Chapter 3

Calcium Model

Biological cells use highly regulated homeostasis systems to keep a very low cytosolic Ca^{2+} level. In normal-growing yeast the cytosolic Ca^{2+} concentration is maintained in the range of $50 - 200nM$ in the presence of environmental Ca^{2+} concentrations ranging from $< 1\mu M$ to $> 100mM$ [11]. To achieve an accurate model, the influences of voltage-dependent calcium channels are added to a basic model for yeast calcium homeostasis. In this model, first described by J. Cui *et al*, the main contributions of calcium transport are defined [12].

In literature, little can be found about modeling calcium channels in Saccaromyces Cerevisiae, most commonly known as budding yeast. Our model of calcium channels in yeast is based on a basic model of sympathetic ganglion 'B' type cells of a bullfrog [19] is used. This is a type of nerve cells that exists in nerve junctions of the orthosympathetic nervous system. At first the complete model of the bullfrog cells was used by C. Balemans, team member of iGEM team of the Eindhoven University of Technology in 2012, to describe the cytosolic Ca^{2+} level in yeast cells [13]. In this research the model is more focused on protein modeling, including the GECO proteins.

The model is made in MATLAB and the code can be found in appendix **??**.

3.1 Calcium homeostatic process in yeast cells

The homeostatis system in yeast cells, as mentioned before, has two basic characteristics. The cytosolic Ca^{2+} concentration is tightly controlled by "zero" steady-state error to extracellular stimuli and the system is relatively insensitive to specific kinetic parameters, due to robustness of such adaptation [14]. This second characteristic will be discussed in more detail in chapter 4.

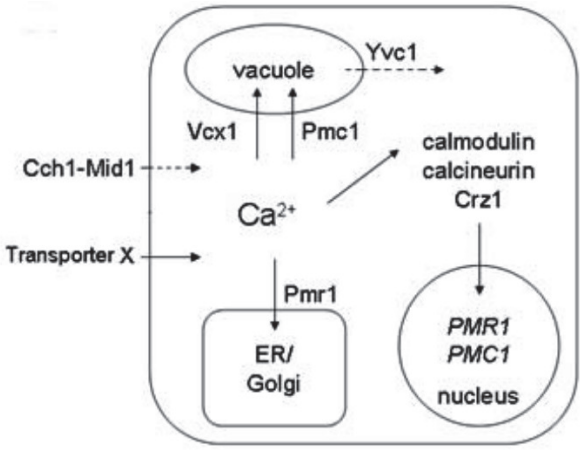

Figure 3.1: A schematic graph illustrating the protein level observations of the yeast calcium homeostasis system. Channel Cch1-Mid1 on the plasma membrane opens only under abnormal conditions. The vacuole can release Ca^{2+} into the cytosol through Yvc1, though only under the abnormal conditions of an extracellular hypertonic shock [12].

Under normal conditions, extracellular Ca^{2+} enters the cytosol through an unknown Transporter X, whose encoded gene has not been identified yet. Cytosolic Ca^{2+} can be pumped into endoplasmic reticulum (ER) and Golgi system through Pmr1 and can be sequestered into the vacuole through Pmc1 and Vcx1. Both the expression and function of Pmc1, Pmr1 and Vcx1 are regulated by calcineurin, a highly conserved protein phosphatase that is activated by Ca^{2+}-bound calmodulin [12].

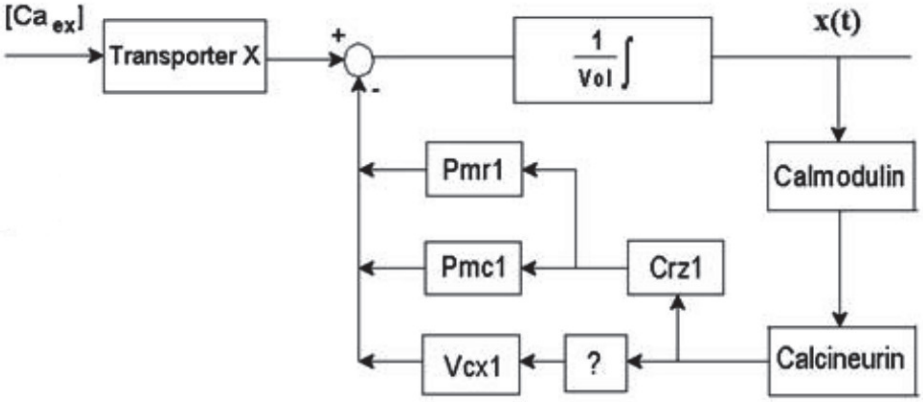

Figure 3.2: Block diagram of calcium homeostasis under normal conditions. Through unknown Transporter X, extracellular Ca^{2+}, denoted by $[Ca_{ex}]$, enters the cell. Proteins Pmr1, Pmc1 and Vcx1 cause feedback control through calmodulin and calcineurin. Crz1 is a transciptional factor and '?' denotes an unknown mechanism [12].

3.2 Feedback modeling

3.2.1 Sensing cytosolic Ca^{2+}

Calmodulin is a Ca^{2+}-binding protein expressed in all eukaryotic cells. The protein binds to cytosolic Ca^{2+} as a response to an increase of the Ca^{2+} level in the cell. A host domain for target proteins, including calcineurin, is activated. In yeast cells, calmodulin can only bind to a maximum of three molecules of Ca^{2+} [15]. A strong cooperativity among the three active sites can be assumed. Therefore the cytosolic Ca^{2+} sensing process can be described as follows:

$$3Ca^{2+} + Calmodulin \rightleftharpoons CaM \tag{3.1}$$

where CaM denotes Ca^{2+}-bound calmodulin. The concentration of Ca^{2+}-bound calmodulin will be denoted as CaM(t) and can be described by mass-action kinetics with forward rate constant k_M^+ and backward rate constant k_M^- as follows:

$$\frac{dCaM(t)}{dt} = k_M^+([CaM_{total}] - CaM(t)) \cdot Ca(t)^3 - k_M^- CaM(t) \tag{3.2}$$

where $[CaM_{total}]$ denotes the total concentration of calmodulin, the sum of Ca^{2+}-free and Ca^{2+}-bound calmodulin.

3.2.2 Calcineurin activation

Upon elevation of cytosolic Ca^{2+}, Ca^{2+}-bound calmodulin binds to the catalytic subunit of calcineurin and activates calcineurin by displacing the carboxyl-terminal autoinhibitory domain. This binding process can be described as:

$$CaM + Calcineurin \rightleftharpoons CaN \tag{3.3}$$

where CaN denotes activated calcineurin. The concentration of Ca^{2+}-bound calneurin will be denoted as CaN(t) and can be described by mass-action kinetics with forward rate constant k_N^+ and backward rate constant k_N^-. Since each molecule of calcineurin binds with one molecule of Ca^{2+}-bound calmodulin, we can derive the following equation:

$$\frac{dCaN(t)}{dt} = k_N^+([CaN_{total}] - CaN(t)) \cdot CaM(t) - k_M^- CaN(t) \tag{3.4}$$

where $[CaN_{total}]$ denotes the total concentration of calcineurin, the sum of Ca^{2+}-free and Ca^{2+}-bound calcineurin.

3.2.3 Gene expression control

Experimental results show that activated calcineurin regulates the production of the proteins Pmc1 and Pmr1 by controlling the synthesis of these two proteins through a transcription

factor Crz1 [16]. Crz1 is a highly phosphorylated protein that can be dephosporylated by activated calcineurin. We assume that only fully dephosphorylated Crz1 molecules in the nucleus are transcriptionally active since this has been shown the case for NFAT1. The mechanism of Crz1 translocation in yeast cells is similar to NFAT (nuclear factor of activated T-cells) translocation in mammalian T-cells. Therefore, some of the parameters stated in this chapter are based on experimental data on NFAT1. A conformational switch model is used to simulate Crz1 translocation, as described by Okamura *et al* [17]. By describing the conformational switch model as a protein network and using the rapid equilibrium approximation [18], we can use the following equation to describe the kinetics of the total nuclear Crz1 fraction which is denoted by Crz(t):

$$\frac{dCrz(t)}{dt} = d \cdot \phi(1/CaN(t)) \cdot (1 - Crz(t)) - f \cdot (1 - \phi(1/CaN(t))) \cdot Crz(t) \tag{3.5}$$

d denotes the import rate constant, f denotes the export rate constant and ϕ denotes the ratio of the fraction of the cytosolic active conformation over the total cytosolic fraction:

$$\phi(y) = 1/(1 + L_0 \cdot \frac{(\lambda y)^{N+1} - 1}{\lambda y - 1} \cdot \frac{y - 1}{(y)^{N+1} - 1}) \tag{3.6}$$

where N is the number of relevant regulatory phosporylation sites, experimental data shows that $N = 13$ in the case of NFAT1. L_0 denotes the basic equilibrium constant and λ is the increment factor. The conformational switch model and deduction of the function $\frac{dCrz(t)}{dt}$ is described in more detail in appendix C.

As mentioned before, Crz1 is a transcription factor which controls the synthesis of Pmc1 and Pmr1. The concentrations of these two proteins are assumed to be proportional to transcriptionally active Crz1 fraction in the nucleus and therefore:

$$[Pmc1] = k_a \cdot h(t) \cdot \theta(1/z(t)) \tag{3.7}$$

$$[Pmc1] = k_b \cdot h(t) \cdot \theta(1/z(t)) \tag{3.8}$$

where $[Pmc1]$ and $[Pmr1]$ denote the concentrations Pmc1 and Pmr1 respectively and k_a, k_b denote the feedback control constants.

For the feedback regulation of activated calcineurin on the synthesis of Vcx1, the process is stil unknown. The only knowledge about this regulation is that the mechanism is possibly posttranslational and the general effect is suppressing. As an approximation this regulation is expressed as follows [12]:

$$[Vcx1] = k_d/(1 + k_c \cdot CaN(t)) \tag{3.9}$$

where k_c and k_d denote the feedback control constants. As stated by this equation, the concentration of Vcx1 will drop when CaN(t) rises.

3.3 Protein modeling

All four involved proteins (Transporter X, Pmc1, Pmr1 and Vcx1) can be described by the Michaelis-Menten kinetics, often used to describe simple enzymatic reactions. For example, the uptake rate of Transporter X, k, can be described by:

$$k = \frac{V_{max} \cdot [Ca_{ex}]}{K_x + [Ca_{ex}]} \tag{3.10}$$

where $V_{max}(\mu M s^{-1})$ is the maximum uptake rate of Transporter X and $K_x(\mu M)$ is the binding constant.

3.3.1 GECO proteins

The iGEM team of the Eindhoven University of Technology in 2012 uses the fluorescent activity of CMV-X-GECO.1 proteins as light-emitting part of the cell, as a result of an increase of the cytosolic Ca^{2+}-level. Therefore, also the kinetics of calcium binding by these G-GECO proteins need to be taken into account. Although three different GECO proteins are used (CMV-R-GECO1, CMV-G-GECO1.1 and CMV-B-GECO1) to provide different colors, only the variant CMV-G-GECO1.1 is discussed. The kinectic characterization of the different GECOs can be found in table 3.1. Upon elevation of cytosolic Ca^{2+}-level, Ca^{2+} binds to the GECO protein, a process that we can described as follow:

$$nCa^{2+} + GECO \rightleftharpoons CaGECO \tag{3.11}$$

where $CaGECO$ denotes Ca^{2+}-bound GECO protein and n the number of Ca^{2+} molecules that binds to the protein. This equation yields a forward rate constant k_{on} and a backward rate constant k_{off} as mentioned in table 3.1.

Protein	$k_{on}(M^{-n}s^{-1})$	$k_{off}(s^{-}1)$	n	$K_{d,kinetic}(nM)$	$K_{d,static}(nM)$
R-GECO1	$9.52 \cdot 10^{9}$	0.752	1.6	484	482
G-GECO1.1	$8.17 \cdot 10^{15}$	0.675	2.6	809	618
B-GECO1	$4.68 \cdot 10^{12}$	0.490	2.0	324	164

Table 3.1: Kinetic characterization of GECO proteins [10].

We can assume $[GECO_{total}]$, the total concentration of GECO (including Ca^{2+}-bound and Ca^{2+}-free form) to be constant. If we further denote the concentration of Ca^{2+}-bound GECO protein as $CaGECO(t)$, then according to the law of mass action, we can derive the time dependence of $CaGECO(t)$ as follows:

$$CaGECO'(t) = k_{on}([GECO_{total}] - CaGECO(t)) \cdot Ca(t)^n - k_{off} CaGECO(t) \tag{3.12}$$

3.4 Ionic Current Flow

The largest flux of calcium ions into the cell occurs via voltage-dependent channels. Equation 3.13 [19] shows the change in calcium concentration in the cell on account of the influx, with $[Ca^{2+}]$ the intracellular calcium concentration (mM), $I_{Ca^{2+}}$ the calcium current (nA) and V_n the volume of the cell (l). Faraday's constant, F, converts the quantity of moles to the quantity of charge for a univalent ion [20].

$$\frac{\partial[Ca^{2+}]}{\partial t} = \frac{-I_{Ca^{2+}}}{2FV_n} \tag{3.13}$$

Generally, ionic currents can be described by Ohm's law [19]:

$$I(t) = g(t, V) \cdot (V - E) \tag{3.14}$$

In this equation, g is the time and voltage dependent conductance (μS), V is the voltage (mV) and E is the Nernst potential (mV). The conductance associated with the channel can be calculated by equation 3.15 [19], with $\bar{g}$ the maximum conductance of the membrane, m the activation variable $(-)$, h the inactivation variable $(-)$ and i and j positive integers.

$$g(t, V) = \bar{g} \cdot m(t, V)^i \cdot h(t, V)^j \tag{3.15}$$

The dynamics of the activation variable can be found using equation 3.16 [19], where m_∞ is the steady state value of m and τ_m is a characteristic time constant (s).

$$\frac{dm(t, V)}{dt} = \frac{m_\infty(V) - m(t, V)}{\tau_m(V)} \tag{3.16}$$

The steady state value of m, m_∞, and the time constant, τ_m, can be described using the Hodgkin-Huxley equations. Equations 3.17 [19] and 3.18 [19] are the Hodgkin-Huxley equation, suited for the calcium influx.

$$\tau_m = \frac{7.8}{e^{+(V+6)/16} + e^{-(V+6)/16}} \tag{3.17}$$

$$m_\infty = \frac{1}{1 + e^{-(V-3)/8}} \tag{3.18}$$

The dynamics of the inactivation variable is given by equation 3.19 [19], a simple Michaelis-Menten equation, with K an equilibrium constant related to the concentration at which inactivation is halfway (μM).

$$h_{Ca^{2+}} = \frac{K}{K + [Ca^{2+}]} \tag{3.19}$$

Equation 3.20 [19] is the Nernst equation for calcium.

$$E_{Ca^{2+}} = 12.5 log \frac{[Ca^{2+}]_{ex}}{[Ca^{2+}]} \tag{3.20}$$

In this equation, $[Ca^{2+}]_{ex}$ is the constant extracellular calcium concentration (μM).

3.5 Cytosolic calcium level

As a result of the uptake models for all the involved proteins, including the GECO proteins, models for the relevant feedback regulation and models for the calcium channels, the main equation for the concentration of cytosolic calcium can be derived:

$$\begin{aligned}\frac{dCa(t)}{dt} = & \underbrace{\frac{V_x \cdot [Ca_{ex}]}{K_x + [Ca_{ex}]}}_{TransporterX} - \underbrace{Crz(t)\theta(\frac{1}{CaN(t)})\frac{V_1 \cdot Ca(t)}{K_1 + Ca(t)}}_{Pmc1} \\ & - \underbrace{Crz(t)\theta(\frac{1}{CaN(t)})\frac{V_2 \cdot Ca(t)}{K_2 + Ca(t)}}_{Pmr1} - \underbrace{\frac{1}{1 + k_c CaN(t)}\frac{V_3 \cdot Ca(t)}{K_3 + Ca(t)}}_{Vcx1} \\ & - \underbrace{n(k_{on} \cdot CaGECO(t) - k_{off} \cdot Ca(t)^n([CaGECO_{total}] - CaGECO(t)))}_{GECO} \\ & - \alpha Ca(t) \end{aligned} \tag{3.21}$$

The final model consists of six equations (Eqs. 3.4, 3.2, 3.12, 3.5, 3.21, 3.16) and six unknowns: $CaN(t)$, $CaM(t)$, $CaGECO(t)$, $Crz(t)$, $Ca(t)$ and $m(t)$.

3.6 Calcium Diffusion

After entering the cell, the calcium will be transported through the cell by diffusion. Since the cell is sphere-like, the diffusion equation will be formulated in spherical coordinates. When the tangential components are neglected, the three-dimensional equation is reduced to a one-dimensional problem, which can be found in equation 3.22 [19].

$$\frac{\partial r[Ca^{2+}]}{\partial t} = D\frac{\partial^2 r[Ca^{2+}]}{\partial r^2} \tag{3.22}$$

In this equation, D is the diffusion constant ($\mu m^2 s^{-1}$) and r is the radius of the cell (μm). Because of the dependence on both space and time and the complexity to model this, the calcium diffusion in the cell is neglected in our first attempt to model calcium dynamics in the cell. However, in order to reliably predict calcium dynamics in the cell, future studies should address the role of diffusion.