

Computer Simulation of MAPK Signal Transduction

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1. Introduction

We predict that computational modeling platforms will soon become standard tools in experimental laboratories involved in the study of complex regulatory networks of various cellular processes—a field of research where an avalanche of genomic, proteomic and other biochemical data have recently been gleaned, and this trend is expected to continue in the foreseeable future. Quantitative kinetic modeling requires one to postulate detailed molecular pathways and to carry out analyses in the context of an integrated dynamic system so that predictions can be made and compared with experimental data as well as aid in the design of future experiments. The speed of modern computers is such that one can perform simulations of many possible models and discriminate against those that are less probable.

The primary objective of this chapter is to guide the newcomer through the basic steps in computational modeling of biochemical reaction networks, starting from a molecular mechanism that is amenable to quantitative description and moving on to coding the kinetic equations in a computer file so that a computer program can then solve the associated dynamical equations. Readers who are already familiar with computer simulations will also benefit from reading our notes on the essential features that we think, based on experimental evidence, must be observed in model mechanisms of mitogen-activated protein kinase (MAPK) signaling. We use the model of Huang and Ferrell (*1*) to illustrate the detailed procedure in our computer simulation of the dynamics and steady-state behavior of MAPK signaling. Our purpose here is not to validate the Huang-Ferrell model but to present a method of computer simulation that could be useful to the experimentalist. Other models are also noted, and it is hoped that the reader can analyze or modify them using the methods learned in this chapter. We also include a short list of computer software available on

From: *Methods in Molecular Biology*, vol. 250: *MAP Kinase Signaling Protocols*
Edited by: R. Seger © Humana Press Inc., Totowa, NJ

the internet and deemed to be user-friendly, especially for biologists who do not have the time to learn the intricacies of computer programming.

2. Materials

The kinetic model we solve in the next section requires a computer program that integrates a system of ordinary differential equations. Because of its speed, ease of use, and many convenient features, we have chosen the software called Berkeley Madonna™ (BM) developed by Robert Macey and George Oster of the University of California at Berkeley. Information on purchasing and downloading this inexpensive program from the internet can be found at www.berkeleymadonna.com. BM can be run in either a Windows or Macintosh environment. BM for Windows requires a personal computer with at least an Intel x86 or compatible processors that operate using Microsoft Windows 95, Windows NT 4.0, or later versions. BM for Macintosh runs on Apple Power Macintosh and 100% compatible computers with a PowerPC CPU. Note that other recommended software platforms are given in **Table 1** and can be used instead of BM (*see Note 1*).

3. Methods

3.1. Creating the Equations File

We use the model mechanism of Huang and Ferrell (*I*) to illustrate the detailed procedure of coding the kinetic equations into BM. The reaction network diagram and a brief summary of the model are given in **Fig. 1** (*see Note 2*). When BM is run for the first time, an equation window is opened wherein the user types the kinetic equations and other computational details of the model. For the Huang–Ferrell mechanism, the corresponding file is given in **Table 2**. Because it is assumed that the total protein concentrations of each level of the cascade are constant (denoted by the parameters *tot1*, *tot2*, and *tot3* in **Table 2**), we classify protein concentrations as either independent or dependent dynamic variables in our kinetic equations. For example, concentration of MKKK (denoted as *mkkk* in **Table 2**) is considered dependent because it could be derived from *tot3* and the concentration of MKKK* (denoted as *mkkks*). The concentrations of MKK-P, MKK-PP, MAPK-P, and MAPK-PP are the other independent variables (whose concentration symbols in **Table 2** are *mkkp*, *mkkpp*, *mapkp*, and *mapkpp*, respectively), and the other dependent species and their corresponding concentrations (in parentheses) are MKK (*mkk*) and MAPK (*mapk*). The individual reaction steps are endowed with the rate expressions identified as *v1*, *vm1*, *v2*, *vm2*, *v3*, *vm3*, *v4*, *vm4*, *v5*, and *vm5* in **Table 2** (*see Note 3*). These rate expressions are assumed to be of the Michaelis-Menten type; for example, $v1 = k1 * e1 * mkkk / (j1 + mkkk)$ in which *j1* is the

Table 1
Computer Simulation Software for Biochemical Reaction Networks

Software	Web site
Berkeley Madonna	www.berkeleymadonna.com/
<i>MCell</i>	www.mcell.cnl.salk.edu/
E-Cell	http://e-cell.org
Virtual Cell	www.nrcam.uchc.edu/index.html
Gepasi	www.gepasi.org/gepasi.html
SCAMP	www.sys-bio.org
Jarnac	www.sys-bio.org
DBsolve	http://websites.ntl.com/~igor.goryanin/
Cellerator	<i>see ref. 7</i>

Michaelis constant, eI is the total concentration of the enzyme catalyzing reaction 1, and kI is a parameter.

The equations file (**Table 2**) has five differential equations (indicated by d/dt , which signifies rate of change with time), the equations of the dependent variables in terms of independent variables, the rate expressions (the vs), the initial values of the independent variables (indicated by “*init*”), and the values of the parameters of the model. In addition, the first few lines of the equations file contain the statements for the integration METHOD used (in this case the integrator for “STIFF” systems, which have widely varying values of parameters; other integrators are available in BM), the initial value of time (STARTTIME), the final value of integration time (STOPTIME), and the time step for integration (DT).

3.2. Running the BM Program

BM provides a good tutorial and user guide so we only give a very brief sample of the software’s capabilities here. To run the program, simply click on the “Run” button on the equations window; the program will then open a window that gives a graph of concentration versus time for selected species (e.g., **Fig. 2** shows *mapkpp* vs *time*). One feature of BM that is quite convenient for exploring the effect of changing parameters is the parameter slider. Click on the “Parameters” menu and select “Define Sliders,” which opens a window where you could select which parameters to vary. **Figure 2** shows the sliders for eI and STOPTIME. It is convenient to choose STOPTIME as a slider when one is interested in following the change in concentrations up to their steady-state levels, which is what we do next.

Table 2

The Equations File for the Huang–Ferrell Model Used by Berkeley Madonna

METHOD STIFF

STARTTIME = 0

STOPTIME = 100

DT = 0.01

$$d/dt(mkks) = v1 - vm1$$

$$d/dt(mkcp) = v2 + vm3 - (vm2 + v3)$$

$$d/dt(mkpp) = v3 - vm3$$

$$d/dt(mapcp) = v4 + vm5 - (vm4 + v5)$$

$$d/dt(mappp) = v5 - vm5$$

$$mkkk = tot3 - mkkks$$

$$mkk = tot2 - (mkcp + mkpp)$$

$$mapk = tot1 - (mapcp + mappp)$$

$$v1 = k1 * e1 * mkkk / (j1 + mkkk)$$

$$vm1 = km1 * e2 * mkkks / (jm1 + mkkks)$$

$$v2 = k2 * mkkks * mkk / (j2 + mkk)$$

$$vm2 = km2 * kcpase * mkcp / (jm2 + mkcp)$$

$$v3 = k3 * mkkks * mkpp / (j3 + mkpp)$$

$$vm3 = km3 * kcpase * mkpp / (jm3 + mkpp)$$

$$v4 = k4 * mkpp * mapk / (j4 + mapk)$$

$$vm4 = km4 * kcpase * mapcp / (jm4 + mapcp)$$

$$v5 = k5 * mkpp * mapcp / (j5 + mapcp)$$

$$vm5 = km5 * kcpase * mappp / (jm5 + mappp)$$

$$init\ mkkks = 0.0$$

$$init\ mkcp = 0.0$$

$$init\ mkpp = 0.0$$

$$init\ mapk = 0.0$$

$$init\ mappp = 0.0$$

$$tot3 = 3.0$$

$$tot2 = 1200.0$$

$$tot1 = 1200.0$$

$$e1 = 0.1$$

$$e2 = 0.3$$

$$kcpase = 0.3$$

$$kcpase = 120.0$$

$$j1 = 300.0$$

$$jm1 = 300.0$$

(continued)

Table 2 (continued)

$j2 = 300.0$
$jm2 = 300.0$
$j3 = 300.0$
$jm3 = 300.0$
$j4 = 300.0$
$jm4 = 300.0$
$j5 = 300.0$
$jm5 = 300.0$
$k1 = 1.0$
$km1 = 1.0$
$k2 = 1.0$
$km2 = 1.0$
$k3 = 1.0$
$km3 = 1.0$
$k4 = 1.0$
$km4 = 1.0$
$k5 = 1.0$
$km5 = 1.0$

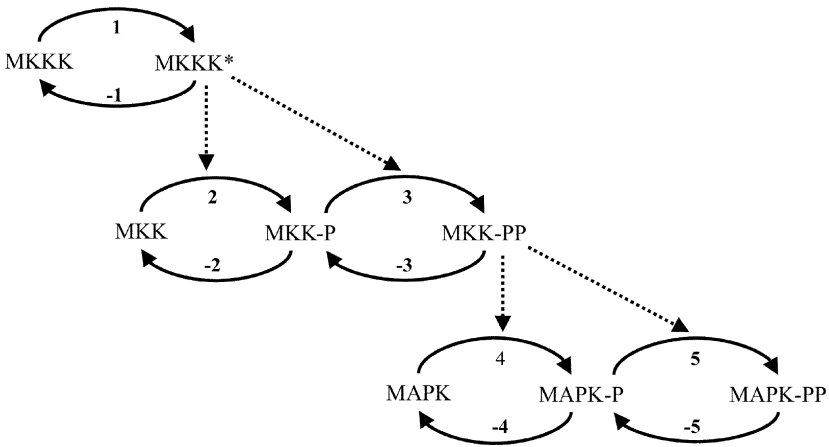


Fig. 1. Huang-Ferrell model for the MAPK cascade. Step 1, the activation of MKKK, is catalyzed by an enzyme E1; the reverse, step -1, is catalyzed by an enzyme E2. Steps 2 and 3 represent the two-collision, nonprocessive mechanism for the double phosphorylation of MKK; these steps are catalyzed by MKKK* while their reverse are assumed to be catalyzed by a phosphatase KKPase. Likewise, steps 4 and 5 represent the double phosphorylation of MAPK catalyzed by the active MKK-PP, and the reverse steps are catalyzed by a phosphatase KPase.

Berkeley Madonna - Untitled2

File Edit Viewchart Model Compute Graph Parameters Window Help

Untitled2 - E equations

```

METHOD STIFF
STARTIME = 0
STOPTIME = 100
DT = 0.01

d/dt(mkks) = v1*vm1
d/dt(mkpp) = v2+vm3-(vm2+v3)
d/dt(mkpp) = v3-vm3
d/dt(mapkp) = v4+vm5-(vm4+v5)
d/dt(mapkpp) = v5-vm5

mkks = tot3-mkks
mkpp = tot2-(mkkp+mkpp)
mapkp = tot1-(mapkp+mapkpp)

v1 = k1*e1*mkks/(1+mkks)
vm1 = km1*e2*mkks/(jrn1+mkks)
v2 = k2*mkks*mkks/(j2+mkks)
vm2 = km2*kpase*mkpp/(jm2+mkpp)
v3 = k3*mkks*mkpp/(j3+mkpp)
vm3 = km3*kpase*mkpp/(jm3+mkpp)
v4 = k4*mkpp*mapk/(j4+mapk)
vm4 = km4*kpase*mapkp/(jm4+mapkp)
v5 = k5*mkpp*mapkp/(j5+mapkp)
vm5 = km5*kpase*mapkpp/(jm5+mapkpp)

init mkks = 0.0
init mkpp = 0.0
init mkpp = 0.0
init mapkp = 0.0
init mapkpp = 0.0

tot3 = 3.0
tot2 = 1200.0
  
```

Run

Page 1

Run 1: 8019 steps in 0.226 seconds

mapkpp

TIME

Run 1: 8019 steps in 0.226 seconds

Run 10x Run 10x

Untitled2 - Sliders

e1 = 0.1

STOPTIME = 8000

Ready

Berkeley Madonna - ...

2:54 PM

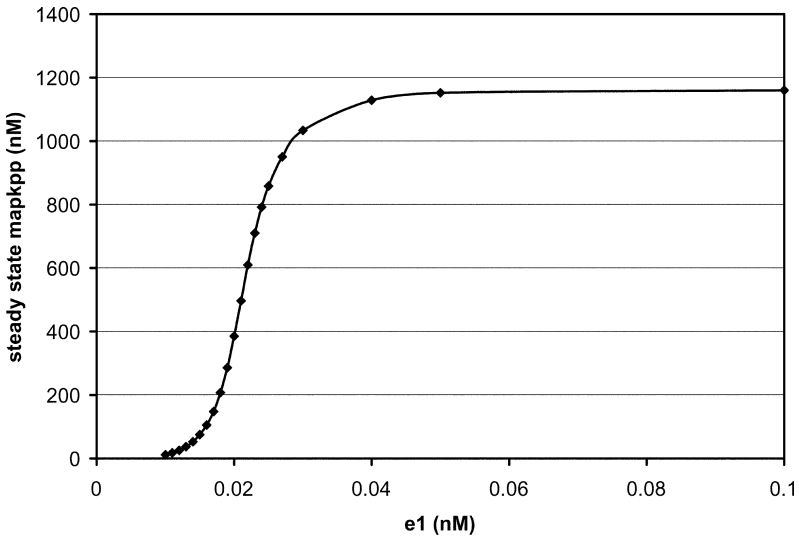


Fig. 3. Graph of steady-state value of *mapkpp* (concentration of active MAPK) as a function of signal strength (here taken as the concentration of first enzyme in the cascade, *e1*).

3.3. Steady-State Levels of Active MAPK vs Input Signal

Using the parameter sliders, we can simulate the steady-state response of the MAPK cascade (as indicated by the steady-state levels of *mapkpp*) to different strengths of signal (here taken as directly proportional to the value of the parameter *e1*, the concentration of the enzyme that catalyzes the activation of MKKK). One would repeat the run shown in **Fig. 2** for various *e1*, vary STOPTIME so that *mapkpp* has enough time to level off, and then determine the steady-state value of *mapkpp* (click on the readout button located just above the graph and drag/click the crosshair to the end of the curve to determine the steady-state value). A graph of the steady-state *mapkpp* vs *e1* is shown in **Fig. 3**, which is identical to one of Huang and Ferrell’s (1) results (their Fig. 2) after some rescaling. This sharp sigmoidal curve corresponds to a Hill coefficient of 4.9, which demonstrates the ultrasensitivity of the MAPK cascade to changes in signals (such as *e1* here) when these are at levels within the switching region. Using parameter sliders, the user can vary as many parameters as desired to

Fig. 2. (previous page) BM simulation environment showing a graph (foreground window) that is automatically generated by clicking the “Run” button on the Equations window (background). Parameter sliders that allow the user to vary *e1* and STOPTIME are shown below the graph.

explore their influence on the dynamic and steady-state behavior of the cascade. See **Note 4** for other possible projects.

4. Notes

1. **Table 1** provides a short list of computer simulation software that can be downloaded from the Internet. *MCell* is a general Monte Carlo simulator that takes into account three-dimensional subcellular architecture in models of ligand diffusion and signal transduction. *MCell* has been used by its developers to simulate the microphysiology of synaptic transmission. The aim of the E-Cell project is to create a modeling and simulation environment that may allow the user to predict the dynamics of living cells through integrative models incorporating gene regulation, and metabolic and signaling pathways. To date, *E-Cell* has been used to construct a model of a hypothetical cell having a minimal gene set. Virtual Cell is another general computational framework for simulating cellular physiology and also takes into account subcellular spatial geometries, both user defined and empirically derived. Gepasi is a software package intended to simulate the kinetics of biochemical reaction networks and includes tools such as data fitting and optimization, metabolic control analysis, and linear stability analysis. SCAMP is another general purpose simulator of metabolic and chemical reaction networks; this freeware also carries out calculations involved in metabolic control analysis. Jarnac is essentially a rewrite of SCAMP with the added feature, among others, of a designer that enables users to build models visually. Another freeware is DBsolve, which provides an integrated development environment for metabolic, enzymatic, and receptor-ligand binding simulation. Cellerator is an automatic model generator that allows computerized construction of the differential equations, which is needed especially when there is a combinatorial explosion on the number of possible states of protein complexes (see **Note 4** on modeling involving scaffolds).
2. Implicit in the differential equations used for the Huang–Ferrell model are the assumptions that the numbers of all molecular species involved are large so that we can follow the time evolution of their concentrations in a deterministic manner (i.e., through the continuous differential equations), and that the reactions occur in a spatially homogeneous solution. In fact, both of these assumptions may not apply in actual cellular systems, and more realistic but computationally intensive calculations, such as those carried out by *MCell* (see **Table 1**), should be performed. Gibson and Mjolsness (2) provide an excellent summary of various levels of modeling biochemical networks.
3. The kinetic equations in **Table 2** differ from those used by Huang and Ferrell (1) in that we have assumed that the Michaelis-Menten rate expression applies to each reaction step in **Fig. 1**. Huang and Ferrell considered the detailed enzymatic mechanism—i.e., $E + S \leftrightarrow ES \rightarrow E + P$ (where E = active enzyme, S = substrate, ES = enzyme-substrate complex, and P = product), for each reaction step. If the interest is on steady-state behavior such as in **Fig. 3**, our simplification will give the same results as Huang and Ferrell's.

4. Asthagiri and Lauffenburger (3) have recently carried out a computational study of feedback effects, both positive and negative, on the dynamics of signal transduction. Their approach involves breaking down the complex signaling network into modules, namely the receptor-ligand processing module, the adaptor chain module, and an enzyme-cascade module (of which the MAPK cascade is an example). Negative feedback among these modules can generate signal adaptation as well as sustained concentration oscillations. Kholodenko (4) has utilized a quantitative measure of global sensitivity of a MAPK cascade and showed that a negative feedback lowers this sensitivity whereas a positive feedback increases it. Other factors that could significantly affect signal propagation include association between members of a MAPK cascade (e.g., see ref. 5) and the presence of scaffold proteins that bind the members of the kinase cascade; Levchenko et al. (6) have recently modeled the latter.

Acknowledgments

We thank Andre Levchenko for helpful discussions. B.D.A. thanks Mark Borisuk and John Doyle for making his sabbatical visit to Caltech possible.

References

1. Huang, C-Y. F. and Ferrell, J. E., Jr. (1996) Ultrasensitivity in the mitogen-activated protein kinase cascade. *Proc. Natl. Acad. Sci. USA* **93**, 10,078–10,083.
2. Gibson, M. A. and Mjolsness, E. (2001) Modeling the activity of single genes, in *Computation Modeling of Genetic and Biochemical Networks* (Bower, J. M. and Bolouri, H., eds.), MIT Press, Cambridge, MA, pp. 1–48.
3. Asthagiri, A. R. and Lauffenburger, D. A. (2001) A computational study of feedback effects on signal dynamics in a mitogen-activated protein kinase (MAPK) pathway model. *Biotechnol. Prog.* **17**, 227–239.
4. Kholodenko, B. N. (2000) Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *Eur. J. Biochem.* **267**, 1583–1588.
5. Cheng, J., Yang, J., Xia, Y., Karin, M., and Su, B. (2000) Synergistic interaction of MEK kinase 2, c-Jun N-terminal kinase (JNK) kinase 2, and JNK1 results in efficient and specific JNK1 activation. *Mol. Cell. Biol.* **20**, 2334–2342.
6. Levchenko, A., Bruck, J., and Sternberg, P. W. (2000) Scaffold proteins may biphasically affect the levels of mitogen-activated protein kinase signaling and reduce its threshold properties. *Proc. Natl. Acad. Sci. USA* **97**, 5818–5823.
7. Shapiro, B. E., Levchenko, A., and Mjolsness, E. (2002) Automatic model generation for signal transduction with applications to MAPK pathway, in *Foundations of Systems Biology* (Kitano, H., ed.), MIT Press, Cambridge, MA, pp. 145–162.

