MATLAB project: calcium oscillations

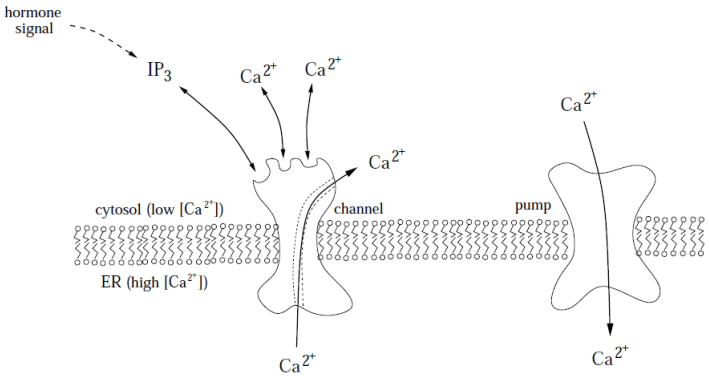
Many types of animal cells use calcium ions, $Ca2^+$, as part of signal transduction cascades. Calcium is used to trigger, for example, the initiation of embryonic development in fertilized egg cells, the contraction of muscle cells, and the secretion of neurotransmitters from neurons.

Calcium signals are sent by rapid spikes in cytosolic $Ca2^+$ concentration. Cells that employ these signals normally have low levels of cytosolic calcium (about 10-100 nM). These low levels are maintained by ATP-dependent pumps that export cytosolic $Ca2^+$ both out of the cell and into the endoplasmic reticulum (ER). The concentration of $Ca2^+$ in the ER can reach as high as 1 mM (106 nM). Signaling pathways open calcium channels in the ER membrane, leading to rapid (diffusion-driven) surges in cytosolic calcium levels.

However, because calcium is involved in many cellular processes, persistent high concentrations can be detrimental. (For example, failure to remove calcium from muscle cells keeps them in a state of constant tension. This is what causes rigor mortis.) Some cells that use calcium as an intracellular signaling molecule avoid persistently high $Ca2^+$ concentrations by generating oscillations in calcium levels. The frequency of the oscillations is dependent on the intensity of the signal, while the amplitude is roughly constant. The downstream cellular response is dependent on the oscillation frequency.

We will consider an instance of this frequency-encoding mechanism in mammalian liver cells. These cells respond to certain hormones with the activation of G-protein-coupled receptors. The G-protein triggers a signaling pathway that results in production of inositol 1,4,5-triphosphate (IP3). These IP3 molecules bind a receptor that is complexed with a calcium channel in the membrane of the ER.

The IP3 binding event exposes two receptor sites at which $Ca2^+$ ions can bind. These two sites have different affinities for $Ca2^+$. At low concentration only one site is occupied, while at higher concentrations both sites are bound. The calcium binding events have opposing effects on the receptor-channel complex. Binding of the first calcium ion causes the channel to open, allowing $Ca2^+$ to flow into the cytosol. Binding of the second ion causes the channel to close. This interplay of positive and negative feedback generates oscillations in the cytosolic $Ca2^+$ concentration, as follows. When cytosolic calcium levels are low, the channels are primarily in the open state, and so $Ca2^+$ rushes into the cytosol from the ER. When high calcium levels are reached, the channels begin to shut. Once most of the channels are closed, the continual action of the $Ca2^+$ pumps eventually causes a return to low cytosolic [$Ca2^+$], from which the cycle repeats.



Calcium-induced calcium release. A G-protein pathway (not shown) responds to a hormone signal by inducing production of IP3, which activates calcium channels in the ER membrane. These channels bind $Ca2^+$ ions at two sites. The first binding event causes the channel to open; the second causes it to close. Calcium pumps continually pump $Ca2^+$ ions from the cytosol to the ER.

In 1993 Hans Othmer and Yuanhua Tang developed a model of this pathway that focuses on the behavior of the channel, described in:

H.G. Othmer, Signal transduction and second messenger systems. In Case Studies in Mathematical Modelling: Ecology, Physiology, and Cell Biology, H.G. Othmer, F.R. Adler, M.A. Lewis and J. C. Dallon, eds, 99-126, 1996

Taking I =[IP3] as the system input, the receptor binding events are described by:

$$\mathrm{I} + \mathrm{R} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{RI} \qquad \mathrm{RI} + \mathrm{C} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \mathrm{RIC}^+ \qquad \mathrm{RIC}^+ + \mathrm{C} \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} \mathrm{RIC}^+\mathrm{C}^-$$

where

R is the receptor-channel complex,

C is cytosolic calcium,

RI is the IP3-bound receptor-channel complex,

RIC^+ is the open (one $\mathrm{Ca2}^+$-bound) channel,

and $\mathrm{RIC}^+\mathrm{C}^-$ is the closed (two $\mathrm{Ca2}^+$-bound) channel.

The rate of diffusion of calcium into the cytosol depends on the concentration of calcium in the ER (denoted $[\mathrm{C_{ER}}]$, and held fixed) and the abundance of open channels. The rate of diffusion is proportional to the difference in concentration between the two compartments. This transport rate is modeled as

$$\text{rate of } \mathrm{Ca2}^+ \text{ diffusion into the cytosol} = v_r(\gamma_0 + \gamma_1[\mathrm{RIC}^+])([\mathrm{C_{ER}}] - [\mathrm{C}])$$

where v_r is the ratio of the ER and cytosolic volumes, and γ_0 characterizes a channel-independent "leak."

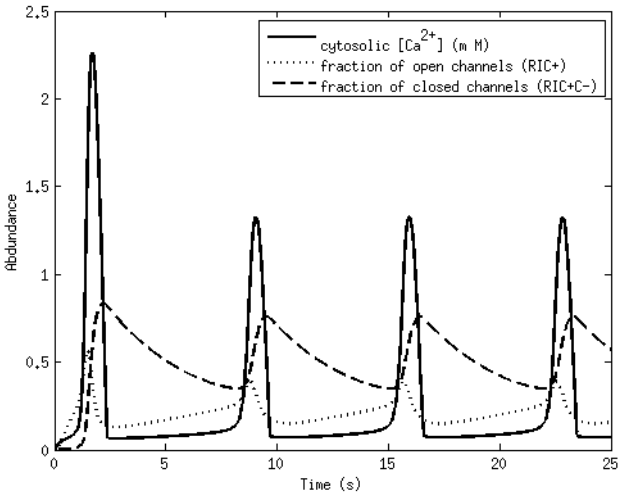
Calcium is continually pumped from the cytosol to the ER. Assuming strong cooperativity of calcium uptake, the pumping rate is modeled as

$$\text{rate of } \mathrm{Ca2}^+ \text{ pumping out of the cytosol} = \frac{p_1[\mathrm{C}]^4}{p_2^4 + [\mathrm{C}]^4}$$

for parameters p_1 and p_2. The complete model is then:

$$\begin{aligned}
d/dt[\mathrm{R}] &= -k_1[I]\cdot[\mathrm{R}] + k_{-1}[\mathrm{RI}] \\
d/dt[\mathrm{RI}] &= -(k_{-1} + k_2[\mathrm{C}])\cdot[\mathrm{RI}] + k_1[I]\cdot[\mathrm{R}] + k_{-2}[\mathrm{RIC}^+] \\
d/dt[\mathrm{RIC}^+] &= -(k_{-2} + k_3[\mathrm{C}])\cdot[\mathrm{RIC}^+] + k_2[\mathrm{C}]\cdot[\mathrm{RI}] + k_{-3}[\mathrm{RIC}^+\mathrm{C}^-] \\
d/dt[\mathrm{RIC}^+\mathrm{C}^-] &= k_3[\mathrm{C}]\cdot[\mathrm{RIC}^+] - k_{-3}[\mathrm{RIC}^+\mathrm{C}^-] \\
d/dt[\mathrm{C}] &= v_r(\gamma_0 + \gamma_1[\mathrm{RIC}^+])([\mathrm{C_{ER}}] - [\mathrm{C}]) - \frac{p_1[\mathrm{C}]^4}{p_2^4 + [\mathrm{C}]^4}
\end{aligned}$$

The following simulation in illustrates the system's oscillatory behavior. When the calcium level is low, the concentration of open channels increases, followed by a rapid increase in $[\mathrm{Ca2}^+]$. Once the calcium concentration rises, the channels close, and the calcium level falls, setting up a new cycle.



Calcium oscillations. This simulation shows the oscillatory behavior of the system. When calcium levels are low, channels open, letting more Ca2^+ *into the cytosol. As calcium levels rise, channels begin to close, leading to a drop in cytosolic Ca*2^+ *levels. The IP3 concentration is fixed at 2* μM*. Parameter values used : (in* $1/\mu M$ *1/s)* $k_1 = 12, k_2 = 15, k_3 = 1.8$*; (in 1/s)* $k_{-1} = 8, k_{-2} = 1.65, k_{-3} = 0.21, \gamma_0 = 0.1, \gamma_1 = 20.5; [C_{\mathrm{ER}}] = 8.37 \mu M$*,* $p_1 = 8.5$ μM *1/s,* $p_2 = 0.065$ μM*,* $v_r = 0.185$*.*

Problems

1. For this problem, you will use the MATLAB program "calcium_cytosolic" which was provided by the instructor.

   You have to place the file calcium_cytosolic.m in a folder that MATLAB will find. To run the program, just type "calcium_cytosolic" in MATLAB.

   Please print and hand-in the corresponding plots. It is OK to give a very approximate answer to the questions about frequency.

   (a) Look at the program listing. (Open the file calcium_cytosolic.m using MATLAB or your favorite text editor.) What are the initial concentrations for the variables? You don't need to "know MATLAB" to read the file and figure this out!

   (b) The program starts with an IP3 concentration that changes from $I = 0$ to a new value of I at time $t = 20$. With the new I set to 1, you will observe that, after stimulation, the system settles into an oscillation in cytosolic calcium with a frequency of roughly 0.14 1/s. This is estimated as follows: on an interval of length 100 seconds, from time $t = 25$ to $t = 125$ (more or less!) we have about 14 complete oscillations, and 14/100 = 0.14.

   (c) Modify the program to set the value after $t = 20$ to $I = 0.7$. (Open the file calcium_cytosolic.m using MATLAB or your favorite text editor, and change the line where I was set to 1.) Now run the program, and answer: what frequency of oscillations results now?

   (d) Repeat with $I = 2$.

   (e) Repeat with $I = 10$.

   (f) Speculate on what happens if the input I is set really large. Does the frequency tend to infinity?

   (g) Repeat with $I = 0.5$. What do you see now?

   (h) Suppose now that (perhaps because of a mutation), calcium cannot bind anymore to the open channels, so as to close them. Answer: which parameter k_i has to be changed to zero in order to model this new system?

   (i) Now set the parameter k_i from the previous part to zero, and run the program again (with $I = 1$). **Make sure** to change the "2.5" in the line:

   ```
   axis([0 120 0 2.5])
   ```

   to something large, let us say 10, before running the program. (This sets the y axis to be between 0 and 10.) Interpret in words what you see.

2. Calcium-induced calcium release: frequency encoding. Run simulations to explore the dependence of frequency and amplitude on the strength of the input. Prepare plots showing frequency and amplitude as a function of model input I over the range 1-10 μM. Does the amplitude vary significantly over this range? What about for higher input levels?

3. Calcium-induced calcium release: parameter balance. The model's oscillatory behavior depends on a balance between the positive and negative effects of calcium binding. Oscillations are easily lost if parameter values vary. Explore this sensitivity by choosing one of the model parameters and determining the range of values over which the system exhibits oscillations

(with I =1 μM). Provide an intuitive explanation for why oscillations are lost outside the range you have identified.

4. Calcium-induced calcium release: frequency decoding. One mechanism by which cells can "decode" the information encoded in calcium oscillations is by the activity of $Ca2^{+}$/calmodulin-dependent protein kinases (CaM-kinases). Calmodulin is a protein that mediates a number of calcium-dependent cellular processes. It has four high-affinity $Ca2^{+}$ binding sites, and is activated when saturated by $Ca2^{+}$. CaM-kinases are activated (by autophosphorylation) upon binding to active calmodulin. The CaM-kinaseactivityis "turned off" byphosphatases. Extend the model to include CaM-kinase activity, and verify that, for persistent oscillations, the higher the frequency of$Ca2^{+}$ oscillations, the higher the average CaM-kinase activity level. To keep the model simple, suppose that four calcium ions bind calmodulin simultaneously (i.e. with high cooperativity), and that CaM-kinase autophosphorylation occurs immediately upon calmodulin binding. (Hint: the frequency-dependent effect is strongest when the time-scales of deactivation of calmodulin and CaM-kinase are slow, so that high-frequency inputs cause near-constant activity levels.)