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Method Article

Methodology for development of single cell dendritic spine (SCDS) synaptic tagging and capture model using Virtual Cell (VCell)☆

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ABSTRACT

Single cell dendritic spine modelling methodology has been adopted to explain structural plasticity and respective change in the neuronal volume previously. However, the single cell dendrite methodology has not been employed previously to explain one of the important aspects of memory allocation i.e., Synaptic tagging and Capture (STC) hypothesis. It is difficult to relate the physical properties of STC pathways to structural changes and synaptic strength. We create a mathematical model based on earlier reported synaptic tagging networks. We built the model using Virtual Cell (VCell) software and used it to interpret experimental data and investigate the behavior and characteristics of known Synaptic tagging candidates.

- We investigate processes associated with synaptic tagging candidates and compare them to the assumptions based on the STC hypothesis.
- We assess the behavior of several reported synaptic tagging candidates against the requirements outlined in the synaptic tagging hypothesis.

Specifications table

Subject area:	Bioinformatics
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Name of your method:	*Single cell dendritic spine (SCDS) STC-model*
Name and reference of original method:	*Rangamani, P., Levy, M. G., Khan, S., & Oster, G. (2016). Paradoxical signaling regulates structural plasticity in dendritic spines. Proceedings of the National Academy of Sciences, 113(36), E5298-E5307.*
Resource availability:	www.vcell.org *(software resource)* https://drive.google.com/drive/folders/1ORCvvjLW_BhRipzZnlknBKiEZYpK7bni?usp=sharing *(SBML Code resource)*

Background

According to the Synaptic Tagging and Capture (STC) theory, long-term potentiation (LTP) is maintained by the coordination of two associated but independent neuronal pathways [20]. When LTP or long-term depression (LTD) is induced, a time-limited and input-specific synaptic tag is formed. When the initial stimulation is modest enough to initiate protein synthesis (weak stimulus), a

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transitory and brief form of plasticity is exhibited. Protein synthesis is activated in response to intense stimulation (strong stimulus); this procedure resulted in the capture of plasticity-related proteins (PRPs) in activated synapses and aids in the maintenance of plasticity. Late-LTP (Late long-term potentiation) will be maintained by weakly tetanized synapses in area CA1 of the hippocampus that typically displays long-term potentiation lasting 3 h if the weak stimulation takes place just before or after strong stimulation of an independent but convergent set of synapses in CA1. The synaptic tagging and capture theory explains this heterosynaptic impact on persistence by distinguishing between local synaptic tagging processes and cell-wide mechanisms involved in the synthesis, distribution, and capture of plasticity-related proteins (PRPs).Transient versions of LTP (or LTD) and synaptic tagging occur in the absence of protein synthesis or are resistant to protein synthesis inhibitors [14]. Only long-term types of synaptic plasticity rely on PRP capture [13].

Considering that only strong stimulation promotes protein synthesis, one might conclude that there exists a threshold level for translation and, most likely, transcription that specifies the maintenance of synaptic alterations. At contrast, weak stimulation may also trigger a modest amount of translation, and the supply of PRPs is insufficient for LTP stabilization in activated synapses [5]. It is still unknown if the translation trigger is discrete or continuous. It is intriguing to investigate the implications and experimental pattern that led to the STC hypothesis [6].

Model development

We create a model for a single dendritic spine to investigate synaptic tagging possibilities based on five fundamental synaptic tag requirements. Our STC model investigates the important chemical networks that underpin the STC as well as the crucial properties of a synaptic tag. We create a mathematical model based on experimental findings and observations to portray a molecular network of critical STC pathways. We include in the model important memory molecules that have previously been deemed to be essential for STC pathways in previous research [2,12].

We perform computational experiments to the test and assess the characteristics of the proteins that are involved in the STC process. Postsynaptic electrical stimulation inputs representing Ca^{2+} influx via open NMDARs channels excite the STC model. CaMKII activation, the Cofilin-F-actin pathway, PKA activation, and the ERK pathway are all triggered by Ca^{2+} influx.

To investigate the impacts of Ca^{2+} dynamics on the single dendritic spine and make the simulation more robust, we developed certain assumptions about the STC mechanism and included them into our STC model. The following are the most significant and critical model assumptions:

1. We consider that all biochemical response networks in CA1, a hippocampus area of the brain, are relevant for a single dendritic spine. A single dendritic spine in CA1, contains the whole network of metabolic events. We built a dynamic deterministic model based on the premise that the cellular components are available in adequate quantity and with known parameters to characterize the quantities of distinct molecular species. The kinetic model parameters included in the models are derived from previously published and validated experimental research [10,15,22].
2. We assume the single dendritic spine in the model to be a well-mixed compartment to observe the successive increase of the concentrations of the individual components. To represent each biochemical interaction, we employ Michaelis-Menten kinetics for enzyme-catalyzed events or mass action kinetics for association and dissociation processes. Using the Virtual Cell modelling platform, we build the whole reaction network (http://www.vcell.org) [21].
3. We disregard the CaMKII subunits' switching dynamics between autoinhibited states. The reason being that the CaMKII isoforms have different switching rates, which are not taken into consideration by the STC model. In contrast to the gradual binding between both the CaMKII and Ca2+-CaM complex, which includes targeting and spatial mobility, we assume that switching of the CaMKII subunits quickly reaches a constant ratio. As a result, the constant ratio may be reflected by the binding rate between the CaMKII subunits and the Ca2+-CaM complex.

Modelling platform: the virtual cell (VCell)

Virtual Cell (VCell) is an open-source, web-based modelling, and simulation programme [21]. It is available for free download at http://vcell.org/. The VCell project is based on a remote computational system that allows for model construction and analysis. Users may run simulations and solve mathematical equations. Computational biologists may easily access the VCell software suite [17]. The programme offers users how to understand and arrange the physical and numerical functions associated with constructing models and generating outcomes from them. Clients can use VCell to collaborate, communicate, share, and obtain outside resources. Furthermore, user-server execution allows clients to conduct large and complex simulations without having to access their high-performance computing equipment, contemporary mathematical instruments, and/or programming libraries. Furthermore, VCell allows users to create models and simulate metabolic processes, electrophysiology, and membrane transport.

Because VCell includes a 3D capability (which allows to examine the environment in 3D), it allows researchers to combine the actual exploratory geometries. As a result, the effects of flow and diffusion may be unambiguously combined into models, and simulations can yield solutions to the corresponding partial differential equations. In the VCell, an easy graphical interface integrates options for database access, compartment topology determination, geometry specification, chemical reaction input, species definitions, initial conditions, transport components, and computational boundaries. In addition to the graphical model structure interface, VCell features a numerical interface that allows scientists to study and expand models using solely numerical information. Using Virtual Cell Mathematics Description Language, it considers the immediate section of numerical circumstances that depict a model

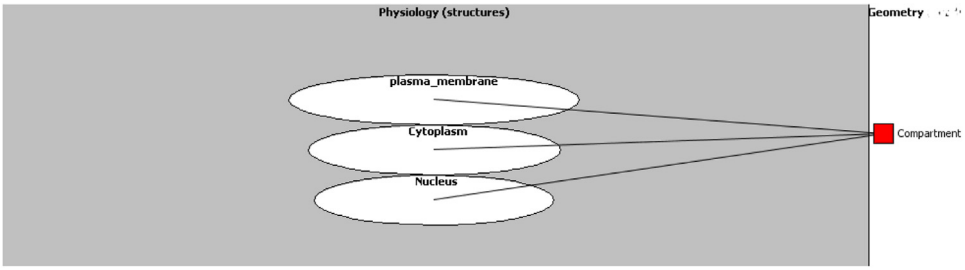

Fig. 1. Single dendritic postsynaptic neuron structural mapping in Virtual Cell software. There are three compartments, including the plasma membrane, cytoplasm, and nucleus, represented in the physiology section.

Table 1
Model geometry, pre-defined functions, and constants

Description	Symbol	Value	Refs.
Plasma membrane	plasma_membrane	1414 μm3	[15]
Nucleus	Nucleus	113.09 μm3	[1]
Cytoplasm	Cytoplasm	500 μm3	[15]

(VCMDL). The computed expression is transformed into C++ code, which is then transmitted to the numerical solvers. To summarize, researchers are not required to build programming code for each new showing activity. Furthermore, the VCMDL language of a given model may be generated from a model created within the graphical biological interface. This dual interface allows modelling and experimental researchers to engage and collaborate.

Another significant and original feature of the VCell programme is the depiction of three generalization boundaries in the modelling scheme. These limits follow a pattern like that of an experimental investigation. Fig. 1 depicts the organization of the modelling process within the VCell programme. The "Physiology" is the first tiered boundary-like structure. This border includes the representation of molecular species and hypothetical interactions restricted to cell structures. In VCell, cell structures are defined as membrane-bounded compartments and topological configurations of membranes. VCell identifies biochemical reactions in the cell's volumetric compartments or in membranes.

The biochemical and biophysical aspects of these interactions are captured as rate laws for responses and transitions by the physiology boundary in VCell. As a result, mass action and Michaelis-Menten rate laws are available, as are discretionary user-defined generic dynamic expressions [9]. Membrane transport kinetics can be estimated using molecular transition expressions or the electric current for ions. Transport kinetics can be represented as either basic electrophysiological principles or user-defined chemical flow [7].

Model organization in VCell

Construction of single cell dendritic spine (SCDS) STC – model in VCell

A cellular physiological model is a collection of cell structures, species, relationships, and diffusivity [3]. These principles are sufficient in terms of cell structure to develop non-spatial, compartmental models of biological pathways. The purpose is to depict physiology regardless of spatial domain (cell structure and scale) or distinctive experimental context, ensuring that the following biochemical processes are flexible and may be implemented into other models with minimal alterations.

Specifying cellular structure and geometry

The SCDS STC – model comprises three mutually exclusive compartments (nucleus, cytoplasm, and plasma membrane) within single dendritic cell. The compartments are 3-D volumetric spaces that are divided by membranes, which are two-dimensional surfaces. Every structure can contain molecular species and a series of reactions that characterize the biological activity of those species [11].

To describe the morphological characteristics of a single cell dendritic spine, multiple features that span several orders of magnitude in scale must be included [16]. Various imaging modalities, such as wide field, confocal, or electron microscopy, can capture the geometry. Our model incorporates tiny structures such as intracellular proteins, kinases, phosphatases, and other enzymes that rely on the capacity to resolve such anatomical structures experimentally and the spatial scale of interest.

In the model, we assume that the volume of the plasma membrane is- 1414 μm3 (Table 1). In contrast, cytoplasm and nucleus volume is considered- 500 μm3 and- 113.09 μm3, respectively. This assumption is based on previous single dendritic spine studies [15]. The values of pre-defined constants and functions are listed in Table 1.

Specifying molecular species

The second and most crucial stage is to identify and establish molecular species for the three compartments stated above. The molecular species reflect any different state required to characterize the cell's biochemistry, such as molecular conformation or receptor bound vs receptor unbound. Multiple compartments, membranes, or filaments can be used to define the species. In the model we included a total of 83 molecular species in the model which includes 73 species in the cytoplasm and 10 in the nucleus. The species can engage in reactions, fluxes, and diffusion and are represented by concentration and diffusion constants. Diffusion constants are used to determine species diffusion for each cellular compartment or membrane.

Specifying biochemical reactions and reaction kinetics

The biochemical reactions explain reaction kinetics and stoichiometry in detail. The model's reactions are either a set of linked reaction mechanism that occur at or near a single cell structure or transmembrane fluxes. Each reaction step in the model correlates to a particular cellular structure. The stoichiometry of a step is defined by products, catalysts, and reactants, all of which are related to species in a certain cellular structure [19].

Reaction steps in a compartment only contain the species found within that compartment, but reaction steps on a membrane include both the species on the membrane and the species found in the compartments next to the membrane. In the model, the reaction rates and fluxes are quantitatively represented by arbitrary algebraic formulae in terms of parameters and species concentrations [18].

The reaction kinetics include general, mass action, and Henri Michaelis – Menten (reversible and irreversible) kinetics. Depending on the nature, previous research, and condition of the reactions, we assign reactions kinetics to each biochemical reaction. We added a parameter to each equation and defined the parameter's expression, which must be spatially independent. When defined under initial conditions in the Virtual Cell's application component, an expression can have spatially dependent variables. Depending on the type of reaction and condition, the parameter may be a variable, a constant, or a mixture of both. Our model contains 61 biochemical reactions including 9 reactions based on mass action, 22 reactions based on Henri – Michaelis – Menten (all reversible), and 30 reactions based on general kinetics. All 6 reactions in the nucleus compartment assigned general kinetics. The mass action kinetics in VCell automatically prompted to provide forward (K_f) and reverse rate (K_r) constants for the relevant species, whereas, for Michaelis – Menten reactions we provide Vmax (Maximum velocity/rate of the reaction) and Km (substrate concentration). The values for kinetic parameters and rate constants can be accessed from Khan et al. recently published research [10].

Specifications and protocols for SCDS STC – model

For the model specification and establishment of experimental protocols it is necessary that geometry of the compartments is properly mapped to the physiology. We use the application component of the VCell software to accomplish this. The application component consists of structure mapping, initial conditions, reaction mapping, electrical mapping, and simulations. For our model we only used structural mapping, reaction mapping and simulation application to establish computational protocols. The initial concentrations and conditions for the species can be accessed from Khan et al. research published recently in 2022 [10].

We set the concentrations to be fixed and confine the concentration of a variable or variables to a certain region or regions within the model. We use following equation to represent initial Ca^{2+} with time dependent component:

$$\begin{pmatrix}((12.0 * (t > 6.0))\&\&(t < 8.0)) + ((12.0 * (t > 10.0))\&\&(t < 12.0)) + ((12.0 * (t > 14.0))\&\&(t < 16.0)) \\ +((12.0 * (t > 18.0))\&\&(t < 20.0))\end{pmatrix} \quad (1)$$

This describes the calcium influx is variable and time-dependent i.e., influx of 12 μM of Ca^{2+} concentration between 6 and 8 s, 10–12 s, 14–16 s, and 18–20 s from the start of simulation.

Similarly, we express the initial concentration as an equation for the following species in the model:

$$\mathrm{MAPK\_P} = (\mathrm{MAPK\_Total} - \mathrm{MAPK} - \mathrm{MAPK\_PP}) \quad (2)$$

$$\mathrm{MAPKK\_P} = (\mathrm{MAPKK\_Total} - \mathrm{MAPKK} - \mathrm{MAPKK\_PP}) \quad (3)$$

$$\mathrm{MAPK_s\_P} = (\mathrm{MAPK\_Total} - \mathrm{MAPKs} - \mathrm{MAPKs\_PP}) \quad (4)$$

$$\mathrm{AC_0} = (\mathrm{AC\_Total} - \mathrm{AC4} - \mathrm{AC3} - \mathrm{AC2} - \mathrm{AC1}) \quad (5)$$

$$\mathrm{Raf\_P} = (\mathrm{Raf\_Total} - \mathrm{Raf}) \quad (6)$$

As part of reaction mapping, we considered fast kinetics as an option, because by choosing the option we can designate the subset of reactions that have fast kinetics, and the fast system will be constructed and incorporated into mathematical description automatically.

Table 2
Simulation protocol for the model.

Maximum Timestep (sec)	Output (sec)	Relative Tolerance	Absolute Tolerance	Sensitivity Analysis
1.0×10^{-4}	every 1 sec	1.0×10^{-9}	1.0×10^{-9}	No

Model simulations

Once Initial conditions and reaction mapping is completed, we establish simulation protocol as per the STC computational experimental requirements. We chose combined IDA/CVODE (implicit differential algebraic/C-language variable coefficient ordinary differential equation) solver. The kinetics of the processes are represented by ordinary differential equations (ODE) for single point approximations, which are then sent to an interpreted ODE solver. This equation system is solved with one of many integration strategies. In most cases, the set of nonlinear ODE equations may be solved in seconds. It enables interactive parameter adjustment as well as rapid estimation of the effect over time.

The compartmental simulation runs a single point calculation based on the physiological model and geometric parameters, surface to volume ratios, and volume fractions. We ran the simulation for 1200 seconds with the settings given in Table 2.

The simulation is obtained through the "Native simulation results" and correlation results can be obtained by selecting two more variables. We export the simulation results and perform analysis on our model.

Our findings show the early establishment and degradation of a synaptic tag in a single dendritic spine in response to Ca^{+2} activation. The SCDS STC - model consists of three steps: (1) Kinase activity in response to first stimulation (CaMKII, PKA, MAPK/ERK); (2) initial tag setup and its influence on total TAG strength; and (3) induction of L-LTP with protein synthesis. We utilise the model to study the nature of each tagging candidate (Tag-1, Tag-2, Tag-3, and Tag-4) in relation to the synaptic tagging hypothesis's requirements.

Conclusion

Here we presented methodology for SCDS STC – model, an open-accessible model built-in VCell, that aims to ease the analysis of synaptic tagging and capture hypothesis. It provides a starting point, model physiology, reaction mapping, model compartmentalisation, ODE solvers and applications in VCell. We provide an open-source code that can easily be used in the form of System biology markup language (SBML) [8].

For our model, VCell software creates a reaction network based on the least possible set of reaction rules. It is incredibly useful here because the SCDS STC – model include few reaction categories which are replicated many times for each of the different cell structures. Our model in VCell catches the biochemical and biophysical properties of interactions as rate laws for responses and transitions [4]. Consequently, mass action and Michaelis-Menten rate laws can be used, as well as optional user-defined generic dynamic expressions. Membrane transport kinetics can be estimated using molecular transition articulations or, for ions, the electric current. Transport kinetics can be represented as either basic electrophysiological principles or as researcher-defined chemical flow.

Ethics statements

MethodsX has ethical guidelines that all authors must comply with. In addition, we ask you to complete the relevant statement(s) below. Please delete those which are not relevant to your work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Raheel Khan: Conceptualization, Methodology, Software, Writing – original draft. **D. Kulasiri:** Conceptualization, Methodology, Software, Writing – original draft. **S. Samarasinghe:** Supervision.

Data availability

No data was used for the research described in the article.

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