A Diffusion-Reaction Model of Signal Transduction by MAPK Cascades

Bo Hu*
Center for Theoretical Biological Physics, Department of Physics
University of California, San Diego, La Jolla, CA, 92122
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The mitogen-activated protein kinase (MAPK) cascade is a highly conserved signaling module comprised of three sequentially activated protein kinases, which are rapidly phosphorylated near the membrane and dephosphorylated in the cytosol. It regulates various important biological processes including differentiation, proliferation, and synaptic plasticity. In this paper, a diffusion-reaction model is proposed to study this signal transduction pathway. It has been demonstrated before by quantitative analysis that the spatial separation of activation and deactivation of MAPK kinases will lead to a spatially precipitous gradient of active MAPKs, which may be useful to enhance local concentrations of MAPK-dedicated proteins, and hence promote signaling efficiency. On the other hand, this finding also poses a problem: how can signals concentrated near the membrane be effectively transmitted to the nucleus where necessary transcriptional programs have to be initiated? Several additional mechanisms like endocytic trafficking and MAPK dimerization may be responsible. The model is also extended to examine the possible contribution of phosphorylation-dependent MAPK homodimerization to the nuclear transport of MAPKs.

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I. INTRODUCTION

As a pivotal module in many signaling pathways, the mitogen-activated protein kinase (MAPK) cascade regulates various cellular activities, such as cell mitosis, differentiation, and synaptic plasticity [1–4]. It transmits extracellular signals from the plasma membrane to cytoplasmic and nuclear targets through sequential activation of MAP kinase kinase kinase (MAPKKK, also know as MEKK), MAP kinase kinase (MAPKK, or MEK) and MAP kinase (MAPK, or ERK) [4–6]. As well conserved over evolution, MAPK module has been employed in many different cellular contexts for distinct purposes [6]. Despite so versatile, a common feature of MAPK signaling is generally appreciated: MAPKKK, MAPKK, and MAPK are all phosphorylated/activated in proximity to the plasma membrane upon certain stimuli, while they are dephosphorylated/deactivated by MAPK phosphatases in the cytosol [7]. The fact that activation and deactivation of signaling proteins are spatially separated creates a nontrivial spatiotemporal pattern of active MAPKs which encodes important biological information for its regulation of downstream processes. In this paper, a mathematical model based on diffusion-reaction equations is proposed to revisit the signal transduction mechanism of MAPK cascades.

Quantitative analysis reveals that the spatial separation of a membrane-bound kinase and a cytosolic phosphatase potentially results in exponentially precipitous gradients of target phosphoproteins, which may have important biological implications [7]. For example, the signaling proteins may be effectively restricted to a narrow domain below the plasma membrane, which will enhance their local concentration and therefore signaling efficiency. In real cells with real geometries and heterogenous distributions of kinases and phosphatases, extremely complex spatial patterns of phosphoproteins may be expected. Also, this implies potential control of signaling pathways via cell size and shape [8]. Moreover, asymmetrical stimulation of phosphoproteins may result in their polar distribution, which might be useful for cell to sense extracellular gradients. It is worth remarking that the analysis here applies not only to phosphoproteins like MAPK proteins but also to any reversible protein modification where the forward and reverse reactions are spatially separated, e.g. G-protein.

II. MATHEMATICAL MODEL

As the first step, consider the activation process of one phosphoprotein, e.g. MAPKKK (MEKK) in the setting of MAPK cascade. The cytosolic protein of interest binds to one of N_f free binding sites (e.g. provided by receptors

*Electronic address: bhu@physics.ucsd.edu

or scaffold proteins) on the plasma membrane with association rate k_{on} and dissociation rate k_{off}. It is activated only when membrane-bound with activation rate k_{act}, and deactivated in the cytosol with rate constant k_p. After activation, the phosphoprotein can falls off from the membrane with association rate k^*_{off}, and rebind to membrane with k^*_{on}. Let A denote the cytosolic concentration of inactive phosphoproteins, and A^* be the concentration of active phosphoproteins. For now, it is assumed that the activation status of the protein does not affect the mobility coefficient of the protein in cytoplasm (D). On the membrane, define densities of membrane-bound protein in the active (N_b) and inactive (N^*_b) states. The density of binding sites (free or occupied) on the membrane $N = N_b + N^*_b + N_f$ depends on the location and strength of extracellular stimuli. The lateral motility of membrane-bound protein can be neglected. Then it is straightforward to write down the following partial differential equations for this system:

$$\frac{\partial A}{\partial t} = D\nabla^2 A + k_p A^* \tag{1}$$

$$\frac{\partial A^*}{\partial t} = D\nabla^2 A^* - k_p A^* \tag{2}$$

$$\frac{\partial N_b}{\partial t} = -k_{off} N_b - k_{act} N_b + k_{on} N_f A \tag{3}$$

$$\frac{\partial N^*_b}{\partial t} = -k^*_{off} N^*_b + k_{act} N_b + k^*_{on} N_f A^* \tag{4}$$

with boundary conditions:

$$D\frac{\partial A}{\partial \overrightarrow{n}} = k_{off} N_b - k_{on} N_f A \tag{5}$$

$$D\frac{\partial A^*}{\partial \overrightarrow{n}} = k^*_{off} N^*_b - k^*_{on} N_f A^* \tag{6}$$

$\overrightarrow{n}$ is the outward normal vector. At steady state, one obtains:

$$D\nabla^2 (A + A^*) = 0 \tag{7}$$

with boundary condition

$$D\frac{\partial (A + A^*)}{\partial \overrightarrow{n}} = 0 \tag{8}$$

Therefore, $A_0 = A + A^*$ is constant over space, which is intuitively true. We are interested in the concentration of active phosphoproteins, which in steady state satisfies:

$$D\nabla^2 A^* = k_p A^* \tag{9}$$

and on the boundary

$$D\frac{\partial A^*}{\partial \overrightarrow{n}} = k_{act} N_b = \frac{NA}{h_1 A_0 + h_2 K^* + (h_2 - h_1) A^*} \tag{10}$$

where

$$h_1 = \frac{1}{k_{act}} + \frac{1}{k_{off}}$$

$$h_2 = \frac{k^*_{on}}{k_{on}} \left(\frac{1}{k^*_{off}} + \frac{k_{off}}{k^*_{off}} \frac{1}{k_{act}}\right)$$

$$K^* = \frac{k^*_{off}}{k^*_{on}}$$

If one further assumes that the activation status of phosphoprotein does not affect its kinetics of membrane binding, i.e. $k_{on} = k^*_{on}$ and $k_{off} = k^*_{off}$, then $h_1 = h_2$, and the boundary condition is simplified as follows:

$$D\frac{\partial A^*}{\partial \overrightarrow{n}} = \frac{NA}{h_1 (A_0 + K^*)} \equiv gA \tag{11}$$

here g is a "lumped" parameter that reflects the signaling efficiency of certain membrane region, which depends on the strength of the extracellular stimuli and molecular kinetics of membrane binding/modification. For ideal cell geometry like a spherical cell, equation (9) is exactly solvable. Since there is no diffusion flux at the center of the cell $\partial A^*/\partial r|_{r=0} = 0$, the steady-state solution for a spherical cell with radius R and uniform N reads as follows (no angular dependence due to symmetry):

$$A^*(r) = \frac{const \cdot (\exp(r/L) - \exp(-r/L))}{r} \tag{12}$$

where $L = \sqrt{D/k_p}$, called decay length, defines how fast the concentration decays over distance from the membrane. The constant factor in $A^*(r)$ is determined by boundary condition:

$$const = \frac{gA_0R^2}{[3D(1/L - 1/R) + gR]\exp(R/L) + [3D(1/L + 1/R) - gR]\exp(-R/L)} \tag{13}$$

Therefore, the phosphoprotein concentration almost exponentially decreases toward the cell interior, see Fig. 1. This result has actually been derived in previous work, where the relative gradient was also estimated [7]: (1) eukaryotic cell radii vary from 5 to 50 μm; (2) k_p is the first-order phosphatase reaction rate constant (typically, 0.1-100 s^{-1}); (3) the diffusion coefficient D for globular proteins ranges from 1 to $10 \mu m^2$. From the above values, it is estimated that the decay length L will vary broadly from $0.1 \mu m$ to $10 \mu m$. Recent experimental results confirm the existence of gradient decay lengths on the scale of $1 \mu m$ in both *Xenopus* extracts and in living cells [9, 10].

The above analysis and estimation suggest that most of phosphoproteins activated on the membrane and deactivated in the cytosol will be in general restricted to a narrow space below the membrane. In ERK/MAPK cascade system, MEKK are first recruited to the plasma membrane by upstream membrane-associated kinase Ras. After being activated by Ras, MEKK dissociates from the membrane to cytosol, and phosphorylates MEK, which sequentially phosphorylates ERK. Since theoretical analysis indicates a spatial gradient of phosphorylated MEKK, the concentration profile of active MEK is intuitively heterogenous as well. The clear difference is that the source for activating MEKK is on the boundary, while the source for activating MEK is concentrated near the membrane. To quantitatively examine the spatial pattern of active MEK, one can add two additional diffusion-reaction equations to the original model system:

$$\frac{\partial B}{\partial t} = D'\nabla^2 B - k_{act}^b A^* B + k_p^b B^* \tag{14}$$

$$\frac{\partial B^*}{\partial t} = D'\nabla^2 B^* + k_{act}^b A^* B - k_p^b B^* \tag{15}$$

where D' is the diffusion coefficient for B (unphosphorylated MEK) and B^* (phosphorylated MEK), k_{act}^b is the activation rate of B by A^*, and k_p^b is the first-order phosphatase reaction rate for B^*. The associated boundary conditions for B and B^* are $D'\partial B/\partial \overrightarrow{n} = 0$ and $D'\partial B^*/\partial \overrightarrow{n} = 0$. Numerical solutions indicate that $B^*(r)$ also form a spatial gradient near the membrane, with decay length $L_b > \sqrt{D'/k_p^b}$, see Fig. 2. The average level of B^* is most affected by the factor k_{act}^b, and for biologically realistic values, the level of B^* (MAPKK) is usually quite larger than that of A^* (MAPKKK); in other words, the signal is amplified through the MAPK cascade. In a similar manner, it is effortless to introduce MAPK into the current mathematical model. And predictably, the signal is further amplified, and decays less precipitously than MAPKK or MAPKKK over distance from the membrane.

III. DISCUSSION

Multiple cellular proteins are phosphorylated and dephosphorylated at distinct cellular locations. In the Ras-ERK pathway for example, inactive Raf-1 (MAPKKK) resides in the cytosol. Upon stimulation of cell surface receptors, Raf-1 is translocated from the cytosol to the plasma membrane by a high affinity binding to GTP-loaded Ras. At the membrane, Raf-1 undergoes a series of activation steps involving interaction with 14-3-3 proteins and phosphorylation on specific tyrosine and serine residues [11]. Although the mechanism of activation is not well understood, the association of Raf-1 with membranes appears to be essential for its activation. The Raf-1 Kinase phosphorylates the cytosolic kinase MAPKK at the plasma membrane, whereas soluble serine/threonine phosphatases dephosphorylate the activated MAPKK [12]. In the cytosol, active MAPKK phosphorylates ERK1,2 (MAPK) and specific ERK phosphatases are localized to the cytosol and nucleus. By spatial segregation of activation and deactivation processes, phosphoproteins will be heterogeneously distributed in the cytosol, with high concentrations in the periplasmic region

near the phosphorylation location and low concentrations at a distance from the plasma membrane. This property actually highlights the regulatory function of plasma membrane, which provides a major platform for processing and transmitting extracellular information. By organizing related signaling proteins on and near the membrane, signaling events can be compartmentalized with the aid of phosphatases, and signal may be more efficiently processed in the virtual compartment [13]. Also, the spatial gradient property implies that signaling pathways may be controlled by cell size and shape [8]. It is well appreciated that cell morphology can effectively affect the spatiotemporal pattern of signaling proteins. Recent computational simulations also demonstrates that cell signaling pathways can theoretically be turned on and off, both locally and globally, in response to alterations in cell size and shape [8].

A puzzle may also arise from the above analysis, which implies that the levels of activated MEK and ERK will drop almost exponentially toward the cell interior, without additional mechanism for signal propagation through MAPK cascade. According to previous analysis, at distances larger than several μm from the plasma membrane, the phosphorylation signal should decrease to subthreshold levels, provided that the cytosolic phosphatases activity is normally high. Recall that eukaryotic cell radii R can vary from 5 μm to 50 μm, passive diffusion may be not sufficient for active MAPKs to locate in the nucleus [13]. We discuss two additional mechanisms that may be involved in this problem. First, experimental documents show that phosphorylated MAPKs tend to homodimerize with phosphorylated or unphosphorylated MAPKs, and the MAPK homodimers can be actively transported into the nucleus through some unknown mechanism [14]. The mathematical model has been extended to study the role of dimerization in changing the spatiotemporal pattern of active MAPKs and facilitating their nuclear transport. Some primitive result is shown in Fig. 3. Second, a novel role of endocytosis in turning on activation of the ERK/MAPK cascade by cell surface receptors was first reported for the EGF receptor [15]. Subsequent experimental evidence points to an essential role of receptor and MEK endocytosis in the activation of MAPK cascades, which may suggest that trafficking of signaling intermediates within endocytic vesicles may be an efficient way of propagating the signal [16]. Endocytic trafficking of activated MEK may help to avoid the formation of steep spatial gradients of phosphorylated MEK and ERK, since the spatial separation of kinases and phosphatases is avoided.

As a last word, the traditional perspective that plasma membrane as well as endosome is the unique platform of receptor-mediated signaling has been challenged by the surprising observation that Ras signaling also occur on Golgi, ER, and ER [17]. Such additional complexity of MAPK signaling may also be partly responsible for the nuclear localization of activated ERKs. The author thanks Prof. Gert Cauwenberghs for his motivating this research and his valuable instruction in the course BGGN260 Winter07.

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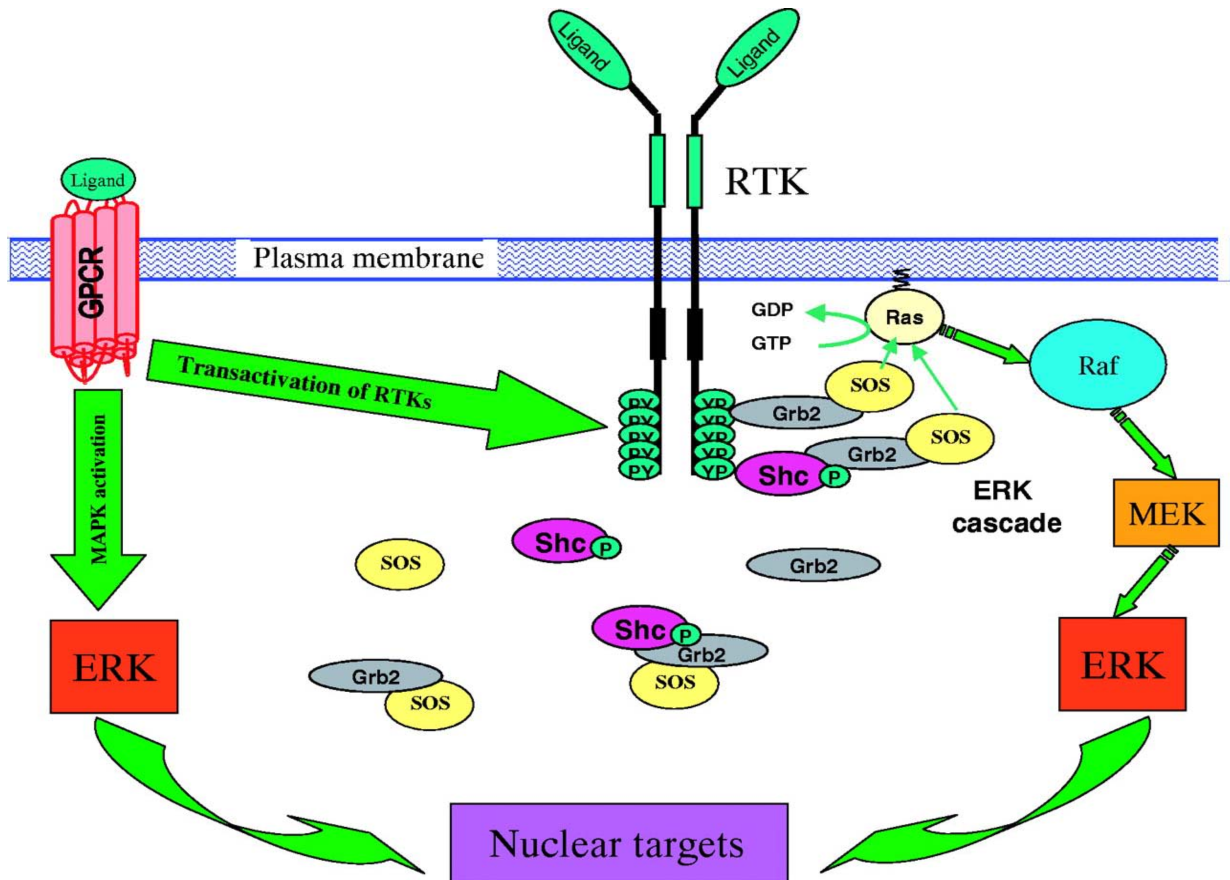

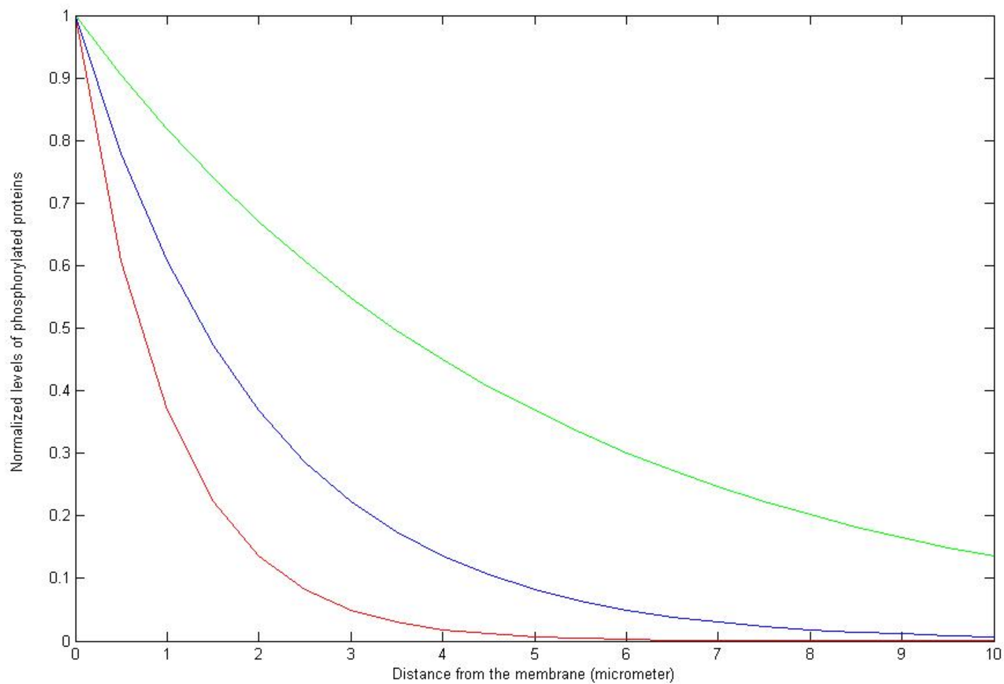

Fig. 1: Steady-state spatial distribution of phosphoproteins. Red curve corresponds to the decay length L=1, blue curve L=2, and green curve L=5; the cell radius is kept as 10 micron here. All the curves show how the phosphorylated fraction decreases with the distance from the plasma membrane (where the protein is phosphorylated) into the cell interior (where it is dephosphorylated by homogeneously distributed phosphatases). For convenience of comparison, all their boundary values have been normalized to be 1.

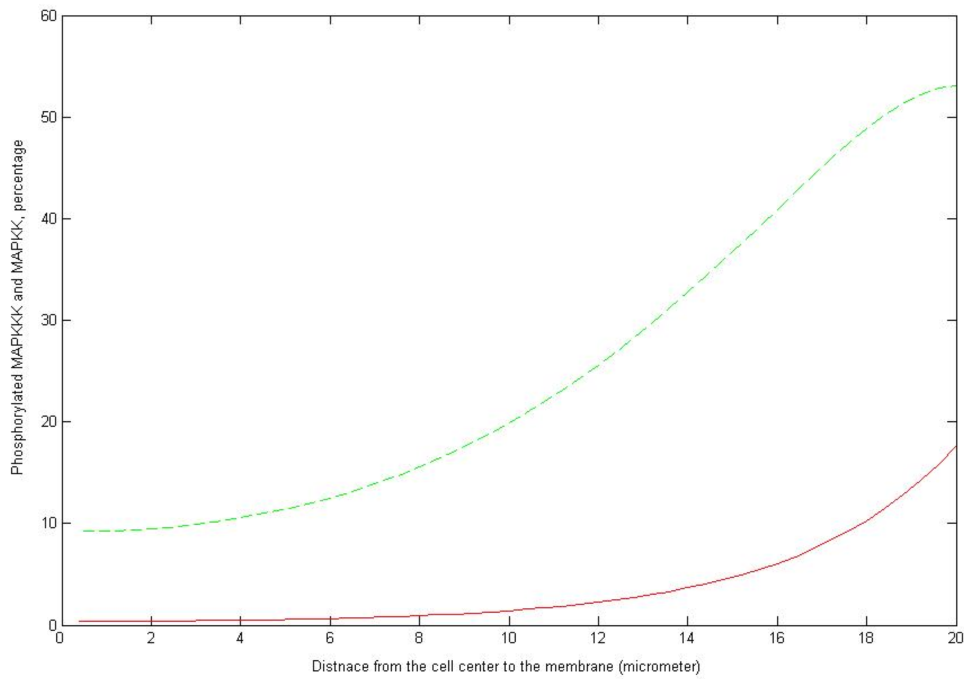

Fig. 2: Percentage of phosphorylated MAPKKK (Red) and MAPKK (Green) as a function of distance from the cell center to the membrane. Cell radius is chosen as 20 micron. Both kinases are supposed to have the same motility coefficients 10 (micron^2/s), and the same dephosphorylation rate k_p=1 (/s). Here the other parameters are g = 1 (/s) and Kact=0.1 (/s).

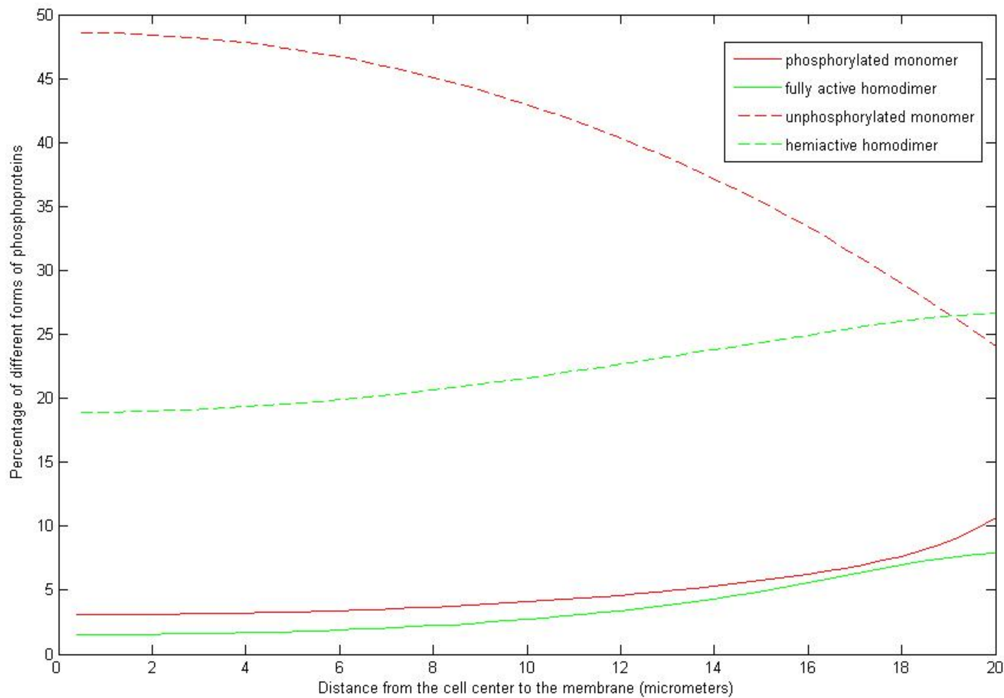

Fig. 3: Percentage of different forms of phosphoproteins when considering homodimerization. Cell radius is chosen as 20 micron. Both kinases are supposed to have the same motility coefficients: 10 (micron^2/s) for monomers and 6 (micron^2/s) for dimers, and the same dephosphorylation rate k_p=0.1 (/s) for all the active forms. Here the other parameters are as such: g = 1 (/s), Kon=0.1 (nM/s), and Koff=0.75 (/s).