Title:

Computational models for describing Ras nanocluster dynamics and PRMT5-mediated modulation.

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Description:

The computational models implemented and exploited for the different analysis performed in the thesis "*Spatiotemporal regulation of Ras-driven MAPK signaling: a stochastic and computational approach to Ras nanocluster dynamics and PRMT5-mediated modulation*" were defined and simulated using a stochastic approach similar to Gillespie's Stochastic Simulation Algorithm (SSA), in which the biochemical reactions of the signaling cascade are treated as a set of discrete stochastic events [1-3]. Each event is modeled as a Poissonian process with its occurrence frequency characterized by the corresponding reaction rate. All simulations were implemented in the Fortran programming language, specifically using the GNU Fortran 77 compiler.

Two different basic configurations of the EGF-Ras-ERK MAPK signaling pathway have been modelled and simulated: i) a configuration based on the classical three-tiered cascade architecture comprising the core signaling module Raf-MEK-ERK, and ii) a configuration that localizes all pathway components and signaling events within discrete, membrane-associated nanoclusters.

The different models are listed below.

• **Basic cascade model** (Basic Ras-ERK MAPK cascade model).

This stochastic model of the three-tiered Ras-ERK MAPK cascade is based on two foundational deterministic models of this signaling pathway: the basic cascade model developed by *Huang and Ferrell, 1996* [4] and the extended model described by *Sturm et al., 2010* [5]. The Epidermal Growth Factor (EGF) functions as the input stimulus, initiating a downstream cascade across three kinase tiers: Raf as a MAPKKK (or KKK), MEK as a MAPKK (or KK), and ERK as a MAPK (or K). The concentration of doubly phosphorylated ERK ($_{pp}$ERK) represents the output response of the signaling cascade. The proposed model incorporates the dephosphorylation activity of corresponding phosphatases, and a negative feedback loop from the output response of the cascade ($_{pp}$ERK) to the Raf activation process. This last regulatory mechanism is defined in a manner consistent with the dual negative feedback system of the *Sturm et al., 2010* model [5], which includes feedback loops from $_{pp}$ERK to SoS and from $_{pp}$ERK to activated Raf.

The variable input parameter of the model is the EGF concentration (represented in the code as $E1_{tot}$) and the output parameter of the model is represented by the extension of $_{pp}$ERK (represented

in the code as K_{pp}).

• **Model A PRMT5** (Ras-ERK MAPK cascade model with EGF-mediated PRMT5 activity).
• **Model B PRMT5** (Ras-ERK MAPK cascade model with ppERK-mediated PRMT5 activity).
Two versions of the **basic cascade model**, which was extended with a regulatory module analogous to the PRMT5 motif proposed by *Andreu-Pérez et al., 2011* [6]. This PRMT5 motif acts as an inhibitor of Raf activity through a methylation process. The PRMT5 activity over the Ras-ERK MAPK cascade is modeled by either (i) a stimulus-dependent configuration, in which PRMT5 is directly activated by the EGF input (**model A PRMT5**), or (ii) a response-dependent configuration, in which PRMT5 is activated by the $_{pp}$ERK output through a positive feedback loop (**model B PRMT5**). The parameter k_{PRMT5} denotes the catalytic rate constant that defines the strength of the active Raf degradation exerted by PRMT5, which is considered as a variable (0.0001, 0.0002, and 0.0006 cell/molecules)·s^{-1}) to tune the strength of its activity. This kinetic parameter is modulated by a dimensionless parameter α_{PRMT5}, which captures the nature of the PRMT5 activation through either (i) the input stimulus EGF for the **model A PRMT5**, defining α_{PRMT5} as the ratio between the applied EGF (EGF_t) and its maximal value (EGF_{max}), or (ii) the output $_{pp}$ERK response of the pathway for the **model B PRMT5**, defining α_{PRMT5} as the ratio between the instantaneous concentration of $_{pp}$ERK ($_{pp}$ERK(t)) and its maximum possible value ($_{pp}ERK_{max}$).
The variable input parameters of the models are the EGF concentration (represented in the code as $E1_{tot}$) and the kinetic parameter k_{PRMT5}. And the output parameter is the time response of the $_{pp}$ERK (represented in the code as K_{pp}), i.e. its concentration over the simulation time.
See *Jurado et al., 2021* [7] for more details of the models.

• **Basic nanocluster model** (Basic K-Ras nanoclusters model).
This stochastic model defines the aggregation of Ras proteins into nanoclusters at the cell membrane, based on the premises proposed by *Harding and Hancock, 2008* [8], which characterize Ras nanoclusters as transient structures with short lifetimes, presenting a dispersed spatial distribution across the membrane and occupying small volumes. The mathematical implementation of the model is based on *Tian et al., 2007* [9], where the EGF-Ras-ERK MAPK system in described by the simplest way, wherein each nanocluster is expressed as a minimal signaling motif based on the generalist Michaelis-Menten formulation. This model includes five reactions: the generation of nanoclusters, the recruitment and activation of signaling components, their unbing from nanoclusters, the generation of $_{pp}$ERK as the output response of the biological system, and the disassembly of nanoclusters. Two of these reactions are stimulus-dependent: the nanocluster formation and the $_{pp}$ERK production, represented by α as a normalized input stimulus (compressed

between 0 and 1, representing the absence of stimulus or its maximum expression, respectively). In this model, nanoclusters transiently accumulate $_{pp}$ERK in local pools (P_{nc}) and undergo disassembly through an extrinsic mechanism defined by a constant rate k_3. All kinetic parameters are fixed according previously published values. The variable input parameter of the model is the normalized input stimulus α (represented in the code as alpha), while the output of the model is the average value of the $_{pp}$ERK concentration (represented in the code as P_t/time).

• **Extended nanocluster model** (K-Ras nanoclusters model with self-regulating lifetimes and local production).

This model is an extended version of the **basic nanocluster model**, where a new mechanism for self-regulation of the nanoclusters lifetime is incorporated to describe their formation and disassembly. This mechanism introduces a new approach of the K-Ras nanoclusters dynamics in which nanocluster activity fully regulates its own lifetime. In this model, nanoclusters accumulate $_{pp}$ERK locally in local pools (P_{nc}) and undergo dissassembly through a self-regulatory process dependent on their own output, with the disassembly rate defined as $k_3 \cdot P_{nc}$. Thus, the nanocluster has a chance of disassembly as soon as it generates a minimum amount of $_{pp}$ERK, and this chance increases with the accumulation of local $_{pp}$ERK. The variable input parameters of the model are the normalized input stimulus α (represented in the code as alpha), the forward reaction rate k_1 that corresponds to the binding of the substrate to the nanocluster (represented in the code by the third register of the vector k, k(3)), and the catalytic constant rate k_2 that corresponds to the kinase phosphorylation process (represented in the code by the fifth register of the vector k, k(5)). The output of the model is the average value of the $_{pp}$ERK concentration (represented in the code as P_t/time).

See *Serrano et al., 2013* [10] for more details of the model.

• **Completed nanocluster model** (K-Ras nanoclusters model with self-regulating size and lifetime dynamics).

This model is a more completed implementation of the K-Ras nanocluster dynamics, where signaling events are localized within each K-Ras nanocluster, as defined in the **extended nanocluster model** [10], but describing these signaling nanoplatforms as cooperative dynamic structures that self-regulate their growth and decay according to their sizes.

The simulated model consists of five reactions. Two reactions describe the formation (*Ra*) and dissociation (*Rf*) of nanoclusters, while the remaining three reactions account for internal processes that regulate nanocluster dynamics: the binding of K-Ras molecules to nanoclusters (*Rb*) and the unbinding of K-Ras molecules from nanoclusters (*Rc*), which represent the growth and decay of

nanoclusters, respectively, and the local production of $_{pp}$ERK (Re).

The frequency of nanocluster formation and the rate of $_{pp}$ERK generation are input stimulus-dependent, as defined in the **extended nanocluster model** [10]. However, the proposed model is evaluated under a scenario of maximal nanocluster activation, whereby nanoclusters are simulated under conditions of maximum expression of the input growth factor EGF ($\alpha = EGF/EGF_{max} = 1$, represented in the code as alpha). Therefore, nanocluster formation is defined as a function of the pool of active K-Ras monomers available in the cell, whereas nanocluster dissassembly is directly regulated by the local concentration of $_{pp}$ERK.

The main novel contributions of the proposed model are: (i) the nanocluster dissassembly is indirectly regulated by the size of the nanocluster because the local $_{pp}$ERK production is proportional to the nanocluster size; (ii) the rates governing the internal nanoclusters reactions of growth, decay and local $_{pp}$ERK production (Rb, Rc and Re, respectively) are defined as variable parameters depending on the current size of nanoclusters; and (ii) the implementation of these three internal processes are described by Heaviside (or step) functions, suggesting a highly cooperative behavior within the nanocluster dynamics.

The variable input parameters are the kinetic constants related to the formation and dissassembly of nanoclusters, k_1 and k_5 respectively (represented in the code by the first and fifth registers of the vector k, k(1) and k(5) respectively). The output measures of the model are the sizes (both at the time of their death and at their maximum values) and lifetimes of the nanoclusters. The input parameters were considered within a wide range of values to evaluate the distribution of nanoclusters according to their sizes and lifetimes, and to assess the robustness of such measurements.

See *Jurado et al., 2023* [11] for more details of the model.

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