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Spatial Gradients in Kinase Cascade Regulation

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1 Abstract

The spatiotemporal kinetics of proteins and other substrates regulate cell fate and signaling. In this study we consider a reaction-diffusion model of interaction of membrane receptors with two-step kinase cascade. The receptors activate the "upstream" kinase, which may freely diffuse over cell volume and activate the "downstream" kinase, also freely diffusing. Both kinase species and receptors are inactivated by uniformly distributed phosphatases. The positive feedback, key to considered dynamics, arises since the upstream kinase activates receptors. Such mutual interaction is characteristic for immune cell receptors, in particular B-cell receptors.

Based on the proposed model we demonstrated that cell sensitivity (measured as a critical value of phosphatase activity by which a cell may be activated) increases with decreasing motility of receptor-interacting kinases and with increasing polarity of receptors distribution. These two effects are cooperating, the effect of receptors localization at one of the cell poles grows with the decreasing kinase diffusion, and vanishes in the infinite diffusion limit. As the cell sensitivity increases with decreasing diffusion of receptor-interacting kinase, the overall activity of the downstream kinase increases with its diffusion.

In conclusion, the analysis of the proposed model shows that, for the fixed substrate interaction rates, spatial distribution of the surface receptors together with the motility of intracellular kinases, control cell signalling and sensitivity to extracellular signals.

Keywords: reaction-diffusion system, kinase cascade, positive feedback, membrane receptors, protein motility.

2 Introduction

Regulatory networks process cellular signals in time and space enabling cell self-organization (see Kholodenko 2006 and Karsenti 2008 for reviews). The temporal dynamics is coupled with spatial gradients of concentrations or activity. For example, kinase cascades can emerge from receptors and transmit signals from the cell membrane to the nucleus. In this case, the gradient of active kinase activity develops since phosphorylation and dephosphorylation proceed at different cellular locations, respectively cell membrane and cell volume. Due to the estimations of Brown and Kholodenko (1999), basing on measured values of protein diffusion coefficients and phosphatase activities, gradients of kinase activity are potentially very large. In a simple system in which kinase molecules are phosphorylated at the cell membrane and dephosphorylated by a phosphatase molecules located homogeneously in the cell cytosol (analyzed by Brown and Kholodenko 1999) small diffusion implies high gradient and low kinase activity in the cell center. The problem of receptor-kinase interaction has been also studied in the context of diffusion with obstacles in the stochastic numerical simulations of bacterial chemotaxis (Lipkow et al. 2005). One of the conclusions of Lipkow et al. 2005 is that crowding results in a fall of the apparent diffusion coefficient and at the anterior end, where CheY is phosphorylated, the local concentration of CheYp increases and therefore accelerates the response of the anterior close motor. At the other, posterior, end of the cell, the local CheYp concentration is reduced by the need to diffuse through the obstacles and the responses of motors in this region is consequently delayed.

In this study we consider a reaction-diffusion model of mutual interaction of membrane receptors with two-step kinase cascade. Membrane receptors can bind extracellular ligands, that leads to cascade of molecular processes inside the cell and formation of an active receptor complex. In many cases, receptor activation requires phosphorylation. Almost all G-protein coupled receptors (GPCRs) are regulated by phosphorylation, see Tobin (2008) for review. Engagement of immunoreceptors (TCR, BCR, FcR) leads to activation of different members of the Src kinase family, which includes Lck (for T-cell, Housden et al. 2003), Fyn and Lyn (for B and mast cells, Gauld and Cambier 2004). Src kinases then phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) contained within the immunoreceptors themselves or in receptor-associated molecules, see Abram and Lowell (2007) for review. This may lead to positive feedback, in which active receptors send signal to kinase and in turn are activated by the same kinase species or by one of the downstream kinases. In this study we consider the simplest situation, in which receptors are activated by the same kinase species they activate.

We will show, that in the case of mutual receptor-kinase activation, in a broad range of parameters controlling the process, the cell becomes activated only if the receptor-interacting kinase diffusion is sufficiently small and the receptors are concentrated in the limited area of the membrane.

3 Model formulation

As said, we will assume that membrane receptors bind extracellular ligands, that leads to a cascade of processes and receptor activation. At constant extracellular cytokine concentration, a steady state uniform surface concentration of ligand-bound receptors $P(\theta) = const$ is established. We will assume that the limiting step in the formation of the active receptor complex is its phosphorylation by the Kinase I. In turn, active receptors may activate kinase molecules, that defines the positive feedback in the regulation process. The activated Kinase I may freely diffuse over entire cell volume, and activate Kinase II, which also may freely diffuse over entire cell volume. We assume that both kinase species are inactivated by uniformly distributed phosphatases.

The cell will be modeled geometrically as a ball $B(0, r_0)$ of radius $r_0 = 1$, centered at the origin of the coordinate system. We restrict to the axially symmetric case and from the beginning we will formulate the model in nondimensional units (Kazmierczak and Lipniacki 2009). We will use the following notation **:**

$K(t, r, \theta)$ - the concentration of the active Kinase I

$K_0 = const = 1$ - the total concentration of the Kinase I

$H(t, r, \theta)$ - the concentration of the active Kinase II

$H_0 = const = 1$ - the total concentration of the Kinase II

$R(t, \theta)$ - the surface concentration of the active receptors

$P(\theta) = const$ - the total surface concentration of the ligand bound receptors (active and inactive)

$\Phi_K(t, \theta)$ - the flux of the active Kinase I

The active Kinase I concentration satisfies

$$\frac{\partial K}{\partial t} = \alpha^{-2}\nabla^2 K - K, \tag{1}$$

where α^{-2} is the nondimesional diffusion coefficient. The kinase dephosphorylation rate is set equal 1. The flux of the active kinase results from its phosphorylation by the surface receptors implying the Robin type boundary condition,

$$\Phi_K = R\,(1 - K_b) = \alpha^{-2}\mathbf{n} \cdot (\nabla K)_b, \tag{2}$$

where $\mathbf{n}$ is a unit vector normal to cell surface and subscript b denotes the boundary value for $(r = 1)$. We assume that the limiting step in the receptor activation is its phosphorylation by the Kinase I, that defines the positive feedback in receptor-kinase activation. Receptors are inactivated with rate b due to phosphatase activity

$$\frac{dR}{dt} = K_b(P - R) - bR. \tag{3}$$

The Kinase II diffuses with diffusion coefficient α_2^{-2} and it is activated by the Kinase I with rate c, and inactivated by the phosphatase with the rate 1. Its concentration thus satisfies

$$\frac{\partial H}{\partial t} = \alpha_2^{-2}\nabla^2 H + cK(1-H) - H, \tag{4}$$

In the further consideration we will assume that all the reaction rate coefficients, b, c, and diffusion coefficients α^{-2}, α_2^{-2} are positive. The aim of the present study is to analyze the steady state solutions of the above system with respect to kinases diffusion and distribution of receptors which will be assumed axisymmetric

$$P_i(\theta) = p_i^{-1}(1+\cos\theta)^i, \quad \text{where} \quad p_i = (4\pi)^{-1}\int (1+\cos\theta)^i = \frac{2^i}{i+1}. \tag{5}$$

According to the above assumption the total amount of receptors is fixed and equals 4π, but their distribution may have lower or higher polarity with respect to the coefficient i; in the following we will refer to i as a polarity coefficient. The spherically symmetric receptor distribution $P_0(\theta) = 1$ will serve us as a reference.

Remarks and basic intuitions

For the sake of simplicity of model analysis most of the reaction rate coefficients are implicitly set equal 1. Nevertheless the model has sufficient generality to analyze the interplay between the kinase diffusion and polarity of receptors distribution in the control of cell activation.

The kinetics of Kinase II is not coupled back with the Kinase I and receptors. This activity of the Kinase II can be thus considered as a final readout, measuring the cell activity.

The parameter b controlling receptors inactivation will be referred to as phosphatase activity. Intuitively the higher is the phosphatase activity the lower is cell activity, and for sufficiently high b (and other parameters fixed) cell may not be activated. Provided that there exists some activity of the Kinase I ($K > 0$), the activity of the downstream kinase H will grow with is phosphorylation coefficient c.

3.1 Summary of results for spherically case

Since the, analyzed previously, spherically symmetric solutions (for single kinase regulation, say Kinase I) will serve as a reference, we recall here the main results (Kazmierczak and Lipniacki 2009).

Limit of infinite diffusion $\alpha \to 0$, $K = K(t)$, $R = R(t)$.

For the infinite diffusion ($\alpha = 0$), the active kinase concentration is uniform, and the system of Eqs. 1-3 is equivalent to the system of two ordinary equation, for $K(t)$, $R(t)$,

$$\frac{dK}{dt} = 3R\,(1-K) - K, \quad \frac{dR}{d\tau} = K(1-R) - R. \tag{6}$$

The system 6 has only one nonnegative steady state $\{K_0, R_0\}$,where
$\{K_0, R_0\} = \{0, 0\}$ for $b \leq 3$
$\{K_0, R_0\} = \{(3-b)/4, (3-b)/3b)$, for $b > 3$
i.e. it has the positive stable steady state solution only for $b > 3$

Finite diffusion

For finite diffusion ($\alpha > 0$), the stable solution $K(r)$ is given by

$$K(r) = \frac{K_c \left(e^{\alpha r} - e^{-\alpha r}\right)}{2r\alpha}. \tag{7}$$

where $K_c = K(0)$ is the active kinase concentration in cell center. $K_c = \max(0, K_c^*)$, where

$$K_c^* = \frac{2\alpha e^{\alpha}\left(be^{2\alpha}\left(1-\alpha\right) - b(\alpha+1) + \alpha^2\left(e^{2\alpha}-1\right)\right)}{\left(e^{2\alpha}-1\right)\left(1+\alpha-\alpha^2+e^{2\alpha}(\alpha+\alpha^2-1)\right)}. \tag{8}$$

The consequence of Eq. 8 is that the critical phosphatase activity b_{crit} above which cell may not be activated ($K(r) = K_c = 0$) is the growing function of α. In other words, due to mutual kinase and receptors interaction, the smaller is the kinase diffusion the easier is to activate both kinases and receptors.

It is interesting that, while for small b (approximately for $b \leq 2$), K_c decreases with growing α, for larger values of b (approximately for $b \geq 2$), $K_c(\alpha)$ has a maximum for some $\alpha_0(b) > 0$. The existence of such an "optimal" α_0 is due to interplay of two counter-effects**:**

(1) large diffusion (small α) speeds translocation of active kinases, so they have a larger chance to remain phosphorylated until they reach the cell center,

(2) simultaneously large diffusion attenuates the positive feedback coupling kinases with receptors.

4 Results

The preliminary analysis shown in Fig. 1 indicates that for $\alpha = 2$ and $b = 4$ and the uniform distribution of receptors $P_0(\theta) = 1$, the only steady state stable solution is $R \equiv K \equiv H \equiv 0$ corresponding to inactive cell. In contrast the nonuniform distribution of receptors $P_i(\theta) = \frac{i+1}{2^i}(1+\cos\theta)^i$ $(i = 2, 4, 8)$ results in positive although highly nonuniform activity of Kinase I. However, even this nonuniform distribution of active Kinase I may lead to the high and relatively uniform activity of Kinase II, provided that its activation coefficient c and its diffusion coefficient α_2^{-2} are sufficiently large. This is why in further analysis we concentrate on criteria of positiveness of stable steady state solution of the system of mutually interacting receptors and Kinase I without putting much attention on the absolute value of Kinase I activity.

In Fig. 2 we numerically determined the critical value of phosphatase activity $b_{crit}(i,\alpha)$ below which kinase I may be activated, (i.e. $K \not\equiv 0$), as a function of coefficient α and three values of the polarity coefficient i; 0, 2 and 4. The critical value of phosphatase activity b_{crit} may be considered as a measure of cell sensitivity; with high b_{crit} value indicating high sensitivity. The analysis showed in Fig. 2 suggests that

(**Hypothesis I**) for any $i \geq 0$,

1) $b_{crit}(i,\alpha)$ is a monotonically growing function of α and

2) $b_{crit}(i,\alpha) \to \infty$ for $\alpha \to \infty$.

The effect of receptor concentration increases with decreasing diffusion (increasing α); Particularly in the limit of the infinite diffusion ($\alpha = 0$), $b_{crit}(i) = 3$, for any $i \geq 0$, i.e. the polarity i has no effect on cell sensitivity.

In analysis presented in Fig. 3 we determine, how the critical value of phosphatase activity $b_{crit}(i,\alpha)$ changes with polarity coefficient i, for three values of α; 1, 4 and 10. This analysis suggests that

(**Hypothesis II**) for any $\alpha > 0$,

1) $b_{crit}(\alpha,i)$ is a monotonically growing function of i and

2) $b_{crit}(\alpha,i) \to \infty$ as $i \to \infty$.

In Fig. 4 we determine the critical amount of receptors $P_{crit}(i,\alpha) = \int P_i(\theta)$ above which the cell may be stably activated. As one could already expect also from the analysis presented in Fig. 3, for any

(**Hypothesis III**) $P_{crit}(i,\alpha)$ is a monotonically decreasing function of polarity i, and $P_{crit}(i,\alpha) \to 0$ for $\alpha > 0$.

Finally, we analyze how the total activity of Kinase II $\int HdV$ (which can be considered as a readout of the transduction pathway) depends on its diffusion coefficient α_2^{-2}. The analysis presented in Fig. 5 suggests the that

(**Hypothesis IV**) $\int HdV$ is a monotonically growing function of the activation coefficient c and diffusion constant α_2^{-2}.

Remark The limits $\alpha \to \infty$ and $i \to \infty$ should be considered with caution. Thus, for fixed $i \geq 0$, $K(r,\theta) \to K_i(r,\theta)$ as $\alpha \to \infty$ pointwise, where $K_i(r,\theta) = 0$ for $r < 1$ and $K_i(1,\theta) > 0$ for $\theta > -\pi$. Similarly, for fixed $\alpha > 0$, $K(r,\theta) \to 1$ for $(r,\theta) = (1,0)$ and $K(r,\theta) \to 0$ for $(r,\theta) \neq (1,0)$ as $i \to \infty$.

5 Discussion

Dynamics of molecular pathways is determined by both, chemical reaction rules and localization of substrates that in turn is governed by diffusion or transport. We considered here a spatial model of activation of the two-step kinase cascade by non-uniformly distributed membrane receptors. We assume the mutual interaction of receptors and kinases. The Kinase I molecules, activated by the receptors, may freely diffuse in the cell

volume (where they are dephosphorylated) and activate the downstream Kinase II, which also freely diffuse inside the cell. The positive feedback, considered in the model, arises since receptor activated Kinase I, may in turn activate receptors. The presence of the positive feedback causes that the concentrations of the active Kinases I and II in the cell volume are nontrivial functions of the diffusion coefficient and the polarity in receptors distribution. As demonstrated earlier (Kazmierczak, Lipniacki 2009) the decreasing diffusion of receptors-interacting kinase may lead to increased cell sensitivity - in particular, it increases the critical phosphatase activity at which the kinase may be activated.

Here, we showed that for the finite diffusion receptors-interacting kinase (Kinase I), the localization of the receptors at one pole of the cell eases the cell activation, i.e. increases the critical phosphatase activity at which cell may be activated (Figs. 1 and 3). In other words the cell sensitivity increases most if, simultaneously, the diffusion of receptor-interacting kinase is lowered and receptors are localized in the small subdomain of the cellular membrane. The effect of receptors localization vanishes if the diffusion of receptor-interacting kinase grows, and completely vanishes in the limit of infinite receptor-interacting kinase diffusion (Fig 2). On the other hand the high diffusion of the down stream kinase (Kinase II) increases not only the uniformity of the active kinase distribution but also the total level of active kinases (Figs. 1 and 4). This suggest that optimal strategy for increasing cell sensitivity is to:

(1) localize receptors in a small subdomain of the cell membrane

(2) lower the motility of receptor-interacting kinase (which will increase the strength of the positive feedback of receptor-kinase interaction)

(3) increase the motility of downstream kinases, which distribute the signal form the receptor-interacting kinases over the cell.

In living cells the diffusion and thus spatiotemporal localization of substrates can be controlled in a number of ways. Molecules can bind to a larger molecules of lower motility such as buffers or scaffolds. Various proteins can also be recruited to the cell membrane and other structural elements directly or with help of the, so called, anchoring proteins. On the cell membrane receptors can form larger complexes of lower motility, or get localized within lipid rafts. Relevant to our considerations, cell membrane can create microdomains which trap signalling molecules, like Src family kinases, that activate TCR, BCR or FcR receptors, Douglass and Vale (2005).

Our simple model provides an example in which kinase diffusion and spatial distribution of membrane receptors control cooperatively strength of the feedback regulation and the cell sensitivity to extracellular signal. In the considered model, for a broad range of parameters the cell can be activated only when the kinase diffusion coefficient is sufficiently small, and receptors are localized in the one pole of the cell membrane.

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References

Abram, C.L., Lowell, C.A., 2007. Convergence of immunoreceptor and integrin signaling. Immunological Reviews 218:29–44.

Brown, G.C., Kholodenko, B.N., 1999. Spatial gradients of cellular phospho-proteins, FEBS Lett. 457:452-454.

Douglass, A.D., Vale, R. D., 2005. Single-molecule microscopy reveals plasma membrane microdomains created by protein-protein networks that exclude or trap signaling molecules in T cells, Cell 121:937-950.

Gauld, S.B., Cambier, J.C., 2004. Src-family kinases in B-cell development and signaling. Oncogene 23:8001–8006.

Housden, H.R., Skipp, P.J.S., M. P. Crump, M.P., Broadbridge, R.J., Crabbe, T., Perry, M.J., Gore, M.G., 2003. Investigation of the kinetics and order of tyrosine phosphorylation in the T-cell receptor ξ chain by the protein tyrosine kinase Lck, Eur. J. Biochem. 270, 2369–2376.

Karsenti E., 2008. Self-organization in cell biology: a brief history. Nat. Rev. Mol. Cell Biol. 9:255-262.

Kazmierczak B., Lipniacki T., 2009. Regulation of kinase activity by diffusion and feedback. J. Theor. Biol. 259, 291–296.

Kholodenko B.N., 2006. Cell-signalling dynamics in time and space. Nat. Rev. Mol. Cell Biol. 7:165-174.

Lipkow, K., Andrews, S.S., Bray, D., 2005. Simulated diffusion of phosphorylated CheY through the cytoplasm of Escherichia coli. J. Bacteriol 187:45-53.

Tobin, A.B., 2008. G-protein-coupled receptor phosphorylation: where, when and by whom. British Journal of Pharmacology 153: S167–S176.

Figure captions

Fig. 1. Steady state spatial distribution of active Kinase I and Kinase II for $\alpha = 2$, $b = 4$, $\alpha_2 = 1$, $c = 10$ and three receptors distributions $P_2(\theta) = (3/4)(1+\cos\theta)^2$, $P_4(\theta) = (\frac{5}{16})(1+\cos\theta)^4$, $P_8(\theta) = (\frac{9}{2^8})(1+\cos\theta)^8$. Let us notice that for the spherically symmetric distribution of receptors $P_0(\theta) = 1$, and the same α and b, the Kinase I (and thus Kinase II) remain inactive, $R \equiv K \equiv H \equiv 0$.

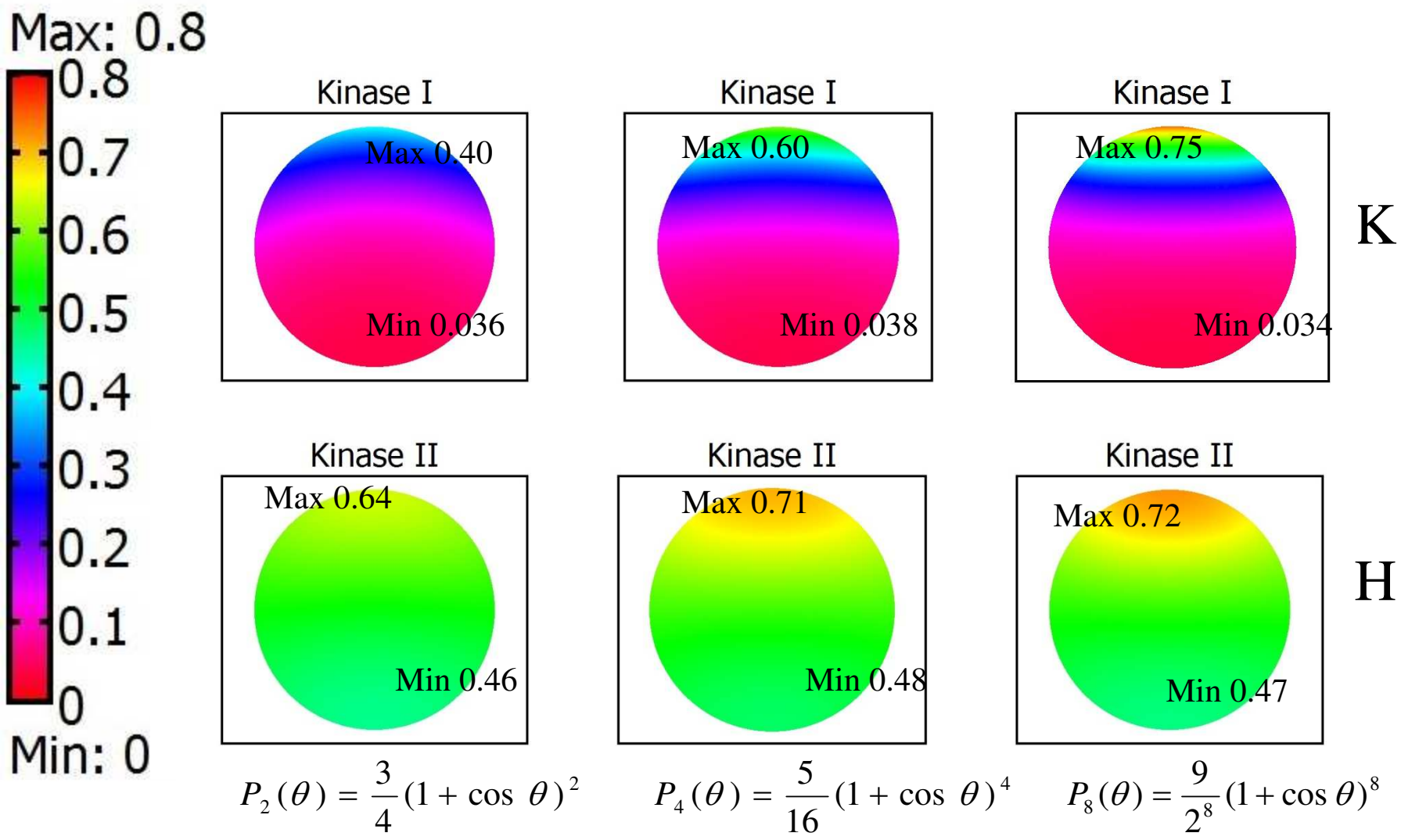
Fig. 2. Critical $b_{crit}(\alpha)$ for three values of polarity coefficient i: 0, 2 and 4; for $b > b_{crit}(\alpha)$ the unique steady state solution is identically zero, $R \equiv K \equiv H \equiv 0$ (the cell may not be stably activated).

Fig. 3. Critical phosphatase activity $b_{crit}(i)$ for three values of α: 1, 4 and 10; for $b > b_{crit}(i)$ the unique steady state solution is identically zero, $R \equiv K \equiv H \equiv 0$.

Fig. 4. The critical amount of receptors P_{crit} as a function of the polarity i for $\alpha = 10$; for $\int P(\theta)dS < P_{crit}$ the unique steady state solution is identically zero, $R \equiv K \equiv H \equiv 0$.

Fig. 5. The spatial distribution of active kinase I for $i = 4$, $\alpha = 6$, and $b = 10$, and corresponding spatial distribution of kinase II for $c = 10$, and $\alpha_2 = 0.5$, $\alpha_2 = 2$, $\alpha_2 = 6$. Upper right panel: total activity of kinase II, $\int H(\alpha_2)dV$ for $i = 4$, $\alpha = 6$, and $b = 10$, and three values of kinase II activation coefficient c: 2, 5 and 10.

Fig. 1



$$P_2(\theta) = \frac{3}{4}(1+\cos\theta)^2 \qquad P_4(\theta) = \frac{5}{16}(1+\cos\theta)^4 \qquad P_8(\theta) = \frac{9}{2^8}(1+\cos\theta)^8$$

For $P_0(\theta) = 1,\ \mathrm{K} \equiv \mathrm{H} \equiv 0$

Fig. 2

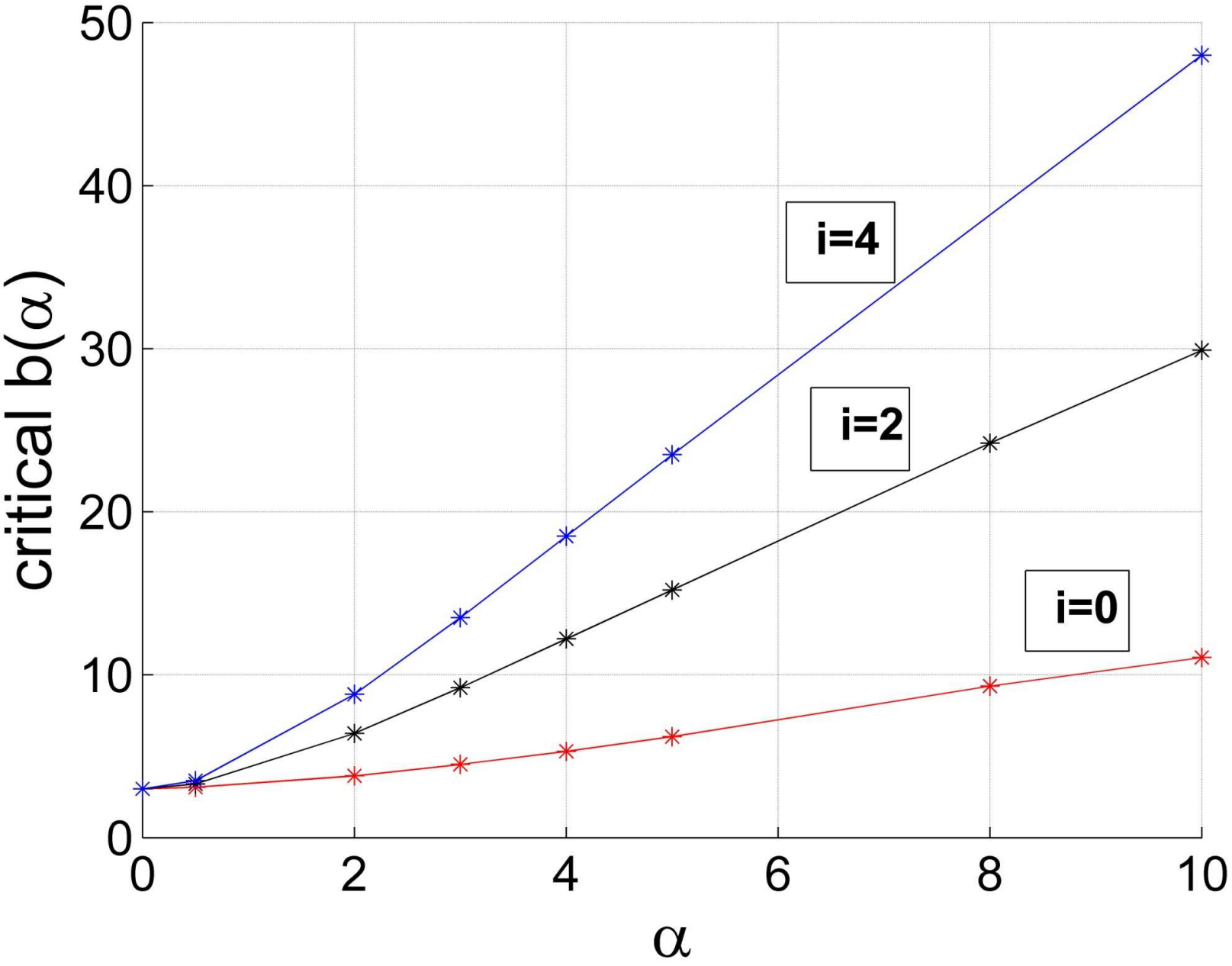


Fig. 3

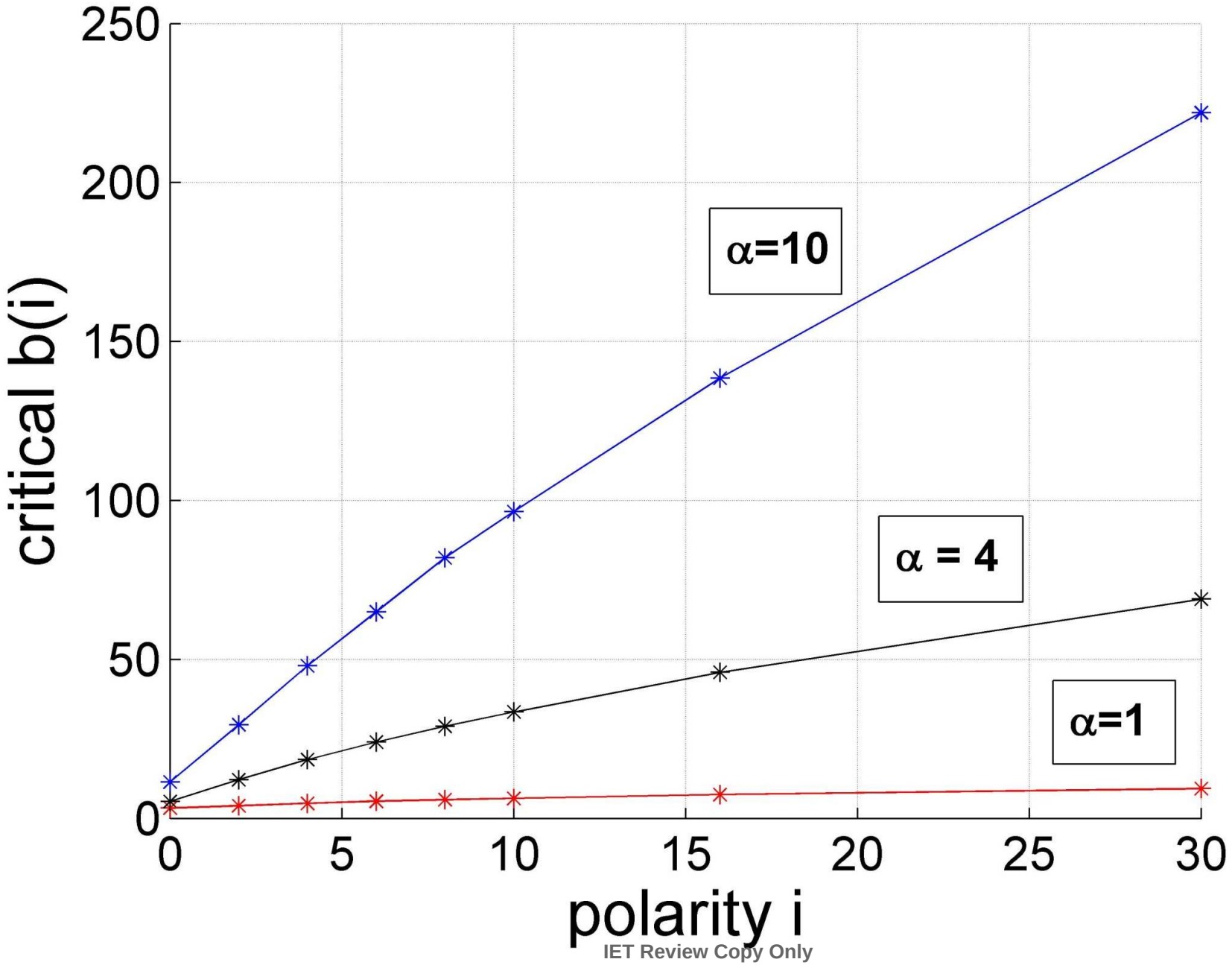


Fig. 4

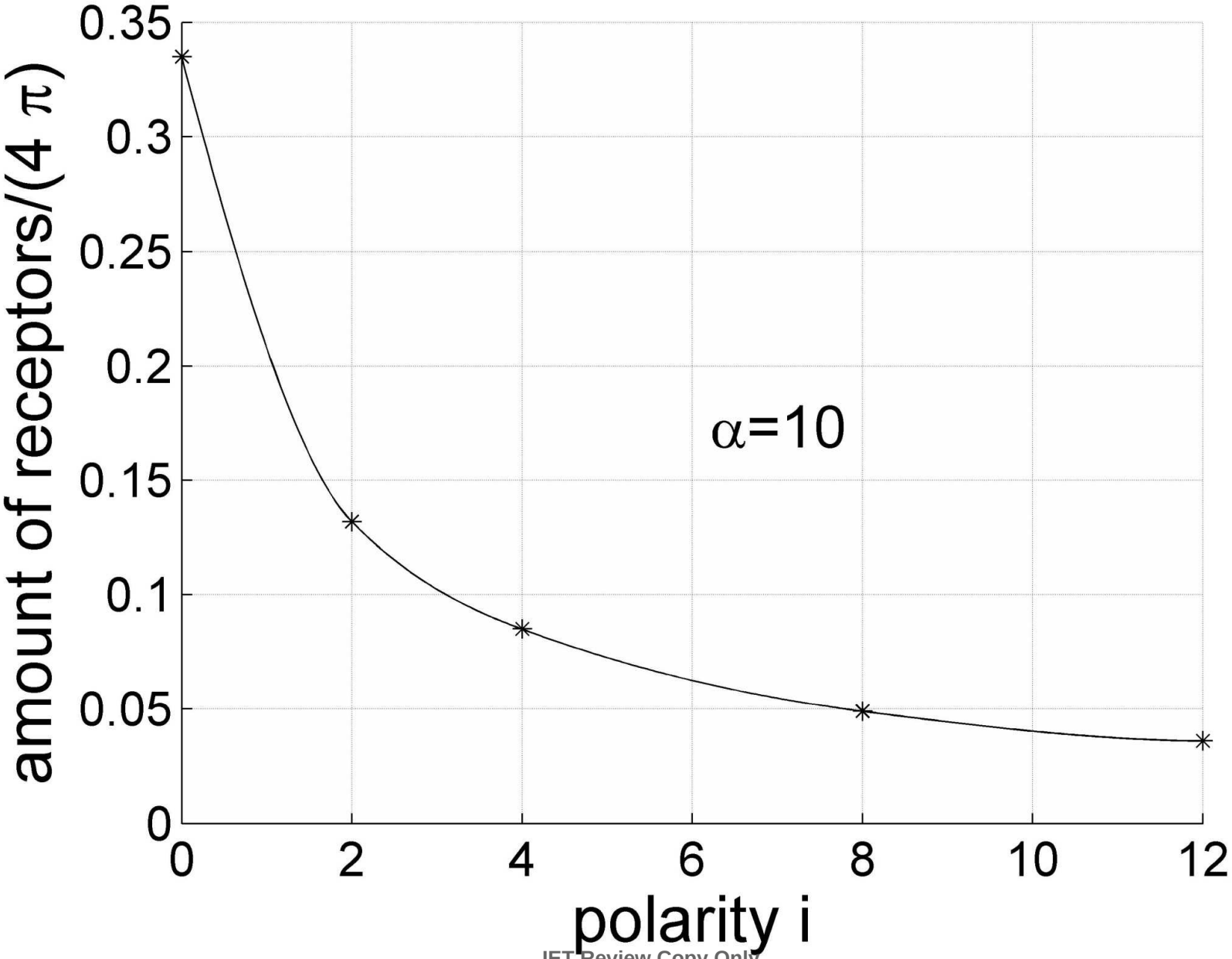
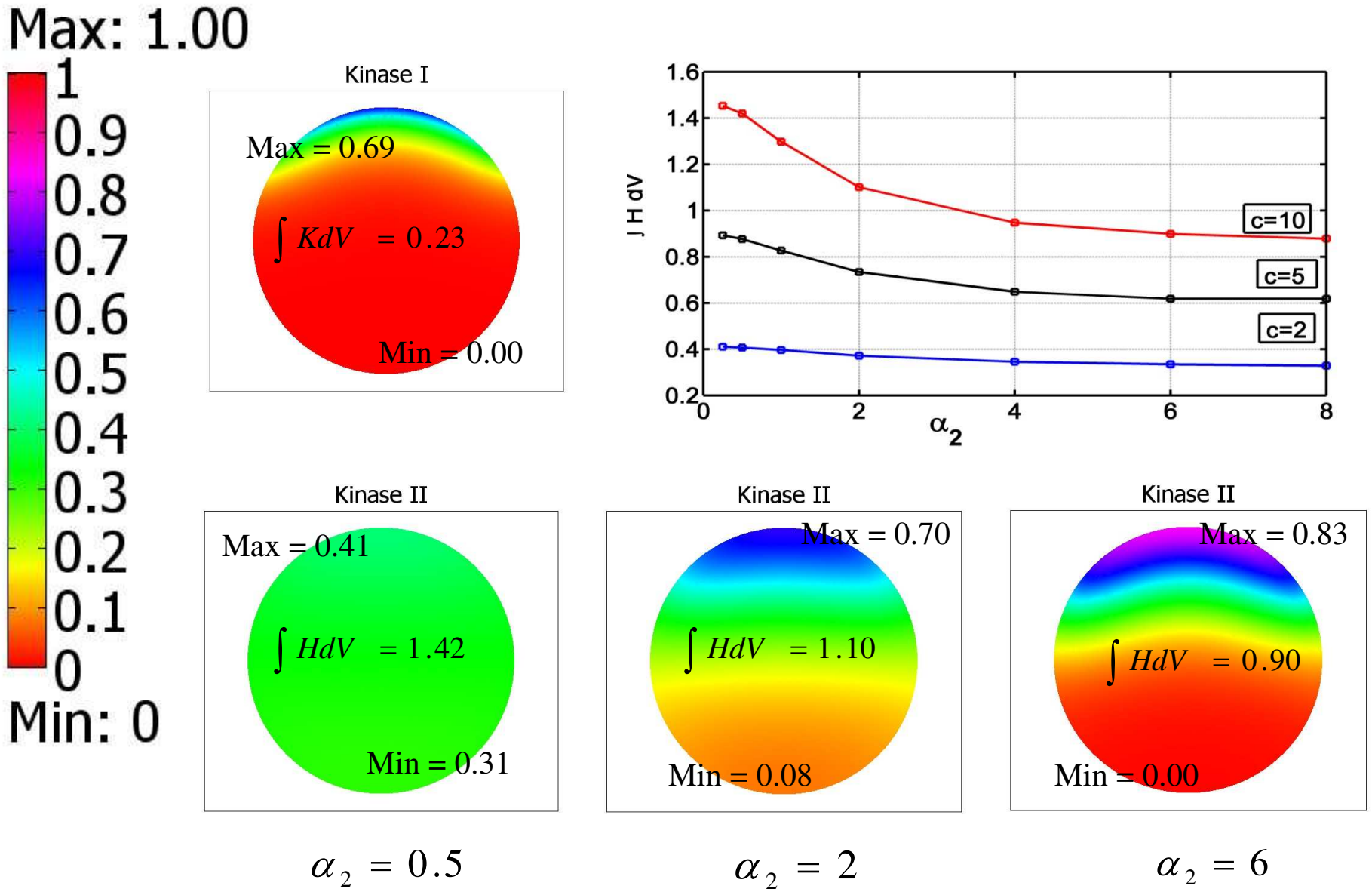


Fig. 5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