***S1 APPENDIX: 1-Dimensional Continuum Functional Barrier Model***

In this appendix, we derive the 1-dimensional (1D) continuum functional barrier model and provide an analytical solution for the steady state concentration of cAMP.

Our 3-dimensional stochastic functional barrier model of cAMP diffusion consisted of a single caveolar domain () flanked by extra-caveolar a space for a total of nm. (See figure 1D). cAMP freely diffused in space. βARs and AC5/6 were placed in the plasma membrane associated with caveolar domains and generated a flux of cAMP into the domain. Otherwise boundary conditions were taken to be no flux. PDE molecules were placed in the plane as a functional barrier, where the direction was defined to be orthogonal to the membrane. PDE and cAMP molecules could react if the distance between them was less than an "interaction radius" of . The reaction kinetics between cAMP and PDE were given by

where , , and 34.

Even with the smallest cAMP diffusion coefficient considered in this manuscript (10 m2/s), the diffusion length (2) of cAMP on relevant time scales (1-10 seconds) is much larger than the length scale of the cross-sectional area of the microdomain domain () and the cAMP-PDE interaction radius . This leads to a nearly uniform concentration of cAMP in planes parallel to the plasma membrane along the microdomain (fixed ), and therefore the effective concentration of cAMP as a function of can be approximated by a 1D continuum model.

In the 1D continuum model, cAMP dynamics within the microdomain are described by the diffusion equation

where is the concentration of cAMP (in ), is the diffusion constant of cAMP (in ), is the length of the microdomain (), is time (in ), and is the distance along the microdomain (in ). The reaction term accounts for the effects of the PDE barrier. This reaction term is localized to a barrier region centered at the position of the PDE molecules () and has a thickness of twice the cAMP-PDE interaction radius (), i.e.,

 is the effective concentration of PDE (in ) in the reaction region, i.e., the number of unbound PDE molecules divided by the volume of the PDE-cAMP interaction region. Mass action kinetics for reaction scheme provide the magnitude of reaction term is given by the

where is the total concentration of PDE (bound or unbound) in the reaction region, and the dynamics of the PDE

where is the average cAMP concentration within the barrier region

To complete the 1D continuum model, the boundary conditions are set to account for the influx of cAMP at the plasma membrane () and to correspond to zero flux at the cytosolic end of the microdomain (

where is the flux of cAMP into the microdomain due to βAR activity (),

Because the interaction radius is much smaller than the diffusion length, the thickness of the PDE barrier can be reduced to a point at in a manner that preserves the strength of effect of the reaction. That is, can be well approximated by a delta-function and . Therefore, the full 1D functional barrier model is

Setting in the system , we obtain equations for the steady state concentration of cAMP along the microdomain, , and the steady state effective concentration of PDE at the barrier, . The presence of the delta-function at in the reaction term requires that we solve the system on and , and then apply the boundary conditions and appropriately match the solutions in these two regions at .

(i) Within the microdomain with , the steady state equation for cAMP concentration is

and therefore

for some constants and .

(ii) The boundary conditions and imply that and , respectively.

(iii) Because the cAMP concentration must be continuous at all including , .

(iv) The difference in the flux of cAMP into and out of must be balanced by the effects of the PDE-cAMP reaction,

(Note the this condition can be obtained by integrating the steady state differential equation for cAMP across the delta-function) Substituting the information from (i)-(iii) into this expression yields

(v) Finally, the steady state equation for the concentration of unbound PDE is

We obtain and by solving equations and

and

Therefore,

where

and

This implies that there is a linear decay in cAMP concentration from the plasma membrane () to the PDE barrier at and is constant beyond the barrier.

Note that the effective concentration of PDE in the above expression is taken with respect to the width of the cAMP-PDE interaction region . In the main manuscript, despite all of the PDE molecules being located at , the effective concentration of PDE is taken with respect to the region between the plasma membrane and the PDE barrier at at . Therefore, the expression in the main manuscript has in place of .

The compartmentation ratio (as described in the main manuscript is

This expression reveals that

(i) As the cAMP production rate increases towards a critical value , the compartmentation approaches 0, i.e., no compartmentation. For , PDE is saturated and cAMP grows unboundedly, i.e., there is no steady state.

(ii) For , the compartmentation ratio increases as the cAMP production rate decreases. In the limit of going to 0, approaches

which provides an *upper limit* to compartmentation. For all parameter sets use in the manuscript, is small compared except at small PDE concentrations, and plots of vs. are nearly indistinguishable from those of vs. on the scale used in Figure 5. This agrees with tOK very low sensitivity in response to changes in reported in the main text.