Published in final edited form as:

Sci Signal. ; 4(192): tr12. doi:10.1126/scisignal.2001970.

Developing models in Virtual Cell

Susana R. Neves*

Department of Pharmacology and Systems Therapeutics and Systems Biology Center New York, Mount Sinai School of Medicine, New York, NY 10029, USA

Abstract

This Teaching Resource provides lecture notes, slides, and a student assignment for a two-part lecture on mathematical modeling using the Virtual Cell environment. The lectures discuss the steps involved in developing and running simulations using Virtual Cell, with particular focus on spatial partial differential equation models. We discuss how to construct both ordinary differential equation models, in which the cytoplasm is considered a well-mixed cellular compartment, and partial differential equation models, which calculate how chemical species change as a function of both time and location. The Virtual Cell environment is especially well suited for models that explore spatial specificity of cellular reactions. Partial differential equation models in Virtual Cell can give rise to simulations using predefined cellular geometries, which enable direct comparison with imaging data. These models address questions regarding the regulatory capability arising from spatial organization of the cell. Examples are provided of studies that have successfully exploited the Virtual Cell software to address the spatial contribution to signaling.

Copyright 2008 by the American Association for the Advancement of Science; all rights reserved.

*Corresponding author. susana.neves@mssm.edu.

Educational Details

Learning Resource Type: Lecture, assignment, digital presentation

Context: Graduate

Intended Users: Teacher, learner

Intended Educational Use: Learn, plan, teach

Discipline: Biochemistry, cell biology, education, enzymology

Technical Details

Software: VCell Software

Requirements: Platform independent

Web site: http://www.nrcam.uchc.edu/vcell_software/login.html

Format: PowerPoint (.ppt)

Size: 5.43 MB

Requirements: Microsoft PowerPoint

Format: PDF (.pdf)

Requirements: Adobe PDF Reader

Limits for Use

Cost: Free

Rights: This material may be downloaded for noncommercial, course-teaching purposes only, provided credit to *Science Signaling* is included by listing the citation for the Teaching Resource.

Supplementary Materials

<http://stke.sciencemag.org/cgi/content/full/sigtrans;4/192/tr12/DC1>

Slides. Developing Models in Virtual Cell. Answer key. A PDF of the answers is available upon request.

Citation: S. R. Neves, Developing models in Virtual Cell. *Sci. Signal*. **4**, tr12 (2011).

Keywords

Dynamical systems; mathematical modeling; differential equations; protein kinase signaling; Virtual Cell; simulation

Summary of Lecture

As mathematical modeling becomes an increasingly important method for quantitative insight into cell biology and physiology, software packages that simplify this process become valuable tools. These lectures introduce students to such a tool, the Virtual Cell environment, developed by Les Loew and colleagues at the University of Connecticut Health Center over the past decade (Slides 1 to 3) (1–8). The lectures focus specifically on the use of Virtual Cell (VCell) to study the spatial regulation of cellular biochemistry. VCell is constantly improved, and the most recent versions of manuals and tutorials are available at http://vcell.org/vcell_software/user_guide.html.

VCell is a modeling software package that allows the intuitive development of mathematical models. After briefly listing the requirements for running VCell, the lecture describes the steps involved in developing a simple kinetic model of a single cellular compartment. These steps include (i) generation of the connectivity map of all the molecular species (molecules and their biochemically modified versions); (ii) conversion of the connectivity map to a mathematical description of the binding or enzymatic reactions (with their respective kinetic parameters); (iii) implementation of initial conditions (such as initial concentrations); and (iv) use of input:output relations to constrain the model (9, 10) (Slide 4). Model construction in VCell follows a hierarchical progression (Slide 5). At the highest level, a parent model (called the Bio-Model) contains all the molecular species, their respective connectivity as described by biochemical reactions, and the kinetic parameters defining the reaction rates. Within the parental BioModel are applications. A single BioModel may contain several applications that may explore the system in a deterministic or stochastic and/or spatial or compartmental manner. Within applications, simulation-dependent conditions are specified, such as (i) numerical solver, time step, and duration of simulation; (ii) initial concentrations; and (iii) changes to the kinetic parameters defined in the parental BioModel.

The lecture next provides a step-by-step demonstration of how to construct a relatively simple model consisting of a system of ordinary differential equations (ODEs). This ODE model is most often a necessary prelude to the construction of a spatially explicit partial differential equation (PDE) model. Slides 6 to 12 illustrate the inclusion of cellular compartments, molecules, and biochemical reactions within a BioModel. Reactions are set up by right-clicking on the cellular compartment of interest and selecting “reactions” from the menu. Place the reaction icon (yellow) in the appropriate cellular compartment and use the connector tool to link the reactants (connect to the left of the reaction icon) and products (connect to the right of the reaction icon). For enzyme-catalyzed reactions, connect the enzyme to the center of the reaction icon, and a dashed line will appear (Slide 13). To define the reaction, right-click on the reaction icon and select “properties.” Within this window, the kinetic type and parameters are specified (Slides 14 to 16). Once all the reactions are added to their appropriate cellular compartments, the initial conditions can be included. For an

ODE application, right-click on the BioModel icon on the top right and select “create deterministic application” (Slide 17). The geometry of the cellular compartment is specified in terms of volume and surface area (Slide 18). In the next tab, “initial conditions,” the initial concentrations for each component can be added. Concentrations can be “clamped” if they are buffered (not limiting). An example of a component with a buffered cellular concentration would be ATP in healthy cells (Slide 19). Each reaction can be disabled by clicking off the “enable” button under the “reaction mapping” tab (Slide 20). This feature is helpful during the troubleshooting process of a model. Under the “simulation” tab, select “new” then “edit” (Slide 21). The new window allows the testing of different combinations of parameters and the specification of the length of the simulation, sampling rate, solver, and time step (Slides 22 and 23). The ODE application also includes tools for viewing, plotting, and exporting the simulation results (Slides 24 to 26).

For more complex problems, VCell allows the development of multicompartment ODE models. A simple example is a model that simulates reactions occurring in the cytoplasm in addition to reactions in an organelle such as the nucleus. Defining such a model requires assigning the reactions to the appropriate cellular compartment where they occur (specified within the BioModel); the rules governing transfer of species between cellular compartments (also defined within the BioModel); and the volumes and surface areas of each cellular compartment (defined for a given Application). In VCell, the tools provided to solve ODE models, which assume well-mixed cellular compartments, are similar to those provided by other computing environments.

VCell is especially valuable for problems that require PDEs—that is, systems for which concentrations within cellular compartments can evolve as functions of both space and time, and initial concentrations may be nonhomogeneous (Slides 27 to 29). VCell employs a uniform finite volume solver to solve PDEs and provides specialized tools that allow for models utilizing complex cellular geometries. For instance, images derived from microscopy experiments can be imported and used as geometries in the model. The lecture describes how to define model geometries using experimental images (Slides 30 to 32) and how to create analytic geometries (Slide 33). Gray-scale, 8-bit experimental images in TIFF format are used as the basis for modeling geometries. Within the modeling geometry, each cellular compartment (cytoplasm, nucleus, and so on) is segmented, so that it is represented by a unique gray-scale value. Model cellular compartments (originally specified only in terms of volume and surface area) can then be assigned to the proper cellular morphological substructures. The geometry is then subdivided by VCell into a uniform mesh of elements, with the mesh resolution (number of elements per unit area) defined by the user (Slide 34). The mesh size can be modified under the simulation tab, with smaller mesh sizes providing finer detail but at the expense of increased computational cost. In determining the mesh size, each element within a mesh should represent a region small enough to exclude concentration gradients. Slides 35 and 36 describe the components of a PDE equation. In the case of three-dimensional (3D) geometries, a stack of segmented 2D images is used (Slide 37).

In a PDE model, VCell Applications specify essential information such as (i) diffusion constants of each molecule, (ii) boundary constraints, and (iii) the surface-to-volume ratio of any cellular compartment that is not explicitly defined within a geometry, and (iv) different

cellular geometries. Slides 38 to 41 illustrate examples of how these can be defined in VCell. The diffusion constants for each molecule can be added under the initial conditions tab in the application (Slide 38). In the simulation tab, the time step and element grid are specified. PDE simulations have a size limit of 200 MB, and hence, it may be necessary to adjust time steps and element mesh (Slides 39 and 40). In the simulation window, the results are found under the “View Data” tab. VCell allows the visualization of the simulation results, either as a snapshot of the color-coded distribution of the species within the geometry or as concentration—as a function of time or space plot (Slide 41). Kymographs are also available, consisting of a line scan across the geometry over time.

Diffusion coefficients must be assigned for each molecule included in the PDE model in addition to the parameters needed for an ODE-based model. Diffusion coefficients can be experimentally determined by techniques such as fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS). FRAP involves the overexpression of a fluorescently tagged protein and the monitoring of its fluorescence in live cells. The diffusivity of a protein can be determined by exciting a small defined subregion of the cell until all the fluorescence from the tagged molecules has disappeared (a phenomenon called photobleaching) (Slides 42 and 43). The photobleached region is then monitored as nonphotobleached tagged molecules from neighboring regions repopulate it. This rate of recovery of fluorescence in the photobleached region can be used to derive diffusion coefficients. VCell contains a FRAP module that can be used create reaction or diffusion models to extrapolate diffusion coefficients from FRAP experimental data.

FCS is another technique that measures single-molecule diffusivity (Slide 44). FCS measures the fluctuation of fluorescently tagged proteins as they move in and out of a small defined volume within the cell. These fluorescence-intensity fluctuations are then correlated over time and converted into diffusion coefficients. Also, given the fluorescence intensity, the average number of molecules within the volume can be calculated. Kinetic information of binding reactions to nonfluorescent species can also be obtained, because these events will affect motility of the tagged protein. Diffusion coefficients may not be readily available for all molecules in the model, and these unknowns must be sometimes approximated using known diffusion coefficients of other proteins (Slide 44). Green fluorescent protein (GFP) can be used as a reference, and the diffusion coefficient can be calculated with the following equation:

$$D = D_{GFP} \left[\frac{MW}{MW_{GFP}} \right]^{1/3}$$

where $D_{GFP} = 25 \mu\text{m}^2/\text{s}$, and the molecular mass of GFP $MW_{GFP} = 27 \text{ kD}$.

Signal flow within the intact cell can be visualized using probes based on Förster resonance energy transfer (FRET) (Slide 46). FRET is defined as the measurement of energy transfer between a donor and an acceptor fluorophore with overlapping spectra but distinct emission peaks. FRET can measure enzymatic reactions such as phosphorylation, accumulation of second messengers such as adenosine 3',5'-mono-phosphate (cAMP) and Ca^{++} , or dynamics

of binding reactions. Comparing reaction dynamics data obtained by FRET with simulations may require the explicit description in the model of the reaction(s) involving the exogenously expressed FRET reporter (Slide 47). Time courses and spatial plots can be used to compare the changes in concentration across time and space in both the simulations and the experiments.

An important feature of VCell is the ability to include “Modifiers” as Boolean variables. These specify special cases, such as where a molecule is available, only for part of the length of the simulation or only within a certain region of the geometry (Slides 48 to 51). An example may be the application of a ligand at a certain time in the simulation. VCell can also estimate parameters (Slides 52 to 54).

The lecture concludes with several examples of studies that have used the VCell environment to run simulations with PDE models, sometimes with complex and biologically realistic geometries that are difficult to simulate with custom-written software (Slides 55 to 58) (11–14).

The homework assignment requires the students to set up both ODE and PDE models in the VCell environment. Besides allowing the students to gain proficiency using the software, the assignment provides insight into how surface area-to-volume ratio influences the dynamics of intracellular signaling.

Problem Set

Introductory Details

A now-classic paper by Bhalla and Iyengar (15) presents mathematical models of several well-known biochemical signaling “modules.” The paper showed that individual modules may exhibit simple and straightforward behaviors, but connections between modules allow for more complex and counterintuitive responses to biochemical stimuli. Here, you will implement a simple module to gain expertise with the VCell environment.

Student Assignment: Part 1

Simulate the activation of the mitogen-activated protein kinase (MAPK) cascade in response to activated Raf-1. Figure 1 shows the reaction scheme: Input this into VCell as an ODE model. For clarity, the same reactant may be represented multiple times in a given reaction scheme but should be modeled only as a single reactant. The initial concentrations are shown in Table 1 (units are μM). The enzymatic parameters are shown in Table 2 (k_m : units, μM ; k_{cat} : units, per s). Assume a cell with a diameter of 10 μm .

1. Explore the relation between activated Raf-1 and dually phosphorylated MAPK (PP-MAPK). Vary the initial concentration of activated Raf-1 (GTP.Ras. Raf*) from 0.02 μM to 1 μM (test at least five initial concentrations). Plot time versus [PP-MAPK]. Plot [active Raf] versus [PP-MAPK]. Create a dose-response curve for active MAPK and determine the median effective concentration (EC_{50}) value.

2. Vary the concentration of MAPK phosphatase 1 (MKP1) (range 0.0001 to 0.05) or protein phosphatase 2A (PP2A) (range 0.05 to 0.5) in response to varying activated Raf-1. Plot.
3. Which phosphatase is a more effective inhibitor of MAPK? Is there a range of concentrations of active MAPK at which one phosphatase is more effective than the other?

Student Assignment: Part 2

For Part 1, you assumed that all species were present in a well-mixed cytoplasm. In reality, however, activated Raf-1 is present in the cell membrane. These simulations will explore how this factor, coupled with the geometry of the cell, influences the downstream signals predicted by the model.

Again, simulate the activation of the MAPK cascade in response to activated Raf-1. This time, place activated Raf-1 on the membrane (units should now be molecules/ μm^2). Adjust any reaction kinetic parameters with the appropriate units. Include diffusion coefficients. The initial concentrations are shown in Table 1 (units are μM). The enzymatic parameters are shown in Table 2 (K_m : units, μM ; k_{cat} : units, per s).

Create two spatial models that assume two different cellular geometries: Use 2D analytical geometries of a circle (with a diameter of 20 μm) and a rectangle (with dimensions $w = 3 \mu\text{m}$, $l = 63 \mu\text{m}$).

1. Explore the relation between activated Raf-1 and dually phosphorylated MAPK in both geometries. Set the initial concentration of activated Raf-1 (GTP.Ras.Raf*) to 200 molecules/ μm^2 .
2. Are the cytoplasmic concentrations of dually phosphorylated MAPK higher in the long, thin rectangle or in the circular geometry? Given that all kinetic biochemical parameters are identical in the two simulations, any differences must result from the cellular geometry. How can you explain the observed differences?

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The author thanks E. Sobie for critical reading of the manuscript. **Funding:** This work was supported by Systems Biology Center of New York (PSDGM071558) and NIH (5R01DK087650).

References and notes

1. Slepchenko BM, Loew LM. Use of virtual cell in studies of cellular dynamics. *Int. Rev. Cell. Mol. Biol.* 2010; 283:1–56. [PubMed: 20801417]
2. Schaff J, Fink CC, Slepchenko B, Carson JH, Loew LM. A general computational framework for modeling cellular structure and function. *Biophys. J.* 1997; 73:1135–1146. [PubMed: 9284281]
3. Loew LM, Schaff JC. The Virtual Cell: A software environment for computational cell biology. *Trends Biotechnol.* 2001; 19:401–406. [PubMed: 11587765]

4. Moraru II, Schaff JC, Slepchenko BM, Blinov ML, Morgan F, Lakshminarayana A, Gao F, Li Y, Loew LM. Virtual Cell modelling and simulation software environment. *IET Syst. Biol.* 2008; 2:352–362. [PubMed: 19045830]
5. Schaff JC, Slepchenko BM, Loew LM. Physiological modeling with virtual cell framework. *Methods Enzymol.* 2000; 321:1–23. [PubMed: 10909048]
6. Slepchenko BM, Schaff JC, Carson JH, Loew LM. Computational cell biology: Spatiotemporal simulation of cellular events. *Annu. Rev. Biophys. Biomol. Struct.* 2002; 31:423–441. [PubMed: 11988477]
7. Slepchenko BM, Schaff JC, Macara I, Loew LM. Quantitative cell biology with the Virtual Cell. *Trends Cell Biol.* 2003; 13:570–576. [PubMed: 14573350]
8. Loew LM. The Virtual Cell project. *Novartis Found. Symp.* 2002; 247:151–160. discussion 160–161, 198–206, 244–252. [PubMed: 12539954]
9. Neves SR, Iyengar R. Modeling of signaling networks. *Bioessays.* 2002; 24:1110–1117. [PubMed: 12447976]
10. Neves SR, Iyengar R. Models of spatially restricted biochemical reaction systems. *J. Biol. Chem.* 2009; 284:5445–5449. [PubMed: 18940805]
11. Brown SA, Morgan F, Watras J, Loew LM. Analysis of phosphatidylinositol-4,5-bisphosphate signaling in cerebellar Purkinje spines. *Biophys. J.* 2008; 95:1795–1812. [PubMed: 18487300]
12. Fink CC, Slepchenko B, Moraru II, Watras J, Schaff JC, Loew LM. An image-based model of calcium waves in differentiated neuroblastoma cells. *Biophys. J.* 2000; 79:163–183. [PubMed: 10866945]
13. Smith AE, Slepchenko BM, Schaff JC, Loew LM, Macara IG. Systems analysis of Ran transport. *Science.* 2002; 295:488–491. [PubMed: 11799242]
14. Neves SR, Tsokas P, Sarkar A, Grace EA, Rangamani P, Taubenfeld SM, Alberini CM, Schaff JC, Blitzer RD, Moraru II, Iyengar R. Cell shape and negative links in regulatory motifs together control spatial information flow in signaling networks. *Cell.* 2008; 133:666–680. [PubMed: 18485874]
15. Bhalla US, Iyengar R. Emergent properties of networks of biological signaling pathways. *Science.* 1999; 283:381–387. [PubMed: 9888852]

Fig. 1.
Activation of the MAPK cascade in response to activated Raf-1. S, Ser; T, Thr; Y, Tyr; and P, phospho.

Table 1

Initial concentrations. MAPKK, MAPK kinase.

Name of enzyme	Concentration (μM)
MKP1	0.0032
PP2A	0.224
MAPKK	0.18
MAPK	0.36

Table 2

Enzymatic parameters.

Reaction #	$[K_m]$ (μM)	k_{cat} (/s)
1	0.159091667	0.105
2	0.159091667	0.105
3	15.6565	6
4	15.6565	6
5	0.046296667	0.15
6	0.046296667	0.15
7	0.066666667	1
8	0.066666667	1