Identification of the key parameters in a mathematical model of PAR1-mediated signaling in endothelial cells

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\section*{Abstract}

Biophysical models are often populated by a large number of input parameters that are difficult to predict or measure experimentally. The validity and robustness of a given model can be evaluated by a sensitivity test to its input parameters. In this study, we performed local (based on a Taylor-like method) and global sensitivity (based on Monte Carlo filtering techniques) analyses of a previously derived PAR1-mediated activation model of endothelial cells. This activation model previously demonstrated that peptide-activated PAR1 has a different receptor/G-protein binding affinity that favors $G_\alpha_1$ activation over $G_\alpha_{12/13}$ by approximately 800-fold. Interestingly, the present study shows that the parameter regulating the binding rate of activated PAR1 to $G_\alpha_{12/13}$ is indeed important to obtain the expected RhoGTP response. Moreover, we show that the parameters representing the rate of PAR1 deactivation and the rate of PAR1 binding to $G_\alpha_q$, are the most important parameters in the system. Finally, we illustrate that the kinetic model considered in this study is robust and we provide complementary insights into the biological meaning and importance of its kinetic parameters.

\section*{Introduction}

Mathematical models of biophysical phenomena often involve a large number of input parameters \cite{1-5}, such as reaction rates and initial concentrations, that must be either measured experimentally or inferred from similar biological systems. Moreover, experimentally measured parameters carry uncertainties due to experimental limitations, statistical analysis and different experimental conditions. One of the major goals in systems biology is to estimate how sensitive a computational model is to variations of its parameters.
and study the effect of the parameter uncertainties on the model response. Sensitivity analysis aims at determining which parameters have the most influence on a predicted system behavior [6–9]. When the notion of “influence” is made quantitatively precise, sensitivity analysis can constitute a reliable robustness test for computational models [10].

In this study, we performed sensitivity analysis on the mathematical model of PAR1-mediated activation of endothelial cells of [4]. A schematic representation of the main signaling pathways analyzed in [4] is shown in Fig. 1. A specific PAR1 agonist, SFLLRN, simultaneously activates two classes of G proteins: $\text{G}_q$ and $\text{G}_{12/13}$ [11]. The $\alpha$ subunit of the $\text{G}_q$ protein activates the $\beta$ isoforms of phospholipase C ($\text{PLC}_\beta$), which hydrolyze the lipid phosphatidylinositol 4,5-bisphosphate (PIP$_2$) to generate the second messengers inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG) [12]. The second messenger IP$_3$ regulates the activity of the inositol trisphosphate receptors (IP$_3$R) on the surface of the endoplasmic reticulum (ER), allowing the rapid release of Ca$^{2+}$ into the cytoplasm [13,14]. Simultaneously, the $\alpha$ subunit of the $\text{G}_{12/13}$ protein activates the small GTPase RhoA, known to promote cytoskeletal changes [15].

The signaling pathways described above are also common to a number of transduction systems mediated by G-protein coupled receptors in other cell types. We have recently published a model of PAR1 signaling in platelets ([13]).

In [4], several kinetic parameters had to be inferred because they were unknown and/or difficult to measure. In this work, we analyzed the influence of the chosen parameters to the system’s output. We performed a sensitivity test based on the Taylor expansion of the output functions around the chosen input parameters (local analysis) and a sensitivity test based on Monte Carlo sampling techniques of the system parameters (global analysis). Our results show that although the two techniques implemented are conceptually different, they lead, at least qualitatively, to consistent results. We show that the system is not sensitive to the majority of the parameters chosen in [4] and we identify three important nodes in the model. These results represent a further test for the validity of the model in [4]. Additionally, this analysis will help design more refined computational models, and will have the ultimate goal of identifying influential parameters and key signaling nodes with possible applications in biology and medicine.
Figure 1. Pictorial representation of PAR1-mediated signaling pathways in endothelial cells. Upon cell stimulation by a PAR1 agonist peptide such as SFLLRN, PAR1 simultaneously activates the two classes of G proteins, G_q and G_{12/13}. The α subunit of the G_q protein activates the β isoforms of phospholipase C (PLC_β), which hydrolyze the lipid phosphatidylinositol 4,5-bisphosphate (PIP2) to generate the second messengers inositol 1,4,5-trisphosphate (IP3). IP3 then regulates the activity of the inositol trisphosphate receptors (IP3R) on the surface of the endoplasmic reticulum (ER) allowing the rapid release of Ca^{2+} into the cytoplasm. Simultaneously, the α subunit of the G_{12/13} protein activates the small GTPase Rho to promote cytoskeletal changes. Reproduced from [4].

Methods

Sensitivity Analysis by Taylor Expansion

Consider a mathematical model defined by the following q-dimensional system of ordinary differential equations (ODEs) with initial conditions y₀,

\[
\begin{align*}
\dot{y} &= f(y, k) \\
y₀ &= y(0),
\end{align*}
\]

where \( y = (y_1, \ldots, y_q) \) is the q-dimensional vector of system states and \( k = (k_1, \ldots, k_n) \) is the n-dimensional vector of input parameters. A simple estimate of the effect of the uncertainties in \( k \) on \( y \),
Table 1. The sensitivity coefficients calculated by the Taylor-like formula at the peak time $t_i$ for Ca$^{2+}$ and RhoGTP

|   | Ca$^{2+}$ | RhoGTP | FUNCTIONAL ROLE |
|---|-----------|--------|-----------------|
| 1 | 0.000     | 0.002  | 0.012 0.038     |
| 2 | 0.000     | 0.000  | 0.000 0.000     |
| 3 | -0.001    | 0.000  | 0.000 0.018     |
| 4 | 0.008     | 0.010  | 0.005 0.003     |
| 5 | 0.000     | 0.000  | 0.000 0.000     |
| 6 | 0.000     | 0.000  | 0.000 0.000     |
| 7 | 0.000     | 0.000  | 0.000 0.000     |
| 8 | 0.000     | 0.000  | 0.000 0.000     |
| 9 | 0.000     | 0.000  | 0.000 0.000     |
| 10| 0.000    | 0.000  | 0.000 0.000     |
| 11| 0.000    | 0.000  | 0.000 0.000     |
| 12| 0.000    | 0.000  | 0.000 0.000     |
| 13| 0.000    | 0.000  | 0.000 0.000     |
| 14| 0.000    | 0.000  | 0.000 0.000     |
| 15| 0.000    | 0.000  | 0.000 0.000     |
| 16| 0.000    | 0.000  | 0.000 0.000     |
| 17| 0.000    | 0.000  | 0.000 0.000     |
| 18| 0.000    | 0.000  | 0.000 0.000     |
| 19| 0.000    | 0.000  | 0.000 0.000     |
| 20| 0.000    | 0.000  | 0.000 0.000     |
| 21| 0.000    | 0.000  | 0.000 0.000     |
| 22| 0.000    | 0.000  | 0.000 0.000     |
| 23| 0.000    | 0.000  | 0.000 0.000     |
| 24| 0.000    | 0.000  | 0.000 0.000     |
| 25| 0.000    | 0.000  | 0.000 0.000     |
| 26| 0.000    | 0.000  | 0.000 0.000     |
| 27| 0.000    | 0.000  | 0.000 0.000     |
| 28| 0.000    | 0.000  | 0.000 0.000     |
| 29| 0.000    | 0.000  | 0.000 0.000     |
| 30| 0.000    | 0.000  | 0.000 0.000     |
| 31| 0.000    | 0.000  | 0.000 0.000     |
| 32| 0.000    | 0.000  | 0.000 0.000     |
| 33| 0.000    | 0.000  | 0.000 0.000     |
| 34| 0.000    | 0.000  | 0.000 0.000     |
| 35| 0.000    | 0.000  | 0.000 0.000     |
| 36| 0.000    | 0.000  | 0.000 0.000     |
| 37| 0.000    | 0.000  | 0.000 0.000     |
| 38| 0.000    | 0.000  | 0.000 0.000     |
| 39| 0.000    | 0.000  | 0.000 0.000     |
| 40| 0.000    | 0.000  | 0.000 0.000     |
| 41| 0.000    | 0.000  | 0.000 0.000     |
| 42| 0.000    | 0.000  | 0.000 0.000     |
| 43| 0.000    | 0.000  | 0.000 0.000     |

The sensitivity coefficients $S_{ij}(t, \bar{k}) = \left. \frac{\partial \ln(y_i)}{\partial \ln(k_j)} \right|_{k=\bar{k}}$ calculated at the nominal peak time $t_i$ for $y_i = \text{Ca}^{2+}$ and variations $\Delta k = \pm 5\%$ (columns two and three) and $y_i = \text{RhoGTP}$ and variations $\Delta k = \pm 5\%$ (columns four and five). Sensitivity coefficients larger than 10% are in bold font. The nominal peak time $t_i$ is defined in (5) and the coefficients $S_{ij}$ are defined in (3).

that is, the variation of $y$ caused by a variation $\Delta k$ in $k$ is

$$\Delta y(t, k) = y(t, k + \Delta k) - y(t, \bar{k}),$$

where $y(t, \bar{k})$ is a solution of (1) at time $t$ with parameters $k = \bar{k}$. Assuming $|\Delta k| \ll 1$, by the Taylor's
expansion in the variables $k$ about $\bar{k}$,

$$\Delta y(t, k) = D(t, \bar{k})\Delta k + O(|\Delta k|^2)$$  \hspace{1cm} (2)$$

where $O(|\Delta k|^2)$ is a $q$-dimensional vector infinitesimal of order no less than 2 with respect $|\Delta k|$, and $D(t, \bar{k})$ is the $n \times q$ matrix of the partial derivatives at $\bar{k}$ of entries

$$D(t, \bar{k}) = \left( \frac{\partial y_i}{\partial k_j}(t, k) \right) \bigg|_{k=\bar{k}} \quad i = 1, \ldots, q \quad j = 1, \ldots, n$$

Then one takes a dimensionless version of $D(t, \bar{k})$ as a measure of the sensitivity of the system at time $t$, with respect to the set of parameters $k$. Precisely one introduces a sensitivity matrix $S(t, \bar{k})$ of entries

$$S_{ij}(t, \bar{k}) = \frac{\partial \ln(y_i)}{\partial \ln(k_j)} \bigg|_{k=\bar{k}} = \frac{\bar{k}_j}{y_i(t, \bar{k})} \frac{\partial y_i(t, k)}{\partial k_j} \bigg|_{k=\bar{k}}$$  \hspace{1cm} (3)$$

and takes $S_{ij}(t, \bar{k})$ as a measure of the sensitivity of the system state $y_i(t, k)$ at time $t$ with respect to the kinetic parameter $k_j$ about the nominal values $\bar{k}$. In the context of the kinetic model in [4] we selected the states

$$y_i(t, k) = [Ca^{2+}](t, k)$$

$$y_i(t, k) = [RhoGTP](t, k)$$  \hspace{1cm} (4)$$

and computed $S_{ij}(t_i, \bar{k})$ at time $t_i$ where the nominal $[Ca^{2+}](t, \bar{k})$ and $[RhoGTP](t, \bar{k})$ attain their maximum values, e.g.,

$$y_i(t_i, \bar{k}) = \max_t y_i(t, \bar{k})$$  \hspace{1cm} (5)$$

The sensitivity coefficients $S_{ij}(t_i, \bar{k})$ for $Ca^{2+}$ are reported in Table 1 in columns two and three along those for RhoGTP in columns four and five. The partial derivatives $\partial y_i/\partial k_j$ were approximated by discrete differences with values of $\pm 5\%$ of the nominal values and indicated respectively with the symbols $S_+$ and $S_-.$

The total output of $y(\cdot, k)$ over the average time course $T$ of the experiment, is

$$z(k) = \int_0^T y(t, k)dt.$$  \hspace{1cm} (6)$$
Table 2. The sensitivity coefficients calculated by the Taylor-like formula over the course of the entire simulation $T = 600$ s for Ca$^{2+}$ and RhoGTP

| k  | $\Sigma_i$ (Ca$^{2+}$) | $\Sigma_j$ (Ca$^{2+}$) | $\Sigma_i$ (RhoGTP) | $\Sigma_j$ (RhoGTP) | FUNCTIONAL ROLE |
|----|------------------------|------------------------|---------------------|---------------------|----------------|
| 1  | 0.000                  | 0.000                  | -0.001              | -0.017              | PAR1 activation by SFLLRN |
| 2  | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 3  | 0.000                  | 0.000                  | -0.003              | -0.011              |                |
| 7  | 0.000                  | 0.000                  | -0.449              | -0.443              | PAR1 deactivation |
| 8  | 0.000                  | 0.000                  | 0.021               | 0.040               | PAR1 binding Gq |
| 9  | 0.000                  | 0.000                  | -0.007              | -0.007              |                |
| 10 | 0.000                  | 0.000                  | -0.001              | -0.012              | Gq releasing GDP |
| 11 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 12 | 0.000                  | 0.000                  | -0.001              | -0.001              | Gq binding GTP |
| 13 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 14 | 0.000                  | 0.000                  | -0.006              | -0.016              | Gq releasing $p\gamma$ |
| 15 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 16 | 0.000                  | 0.000                  | 0.000               | 0.000               | Gq deactivation |
| 17 | 0.000                  | 0.000                  | 0.011               | 0.000               | PLC binding GqGTP |
| 18 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 19 | 0.000                  | 0.000                  | -0.022              | 0.002               | PLC5 hydrolyzing GqGTP |
| 20 | 0.000                  | 0.000                  | 0.000               | 0.000               | PLC5 releasing GqGDP |
| 21 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 22 | 0.000                  | 0.000                  | 0.001               | 0.019               | PLC5 binding PIP2 |
| 23 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 24 | -0.051                 | -0.057                 | -0.009              | 0.031               | Consumption of IP3 |
| 25 | -0.043                 | -0.429                 | 0.429               |                  |
| 26 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 27 | 0.000                  | 0.000                  | -0.070              | -0.071              |                |
| 28 | 0.000                  | 0.000                  | 0.068               | 0.074               | G13 releasing GDP |
| 29 | 0.000                  | 0.000                  | 0.000               | 0.000               | G13 binding GTP |
| 30 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 31 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 32 | 0.000                  | 0.000                  | 0.000               | 0.000               | G13 deactivation |
| 33 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 34 | -0.199                 | -0.202                 | 0.429               | 0.434               |                |
| 35 | 0.000                  | 0.000                  | 0.064               | 0.069               | GEF binding G13GTP |
| 36 | 0.000                  | 0.000                  | -0.066              | -0.066              |                |
| 37 | 0.000                  | 0.000                  | -0.488              | -0.504              | GEF hydrolyzing G13GTP |
| 38 | 0.000                  | 0.000                  | 0.000               | 0.000               | GEF releasing G13GDP |
| 39 | 0.000                  | 0.000                  | 0.000               | 0.000               |                |
| 40 | 0.000                  | 0.000                  | -0.030              | -0.030              |                |
| 41 | 0.000                  | 0.000                  | 0.029               | 0.031               | Rho activation |
| 42 | 0.000                  | 0.000                  | -0.001              | -0.001              |                |
| 43 | -0.001                 | -0.001                 | 0.000               | 0.000               |                |

The sensitivity coefficients $\Sigma_{ij}(\bar{k}) = \left. \frac{\partial z_i}{\partial k_j} \cdot \frac{\partial z_i}{\partial \bar{k}_j} \right|_{k=\bar{k}}$ with $z(\bar{k}) = \int_0^T y(t, \bar{k}) dt$ for $y_i = \text{Ca}^{2+}$ and variations $\Delta k = \pm 5\%$ (columns two and three) and $y_i = \text{RhoGTP}$ and variations $\Delta k = \pm 5\%$ (columns four and five). Sensitivity coefficients larger than 10\% are in bold font.

For the states in (4) we chose in our simulations $T = 600$s. The vector $z(\bar{k})$ is independent of time and it can be expanded in Taylor’s series with respect to $k$, as in (2) with $D(t, \bar{k})$ replaced by its time integral over $(0, T)$. The sensitivity coefficients of $z(\bar{k})$ about the nominal values $\bar{k}$ are

$$
\Sigma_{ij}(\bar{k}) = \left. \frac{\partial z_i}{\partial k_j} \right|_{k=\bar{k}}.
$$

(7)
The sensitivity coefficients for Ca$^{2+}$ are reported in Table 2 in columns two and three along those for RhoGTP in columns four and five. The partial derivatives $\partial y_i / \partial k_j$ were approximated by discrete differences with values of $\pm 5\%$ of the nominal values and indicated respectively with the symbols $\Sigma_+$ and $\Sigma_-.

The nominal values of the parameters $k_j$ used in the sensitivity analysis described above are given in Table 3.

**Monte Carlo Filtering Sensitivity Analysis**

The method is based on estimating the uncertainties distributions $p(k) = \{p_1(k_1), \ldots, p_n(k_n)\}$ of the parameters $k = (k_1, \ldots, k_n)$, each ranging over the intervals

$$k_j \in (k_{j}^{\text{min}}, k_{j}^{\text{max}}), \quad j = 1, \ldots, n$$

and cumulatively generating the probability measure

$$p(k)dk = p_1(k_1) \cdots p_n(k_n) dk_1 \cdots dk_n$$

over the space of parameters

$$\prod_{j=1}^{n} (k_{j}^{\text{min}}, k_{j}^{\text{max}}).$$

If these ranges and uncertainties distributions were known, regarding each of the $y_i(t, k)$ as a random variable depending on the random $k$ one computes the mean

$$\langle y_i(t) \rangle = \int \cdots \int_{k_{j}^{\text{min}}}^{k_{j}^{\text{max}}} y_i(t, k) p(k) dk$$

and, the variance

$$\sigma_i^2(t) = \langle y_i^2(t) \rangle - (\langle y_i(t) \rangle)^2,$$

where

$$\langle y_i^2(t) \rangle = \int \cdots \int_{k_{j}^{\text{min}}}^{k_{j}^{\text{max}}} y_i^2(t, k) p(k) dk.$$
Table 3. Symbolic reaction schemes and effective kinetic parameters used in the simulations of the PAR1-mediated activation model of endothelial cells proposed in [4].

| Symbolic Reactions | \(k_\text{on}\) | \(k_\text{off}\) | References |
|--------------------|----------------|----------------|------------|
| \(\text{Agonist} \rightarrow \text{PAR1} \equiv \text{PAR1}^*\) | \(6.00\times10^4 \text{ M}^{-1}\text{s}^{-1}\) (\(k_1\)) | \(1.00\times10^{-3} \text{ s}^{-1}\) (\(k_2\)) | [4] |
| \(\text{PAR1}^* \rightarrow \text{null}\) | \(2.00\times10^1 \text{ s}^{-1}\) (\(k_3\)) | | [4] |
| \(\text{PAR1}^* \rightarrow \text{null}\) | \(2.00\times10^1 \text{ s}^{-1}\) (\(k_7\)) | | [4] |

**Reactions governing PAR1 activation**

| \(\text{PAR1}^* + \text{G}_\alpha\text{GDP} \cdot \beta\gamma \equiv \text{PAR1}^*. \cdot \text{G}_\alpha\text{GDP} \cdot \beta\gamma\) | \(1.00\times10^8 \text{ M}^{-1}\text{s}^{-1}\) (\(k_9\)) | \(1.00 \text{ s}^{-1}\) (\(k_3\)) | [17] |
| \(\text{PAR1}^* \cdot \text{G}_\alpha\text{GDP} \cdot \beta\gamma + \text{G}_\alpha\text{GTP} \equiv \text{PAR1}^* \cdot \text{G}_\alpha\text{GTP} \cdot \beta\gamma\) | \(5.00 \text{ s}^{-1}\) (\(k_{12}\)) | \(1.00\times10^6 \text{ M}^{-1}\text{s}^{-1}\) (\(k_{11}\)) | [17] |
| \(\text{PAR1}^* \cdot \text{G}_\alpha\text{GTP} \cdot \beta\gamma \equiv \text{PAR1}^* + \text{G}_\alpha\text{GTP} \cdot \beta\gamma\) | \(2.00 \text{ s}^{-1}\) (\(k_{14}\)) | \(1.00\times10^7 \text{ M}^{-1}\text{s}^{-1}\) (\(k_{15}\)) | [17] |
| \(\text{G}_\beta\text{GTP} \rightarrow \text{G}_\beta\text{GDP}\) | \(2.00\times10^2 \text{ s}^{-1}\) (\(k_{16}\)) | & [18,19] |

**Reactions governing \(G_\beta/13\) activation**

| \(\text{PLC}\beta \cdot \text{G}_\beta\text{GTP} \equiv \text{PLC}\beta \cdot \text{G}_\beta\text{GTP}\) | \(5.00\times10^2 \text{ M}^{-1}\text{s}^{-1}\) (\(k_{17}\)) | \(5.00 \text{ s}^{-1}\) (\(k_{18}\)) | [20,21] |
| \(\text{PLC}\beta \cdot \text{G}_\beta\text{GTP} \rightarrow \text{PLC}\gamma \cdot \text{G}_\beta\text{GDP}\) | \(1.50 \text{ s}^{-1}\) (\(k_{20}\)) | | [20,21] |
| \(\text{PLC}\beta \cdot \text{G}_\beta\text{GDP} \equiv \text{PLC}\beta \cdot \text{G}_\beta\text{GDP}\) | \(1.00\times10^5 \text{ s}^{-1}\) (\(k_{26}\)) | \(1.00\times10^4 \text{ M}^{-1}\text{s}^{-1}\) (\(k_{21}\)) | [22] |
| \(\text{PLC}\beta \cdot \text{G}_\beta\text{GTP} + \text{PIP2} \equiv \text{PLC}\beta \cdot \text{G}_\beta\text{GTP} \cdot \text{PIP2}\) | \(1.00\times10^4 \text{ M}^{-1}\text{s}^{-1}\) (\(k_{22}\)) | \(1.00 \text{ s}^{-1}\) (\(k_{23}\)) | [22] |
| \(\text{PLC}\beta \cdot \text{G}_\beta\text{GTP} \cdot \text{PIP2} \rightarrow \text{PLC}\gamma \cdot \text{G}_\beta\text{GTP} \cdot \text{IP3}/\text{DAG}\) | \(1.00\times10^2 \text{ s}^{-1}\) (\(k_{24}\)) | | [22] |
| \(\text{IP3} \rightarrow \text{null}\) | \(2.40\times10^2 \text{ s}^{-1}\) (\(k_{25}\)) | | [4] |

**Reactions governing \(Ca^{2+}\) mobilization**

As described previously

| \(\text{RhoGTP} \rightarrow \text{RhoGDP}\) | \(4.00\times10^3 \text{ s}^{-1}\) (\(k_{30}\)) | | [14] |

**Reactions governing GTP hydrolysis**

| \(\text{GTP} \rightarrow \text{null}\) | \(2.40\times10^2 \text{ s}^{-1}\) (\(k_{36}\)) | | [4] |

The nominal values of the parameters adopted in the PAR1-mediated activation model of endothelial cells proposed in [4]. The kinetic constants are numbered sequentially starting from the first \(k \rightarrow (k_1)\) and proceeding in reading order with the exception of reaction “PAR1* → null” which is regulated by the constant \(k_7\).
The variance is a measure of the influence, and relative importance, of the input $k$ to the output $y(t, k)$ (10).

In practice the process is implemented in a less quantitative way, by trading the information coming from the variances $\sigma_i(t)$ with those coming from a biologically motivated objective functional, $g_{obj}$ depending on one or several $y_i(t, k)$ [20]. Having chosen an objective functional $g_{obj}$, one identifies a biologically acceptable range for the objective function $g_{obj}$. For example for a threshold value $g^{\text{thres}}$ of the objective functional $g_{obj}$ one might define [20]

$$
\begin{align*}
  g_{obj} \leq g^{\text{thres}} & \quad \text{as the acceptable range} \\
  g_{obj} > g^{\text{thres}} & \quad \text{as the non acceptable range.}
\end{align*}
$$

(9)

Then for each $k_j$ one determines the probability distributions $f_1(k_j)$ and $f_2(k_j)$ of those values of $k_j$ that output the system in the acceptable or non acceptable range respectively. For each of these, and for each $k_j$, calculate the cumulative frequency distributions

$$
cf_\ell(k_j) = \int_{k_{min}}^{k_{max}} f_\eta(\eta) d\eta, \quad \ell = 1, 2
$$

(10)

and calculate the Kolmogorov-Smirnov coefficients $d_{1,2}(k_j)$ by the maximum distance function

$$
d_{1,2}(k_j) = \sup_{k_j \in [k_{min}, k_{max}]} |cf_1(k_j) - cf_2(k_j)|.
$$

(11)

These coefficients are taken as a measure of the relative importance of each parameter $k_j$ on the model output. The larger the value of $d_{1,2}(k_j)$, the more important is $k_j$ in producing the pre-defined system output [6–8, 26].

In the context of the kinetic model of [4] the method is implemented as follows:

1. Select nominal values $\bar{k} = (\bar{k}_1, \ldots, \bar{k}_n)$ as those originally adopted in the model of [4] (see Table 3). As ranges in [5] we take intervals spanning from $1/10$ to $10$ times these nominal values, e.g.,

$$
k_{j \max} = 10\bar{k}_j, \quad \text{and} \quad k_{j \min} = \frac{1}{10}\bar{k}_j.
$$
On a log\(_{10}\) scale these are symmetric intervals about \(\log_{10} \bar{k}_j\).

2. Generate \(M\) random \(n\)-tuples of numbers

\[
k_m = (k_{1,m}, \ldots, k_{n,m}) \quad \text{for } m = 1, \ldots, M
\]

by uniformly sampling the \(\ln k_j\) from their respective symmetric uncertainty ranges defined above. In the simulations we used \(M = 10,000\). Different sampling distributions (e.g. gaussian, exponential) were seen not to qualitatively affect the results.

3. Solve the system (1) for each random choice of \(k_m\), and compute the functions \(y(t, k_m)\). Solve also (1) for the nominal values \(k = \bar{k}\) to get the functions \(y(t, \bar{k})\). Then for each \(i = 1, \ldots, q\) introduce two kinds of objective output functions of the \(m\)th trial as follows:

**Objective Function at Times \(t_i\):**

\[
g_{\text{obj; } i}(t_i; m) = [y_i(t_i, k_m) - y_i(t_i, \bar{k})]^2
\]

where \(m \in \{1, \ldots, M\}\) is the \(m\)th random trial described in the previous step and the times \(t_i\) are defined in (5). For the states in (4), this function measures the variations of the of \([\text{Ca}^{2+}](t_{\text{Ca}^{2+}}, k)\) and \([\text{RhoGTP}](t_{\text{Rho}}, k)\), from their nominal maximum values. (see Table 4 columns two and three respectively).

**Objective Function for Total Time:**

Let \(z(k)\) be the total state output as in (6) and for \(i = 1, \ldots, q\) set

\[
G_{\text{obj; } i}(m) = [z_i(k_m) - z_i(\bar{k})]^2
\]

where \(m \in \{1, \ldots, M\}\) is the \(m\)th random trial. For the states in (4), the function \(G_{\text{obj; } i}(m)\) measures the perturbation of the total outputs of \(\text{Ca}^{2+}\) and \(\text{RhoGTP}\) over the time course \(T\) of the experiment, from their nominal values. The results are compared in Table 5 in columns two and three.
Table 4. The sensitivity coefficients calculated by Monte Carlo filtering at the nominal peak time $t_i$ for $\text{Ca}^{2+}$ and RhoGTP

| $k$ | $\text{Ca}^{2+}$ | $\text{RhoGTP}$ | FUNCTIONAL ROLE |
|-----|-----------------|-----------------|-----------------|
| 1   | 0.095           | 0.084           | PAR1 activation by SFLLRN |
| 2   | 0.031           | 0.026           |                 |
| 3   | 0.113           | 0.093           |                 |
| 7   | 0.160           | 0.285           | PAR1 deactivation |
| 8   | 0.091           | 0.153           | PAR1 binding Gq |
| 9   | 0.037           | 0.043           | Gq releasing GDP |
| 10  | 0.092           | 0.041           |                 |
| 11  | 0.017           | 0.022           | Gq binding GDP |
| 12  | 0.018           | 0.021           | Gq binding GTP |
| 13  | 0.041           | 0.019           |                 |
| 14  | 0.153           | 0.061           | Gq releasing $\beta\gamma$ |
| 15  | 0.023           | 0.012           |                 |
| 16  | 0.026           | 0.024           | Gq deactivation |
| 17  | 0.024           | 0.033           | PLC$\beta$ binding GqGTP |
| 18  | 0.030           | 0.038           |                 |
| 19  | 0.421           | 0.034           | PLC$\beta$ hydrolizing GqGTP |
| 20  | 0.042           | 0.013           | PLC$\beta$ releasing GqGDP |
| 21  | 0.029           | 0.029           |                 |
| 22  | 0.394           | 0.017           | PLC$\beta$ binding IP$\gamma$2 |
| 23  | 0.031           | 0.030           |                 |
| 24  | 0.034           | 0.021           | PLC$\beta$ hydrolizing IP$\gamma$2 |
| 25  | 0.413           | 0.018           | Consumption of IP$3$ |
| 26  | 0.035           | 0.391           | PAR1 binding G13 |
| 27  | 0.016           | 0.105           |                 |
| 28  | 0.012           | 0.008           | G13 releasing GDP |
| 29  | 0.018           | 0.026           |                 |
| 30  | 0.042           | 0.027           | G13 binding GTP |
| 31  | 0.017           | 0.015           |                 |
| 32  | 0.038           | 0.019           | G13 releasing $\beta\gamma$ |
| 33  | 0.024           | 0.048           |                 |
| 34  | 0.017           | 0.073           | G13 deactivation |
| 35  | 0.021           | 0.158           | GEF binding G13GTP |
| 36  | 0.024           | 0.196           |                 |
| 37  | 0.020           | 0.170           | GEF hydrolizing G13GTP |
| 38  | 0.016           | 0.043           | GEF releasing GEFGDP |
| 39  | 0.026           | 0.025           |                 |
| 40  | 0.042           | 0.385           | GEFG13GTP binding RhoGDP |
| 41  | 0.024           | 0.104           |                 |
| 42  | 0.029           | 0.031           | Rho activation |
| 43  | 0.023           | 0.350           | Rho deactivation |

The sensitivity coefficients measured as Kolmogorov-Smirnov distances $d_{1,2}$ estimated in the ranges $[1/10, 10]$ at the nominal peak time $t_i$ for $y_i = \text{Ca}^{2+}$ (column two) and $y_i = \text{RhoGTP}$ (column three). Sensitivity coefficients larger than 10% are in bold font. The nominal peak time $t_i$ is defined in (5) and the objective function used in the simulations is defined in (12).

4. Introduce threshold values

$$g_{\text{obj},i}^{\text{thres}}(t_i) = \frac{1}{M} \sum_{m=1}^{M} g_{\text{obj},i}(t_i; m)$$

$$G_{\text{obj},i}^{\text{thres}} = \frac{1}{M} \sum_{m=1}^{M} G_{\text{obj},i}(m).$$

(14)

The $m$th random trial and its parameters $k_m$ are deemed acceptable according to the criterion in (9) for each of these objective functions and their respective threshold values. On the basis of this classification, generate the probability distribution functions $f_1(k_j)$ and $f_2(k_j)$ of acceptable and unacceptable values,
Table 5. The sensitivity coefficients calculated by Monte Carlo filtering over the course of the entire simulation $T = 600$ s for $\text{Ca}^{2+}$ and RhoGTP

| $k$ | $\text{Ca}^{2+}$ | $\text{RhoGTP}$ | FUNCTIONAL ROLE |
|-----|------------------|-----------------|-----------------|
| 1   | 0.042            | 0.032           | PAR1 activation by SFLLRN |
| 2   | 0.034            | 0.029           |                     |
| 3   | 0.046            | 0.061           |                     |
| 7   | 0.171            | 0.208           | PAR1 deactivation   |
| 8   | 0.109            | 0.049           | PAR1 binding Gq     |
| 9   | 0.041            | 0.017           | Gq releasing GDP    |
| 10  | 0.044            | 0.017           |                     |
| 11  | 0.017            | 0.018           |                     |
| 12  | 0.046            | 0.021           | Gq binding GTP      |
| 13  | 0.020            | 0.029           |                     |
| 14  | 0.034            | 0.023           | Gq releasing $\beta\gamma$ |
| 15  | 0.030            | 0.019           |                     |
| 16  | 0.031            | 0.038           | Gq deactivation     |
| 17  | 0.021            | 0.050           | PLCβ binding GqGTP  |
| 18  | 0.020            | 0.028           |                     |
| 19  | 0.157            | 0.022           | PLCβ hydrolyzing GqGTP |
| 20  | 0.035            | 0.030           | PLCβ releasing GqGDP |
| 21  | 0.042            | 0.011           |                     |
| 22  | 0.085            | 0.020           | PLCβ binding PIP2   |
| 23  | 0.020            | 0.013           |                     |
| 24  | 0.028            | 0.018           | PLCβ hydrolyzing PIP2 |
| 25  | 0.216            | 0.043           | Consumption of IP3   |
| 26  | 0.032            | 0.141           | PAR1 binding G13    |
| 27  | 0.034            | 0.041           |                     |
| 28  | 0.026            | 0.044           | G13 releasing GDP   |
| 29  | 0.031            | 0.045           |                     |
| 30  | 0.034            | 0.028           | G13 binding GTP     |
| 31  | 0.022            | 0.024           |                     |
| 32  | 0.050            | 0.021           | G13 releasing $\beta\gamma$ |
| 33  | 0.020            | 0.017           |                     |
| 34  | 0.038            | 0.066           | G13 deactivation    |
| 35  | 0.034            | 0.058           | GEF binding G13GTP  |
| 36  | 0.029            | 0.111           |                     |
| 37  | 0.033            | 0.125           | GEF hydrolyzing G13GTP |
| 38  | 0.055            | 0.011           | GEF releasing G13GDP |
| 39  | 0.034            | 0.016           |                     |
| 40  | 0.044            | 0.105           | GEFG13GTP binding RhoGDP |
| 41  | 0.045            | 0.052           |                     |
| 42  | 0.052            | 0.039           | Rho activation      |
| 43  | 0.037            | 0.248           | Rho deactivation    |

The sensitivity coefficients measured as Kolmogorov-Smirnov distances $d_{1,2}$ estimated in the ranges $[1/10, 10]$ $\bar{k}_j$ over the course of the entire simulation $T = 600$ s for $y_i = \text{Ca}^{2+}$ (column two) and $y_i = \text{RhoGTP}$ (column three). Sensitivity coefficients larger than 10% are in bold font. The objective function used in the simulations is defined in (13).

relative to $g_{\text{obj};i}(t_i; m)$ and $G_{\text{obj};i}(m)$ respectively.

5. Calculate the cumulative frequency distributions $c_f(k_j)$ as in (10) relative to each objective function, and the corresponding Kolmogorov-Smirnov coefficient $d_{1,2}(k_j)$ as in (11). The larger the value of $d_{1,2}(k_j)$ the higher the sensitivity of the system to the variation of the corresponding parameter.

All the calculations were performed on a MATLAB (R2009b, The Mathworks, Natick, MA) platform.
### Times to Peak and Peak Values

Table 6. The sensitivity coefficients calculated by Monte Carlo filtering for the peak values of $Ca^{2+}$ and RhoGTP

| $d_{1,2}$ | $Ca^{2+}$ | RhoGTP | FUNCTIONAL ROLE |
|-----------|-----------|--------|-----------------|
| 1         | 0.091     | 0.082  | PAR1 activation by SFLLRN |
| 2         | 0.036     | 0.027  |                     |
| 3         | 0.102     | 0.100  |                     |
| 4         | 0.218     | 0.280  | PAR1 deactivation |
| 5         | 0.160     | 0.120  | PAR1 binding Gq    |
| 6         | 0.038     | 0.039  |                     |
| 7         | 0.073     | 0.014  | Gq releasing GDP   |
| 8         | 0.026     | 0.012  |                     |
| 9         | 0.028     | 0.026  |                     |
| 10        | 0.102     | 0.100  |                     |
| 11        | 0.034     | 0.018  |                     |
| 12        | 0.138     | 0.055  | Gq releasing $\beta\gamma$ |
| 13        | 0.024     | 0.017  |                     |
| 14        | 0.027     | 0.021  |                     |
| 15        | 0.020     | 0.036  | PLC/β binding GqGTP |
| 16        | 0.020     | 0.036  |                     |
| 17        | 0.036     | 0.047  |                     |
| 18        | 0.404     | 0.030  | PLC/β hydrolizing GqGTP |
| 19        | 0.029     | 0.016  |                     |
| 20        | 0.032     | 0.035  |                     |
| 21        | 0.438     | 0.019  | PLC/β binding PIP2 |
| 22        | 0.038     | 0.019  |                     |
| 23        | 0.014     | 0.017  | PLC/β hydrolizing PIP2 |
| 24        | 0.386     | 0.022  | Consumption of IP3 |
| 25        | 0.041     | 0.375  |                     |
| 26        | 0.017     | 0.112  |                     |
| 27        | 0.028     | 0.059  |                     |
| 28        | 0.031     | 0.018  |                     |
| 29        | 0.022     | 0.025  |                     |
| 30        | 0.019     | 0.021  |                     |
| 31        | 0.021     | 0.019  |                     |
| 32        | 0.018     | 0.038  |                     |
| 33        | 0.038     | 0.077  |                     |
| 34        | 0.023     | 0.160  |                     |
| 35        | 0.022     | 0.206  |                     |
| 36        | 0.022     | 0.130  |                     |
| 37        | 0.036     | 0.038  |                     |
| 38        | 0.024     | 0.022  |                     |
| 39        | 0.037     | 0.370  |                     |
| 40        | 0.021     | 0.059  |                     |
| 41        | 0.024     | 0.035  |                     |
| 42        | 0.020     | 0.344  |                     |

The sensitivity coefficients measured as Kolmogorov-Smirnov distances $d_{1,2}$ estimated in the ranges $[1/10, 10]$ for the peak values of $y_i = Ca^{2+}$ (column two) and $y_i = RhoGTP$ (column three). Sensitivity coefficients larger than 10% are in bold font. The objective function used in the simulations is defined in (16) and the peak values are defined in (17).

According to (5) the times $t_i$ are those at when the nominal states $y_{i}(\cdot, \bar{k})$ achieve their peak value. The states $y_{i}(\cdot, k)$ however achieve their peak values at times $t_{i}^{peak}$ which, in general differ from $t_i$ and are in general functions of $k$. Set

$$y_{i}^{max}(k) = y_{i}(t_{i}^{peak}(k), k) = \max_{t} y_{i}(t, k).$$ (15)
Table 7. The sensitivity coefficients calculated by Monte Carlo filtering for the peak times $t_{i}^{\text{peak}}$ of $\text{Ca}^{2+}$ and RhoGTP

| $k$ | $\text{Ca}^{2+}$ | RhoGTP | FUNCTIONAL ROLE |
|-----|-----------------|--------|-----------------|
| 1   | 0.075           | 0.043  | PAR1 activation by SFLLRN |
| 2   | 0.032           | 0.028  |                |
| 3   | 0.031           | 0.034  |                |
| 7   | 0.201           | 0.530  | PAR1 deactivation |
| 8   | 0.154           | 0.441  | PAR1 binding Gq |
| 9   | 0.063           | 0.161  | Gq releasing GDP |
| 10  | 0.081           | 0.076  | Gq binding GTP |
| 11  | 0.071           | 0.032  | Gq deactivation |
| 12  | 0.074           | 0.032  |                |
| 13  | 0.033           | 0.045  |                |
| 14  | 0.199           | 0.338  | Gq releasing $\beta\gamma$ |
| 15  | 0.092           | 0.045  |                |
| 16  | 0.076           | 0.025  | Gq deactivation |
| 17  | 0.052           | 0.042  | PLC\(\beta\) binding GqGTP |
| 18  | 0.033           | 0.032  |                |
| 19  | 0.148           | 0.039  | PLC\(\beta\) hydrolyzing GqGTP |
| 20  | 0.025           | 0.040  | PLC\(\beta\) releasing GqGDP |
| 21  | 0.036           | 0.046  |                |
| 22  | 0.324           | 0.039  | PLC\(\beta\) binding PI\(\alpha\) |
| 23  | 0.051           | 0.040  |                |
| 24  | 0.060           | 0.040  | PLC\(\beta\) hydrolyzing PI\(\alpha\) |
| 25  | 0.318           | 0.022  | Consumption of IP\(3\) |
| 26  | 0.038           | 0.083  | PAR1 binding G13 |
| 27  | 0.023           | 0.039  |                |
| 28  | 0.032           | 0.059  | G13 releasing GDP |
| 29  | 0.040           | 0.030  | G13 binding GTP |
| 30  | 0.027           | 0.046  | G13 deactivation |
| 31  | 0.052           | 0.016  |                |
| 32  | 0.056           | 0.021  | G13 releasing $\beta\gamma$ |
| 33  | 0.047           | 0.068  |                |
| 34  | 0.067           | 0.314  | G13 deactivation |
| 35  | 0.044           | 0.063  | GEF binding G13GTP |
| 36  | 0.064           | 0.083  |                |
| 37  | 0.034           | 0.448  | GEF hydrolyzing G13GTP |
| 38  | 0.029           | 0.044  | GEF releasing G13GDP |
| 39  | 0.030           | 0.042  |                |
| 40  | 0.067           | 0.046  | GEFG13GTP binding RhoGTP |
| 41  | 0.046           | 0.051  |                |
| 42  | 0.060           | 0.034  | Rho activation |
| 43  | 0.029           | 0.069  | Rho deactivation |

The sensitivity coefficients measured as Kolmogorov-Smirnov distances $d_{1,2}$ estimated in the ranges $[1/10, 10]$ for the peak times $t_{i}^{\text{peak}}$ of $y_{i} = \text{Ca}^{2+}$ (column two) and $y_{i} = \text{RhoGTP}$ (column three). Sensitivity coefficients larger than 10% are in bold font. The objective function used in the simulations is defined in (16) and the peak times $t_{i}^{\text{peak}}$ are defined in (15).

Dose responses for different concentrations of agonists are measured at $t_{i}^{\text{peak}}$. In practice, for a set of parameters $k$, which in general is unknown, this is done by recording the experimental outputs $y_{i}^{\text{max}}(k)$. For small variations of $k$ about its nominal vector $\bar{k}$, the sensitivity of $y_{i}^{\text{max}}(k)$ could be theoretically “measured” as in (3), by the sensitivity matrix of entries

$$S_{ij}^{\text{peak}}(\bar{k}) = \left. \frac{\partial \ln y_{i}^{\text{max}}}{\partial \ln(k_j)} \right|_{k=\bar{k}} = \frac{\bar{k}_j}{y_{i}^{\text{max}}(k)} \left\{ \frac{\partial y_{i}(t_{i}^{\text{peak}}(k), k)}{\partial k_j} \left( \frac{\partial t_{i}^{\text{peak}}(k)}{\partial k_j} \right) \right\} |_{k=\bar{k}} + \frac{dy_{i}(t_{i}^{\text{peak}}(k), k) \partial t_{i}^{\text{peak}}(k)}{\partial dt} \frac{\partial t_{i}^{\text{peak}}(k)}{\partial k_j} |_{k=\bar{k}}$$
This formula however requires the form of the functions $k \rightarrow t_i^\text{peak}(k)$, which are in general not know. Alternatively, the analysis can be carried by the filtering method, by introducing two new objective functions:

$$F_{Y_i}^\text{peak}(m) = \left[ y_i^\text{max}(k_m) - y_i(t_i, \bar{k}) \right]^2$$
$$T_{Y_i}^\text{peak}(m) = \left[ t_i^\text{peak}(k_m) - t_i \right]^2$$

where the vector $k_m$ is the output of the $m$th random trial. Threshold values and distribution functions can be defined and determined as above. For $y_i = [\text{Ca}^{2+}]$ and $y_i = [\text{RhoGTP}]$, denote their peak times by $t_{\text{Ca}^{2+}}^\text{peak}$ and $t_{\text{RhoGTP}}^\text{peak}$ respectively, and set

$$[\text{Ca}^{2+}]^\text{max}(k) = [\text{Ca}^{2+}](t_{\text{Ca}^{2+}}^\text{peak}(k), k)$$
$$[\text{RhoGTP}]^\text{max}(k) = [\text{RhoGTP}](t_{\text{RhoGTP}}^\text{peak}(k), k).$$

Then the first of (16) measures the variations of the peak values of $[\text{Ca}^{2+}]$ and $[\text{RhoGRP}]$ from their peak nominal values, whereas the second of (16) measures the variation of their times to peak $t_{\text{Ca}^{2+}}^\text{peak}$ and $t_{\text{RhoGTP}}^\text{peak}$ respectively, from their nominal values $t_{\text{Ca}^{2+}}$ and $t_{\text{RhoGTP}}$. The results are reported in Tables 6 and 7 respectively.

**Results and Discussion**

We performed sensitivity analysis of a previously derived PAR1-mediated activation model of endothelial cells. We used two different techniques, one based on the Taylor expansion of the system’s output around the nominal values of its input parameters $k_j$ and the second based on Monte Carlo sampling techniques of the model parameters. The analysis based on the Taylor expansions (2) and leading to the sensitivity matrices $S_{ij}(t_i, \bar{k})$ in (3) and $\Sigma_{ij}(\bar{k})$ in (7), imposes two restrictions. The first is that $|\Delta k| \ll 1$ with the notion of “smallness” depending on a predefined notion of smallness of $|\Delta y|$. The second is that these matrices measure the relative variation of $y_i$ and $z_i$ with respect to $k_j$, by keeping all the remaining parameters fixed, thereby neglecting the cumulative effects of $\Delta k$.

The analysis based on the Kolmogorov-Smirnov test, does not require the range of $k$ to be small, however it does require that the uncertainty distributions $p(k)$ be known. The method leaves open the choice of the objective function. This on the one hand affords the flexibility in tailoring the objective function to specific experimental processes, and on the other hand allows non quantitative elements in
Figure 2. Cumulative distribution functions of the most influential kinetic parameter $k_7$ for the output $y_i = \text{Ca}^{2+}$. The red curves indicate the distributions relative to the “acceptable” sets of parameters, whereas the blue ones denote those corresponding to the “unacceptable” set of parameters (see Methods). Panel A: Monte Carlo filtering method results at the nominal peak time $t_i$. Panel B: Monte Carlo filtering results for the entire time-course of the simulation, $T = 600$ s. Panel C: Monte Carlo filtering results for the peak values $[\text{Ca}^{2+}]_{\text{max}}$ of the output function. Panel D: Monte Carlo filtering results for the peak times $t_{\text{peak}}$ of the output function. The maximum distances between the acceptable and unacceptable distributions, $d_{1,2}$, are reported in Tables 4–7 respectively and are defined in (11). The nominal peak time $t_i$ is defined in (5) and the objective functions used are respectively (12), (13), and (16) defined in Methods.

The two methods being complementary, we performed a sensitivity analysis by using both of them, on the states in [4] arising from the mathematical model of [4].

First we analyzed the sensitivity of $\text{Ca}^{2+}$ and RhoGTP at their nominal peak values [4] to variations of the parameters $k$. Using the Taylor expansion method, we computed the sensitivity coefficients $S_{ij}(t_i, \mathbf{k})$ introduced in [3] and reported them in Table [1]. Using the Monte Carlo filtering method, starting from the objective function $g_{\text{obj},i}(t_i; m)$ introduced in [12], we computed the Kolmogorov-Smirnov coefficients by the distance function in (11), and reported them in Table [4].
Figure 3. Cumulative distribution functions of the most influential kinetic parameter $k_7$ for the output $y_i = \text{RhoGTP}$. The red curves indicate the distributions relative to the “acceptable” sets of parameters, whereas the blue ones denote those corresponding to the “unacceptable” set of parameters (see Methods). Panel A: Monte Carlo filtering method results at the nominal peak time $t_i$. Panel B: Monte Carlo filtering results for the entire time-course of the simulation, $T = 600$ s. Panel C: Monte Carlo filtering results for the peak values $[\text{RhoGTP}]_{\text{max}}$ of the output function. Panel D: Monte Carlo filtering results for the peak times $t_{i}^{\text{peak}}$ of the output function. The maximum distances between the acceptable and unacceptable distributions, $d_{1:2}$, are reported in Tables 4–7 respectively and are defined in (11). The nominal peak time $t_i$ is defined in (5) and the objective functions used are respectively (12), (13), and (16) defined in Methods.

Then we analyzed how parameters fluctuations affect the total response of $\text{Ca}^{2+}$ and RhoGTP over the whole time course $T$ of the experiments, quantified by the integrated states (6). We computed first the sensitivity coefficients $\Sigma_{ij}(\bar{k})$ in (7), and reported in Table 2. Then, starting from the objective function $G_{\text{obj};i}(m)$ introduced in (13), we computed the Kolmogorov-Smirnov coefficients as in (11) and reported in Table 5.

Finally, by the same Monte Carlo filtering procedure we investigated the sensitivity of the peak values of $\text{Ca}^{2+}$ and RhoGTP and their relative times to peak for cumulative variations of all the parameters $k_j$ in intervals of 2 orders of magnitude with respect to $\bar{k}_j$. The results are in Table 6 and Table 7 respectively.
Figure 4. Cumulative distribution functions of the influential kinetic parameter $k_{26}$ for the output $y_i = \text{RhoGTP}$. The red curves indicate the distributions relative to the “acceptable” sets of parameters, whereas the blue ones denote those corresponding to the “unacceptable” set of parameters (see Methods). Panel A: Monte Carlo filtering method results at the nominal peak time $t_i$. Panel B: Monte Carlo filtering results for the entire time-course of the simulation, $T = 600$ s. Panel C: Monte Carlo filtering results for the peak values $[\text{RhoGTP}]_{\text{max}}$ of the output function. Panel D: Monte Carlo filtering results for the peak times $t_{\text{peak}}$ of the output function. The maximum distances between the acceptable and unacceptable distributions, $d_{1,2}$, are reported in Tables 4–7 respectively and are defined in (11). The nominal peak time $t_i$ is defined in (5) and the objective functions used are respectively (12), (13), and (16) defined in Methods.

As a way of analyzing and comparing the results in these tables, we deemed a parameter important for a given state $y_i(t, k)$ if its fluctuations about the nominal values $\bar{k}$ produced sensitivity coefficients or Kolmogorov-Smirnov coefficients larger than 10%. Equivalently the state $y_i$ was deemed not to be sensitive to variations of those parameters $k_j$ for which the Taylor sensitivity coefficients or the Kolmogorov-Smirnov sensitivity coefficients were smaller than 10%.

The two methods are conceptually different and hence the sensitivity coefficients $S_{ij}(t, \bar{k})$ and $\Sigma_{ij}(\bar{k})$ introduced in (3) and (7) respectively, are not expected to be numerically similar to the Kolmogorov-Smirnov coefficients relative to the same processes. Nevertheless they exhibit similar qualitative results,
in the sense that most of the parameters that are important by the Taylor expansion method, are likewise important by the Monte Carlo filtering method. We first discarded those parameters to which neither Ca\(^{2+}\) and RhoGTP are sensitive by the quantitative criterion indicated above. Then in Table 8 we cross-listed those parameters to which Ca\(^{2+}\) or RhoGTP or both were sensitive. According to the adopted criterion of importance, our analysis shows that \(k_7\) and \(k_8\) are the most important parameters to reproduce the expected system behavior, because they respectively have ten and six entries in Table 8. In Figs. 2 and 3, as an example, we show the cumulative distribution functions \(10\) calculated by Monte Carlo filtering for the parameter \(k_7\) in the case \(y_i = \text{Ca}^{2+}\) and \(y_i = \text{RhoGTP}\) respectively. The red curves represent the distribution of “acceptable” parameters, whereas the blue ones represent the distributions of “unacceptable” parameters. In panel A we report the results relative to the sensitivity test performed with the objective function calculated as in \(12\), panel B shows the results of the Monte Carlo filtering method with the objective function estimated according to \(13\), and panel C and D are relative to the choice of the objective functions in \(16\) respectively. Similarly, in Fig. 4 we show the cumulative distribution functions \(10\) calculated by Monte Carlo filtering for the parameter \(k_{26}\) in the case \(y_i = \text{RhoGTP}\).

Examination of Tables 1–8 permits one to classify the input parameters into three broad categories:

**RhoGTP-Only Sensitivity Parameters (four or more entries in Table 8)**

- \(k_{26}\) PAR1 to G13 binding rate;
- \(k_{36}\) G13GTP-GEF dissociation rate;
- \(k_{37}\) GEF hydrolyzing G13GTP;
- \(k_{40}\) GEF-G13* to Rho binding rate;
- \(k_{43}\) Rho deactivation rate.

Variations of these parameter affect only RhoGTP and its functionals

\[
[RhoGTP](t, k), \quad \int_0^T [RhoGTP](t, k)dt \\
[RhoGTP]^{\text{max}}(k), \quad t_{\text{peak}}^{\text{Rho}}(k)
\]  

\[(18)\]
introduced in (4)–(6), and (17). Neither small variations (Taylor’s expansion), nor large variations (Monte-Carlo filtering) of these parameters about their nominal values affect Ca\(^{2+}\) and its functionals. This is an expected result as these parameters only belong to the RhoGTP module downstream of G13 (see Fig. 1).

Interestingly, our sensitivity analysis found, independently of [4], that \(k_{26}\), which represents PAR1 binding rate to G\(_{12}/13\), is a key factor in this model to reproduce the experimental data. This emerges from two different sensitivity analysis methods and constitutes a further test of validity for the signaling model adopted in [4].

**Ca\(^{2+}\)-Only Sensitivity Parameters (four or more entries in Table 8)**

- \(k_{19}\) rate of hydrolysis of GqGTP by PLC\(\beta\);
- \(k_{22}\) PLC\(\beta\)-PIP2 binding rate;
- \(k_{25}\) IP3 rate of consumption;

Variations of these parameter affect only Ca\(^{2+}\) and its functionals

\[
[Ca^{2+}]_{\text{max}}(k), \quad t_{\text{peak}}^{Ca^{2+}}(k)
\]

introduced in (4)–(6), and (17). Small variations (Taylor’s expansion) or large variations (Monte-Carlo filtering) of these parameters about their nominal values do not affect RhoGTP or its functionals. Again, this is an expected result as these parameters only belong to the Ca\(^{2+}\) signaling module downstream Gq.

The presence of parameters that affect the Ca\(^{2+}\) output but not the RhoGTP output and viceversa is due to the modular structure of the model in [4]. In this model, Gq and G13 pathways are described by two separate computational modules with a common input, i.e. the activated PAR1 (Fig. 1).

The Ca\(^{2+}\) functionals in [19] are not sensitive to small variations of \(k_{25}\) (rate of IP3 consumption) about its nominal value \(\bar{k}_{25}\) (Taylor’s method for \(|\Delta k_{25}| \leq 5\%\)). They are however severely sensitive for variations of \(k_{25}\) in the range \((10^{-1} \bar{k}_{25}, 10\bar{k}_{25})\) (Monte Carlo filtering method).

In all cases however, the notion of “sensitivity” and “relevance” is the same by both methods, pointing to a robustness and self-consistency of the model in [4].
Table 8. List of the most important parameters as deemed by the six different sensitivity tests adopted

| Sensitivity Analysis Method | Taylor $t_i$ | Filtering $t_i$ | Taylor $T$ | Filtering $T$ | Filtering $y_{max}$ | Filtering $t_{max}$ |
|----------------------------|-------------|----------------|----------|--------------|-------------------|------------------|
| $k$ Ca$^{2+}$ RhoGTP Ca$^{2+}$ RhoGTP Ca$^{2+}$ RhoGTP Ca$^{2+}$ RhoGTP Ca$^{2+}$ RhoGTP | $x$ | $x$ | $x$ | $x$ | $x$ | $x$ |
| 3 | | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| 9 | | | | | | |
| 14 | | | | | | |
| 19 | | | | | | |
| 22 | | | | | | |
| 25 | | | | | | |
| 26 | | | | | | |
| 27 | | | | | | |
| 34 | | | | | | |
| 35 | | | | | | |
| 36 | | | | | | |
| 37 | | | | | | |
| 43 | | | | | | |

Comparison of the sensitivity analysis results for the most important parameters reported in bold font in Tables 1–7. The symbols $\times$ mark the importance of a given parameter $k_j$ according to the different sensitivity test. Parameters $k_7$ and $k_8$ are important in the majority of the sensitivity tests performed.
**Ca\textsuperscript{2+}-RhoGTP Sensitivity Parameters**

- \( k_7 \) PAR1 deactivation rate;
- \( k_8 \) PAR1-Gq binding rate;
- \( k_{14} \) rate of release of \( \beta\gamma \) by Gq;

PAR1 acts on the common part of the Ca\textsuperscript{2+} and RhoGTP pathways and accordingly both of them and their functionals are sensitive to both these parameters. While PAR1 deactivation rate \( k_7 \) is important to both Ca\textsuperscript{2+} and RhoGTP, the various components of the system respond differently to variations of this parameter. First, Ca\textsuperscript{2+} and its functionals [19], are not sensitive to small variations of \( k_7 \) about its nominal value \( \bar{k}_7 \) (Taylor’s methods for \( |\Delta k_7| \leq 5\% \)). On the other hand all the Ca\textsuperscript{2+} functionals are very sensitive for variations of \( k_7 \) in the range \( (10^{-1}\bar{k}_7, 10\bar{k}_7) \) (Monte Carlo filtering method).

Contrarily RhoGTP and its functionals are very sensitive to any variation \( k_7 \), small or large, and by whatever method sensitivity is evaluated (Taylor or Monte Carlo filtering).

Finally, we observe that the pathways analyzed in this work are shared in a number of signaling cellular systems. In a recent work, we devised a mathematical model of PAR1-mediated signaling in human platelets [2] that shares several features with the model in [4]. For these reasons and for the generality of the methods adopted in this work, the conclusions of this study can, at least qualitatively, be extended to the model in [2].
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