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CALCIUM OSCILLATIONS IN A SPATIALLY EXTENDED THREE COMPARTMENT CELL MODEL

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ABSTRACT

We derive a spatially extended three compartment cell model for evolution of calcium ions concetrations. To obtain specific form of the fluxes between the compartments, we compare it with the model proposed by Marhl *et al.* (2000). We examine numerically the period and shape of oscillations as a function of diffusion coefficients. We demonstrate a decay of the oscillations at the critical value of diffusion of free calcium ions.

MARHL'S MODEL

Calcium ions Ca^{2+} are used by eukaryotic cells to carry out various physiological functions. Fast changes of calcium concentration in cytosol are intermediately connected with muscle contraction, hormone secretion, increased ATP production, learning, *etc.* An elevated calcium concentration initiates, *e.g.* cell differentiation, proliferation, cell cycle or apoptosis.

The three-compartment Marhl's model [1] (see also [2, 3]) is given by the following nonlinear ordinary differential equations for concentrations c_{Cyt}, c_{Ret}, c_{Mit} of free calcium ions in the cytosol (Cyt), endoplasmic reticulum (Ret) and mitochondria (Mit) as well as the concentration of buffered calcium ions b_{Cyt} in cytosol:

$$\frac{dc_{Cyt}}{dt} = -J_{Ret} - J_{Mit} - k^{+} c_{Cyt}(b^{0}_{Cyt} - b_{Cyt}), \tag{1}$$

$$\frac{db_{Cyt}}{dt} = -k^{-} b_{Cyt} + k^{+} c_{Cyt}(b^{0}_{Cyt} - b_{Cyt}), \tag{2}$$

$$\frac{dc_{Ret}}{dt} = \frac{\beta_{Ret}}{\rho_{Ret}} J_{Ret}, \tag{3}$$

$$\frac{dc_{Mit}}{dt} = \frac{\beta_{Mit}}{\rho_{Mit}} J_{Mit}, \tag{4}$$

where

$$J_{Ret} := \left(J_{pump} - J_{ch} - J_{leak}\right), \quad J_{Mit} := \left(J_{in} - J_{out}\right), \tag{5}$$

$$J_{pump} = k_{pump} c_{Cyt}, \quad J_{ch} = k_{ch} \frac{c^{2}_{Cyt}}{K^{2}_{1} + c^{2}_{Cyt}} \left(c_{Ret} - c_{Cyt}\right), \quad J_{leak} = k_{leak} \left(c_{Ret} - c_{Cyt}\right), \tag{6}$$

$$J_{in} = k_{in} \frac{c^{8}_{Cyt}}{K^{8}_{2} + c^{8}_{Cyt}}, \quad J_{out} = \left(k_{out} \frac{c^{2}_{Cyt}}{K^{2}_{3} + c^{2}_{Cyt}} + k_{m}\right) c_{Mit}. \tag{7}$$

Here J_{pump} is active influx of calcium ions Ca^{2+} into the endoplasmic reticulum provided by SERCA pumps, J_{ch} and J_{leak} are passive effluxes out of reticulum compartment, J_{in} is the mitochondrial active influx and J_{out} is the passive efflux, b^0_{Cyt} is the concentration of all buffers in cytosol. Parameters $k^-, k^+, k_{pump}, k_{ch}, k_{leak}, k_{in}, k_{out}, k_m, K_1, K_2, K_3$ are kinetic constants. The coefficients $\rho_\gamma := V_\gamma/V_{Cyt}$, where V_γ is the volume of the γ-compartment, $\gamma \in \{Ret, Mit\}$. Their occurence in Eqs. (3) and (4) reflect the fact that, given the flux of calcium into a compartment, the speed of the concentration change is inversely proportional to its volume.

The existence of buffering molecules in the reticulum and mitochondria are taken into account implicitly by assuming that the ratio $c_\gamma/(c_\gamma + b_\gamma) =: \beta_\gamma$ is *constant in time*. This is justified by the the assumption that calcium binding and unbinding process achieves very fast its quasi-stationary values. Mathematically, it may be achieved by assuming large values of the coefficcients k^-_γ, k^+_γ corresponding to the coefficients k^-, k^+ in the cytosol.

The relatively high value of the Hill exponent in the flux J_{in} illustrates the fact that mitochondria start to accumulate calcium ions very fast (possibly in a very fast RAM mode) and secrete it very slowly back to the cytosol.

A SPATIALLY EXTENDED MODEL

Let the cell be modelled by a bounded domain $\Omega \subset R^3$. Let $\Omega = \Omega_{Cyt} \cup \Omega_{Ret} \cup \Omega_{Mit}$. Let $\overline{\Omega}_{Ret} \cap \overline{\Omega}_{Mit} = \emptyset$ and that $\partial\Omega \cap (\partial\Omega_{Ret} \cup \partial\Omega_{Mit}) = \emptyset$. We assume that inside the compartment Ω_γ, $\gamma \in \{Cyt, Ret, Mit\}$, the spatio-temporal dynamics of free calcium concentration c_γ and the concentration of buffered calcium ions b_γ, bound by one representative kind of buffering molecules are governed by the following system of equations:

$$\frac{\partial c_\gamma}{\partial t} = D_{c\gamma}\nabla^2 c_\gamma - E_\gamma(c_\gamma, b_\gamma), \tag{8}$$

$$\frac{\partial b_\gamma}{\partial t} = D_{b\gamma}\nabla^2 b_\gamma + E_\gamma(c_\gamma, b_\gamma), \tag{9}$$

where $E_\gamma(c_\gamma, b_\gamma) = -k^-_\gamma b_\gamma + k^+_\gamma c_\gamma(b^0_\gamma - b_\gamma)$, b^0_γ is total concentration of all buffering molecules, k^-_γ, k^+_γ are constants, $D_{c\gamma}$, $D_{b\gamma}$ are diffusion coefficients of free and buffered calcium, k^+_γ - binding and k^-_γ - unbinding kinetic constants in compartment γ. System (8)-(9) is supplemented by the no flux boundary condition on the boundary of Ω (no flow of calcium between the inside and outside the cell) and with Robin conditions for free calcium ions concentrations c_γ and buffering molecules concentrations b_γ on the boundary $\Gamma_{\gamma,Cyt}$ of the cytosol with the compartment γ, $\gamma = Ret, Mit$:

$$D_{cCyt}\mathbf{n}_{Cyt}(x) \cdot \nabla c_{Cyt}(x,t) = \Phi_{\gamma,Cyt}(x,t) \quad \text{on } \Gamma_{\gamma,Cyt}, \tag{10}$$

$$D_{bCyt}\mathbf{n}_{Cyt}(x) \cdot \nabla b_{Cyt}(x,t) = 0 \quad \text{on } \Gamma_{\gamma,Cyt}, \tag{11}$$

where $\mathbf{n}_{Cyt}(x)$ is a normal vector directed to the outside of the cytosol. Condition (11) means that buffering molecules do not leak through the cytosolic membranes.

Likewise the fluxes of free calcium ions and buffering molecules with bound calcium on the boundary of Ω_γ, $\gamma = Ret, Mit$, with the cytosol are equal to:

$$D_{c\gamma}\mathbf{n}_\gamma(x) \cdot \nabla c_\gamma(x,t) = \Phi_{Cyt,\gamma}(x,t) \quad \text{on } \Gamma_{Cyt,\gamma}, \tag{12}$$

$$D_{b\gamma}\mathbf{n}_\gamma(x) \cdot \nabla b_\gamma(x,t) = 0 \quad \text{on } \Gamma_{Cyt,\gamma}, \tag{13}$$

where $\mathbf{n}_\gamma(x)$ denotes a normal vector directed to the outside of the compartment γ at point x.

From the conservation laws we have: $\Phi_{Cyt,\gamma} = -\Phi_{\gamma,Cyt}$ for $\gamma = Ret, Mit$. We assume, that $\Phi_{\gamma,Cyt}(x,t)$, $\gamma = Ret, Mit$, depends analyticaly on the values of concentrations of free calcium $c_{Cyt}(x,t)$ and $c_\gamma(x,t)$ on both sides of the boundary $\Gamma_{\gamma,Cyt}$. This assumption is justified by the fact that the flux depends only on the states of receptors on the separating surfaces (receptors IP_3R on reticular membrane and mitochondrial uniporters).

Remark It is tacitly assumed that the diffusion coefficients of free buffers and those of the buffer molecules which bound calcium in each of the compartments are the same. This allows us to take advantage of the fact that, for the spatially homogeneous initial data, the total concentration of the buffer molecules is invariant in time and space.

REDUCTION TO A COMPARTMENT MODEL

To obtain specific forms of the fluxes $\Phi_{\gamma,Cyt}$, $\gamma = Ret, Mit$, we will reduce the spatially extended model to a compartmental one and compare it with the Marhl's model. Integrating (8), (9) over considered compartment ($\gamma = Ret, Mit$), and applying Gauss-Ostrogradski theorem we obtain:

$$\frac{d\int_{\Omega_\gamma} c_\gamma dV_\gamma}{dt} = \int_{\Gamma_{\gamma,Cyt}} \Phi_{Cyt,\gamma}(c_{Cyt}, c_\gamma) dS_{\gamma,Cyt} - \int_{\Omega_\gamma} E_\gamma(c_\gamma, b_\gamma) dV_\gamma, \tag{14}$$

$$\frac{d\int_{\Omega_\gamma} b_\gamma dV_\gamma}{dt} = \int_{\Omega_\gamma} E_\gamma(c_\gamma, b_\gamma) dV_\gamma. \tag{15}$$

Adding Eqs. (14) and (15) leads to the conservation law for the total calcium in the γ-compartment:

$$\frac{d\int_{\Omega_\gamma} (c_\gamma + b_\gamma) dV_\gamma}{dt} = \int_{\Gamma_{\gamma,Cyt}} \Phi_{Cyt,\gamma}(c_{Cyt}, c_\gamma) dS_{\gamma,Cyt}. \tag{16}$$

Assuming that the diffusion coefficients of free calcium ions are sufficiently large relative to the dimensions of the compartment (or its connected components, e.g., single mitochondria), we can neglect the spatial heterogeneity of calcium ions inside all compartments. Consequently, c_γ, b_γ depend only on time t (and not depend on spatial coordinate x) and thus equations (14), (15) become ordinary differential equations of the form:

$$\frac{dc_\gamma}{dt} = S_{\gamma,Cyt} V_\gamma^{-1} \Phi_{Cyt,\gamma}(c_{Cyt}, c_\gamma) - E_\gamma(c_\gamma, b_\gamma), \tag{17}$$

$$\frac{db_\gamma}{dt} = E_\gamma(c_\gamma, b_\gamma). \tag{18}$$

Assume that the buffers in reticular and mitochondrial compartments are 'fast' and the total amount of buffers is sufficiently large (buffers in 'excess'). To be more precise, that:

1: k_γ^-, k_γ^+ are sufficiently large

2: $b_\gamma^0 >> b_\gamma$

It follows - from condition **1** that: $E_\gamma(c_\gamma, b_\gamma) \cong 0$, and from condition **2** that:

$$-k_\gamma^- b_\gamma + k_\gamma^+ c_\gamma (b_\gamma^0 - b_\gamma) \cong -k_\gamma^- b_\gamma + k_\gamma^+ c_\gamma b_\gamma^0$$

In consequence $b_\gamma \cong K c_\gamma b_\gamma^0$, $K = k_\gamma^+ (k_\gamma^-)^{-1}$, thus $c_\gamma (c_\gamma + b_\gamma)^{-1} \cong (1 + K\, b_\gamma^0)^{-1}$, so approximately

$$\frac{c_\gamma}{c_\gamma + b_\gamma} =: \beta_\gamma \tag{19}$$

With the above assumptions, the ratio of the concentration of free calcium ions inside compartment γ to the total calcium ion concentration (free and bound) can be assumed to be constant.

Thus from (17),(18) we get for the reticular and mitochondrial compartment ($\gamma \in \{Ret, Mit\}$)

$$\frac{dc_\gamma}{dt} = \frac{\beta_\gamma}{\rho_\gamma} \left[\frac{S_{\gamma,Cyt}}{V_{Cyt}} \Phi_{Cyt,\gamma}(c_{Cyt}, c_\gamma) \right], \quad \rho_\gamma := \frac{V_\gamma}{V_{Cyt}}. \tag{20}$$

Such assumptions underly the relative simplicity of the compartment Marhl's model. However, while the assumption of homogeneity of the concentration of free and buffered calcium ions is somehow justified by small dimensions of individual components of non-cytosolic compartments, the assumption of homogeneity of concentrations in the cytosolic compartment is now more problematic because of its connectivity (no subdivision into subcompartments, as in the case of the mitochondrial and reticular component). Integrating (9), (8) over the cytosolic compartment, after the application of Gauss-Ostrogradski theorem and boundary conditions (10), we obtain, analogously as before:

$$\begin{aligned}\frac{d\int_{\Omega_{Cyt}} c_{Cyt}dV_{Cyt}}{dt} &= \sum_{\gamma=Ret,\,Mit}\int_{\Gamma_{\gamma,Cyt}}\Phi_{\gamma,Cyt}(c_{Cyt},c_{\gamma})dS_{\gamma,Cyt} + \\ &\quad -\int_{\Omega_{Cyt}} E_{Cyt}(c_{Cyt},b_{Cyt})dV_{Cyt}, \qquad (21)\\ \frac{d\int_{\Omega_{Cyt}} b_{Cyt}dV_{Cyt}}{dt} &= \int_{\Omega_{Cyt}} E_{Cyt}(c_{Cyt},b_{Cyt})dV_{Cyt}. \qquad (22)\end{aligned}$$

Adding these we come to conservation law as in (16):

$$\frac{d\int_{\Omega_{Cyt}}(c_{Cyt}+b_{Cyt})dV_{Cyt}}{dt} = \sum_{\gamma=Ret,\,Mit}\int_{\Gamma_{\gamma,Cyt}}\Phi_{\gamma,Cyt}(c_{Cyt},c_{\gamma})dS_{\gamma,Cyt}. \qquad (23)$$

Adding equations (16) for $\gamma = Ret$, $\gamma = Mit$ and equation (23) by sides we obtain total conservation law of all calcium ions:

$$\sum_{\gamma=Cyt,Ret,Mit}\frac{d\int_{\Omega_\gamma}(c_\gamma+b_\gamma)dV_\gamma}{dt} = 0.$$

Because $c_\gamma + b_\gamma = c_\gamma\beta_\gamma^{-1}$, $\gamma = Ret,\ Mit$, thus we get:

$$\frac{d\int_{\Omega_{Ret}} c_{Ret}\beta_{Ret}^{-1}dV_{Ret}}{dt} + \frac{d\int_{\Omega_{Mit}} c_{Mit}\beta_{Mit}^{-1}dV_{Mit}}{dt} + \frac{d\int_{\Omega_{Cyt}}(c_{Cyt}+b_{Cyt})dV_{Cyt}}{dt} = 0.$$

Hence, for spatially homogeneous concentrations we obtain the conservation law for the total amount of calcium in the system (see Eq.(1) in [1]):

$$c_{Ret}\frac{\rho_{Ret}}{\beta_{Ret}} + c_{Mit}\frac{\rho_{Mit}}{\beta_{Mit}} + (c_{Cyt}+b_{Cyt}) = C_cV_c^{-1}, \qquad (24)$$

where C_c is the total amount of calcium ions in the system. On the basis of (20) and (21), the assumption of spatial homogeneity leads to ordinary differential equations of the form:

$$\begin{aligned}\frac{dc_{Cyt}}{dt} &= \sum_{\gamma=Ret,\,Mit}\frac{S_{\gamma,Cyt}}{V_{Cyt}}\Phi_{\gamma,Cyt}(c_{Cyt},c_\gamma) - E_{Cyt}(c_{Cyt},b_{Cyt}), \qquad (25)\\ \frac{db_{Cyt}}{dt} &= E_{Cyt}(c_{Cyt},b_{Cyt}), \qquad (26)\\ \frac{dc_{Ret}}{dt} &= \frac{\beta_{Ret}}{\rho_{Ret}}\left[\frac{S_{Ret,Cyt}}{V_{Cyt}}\Phi_{Cyt,Ret}(c_{Cyt},c_{Ret})\right], \qquad (27)\\ \frac{dc_{Mit}}{dt} &= \frac{\beta_{Mit}}{\rho_{Mit}}\left[\frac{S_{Mit,Cyt}}{V_{Cyt}}\Phi_{Cyt,Mit}(c_{Cyt},c_{Mit})\right]. \qquad (28)\end{aligned}$$

Comparison of (25)-(28) with (1)-(4) gives:

$$\Phi_{Cyt,Ret}(c_{Cyt}, c_{Ret}) = \frac{V_{Cyt}}{S_{Ret,Cyt}} J_{Ret}, \tag{29}$$

$$\Phi_{Cyt,Mit}(c_{Cyt}, c_{Mit}) = \frac{V_{Cyt}}{S_{Mit,Cyt}} J_{Mit}, \tag{30}$$

where J_{Ret} and J_{Mit} are given by definitions (5), (6) and (7).

FINAL FORM OF THE MODEL

Assuming that in Ω_γ, $\gamma = Ret, Mit$, $c_\gamma(x,t)/(c_\gamma(x,t) + b_\gamma(x,t)) = \beta_\gamma$ for $(x,t) \in \Omega_\gamma \times [0,\infty)$, we obtain four reaction-diffusion equations of the form:

$$\frac{\partial c_{Cyt}}{\partial t} = D_{cCyt}\nabla^2 c_{Cyt} - E_{Cyt}(c_{Cyt}, b_{Cyt}) \quad \text{in } \Omega_{Cyt}, \tag{31}$$

$$\frac{\partial b_{Cyt}}{\partial t} = D_{bCyt}\nabla^2 b_{Cyt} + E_{Cyt}(c_{Cyt}, b_{Cyt}) \quad \text{in } \Omega_{Cyt}, \tag{32}$$

$$\frac{\partial c_{Ret}}{\partial t} = D_{Ret}\nabla^2 c_{Ret} \quad \text{in } \Omega_{Ret}, \tag{33}$$

$$\frac{\partial c_{Mit}}{\partial t} = D_{Mit}\nabla^2 c_{Mit} \quad \text{in } \Omega_{Mit}. \tag{34}$$

together with the boundary conditions:

$$\begin{aligned}
D_{cCyt}\mathbf{n}_{Cyt}(x)\cdot\nabla c_{Cyt}(x,t) &= 0 && \text{on } \partial\Omega,\\
D_{cCyt}\mathbf{n}_{Cyt}(x)\cdot\nabla c_{Cyt}(x,t) &= -\frac{V_{Cyt}}{S_{\gamma,Cyt}} J_\gamma && \text{on } \Gamma_{\gamma,Cyt},\ \gamma = Ret, Mit,\\
D_{bCyt}\mathbf{n}_{Cyt}(x)\cdot\nabla b_{Cyt}(x,t) &= 0 && \text{on } \Gamma_{\gamma,Cyt},\ \gamma = Ret, Mit,\\
D_{Ret}\mathbf{n}_{Ret}(x)\cdot\nabla c_{Ret}(x,t) &= \left(\frac{\beta_{Ret}}{\rho_{Ret}}\right)\rho_{Ret}\frac{V_{Cyt}}{S_{Ret,Cyt}} J_{Ret}, && \text{on } \Gamma_{Ret,Cyt},\\
D_{Mit}\mathbf{n}_{Mit}(x)\cdot\nabla c_{Mit}(x,t) &= \left(\frac{\beta_{Mit}}{\rho_{Mit}}\right)\rho_{Mit}\frac{V_{Cyt}}{S_{Mit,Cyt}} J_{Mit}, && \text{on } \Gamma_{Mit,Cyt}.
\end{aligned} \tag{35}$$

In equations above, J_{Ret} and J_{Mit} are given by definitions (5), (6) and (7), whereas

$$D_{Ret} = \beta_{Ret}D_{cRet} + (1-\beta_{Ret})D_{bRet}, \quad D_{Mit} = \beta_{Mit}D_{cMit} + (1-\beta_{Mit})D_{bMit},$$

are the effective diffusion coefficients of calcium ions in the reticulum and mitochondria. Eqs. (33) and (34) can be obtained by adding Eqs. (8) and (9) and using approximate identity (19).

Remark The crucial point in the derivation of the above model was the assumption of fast buffers (in excess) in the reticular and mitochondrial compartment. This assumption is commonly used and generaly accepted, though the kinetic constants of binding and unbinding of calcium by buffer molecules are known only very approximately. □

THE RESULTS OF THE NUMERICAL SIMULATIONS

We have carried out our simulations for axially symetric model with the cross-section geometry as shown in Fig.1. The ratios $\frac{\beta_{Ret}}{\rho_{Ret}}$ and $\frac{\beta_{Ret}}{\rho_{Ret}}$ were taken to be equal to 0.25 as in [1]. For simplicity of calculations we assumed additionally that: $D_{cRet} = D_{bRet} = D_{cMit} = D_{bMit} = D_{cCyt}$. Some of the results are presented below. The basic observation is that the oscillations disappear for small values of D_{cCyt}.

Remark In most situations $D_{b\gamma} < D_{c\gamma}$ (or even $D_{b\gamma} << D_{c\gamma}$), nevertheless our simulations give more information about the spatial distribution of calcium than the compartment models (eg. Marhl's model), even for $D_{b\gamma} = D_{c\gamma}$ in the reticular and mitochondrial compartments.

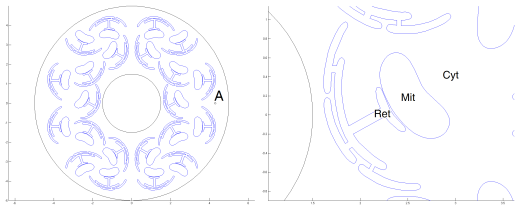

Figure 1. Left panel: The cross-section of the axially symetric geometry used in simulations. The inner disc corresponds to the nucleus. The data acquisition point for plots in Fig.2 is denoted by A. Right panel: The fine structure of the reticulum-mitochondria motif.

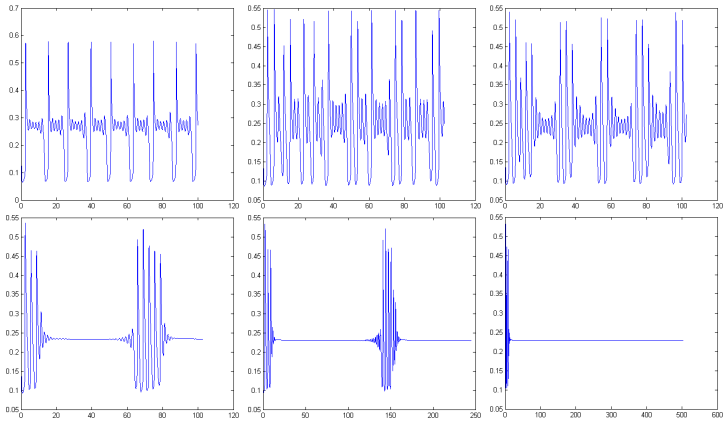

Figure 2. Time courses of free calcium concentration at point A depicted in Fig. 1 for D_{cCyt} equal respectively 100, 20, 17, 15, 14, 13.5 and $D_{bCyt} = 1$ ($\mu m^2/s$). $D_{cRet} = D_{bRet} = D_{cMit} = D_{bMit} = D_{cCyt}$. The values of ρ_{Ret} and ρ_{Mit} were equal to 0.1313, 0.1356 respectively, whereas the ratios β_{Ret}/ρ_{Ret}, β_{Mit}/ρ_{Mit} were both taken as 0.25. The geometrical parameters of the model: $V_{Cyt}/S_{Ret,Cyt} = 0.3073\mu m$, $V_{Cyt}/S_{Mit,Cyt} = 1.0940\mu m$. The other parameters of the model were taken as: $k_{pump} = 20s^{-1}$, $k_{ch} = 4200s^{-1}$, $k_{in} = 300\mu M s^{-1}$, $k_{leak} = 0.05s^{-1}$, $k_{out} = 125s^{-1}$, $k_m = 0.00625s^{-1}$, $K_1 = 5\mu M$, $K_2 = 0.8\mu M$, $K_3 = 5\mu M$, $k^+_{Cyt} = 0.1\mu M^{-1}s^{-1}$, $k^-_{Cyt} = 0.01s^{-1}$, $b^0_{Cyt} = 120\mu M$. Initial values: $c_{Cyt,0} = 0.225\mu M$, $c_{Ret,0} = 0.7\mu M$, $c_{Mit,0} = 0.35\mu M$, $b_{Cyt,0} = 85,575\mu M$.

CONCLUSIONS

For sufficiently large diffusion coefficients D_{cCyt} (exceeding $\cong 100\mu m^2/s$) the model exhibits oscillatory solutions very similar in their structure and period to solutions observed in Marhl's model, *i.e.* relatively regular single peak oscillations of period equal to circa 10s. For decreasing values of D_{cCyt}, this simple structure becomes more complicated. Groups of irregular high peaks are more and more separated by smaller ones untlil the oscillatory solutions cease to exist at $D_{cCyt} \cong 13.5\mu m^2/s$.

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