# A SYSTEM FOR MODELING THE COOPERATIVITY OF RYANODINE RECEPTORS IN CARDIAC MYOCYTES

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**Abstract: A computer system, based on cellular automata, which allows investigating various concepts of the cooperativity between RyR2 mediated by protein FKBP12.6, is presented. Cardiac ryanodine receptors (RyR2), located in endoplasmic reticulum, release the majority of calcium involved in contraction of cardiac myocytes. The RyR2 receptors form homotetrameric structure where each calcium channel is surrounded by four other identical channels. FKBP12.6 is an important regulatory protein which associates with RyR2 providing a link between adjacent RyR2 channels. Experimental evidence shows that FKBP12.6 affects open probability and mean open time of ryanodine receptors. Since the structure of the RyR2 protein is not known, the exact mechanism of the cooperativity between calcium channels and the role of FKBP12.6, has not been revealed. The action of the channels is simulated by Monte Carlo method based on the model proposed by Sobie et al. (S-J model) described by a system of ODEs. S-J model includes only global representation of the cooperativity, which has been replaced by cellular automata to allow modeling local interactions between adjacent RyR2 channels. Graphical representation of the results in the system includes calcium fluxes, RyR2 open probability, and spatio-temporal distribution of the calcium release events.**

## Introduction

Activation of cardiac myocytes is possible due to the rise of $Ca^{2+}$ levels [1]. The majority of calcium is released from sarcoplasmic reticulum (SR) through the calcium channels called ryanodine receptors (RyRs). The channels are located on the surface of the SR, forming clusters of 10-100 channels, in the vicinity of the T-tubules. RyRs are sensitive mostly to the calcium concentration, and their open probability rises at high levels of $Ca^{2+}$. L-type calcium channels (LCCs), called also DHPR channels, are situated on the T-tubules, each in front of the RyRs clusters. Calcium signal is triggered by depolarization of the myocyte plasma membrane, which opens voltage dependent LCCs, allowing calcium into the cell. When calcium flux gets into the region with RyRs, ryanodine receptors open and calcium stored in SR is released, travelling from network SR (NSR) through SR lumen and RyRs. This effect is called calcium-induced calcium release (CICR) [1]-[4]. Then, calcium accumulates in the region between SR and T-tubule. This region is called subspace (SS-SL) or diadic space; each cell has many SS-SLs. Part of the lumenal SR located close to RyRs is called junctional SR (JSR). The more RyRs open the higher calcium concentration in JSR, abruptly increasing the number of subsequently opening RyRs, by the positive feedback. CICR underlies the large, local increase in $Ca^{2+}$, which is called a $Ca^{2+}$ spark, and can be experimentally visualized by detecting the efflux from the loaded with calcium SS-SL to the myoplasm, where calcium binds with troponine C, leading to the myocyte contraction. When calcium concentration in SS-SL reaches 1 mM, its influx stops and sparks are terminated. A mechanism responsible for spark termination is unknown [2], [4].

Rianodine receptors form homotetrameric structures. Each RyR is surrounded by four neighbors, which are attached to its corners. There are three isoforms of RyR channels but RyR2 is the most abundant one in cardiac myocytes. On the cytoplasmic side of SR, RyR2s bind several proteins, e.g. binding proteins FKBP12.6 or calmoduline [5]. On the lumenal side of the SR membrane, RyRs directly or indirectly interact with calsequestrin, triadin and junction. Several authors ([6]-[10]) report that FKBP12.6 protein, which is present at the contact sites of adjacent RyRs, may couple channel gating. Since the RyR structure is not known this hypothesis cannot be fully verified.

Disruption in the mechanism of $Ca^{2+}$ circulation by non-physiological behavior of RyR channels may affect cardiac rhytmicity. For example, it was reported [5] that catecholaminergic polymorphic ventricular tachycardia is caused by mutation localized in the region 1q42-q43 of the first chromosome, coding RyR2.

In this paper, the system modeling action of the RyR channels is presented. The objective of this work is to present a tool for modeling cooperativity between RyRs that will allow to define a formula of coupled gating, which is not available by experimental methods yet. RyR activity is simulated by Monte Carlo method, based on the model proposed by Sobie et al. [2](S-J model) formulated by a system of ODEs. S-J model includes only global representation of the cooperativity, which has been replaced by cellular automata to allow modeling local interactions between adjacent RyR2 channels.


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Cellular automata, introduced by Neumann [11], are mathematical objects defined on the lattice *n* x *n*. Each cell can assume a value from a set of acceptable states; classically it is {0,1}. In each step cells change their states according to the states of their neighbors and to the applied rule of evolution. Spatial layout of exemplary cellular automata is presented in Fig.1. "Life" are classical cellular automata in which each cell, surrounded by 8 neighbors, can assume a value 0 ("dead") or 1 ("alive"). The evolution rule states that a cell survives as long as 2 or 3 neighbors are alive, and the cell can be born if exactly 3 neighbors are alive.

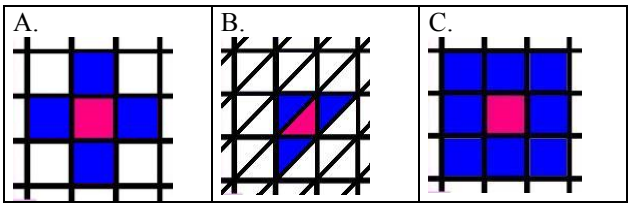


Figure 1: Classical neighborhoods in cellular automata A, B – von Neumann, C – Moore (A, C – rectangular network, B – triangular network).

There are several types of cellular automata. They are usually applied for modeling systems which can be formally described by differential equations. Cellular automata are used to solve problems, in heuristic manner, to avoid errors occurring in numerical solutions of complex non-linear differential equations.

The system presented in this work was tested to verify the hypothesis of the coupled gating of the RyR channels. First, a simple "survival" model of the cooperativity was implemented. Various calcium fluxes, RyR2 open probability, and spatio-temporal distribution of the calcium release events can be obtained in a nice graphical and user-friendly front-end and verified with the experimental characteristics.

**Materials and Methods**

General features of calcium release were modeled based on the system of ordinary differential equations introduced by Sobie et al. [2]. Calcium concentration in subspace is given by

$$\frac{d[Ca^{2+}]_{SS}}{dt} = J_{release} + J_{DHPR} - J_{efflux} - J_{buf}, \quad (1)$$

where $J_{release}$ is a total $Ca^{2+}$ flux through RyR2 cluster, $J_{DHPR}$ is $Ca^{2+}$ flux through L-type calcium channel, $J_{efflux}$ is $Ca^{2+}$ difussion from SS-SL to the myoplasm, $J_{buf}$ represents buffering of $Ca^{2+}$ in SS-SL. $J_{DHPR}$ = 0 if L-channel is closed or described by Eq.2 otherwise

$$J_{DHPR} = -\frac{\bar{I}_{DHPR}}{2FV_{SS}}. \quad (2)$$

$\bar{I}_{DHPR}$ is a current from single L-type channel, $F$ is a Faraday constant, and $V_{SS}$ is a volume of SS-SL.

$Ca^{2+}$ flux through a single RyR channel depends on the concentration gradient between SS-SL and the SR lumen

$$J_{RyR} = D_{RyR}([Ca^{2+}]_{lumen} - [Ca^{2+}]_{SS}). \quad (3)$$

$D_{RyR}$ is diffusion constant through an open RyR.

Total calcium release from all $N$ open RyRs is defined by

$$J_{release} = \sum_{i=1}^{N} RyR^{i}_{open} J_{RyR}, \quad (4)$$

where $RyR_{open}$ equals 0 or 1 depending on the state of the channel.

Calcium is partly buffered in SS-SL by sarcolemmal membrane buffers *SL*, sarcoplasmic reticulum buffers *SR*, and calmoduline *CaM*. Flux decrease due to the buffering is given by

$$J_{buf} = \sum_{i=CaM,SR,SL} k^{i}_{off}([B_i]_{tot} - [B_i]) - k^{i}_{on}[Ca^{2+}]_{SS}[B_i], \quad (5)$$

where $k^{i}_{off}$ and $k^{i}_{on}$ represent rates of dissociation and association of each buffer, respectively. $[B_i]_{tot}$ is a total buffer concentration.

Efflux from the subspace to the bulk myoplasm results from the concentration gradient

$$J_{efflux} = \frac{[Ca^{2+}]_{SS} - [Ca^{2+}]_{myo}}{\tau_{efflux}}, \quad (6)$$

where $\tau_{efflux}$ is a diffusion time constant.

Calcium concentration in lumen depends on the total calcium release $J_{release}$ (Eq. 4) and refilling diffusion $J_{refill}$, from SR tubules into SR lumen

$$\frac{d[Ca^{2+}]_{lumen}}{dt} = \beta_{JSR}(-J_{rel}\frac{V_{SS}}{V_{JSR}} + J_{refil}), \quad (7)$$

$\beta_{JSR}$ represents buffering of the calcium by calsequestrin.

$$J_{refill} = \frac{[Ca^{2+}]_{NSR} - [Ca^{2+}]_{lumen}}{\tau_{refil}}, \quad (8)$$

$\tau_{refil}$ is a diffusion time constant (between NSR and lumen).

Calsequestrin buffering is calculated from rapid buffering approximation [12]

$$\beta_{JSR} = \left[1 + \frac{[B_{CSQ}]_{tot} K_{CSQ}}{(K_{CSQ} + [Ca^{2+}]_{lumen})^2}\right]^{-1}, \quad (7)$$

where $[B_{CSQ}]_{tot}$ is total calsequestrin concentration, $K_{CSQ}$ is a calsequestrin dissociation constant.


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Dynamics of RyR2 channel is modeled as a two-state Markov process. Probablity $P_c$ of closed RyR, and open probability $P_o$ are given as follows

$$\frac{dP_C(t)}{dt} = k_{close} \cdot P_O(t) - k_{open} \cdot P_C(t)$$

$$P_o(t) = 1 - P_c(t), \qquad (10)$$

$k_{close}$ is a transition rate from open to closed state of the channel in the Markov process, $k_{open}$ is a transition rate for the reverse direction, $\Delta t$ is a time step.

In the revised model the rate $k_{close}$ is assumed as independent of the subspace concentration $[Ca^{2+}]_{ss}$ and constant (Table 1). The rate $k_{open}$ depends on the concentration and the equation modeling experimental results yields

$$k_{open} = Cof \cdot CF_{open} \frac{[Ca^{2+}]_{SS}^4}{[Ca^{2+}]_{SS}^4 + K_m^4}[s^{-1}], \qquad (11)$$

Cooperativity factor *Cof* is a variable which, in the revised model, corresponds to dissociation of FKBP12.6. Sensitivity of RyR to $[Ca^{2+}]_{ss}$, represented by $K_m$, is a linearly decreasing function of calcium concentration in lumen

$$K_m = 6.0 - 0.0024[Ca^{2+}]_{lumen}\,[\mu M]. \qquad (12)$$

The averaged coupled gating of RyRs from S-J model, which has an influence on $k_{open}$ and $k_{close}$,, was replaced by cellular automata, in which four neighbors modeling homotetrameric structure of RyR, form von Neuman neighborhood. The cells can assume a value 0 or 1 depending on the rule defined by the system user. Therefore, for each *i*-th channel, $CF_{open}$ assumes an individual value $life^i$ affecting subsequent behavior of the channel.

$$CF_{open} = life^i, \qquad (13)$$

where *life* equals 0 or 1 depending upon the hypothesized cooperativity rule and states of the channel neighbors.

The cooperativity rule is a logical statement, which can be defined by the system user. It takes a form similar to the exemplary rule presented below

$$S_i(t+1) = (S_i(t)\ \&\ No(t) <= Nos) \,|\, (\sim S_i(t)\ \&\ No(t) <= Noo), \qquad (14)$$

where $S_i(t)$ is a state of the RyR channel (0 or 1) at time instant *t*, $No(t)$ is a number of open neighbors, *Nos* and *Noo* define numbers of the RyR's open neighbors necessary for the RyR to: survive open till time $t$+1 provided the channel is open at time *t*, and switch to open state at $t$+1 if it was closed at time *t*, respectively.

Constant parameters of the model are given in Tab.1.

Table 1: Model parameters

| Parameter | Definition | Value |
|---|---|---|
| $\bar{I}_{DHPR}$ | Single L-channel $Ca^{2+}$ current | -0.5 pA |
| $F$ | Faraday constant | 96480 C·mol$^{-1}$ |
| $D_{RyR}$ | $Ca^{2+}$ diffusion constant through an open RyR | 4000 s-1 |
| $V_{SS}$ | SS-SL Volume | $1 \cdot 10^{-13}$ µL |
| $V_{JSR}$ | JSR Volume | $0.3 \cdot 10^{-11}$ µL |
| $[Ca^{2+}]_{myo}$ | $Ca^{2+}$ in myoplasm | 0.1 µM |
| $[Ca^{2+}]_{NSR}$ | $Ca^{2+}$ in NSR | 1000 µM |
| $[B_{CaM}]_{tot}$ | Total calmoduline | 24 µM |
| $[B_{SR}]_{tot}$ | Total SR buffer | 47 µM |
| $[B_{SL}]_{tot}$ | Total SL buffer | 1124 µM |
| $[B_{CSQ}]_{tot}$ | Total calsequestrin | 20 mM |
| $K_{CSQ}$ | Calsequestrin dissociation constant | 0.8 mM |
| $k_{on}^{CaM}$ | Calmoduline association constant | 100µM$^{-1}$s$^{-1}$ |
| $k_{on}^{SR}$ | SR buffer association constant | 115 µM$^{-1}$s$^{-1}$ |
| $k_{on}^{SL}$ | SL buffer association constant | 115 µM$^{-1}$s-1 |
| $k_{off}^{CaM}$ | $Ca^{2+}$-CaM dissociation rate | 38 s$^{-1}$ |
| $k_{off}^{SR}$ | $Ca^{2+}$-SR dissociation rate | 100 s$^{-1}$ |
| $k_{off}^{SL}$ | $Ca^{2+}$-SL dissociation rate | 1000 s$^{-1}$ |
| $\tau_{refil}$ | Time constant for $Ca^{2+}$ diffusion from NSR to lumen | 0.01 s |
| $\tau_{efflux}$ | Time constant for $Ca^{2+}$ diffusion from SS-SL | $7 \cdot 10^{-7}$ s |
| $k_{close}$ | RyR open → closed transition rate | 480 s$^{-1}$ |

The software was created in MATLAB 7.0. To run the model, MATLAB 6.5 or higher version is needed. Typing the command *RyR2sim* in the Matlab Command Window will start modeling RyR2.


ISSN: 1727-1983 

## Results

The main window of the RyR model (Fig. 2) allows starting simulations with default parameters, by choosing the "Start". Changing parameters can be performed by clicking "Options", which opens the window presented in Fig.3.

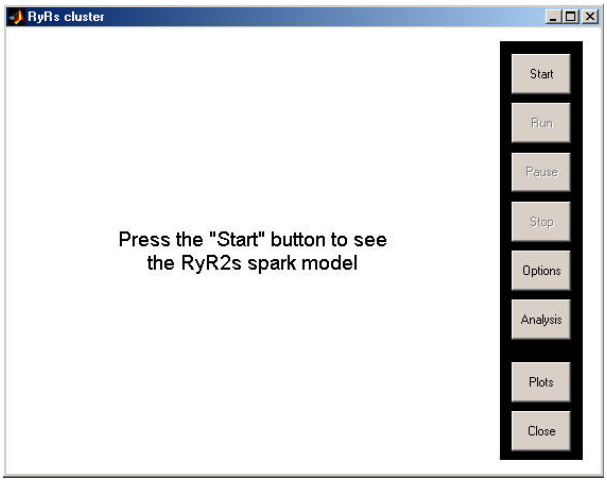


Figure 2: Main panel of the program.

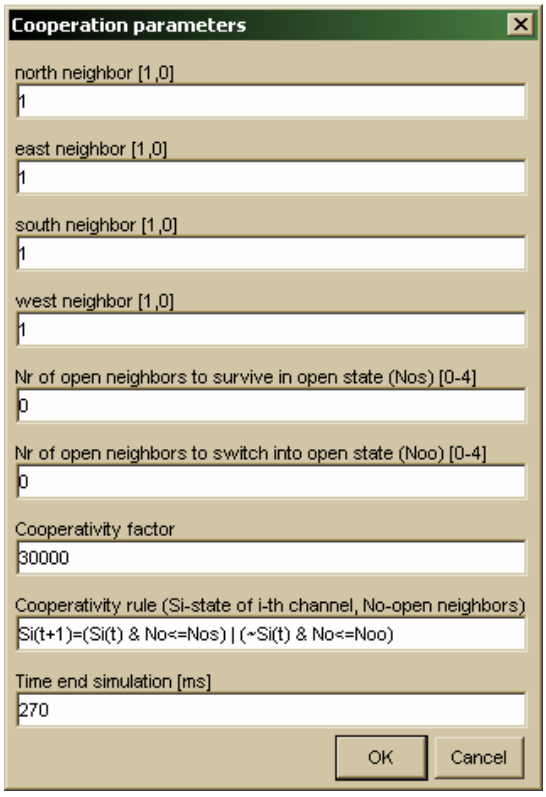


Figure 3: Panel opened from the main window by choosing "Options". Cooperativity rule and other simulation parameters can be defined here.

The rule of evolution for the cellular automata can be defined as presented in Fig. 4.

When simulations are started opening and closing of the RyR channels is displayed, as diamonds switch between grey and red colors (Fig. 5).

(A)

(B)

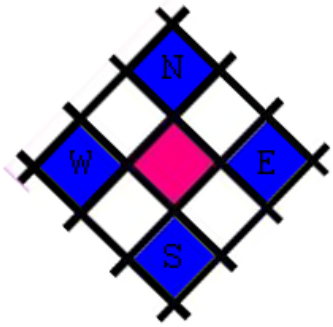


Figure 4: (A) RyR channels and their representation by cellular automata, (B) notation applicable for the cooperativity rule

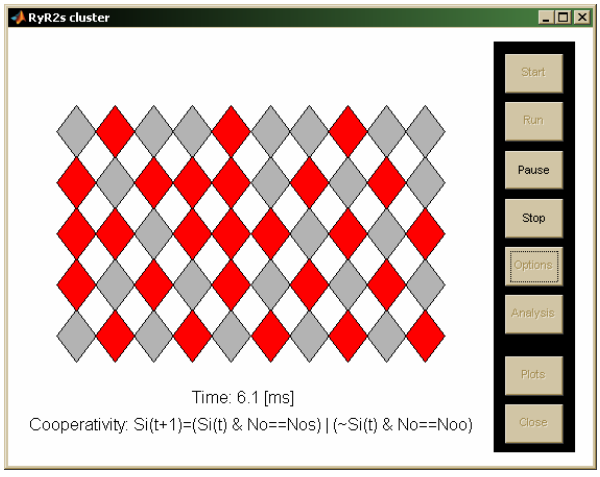


Figure 5: Simulation is running. Red diamonds indicate open RyRs, grey – closed channels.

The simulations can be finished or suspended by choosing "Stop" or "Pause", respectively. The program will ask the user for the file name where data are to be stored. When simulations are complete, the window presented in Fig. 6 will be displayed.


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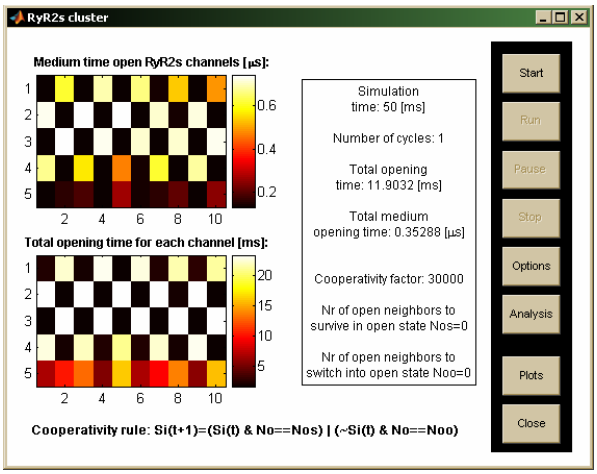


Figure 6: Main panel of the program when simulations are completed and data saved. The charts show statistics of RyRs

Choosing "Plots" from the main window allows plotting the characteristics obtained from the model, an exemplary characteristics is presented in Fig. 7. The characteristics can be plotted for data stored previously by choosing the option "Open" in the window "Plots".

The list of available characteristics (Fig. 8) contains:

| | |
|---|---|
| Calum(t) | lumenal calcium, $[Ca^{2+}]_{lumen}$, |
| Cass(t) | subspace calcium, $[Ca^{2+}]_{SS}$, |
| Open(t) | open probability of RyR, $P_o(t)$, |
| Jbuf(t) | buffered $Ca^{2+}$, $J_{buf}$ |
| Iefflux(t) | $Ca^{2+}$ diffusion current from SS-SL to myoplasm, $I_{efflux}$ |
| Jrel(t) | $Ca^{2+}$ diffusion through RyR2 cluster, $J_{release}$, |
| Jref(t) | $Ca^{2+}$ diffusion from SR network into SR lumen, $J_{refill}$, |
| Bcam(t) | concentration of free calmoduline, $[B_{cam}]$ |
| Bsr(t) | free SR buffer, $[B_{sr}]$ |
| Bsl(t) | free SL buffer, $[B_{sl}]$ |
| Percent | percent of open RyR2, |
| Tot. time | total open time of a single RyR2, |
| Med. time | mean open time of a single RyR2. |

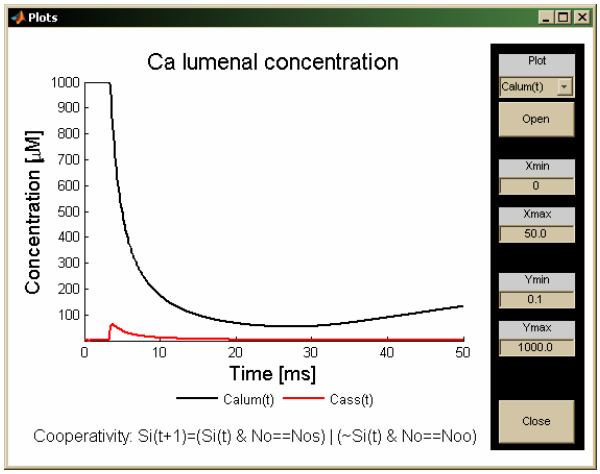


Figure 7: Panel in which all generated characteristics can be visualized


Figure 8: The list of available characteristics

Finally, a detailed spatio-temporal analysis with the original time resolution of 0.1 μs can be reproduced (Fig.9) based on the data stored previously on the disk. Choosing "Analysis" from the main window and defining a new time range in the window "Advanced analysis" will start this action.

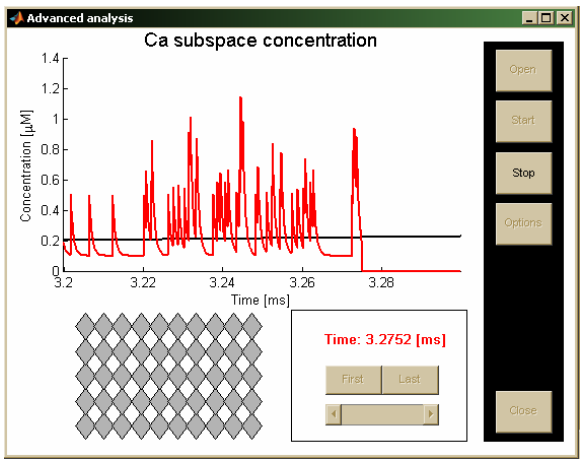


Figure 9: Panel "Advanced analysis" which allows reproducing results with microsecond resolution in an arbitrary range, based on simulations previously stored on the disk.

## Discussion

The computer system allows for studying possible interactions between adjacent ryanodine receptors, modeling the activity of the protein FKBP12.6. Parameters of the hypothetical activity of the protein and possible mathematical description of the cooperativity can be defined and tested. Preliminary


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model experiments, performed by the system, show sensitivity of the calcium release to different modes of the cooperativity. For example, we observed a decrease of calcium spark amplitude when cooperativity was present, consistent with experiments on FKBP12.6 [5]. The model experiments may allow establishing relationship between the rate of amplitude change and the numbers of open neighbors needed for the channel to maintain open, which is not available experimentally, yet. Open probability of the channels, similarly as other variables of the system, proved sensitive to the cooperativity mode. The strength of the cooperativity, represented by *Cof*, can mimick a partial dissociation of FKBP12.6. Preliminary model experiments, performed for 270 ms with the time step 0.1 μs, show an agreement with experiments on partial dissociation. A very interesting feature of the system is its ability to investigate spatio-temporal patterns of the calcium release, which allowed us to observe non-homogenous characteristics of the channels and clustering patterns of their behavior.

## Conclusions

The experiments performed on clusters of ryanodine receptors by means of our modeling system support the hypothesis of the coupled gating of the channels. Simple "survival" model of the cooperativity is capable of producing results that are in line with experimental data, adding information about spatial properties of the calcium release, which is not available experimentally. There is a possibility of extending the simple automata model by increasing their sensitivity to other parameters or using different class of automata. As a result, the system may be capable of reproducing all fine details of experimental data, revealing the mechanisms of the RyR cooperativity.

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