**Kaur, Park et al., "Quantitative model of Ras/phosphoinositide 3-kinase signaling crosstalk based on cooperative molecular assembly"**

**SUPPLEMENT:**

**Local versus average Ras-GTP densities in the plasma membrane**

## 1. Diffusion-limited GEF reaction

As outlined in the Discussion, we postulate that those activated receptors in complex with Ras-GEFs produce much higher local Ras-GTP concentrations than the average. Here we present a preliminary exploration of these ideas, through the use of finite-element modeling (Figure S1); these models were implemented in FEMLAB (COMSOL, Burlington, MA; codes available upon request). First, we suppose that the GEF-catalyzed exchange is diffusion-limited; i.e., the reaction occurs rapidly upon collision of Ras-GDP and receptor-bound GEF molecules in the membrane (Fig. S1A). The basic theory of partially diffusion-limited, reversible reactions in membranes was described in ref. 52, cited in the paper. Defining $N_S^*$ as the local density of Ras-GTP, $N_{S,0}^*$ as the basal Ras-GTP density, and $N_{S,Tot}$ as the total Ras density, a simple model for the conservation of Ras-GTP at steady state is given by

$$\begin{aligned} \frac{\partial n_S^*}{\partial t} &= D_S \nabla^2 n_S^* - k_{GAP} n_S^* = 0; \\ n_S^* &= \frac{N_S^* - N_{S,0}^*}{N_{S,Tot} - N_{S,0}^*}. \end{aligned} \qquad \text{(plasma membrane)} \qquad [S1]$$

$D_S$ is the diffusion coefficient for Ras species, and $k_{GAP}$ is a pseudo-first-order rate constant of the GTPase-accelerated protein (GAP)-catalyzed conversion of Ras-GTP back to Ras-GDP. Thus, we make the approximation that basal exchange and hydrolysis rates are not significantly affected by receptor stimulation (a more complete model would account for Ras-GAP binding to receptors). Membrane-localized GEF enzymes mediate enhanced rates of GDP-GTP exchange, at a location defined by an encounter distance *s*. This provides one boundary condition:

$$-2\pi s D_S \left.\frac{dn_S^*}{dr}\right|_{r=s} = k_{GEF}\left[1 - n_S^*(s)\right]. \qquad [S2]$$

The GEF rate constant, $k_{GEF}$, is cast in terms of the reaction in two dimensions and thus accounts for the induced proximity effect offered by membrane localization. In FEMLAB, the source disk and the surrounding membrane share a boundary, and so a boundary condition is automatically imposed to match concentrations and fluxes on either side. This is reconciled through the subdomain properties of the disk. By setting the diffusion coefficient inside the disk, $D_{disk}$, to a very high value ($D_{disk} = 10^9 D_S$ was used), the flux balance will ensure that $n_S^*$ is constant within the disk. Eq. S2 gives the number of Ras molecules processed per unit time, and so the appropriate steady state balance within the disk is given by

$$\frac{\partial n_S^*}{\partial t} = D_{disk} \nabla^2 n_S^* - \frac{k_{GEF}\left(1 - n_S^*\right)}{\pi s^2} = 0. \quad \text{(source disk)} \qquad \text{[S3]}$$

In other words, the consumption term is specified in FEMLAB as the right-hand side of Eq. S2 divided by the disk area.

In a semi-infinite membrane with free membrane diffusion, the problem has an analytical solution:

$$n_S^* = \frac{\dfrac{k_{GEF}}{D_S} K_0\left(r\sqrt{\dfrac{k_{GAP}}{D_S}}\right)}{2\pi s\sqrt{\dfrac{k_{GAP}}{D_S}} K_1\left(s\sqrt{\dfrac{k_{GAP}}{D_S}}\right) + \dfrac{k_{GEF}}{D_S} K_0\left(s\sqrt{\dfrac{k_{GAP}}{D_S}}\right)}, \qquad \text{[S4]}$$

where $r$ is the radial distance from the center of the Ras-GTP source disk and $K_\nu$ are modified Bessel functions of the second kind, order $\nu$. Numerical solution in FEMLAB under these conditions yields identical results.

## 2. Membrane corralling

Semi-permeable corrals impose other boundary conditions, across which the fluxes are matched, but the concentrations are discontinuous. We modeled corrals in a portion of the membrane as a regular 5 x 5 array of squares, with edge length $L$ (Fig. S1B&C). Denoting $n_{S,i}^*$ and $n_{S,j}^*$ as Ras-GTP concentrations on either side of the boundary, with $n_{S,i}^* > n_{S,j}^*$, the net flux of molecules hopping across the boundary at steady state is given by

$$flux = c\frac{D_S}{L}\left(n_{S,i}^* - n_{S,j}^*\right). \qquad [S5]$$

It has been shown that the constant $c$ is related to the probability of particle transmission upon collision with the boundary [1]. Importantly, $c$ can also be related to a measurable quantity: the apparent diffusion coefficient over μm length scales, $D_{App}$, as determined from fluorescence recovery after photobleaching.

$$\frac{D_{App}}{D_S} = \frac{c}{1+c}; \quad c = \frac{D_{App}}{D_S - D_{App}}. \qquad [S6]$$

To model the jump discontinuity in FEMLAB, transport is modeled as diffusion through a slab with very small thickness $\delta$. Actually, any thickness may be used if the diffusion tensor is specified to give transport only in the direction perpendicular to the corral boundary. Defining $D_{slab}$ as the diffusion coefficient to be specified in the slab, the steady-state flux through the boundary is constant:

$$D_{slab}\nabla^2 n_S^* = 0;$$
$$flux = \frac{D_{slab}}{\delta}\left(n_{S,i}^* - n_{S,j}^*\right). \quad \text{(thin slab)} \qquad [S7]$$

Combining Eqs. S5–S7, the diffusion coefficient in the slab was specified in FEMLAB as

$$D_{slab} = \frac{\delta}{L}\left(1 - D_{App}/D_S\right)^{-1} D_{App}. \qquad [S8]$$

The final boundary conditions are at the outmost edges of the simulated 5$L$ x 5$L$ space. Here we made the following approximations, which are valid for corrals that are far away from the Ras-GTP

[1] Powles, J.G., Mallett, M.J.D., Rickayzen, G., and Evans, W.A.B. *Proc. R. Soc. Lond. A* (1992). 436: 391-403.
Tanner, J.E. *J. Chem. Phys.* (1978). 69: 1748-1754.

source: 1) the Ras-GTP concentration within such a corral is uniform, and 2) that concentration is governed by the diffusion approximation:

$$\frac{\partial n_S^*}{\partial t} = \frac{D_{App}}{r}\frac{\partial}{\partial r}\left(r\frac{\partial n_S^*}{\partial r}\right) - k_{GAP} n_S^* = 0. \qquad \text{[S9]}$$

Here, $r$ is defined as the center-to-center distance between the corral in question and the source corral. Defining $V$ as the net rate of Ras-GTP production (molecules/time) coming across the source corral boundary (defined as $r = L/2$), an approximate solution is thus

$$n_S^*(r) = \frac{V}{4D_{App}}\frac{K_0(\lambda r)}{\lambda L K_1(\lambda L/2)}; \quad \lambda = \sqrt{\frac{k_{GAP}}{D_{App}}}. \qquad \text{[S10]}$$

In FEMLAB, an outer edge boundary condition is thus specified as a convective flux, equivalent to Eq. S5 with $c$ from Eq. S6 and the appropriate $n_{S,j}^*$ from Eq. S10 (with $r = 3L$, $10^{1/2}L$, or $13^{1/2}L$, depending on the position of the edge). The correct value of $V$ was found iteratively, by first guessing a value, determining $V$ based on the resulting solution, and so on. This procedure converges rapidly. The accuracy of Eq. S10 was confirmed through its ability to correctly predict the mean concentration in the corner corrals of the space ($r = 8^{1/2}L$).

## 3. Results

The model is naturally nondimensionalized by making the encounter distance a unit disk ($s = 1$) and specifying $D_S = 1$. Thus, the specified values of $k_{GAP}$, $k_{GEF}$, $D_{App}$, and $L$ are effectively normalized by $D_S/s^2$, $D_S$, $D_S$, and $s$, respectively. The only remaining specification is the location(s) of the source disk(s). In the calculations shown in Fig. S1 below, the center of a single disk was arbitrarily placed at (5,5), with the origin defined at the center of the simulated space.

In Fig. S1A, Ras moves about freely, with diffusion coefficient $D_S$, and the exchange reaction occurs rapidly upon collision ($k_{GEF} = 10D_S$). Away from the receptor, the frequency of conversion back to Ras-GDP here is $10^{-4}D_S/s^2$. Fig. S1B is the same as Fig. S1A, except that Ras diffusion on the macroscopic scale is hindered by the presence of square corrals, $L = 20s$ on a side. The permeability of the corrals is such that, on the μm scale, one would observe an apparent diffusion coefficient of $D_{App} = 0.03D_S$. Finally, Fig. S1C is the same as Fig. S1B, except that the GEF reaction is not diffusion-limited on the microscopic scale ($k_{GEF} = 0.1D_S$). Considering a receptor-GEF density of 1/μm$^2$, the receptors in Fig. S1A–C see $n_S^*$ values of 88%, 99%, and 45%, compared with average plasma membrane values of 11%, 1.2%, and 0.55%, respectively. Recall that $n_S^*$ reflects the percent conversion to Ras-GTP, with the basal Ras-GTP subtracted. Particularly in the case of corralling, then, it is apparent that the Ras-GTP concentration near an activated receptor can be very high even while the average level changes very little from the basal state.

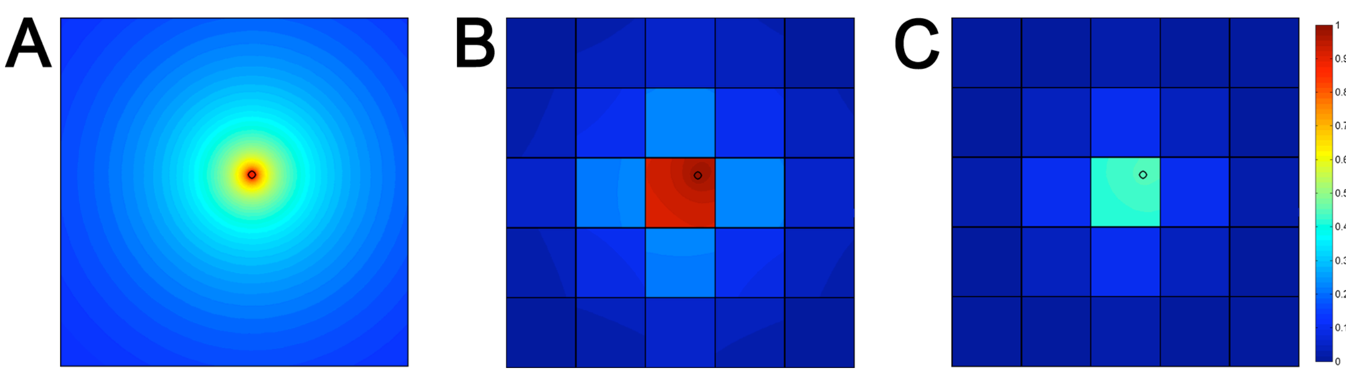


**Figure S1: Finite-element model calculations of Ras-GTP generation in the vicinity of an active receptor-GEF complex.**