the time \(\Delta t\) to the occurrence of the next event from an exponential distribution. The mean of this distribution is given by the inverse of the total rate of all reaction events possible in the present state (attachment
and loss if Ca$^{2+}$ is not attached to the kinase, detachment and phosphorylation otherwise). We then draw the molecules’ next position in $x$-direction from a Gaussian distribution with variance $2D_i\Delta t$, $i = C, K$. Then the actual event is determined and the corresponding action performed. The resulting distribution $P$ is centered around $x = 0$ and more peaked than a Gaussian.

Now consider a kinase that needs to bind to the membrane for activation. The kinase binds to the membrane at rate $\nu_b$ and a membrane-bound kinase unbinds at rate $\nu_u$. It has been shown that following Ca$^{2+}$ stimulation the translocation of cPKC to the membrane is independent of the cytoskeleton [22]. Therefore, we focus our attention on diffusive transport of the kinase. On the membrane, diffusion is reduced compared to transport in the cytoplasm [23]. For simplicity, we assume that a membrane-bound kinase is immobile. All other processes are the same as in the CaM scenario, Fig. 1. We will refer to this case as the PKC scenario and call the kinase PKC.

In the numerical simulations of this process, we now have to account explicitly for the dynamics in the $y$-direction, because only PKC close to the membrane can bind. In the simulation, the boundary is taken into account in the following way [23, 24]: if a diffusion step leads to a position outside the simulation domain, then the particle is binding with a probability that is proportional to the binding rate $\nu_b$. In the opposite case, it is reflected. If the particle stays within the simulation domain then it is still binding to the membrane with a probability that is proportional to the binding rate and to a factor that depends on the distance of the particle to the domain boundary. Explicitly, it is given by $\exp\{-y(t)(y' / DK(t' - t))\}$, where $y(t)$ and $y(t')$ are the $y$-coordinates of the particle at the time $t$ of the previous reaction event and the time $t'$ of the current reaction event. As for the CaM scenario, the distribution $P$ of estimated Ca$^{2+}$ release sites deviates from a Gaussian distribution [1]. Note, also that for the same values of the phosphorylation, attachment, detachment, and loss rates, the distribution is narrower for the PKC scenario compared to the CaM scenario.

In both scenarios, the average total number $N_p$ of phosphorylation events is proportional to the phosphorylation rate and decreases with increasing detachment rate $\nu_d$, Fig. 2a,b. In the CaM scenario, $N_{p,\text{cal}} \propto \nu_u^{-1}$. In the PKC scenario we can observe two different scaling regimes as a function of $\nu_d$. Furthermore, $N_{p,\text{PKC}} \propto \nu_u^{-1}$.

We define the error of the estimate to be given by the variance of the distribution $P$, $\ell^2 = \int dx \hat{x}^2 \hat{P}(\hat{x})$. It decreases with increasing values of $\nu_d$ for $\nu_d \lesssim \nu_u$ and after a possible (weak) increase saturates, Fig. 2c,d. For large enough detachment rates, the error is thus robust against changes in $\nu_d$. As a function of $\nu_l$ it decreases, Fig. 2e,f. In the PKC scenario, the distribution of the estimated position is independent of the values of $\nu_b$ and $\nu_u$ as long as both are non-zero, because we assume membrane-bound particles to be immobile.

We now perform a mean-field analysis of the above processes, where we assume that phosphorylation and transport are independent processes. Let $C$ and $K$ be the respective probability distributions of free Ca$^{2+}$ and of the Ca$^{2+}$-kinase complex in the half space below the membrane. For the CaM scenario, we then have

$$\partial_t C - D_C \Delta C = \nu_b K - \nu_b C - \nu_l C$$

$$\partial_t K - D_K \Delta K = -\nu_d K + \nu_u C$$

with boundary conditions $\partial_y C|_{y=0} = \partial_y K|_{y=0} = 0$. Under the mean-field assumption, the distribution $p$ of phosphorylation events [20] in the limit $t \to \infty$ is given by

$$p(x) = \nu_p \int_0^\infty dy \int_0^\infty dt K(x, y, t).$$

Using the initial conditions $K(x, y, t = 0) = 0$ and $C(x, y, t = 0) = \delta(x)\delta(y)$, where $\delta$ is the Dirac distribution, we can integrate Eqs. (1) and (2) with respect to $t$ from 0 to $\infty$, solve them for $\int_0^\infty dt K(x, y, t)$, and finally obtain $p$. The error is then

$$\ell^2_{\text{CaM}} = \frac{\int_{-\infty}^{\infty} dx x^2p(x)}{\int_{-\infty}^{\infty} dx p(x)} = \ell^2_G + \ell^2_K \left(1 + \frac{\nu_a}{\nu_b}\right).$$

FIG. 1. Determination of the Ca$^{2+}$ entry site as the position of the stimulus through phosphorylation of a target protein. a) Illustration of the CaM scenario, where Ca$^{2+}$ binds directly to a diffusible kinase at rate $\nu_a$, which then phosphorylates at rate $\nu_d$. Ca$^{2+}$ detaches at rate $\nu_v$ from the kinase and is lost from the system at rate $\nu_f$. b) Distribution of the estimated position $\hat{x}$ of Ca$^{2+}$ release. c) Illustration of the PKC scenario, where, for activation, the kinase has to bind to the membrane at rate $\nu_b$. It unbinds at rate $\nu_u$. Other parameters have the same meaning as in (a). d) Distribution of the estimated position $\hat{x}$ of Ca$^{2+}$ release for the PKC scenario. Parameter values in (b) and (d) are $\nu_b/\nu_a = 10$, $\nu_d/\nu_b = 100$, $\nu_f/\nu_b = \nu_a/\nu_p = \nu_v/\nu_f = 1$ and $D_K = 0.01 D_C$. Space has been scaled with $\ell = \sqrt{D_C/\nu_a}$. Red lines in (b) and (d) indicate Gaussian fits to the distributions.
Comparison of the mean-field result with the simulations suggests that this expression is exact, Fig. 2a. It states that the average number of phosphorylation events is small, Fig. 2c,e. It is essentially given by the sum of the variances of CaM and kinase diffusion, where the latter is weighted by one over the probability that CaM is lost from the system rather than attaching to the kinase. The total number of phosphorylation events \( N_{p,\text{CaM}} \) is

\[
N_{p,\text{CaM}} = \int_{-\infty}^{\infty} dx \, p(x) = \frac{\nu_u \nu_p}{\nu_u \nu_d}. \tag{5}
\]

Similarly, we can obtain \( p \) in the PKC scenario. In that case, the boundary condition on the kinase current is given by

\[
D_K \frac{\partial}{\partial y} K(x, y, t)_{y=0} = \nu_b K(x, y = 0, t) - \nu_u k(x, t), \tag{6}
\]

where \( k \) is the distribution of PKC on the membrane. It is governed by

\[
\partial_t k(x, t) = -\nu_b K(x, y = 0, t) + \nu_u k(x, t) \tag{7}
\]

The distribution of the phosphorylation events is now given by \( p = \nu_p \int_0^\infty dt \, k(x, t) \) and we find

\[
\ell_{\text{PKC}}^2 = \frac{1}{2} \left[ \ell_{\text{CaM}}^2 + \ell_C \ell_K \right] \tag{8}
\]

\[
N_{p,\text{PKC}} = \frac{\nu_u}{\nu_d} \left[ 2 \ell_{\text{PKC}}^2 \ell_K \right]^{-1/2} N_{p,\text{CaM}}. \tag{9}
\]

Note that in contrast to the CaM scenario, the average number of phosphorylation events depends on the diffusion constants \( D_C \) and \( D_K \), because only kinases that make it to the membrane can phosphorylate. The mean-field result for \( N_{p,\text{PKC}} \) are exact, Fig. 2b, whereas the expression for the error is appropriate for large values of \( \nu_b \) and \( \nu_k \). Fig. 2d.f. In agreement with the simulation results, \( \ell_{\text{PKC}}^2 \) is independent of the membrane binding and unbinding rates \( \nu_b \) and \( \nu_k \). Let us point out that \( \ell_{\text{PKC}}^2 < \ell_{\text{CaM}}^2 \) for all parameter values, supporting that a membrane-binding kinase is better suited to detect the CaM entry point than a cytosolic kinase.

We now turn to CaM puffs. In Figure 3, we present the error as a function of the number \( N_{\text{CaM}} \) of CaM in
a puff. Note that it does not decrease as $1/N_{\text{Ca}}$. This is due to the fact that in cases, where the number of phosphorylation events per Ca$^{2+}$ is less than 1, some Ca$^{2+}$ do not contribute to the estimate. The error in the CaM scenario presents a minimum for small values of $\nu_l$, a feature that is not shared by PKC, Fig. 3. Note, that for $N_{\text{Ca}} \approx 1000$ the error is more than a factor 10 smaller in the PKC scenario compared to the CaM scenario.

The deviation of the error from scaling as $1/N_{\text{Ca}}$ indicates that the mean-field approach taken above is not appropriate for a Ca$^{2+}$ puff. We will now express the estimated error in the measurement performed by a puff through the distribution of phosphorylation events by one Ca$^{2+}$. For a given distribution $\tilde{p}$ of phosphorylation events resulting from an entire puff, the estimated position $\hat{x}$ of Ca$^{2+}$ release is given by $\hat{x} [\tilde{p}(x)] = \int dx\, x\tilde{p}(x) / \int dx\, \tilde{p}(x)$. It yields zero in our setting. A convenient notation for the variance $\ell_{\text{puff}}^2$ is in form of a path integral

$$\ell_{\text{puff}}^2 = \int \mathcal{D}\tilde{p}(x) \, x^2 [\tilde{p}(x)] \mathcal{P} [\tilde{p}(x)],$$

where the realizations $\tilde{p}$ are understood to be positive integers at each position $x$ and $\mathcal{P}$ is the probability distribution of the realizations.

In the limit, where each Ca$^{2+}$ ion is resulting in phosphorylation at one position at most, phosphorylation at any two different positions results from two different Ca$^{2+}$ ions and are thus independent of each other. Consequently,

$$\mathcal{P} [\tilde{p}(x)] = \prod_x P_{\tilde{p}(x)} (x)$$

with $P_{\tilde{p}(x)} (x)$ being the probability of having $\tilde{p}(x)$ phosphorylation events at $x$. We assume it to be given by a Poissonian distribution with a mean that is equal to the value $p(x)$ of the distribution of phosphorylation events resulting from one Ca$^{2+}$ ion that was calculated above. Explicitly,

$$P_N(x) = \frac{p(x)^N}{N!} e^{-p(x)}.$$

After some calculation, we find

$$\ell_{\text{puff}}^2 = \ell^2 \left[ 1 - e^{-N_{\text{p}}N_{\text{Ca}}} \sum_{n=1}^{\infty} \frac{N_{\text{p}}^n N_{\text{Ca}}^n}{n!n}, \right]$$

where $N_{\text{p}}$ and $\ell^2$ are, respectively, the total number of phosphorylation events and the variance of the corresponding distribution resulting from one Ca$^{2+}$ ion that were calculated above for the CaM- and PKC scenarios. For large $N$ we have $\ell_{\text{puff}}^2 = \ell^2 / (N_{\text{p}}N_{\text{Ca}})$.

In conclusion, we have shown that the spatial distribution of phosphorylation events determines the site of Ca$^{2+}$ increase best when the Ca$^{2+}$-sensitive kinase requires coactivation by membrane binding. In this case, position estimation is optimized if the rate of Ca$^{2+}$ detachment from the kinase is comparable to the phosphorylation rate and if the rate of Ca$^{2+}$ loss from the system is maximal. This finding offers a possible explanation, why cPKCs activate processes of intracellular Ca$^{2+}$ removal [10]. Furthermore, accuracy typically increases for increasing Ca$^{2+}$ detachment rates, but as soon as it is of the same order as the phosphorylation rate, gains in accuracy are minimal or accuracy is even decreasing. In line with this finding, for PKCα the Ca$^{2+}$ detachment rate is about 5 times that of the phosphorylation rate [27]. We also demonstrated that the error in position determination through Ca$^{2+}$ puffs can show a different parameter dependence than for individual Ca$^{2+}$. This effect might even be enhanced in the case of cPKCs, which have been shown to form clusters on the cell membrane [28, 29]. It will be most interesting to explore in the future how our findings impact the specificity of cPKC signaling through the putative mechanism of cPKC localization.

We acknowledge funding through SFB 1027 by Deutsche Forschungsgemeinschaft.

---

[1] A. J. Engler, S. Sen, H. L. Sweeney, and D. E. Discher, Cell 126, 677 (2006).

[2] C. Rosse, M. Linch, S. Kermorgant, A. J. M. Cameron, K. Boeckeler, and P. J. Parker, Nat Rev Mol Cell Bio 11, 103 (2010).
[3] C. Godlee and M. Kaksonen, J. Cell Biol. 203, 717 (2013).
[4] M. Oheim, F. Kirchhoff, and W. Stühmer, Cell Calcium 40, 423 (2006).
[5] P. J. M. Van Haastert and P. N. Devreotes, Nat Rev Mol Cell Bio 5, 626 (2004).
[6] K. Deisseroth, H. Bito, and R. W. Tsien, Neuron 16, 89 (1996).
[7] D. G. Wheeler, C. F. Barrett, R. D. Groth, P. Safa, and R. W. Tsien, J. Cell Biol. 183, 849 (2008).
[8] M. L. Kapsenberg, Nat Rev Immunol 3, 984 (2003).
[9] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, Molecular Biology of The Cell, 5th ed., edited by B. Alberts (Garland Science, 2008).
[10] P. Lipp and G. Reither, Cold Spring Harb Perspect Biol 3 (2011).
[11] J. H. Horne and T. Meyer, Science 276, 1690 (1997).
[12] C. Maasch, S. Wagner, C. Lindschau, G. Alexander, K. Buchner, M. Gollasch, F. C. Luft, and H. Haller, Faseb Journal 14, 1653 (2000).
[13] M. J. Berridge, M. D. Bootman, and H. L. Roderick, Nat Rev Mol Cell Bio 4, 517 (2003).
[14] B. W. Andrews and P. A. Iglesias, PLoS Comput Biol 3, 1480 (2007).
[15] R. G. Endres and N. S. Wingreen, Proc Natl Acad Sci USA 105, 15749 (2008).
[16] B. Hu, W. Chen, W.-J. Rappel, and H. Levine, Phys. Rev. Lett. 105, 048104 (2010).
[17] F. Tostevin, P. R. ten Wolde, and M. Howard, PLoS Comput Biol 3, 763 (2007).
[18] B. Houckmanzadeh, E. Wieschaus, and S. Leibler, Nature 415, 798 (2002).
[19] T. Gregor, D. W. Tank, E. F. Wieschaus, and W. Bialek, Cell 130, 153 (2007).
[20] D. Ellison, A. Mugler, M. D. Brennan, S. H. Lee, R. J. Huebner, E. R. Shamir, L. A. Woo, J. Kim, P. Amar, I. Nemenman, A. J. Ewald, and A. Levchenko, Proc Natl Acad Sci USA 113, E679 (2016).
[21] A. Mugler, A. Levchenko, and I. Nemenman, Proc Natl Acad Sci USA 113, E689 (2016).
[22] X. Hui, B. Sauer, L. Kaestner, K. Kruse, and P. Lipp, Sci. Rep. 7 (2017).
[23] J. Lippincott-Schwartz, E. Snapp, and A. Kenworthy, Nat Rev Mol Cell Bio 2, 444 (2001).
[24] S. S. Andrews and D. Bray, Physical Biology 1, 137 (2004).
[25] R. Erban and S. J. Chapman, Physical Biology 4, 16 (2007).
[26] Note that $p$ is not a probability distribution and typically $\int dx \, p(x) \neq 1$.
[27] E. A. Nalefski and A. C. Newton, Biochemistry 40, 13216 (2001).
[28] M. Bonny, X. Hui, J. Schweizer, L. Kaestner, A. Zeug, K. Kruse, and P. Lipp, Sci. Rep. 6, 36028 (2016).
[29] C. J. Swanson, R. F. Sommese, K. J. Petersen, M. Ritt, J. Karslake, D. D. Thomas, and S. Sivaramakrishnan, PLoS ONE 11, e0162331 (2016).