Learn Utilization of Cell Simulation in 10 Pages

ver. Apr-4-2016

Kazuhisa Ichikawa

True Cell Simulations Inc.

http:// engl.tc-simulations.com/

True Cell Simulations Inc. Tokyo Japan

Contents

Introduction

In a previous e-booklet, “Learn Pathway Simulation in 10 Pages,” I introduced how pathway simulations are run starting with a pathway diagram. However, a second hurdle for experimental researchers is figuring out how to employ simulation in their studies. This second e-booklet shows users how to employ simulation in molecular cell biology.

Here, selected studies from published papers are shown to introduce how to utilize simulation studies. Note that there are many papers that used simulation studies in molecular cell biology. Those illustrated here were arbitrarily selected, but have been chosen as examples of each category shown below. There are several ways to categorize studies, including by the objectives of utilizing simulations. However, categorization by objectives is not realistic because there can be many objectives depending on the study. Therefore, categorization by situational attribute was employed in this e-booklet.

If a simulation study is defined as an approach aimed at explaining phenomena of a cell by clearly defined mechanisms, situations can be categorized into four groups according to whether the phenomenon and mechanism are known or unknown (Table 1). Category 1 may seems to be unnecessary, because both the phenomenon and the underlying mechanisms are known, predicted, or proposed. There seems to be no reason to run a simulation. However, experimental results can be confirmed and strengthened by a simulation that shows congruent results. In addition, simulation can reveal the common mechanisms for phenomena that seem not to share a common origin. These cases are included in Category 1.

Table1 Categorization of situations in utilizing simulation

category	phenomenon	mechanism	utilization ID
1	known/predicted	known/proposed	1, 3
2	known	unknown	4, 5
3	unknown	known	6
4	unknown	unknown	2

Six example papers were selected for explaining these four categories. It is common to find collaborative papers between experimentation and simulation studies include multiple categories from Table1. However, a given paper is referred to in a single category, which might be unwanted by the authors. I would ask them to be forgiving of this inconvenience, as this e-booklet is written to show how to use simulations in molecular cell biology rather than as an introduction to the research results described in the papers.

For convenience of explanation, categories in Table 1 are not shown in sequence. Instead, examples of utilization are shown in sequence of utilization ID. Recently, simulation studies have been employed in drug discovery. These might seem to fit into Category 3. However, applications in drug discovery are shown in the separate Categories 7 and 8 as systems pharmacology.

Utilization 1: Replicate and Reinforce Experiments

Utilization 1 is a case in which experimental findings are reinforced by replicating observations by simulation with definite mechanisms (Category 1). There are many simulation studies performed to this purpose. Among them, an example of ERK oscillation, which is triggered by EGF, is shown here[1].

It was theoretically proposed that activated ERK oscillated by repetitive translocation between the cytoplasm and the nucleus[2], and was later confirmed[3]. The paper shown here first reported the oscillation of GFP-labeled ERK expressed in human mammary epithelial cells at the single cell level, and the following detailed characteristics of oscillation were found:

1) Oscillation period was 15 min on average ranging from 11 to 22 min,
2) Oscillation period did not depend on EGF concentration,
3) Period of upward slope of the oscillation in nuclear ERK was shorter than downward slope, and both of them were independent on EGF concentration: that is the oscillation waveform was independent of EGF concentration.

The paper employed the model shown in Figure 1 to replicate above observations 1)-3)[4]. This model was constructed based on models on feedback regulation of phosphorylated ERK proposed by other laboratories[5]. Model parameters were randomly altered to simulate a scattering of oscillation pattern among cells. Simulation results successfully replicated 1)-3). Thus, detailed characteristics of ERK oscillation that authors found, whose rough behavior was reported before, were replicated by simulating an existing model (Category 1).

A simulation for Category 1 is relatively easy to run, because pathways and mechanisms are known or have been proposed. However, experimental observations are replicated by a different simulation methodology, which can reinforce experimental results found in a paper.

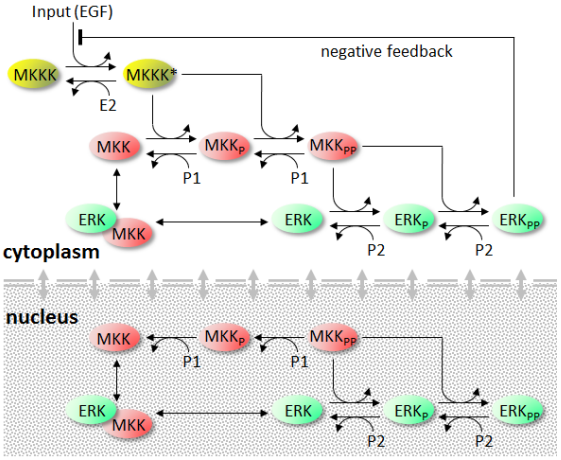

Figure 1 A model for ERK oscillation. Bidirectional arrows indicate protein translocation through nuclear pores.

1 Shankaran, H., et al., Molecular Systems Biology, 5 (2009), 332.

2 Kholodenko, B.N., Eur.J.Biochem., 267 (2000), 1583.

3 Hilioti, Z, et al., Curr.Biol., 18 (2008), 1700.

4 Although a diagram show in Figure 1 distinguishes the cytoplasmic and the nuclear space, there is no spatial discrimination in the simulation. Instead cytoplasmic and nuclear ERK were discriminated by the name such as ERK_c and ERK_n. Thus the simulation was not spatio-temporal 4D but temporal simulation. There are many reports that a simulation is temporal nonetheless, a model is drawn as if it is spatio-temporal. This is largely because software for spatio-temploral 4D simulation such as A-Cell is not available in those studies. One should be careful about this.

5 Paper by Koholodenko, B.N. shown above and by Fujioka, A., et al., J.Biol.Chem., 281 (2006), 8917, etc.

Utilization 2: Propose Possible Mechanisms

In this utilization, phenomenon and mechanism are novel and unknown, respectively (Category 4). If a possible mechanism for a newly found phenomenon is proposed, the impact of the paper will be much higher. In this category, simulation occupies a more important role than in Category 1, because new mechanisms, which are based on experimental observations and existing knowledge, are proposed. There are many studies for this category too. Illustrated here is the oscillation of transcription factor NF-κB as an example[6].

NF-κB resides in the cytoplasm in the absence of a stimulus. The paper reported that nuclear NF-κB (NF-κB$_n$) oscillated by the continuous application of TNFα as a stimulus[7]. NF-κB$_n$ was assayed by EMSA. The following was reported by the analyses of the oscillation:

1) NF-κB$_n$ oscillates with a period of about 2 hours,
2) Oscillation was dampened,
3) There existed negative feedback as proposed in ERK oscillation, and three isoforms of IκB (α, β, ε) were responsible for it with different roles[8].

Although these results seemed similar to the ERK oscillation, NF-κB is a transcription factor, and therefore, a newly translated protein is possibly involved in the mechanism. To explore this, the authors first created a minimal mathematical model of two variables, where feedforward and feedback are included. After showing the emergence of oscillation by this minimal model, they constructed a biological model, where de novo-synthesized IκBα by active NF-κB$_n$ played a role as the negative feedback (Figure 2)[9]. The simulation of this pathway replicated observation 1)-3) shown above[10]. Thus, a newly found phenomenon was replicated by simulation with newly proposed mechanisms.

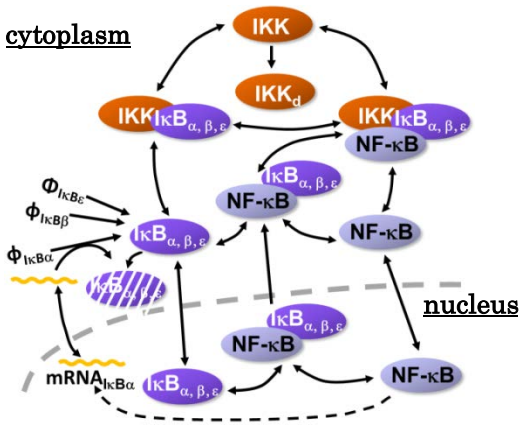

Figure 2 A model for NF-κB oscillation.

In this utilization, running a simulation is far more difficult in comparison to Utilization 1, because the model was created from few clues. If the simulation is successful, the impact of the study is dramatically increased by reinforcing observed phenomenon and showing possible mechanism.

---

6 Hoffmann, A., et al., Science, 298(2002), 1241.

7 After the report by Hoffmann et al, 2002, fluorescence measurement also showed the single cell oscillation of NF-κB$_n$.

8 IκB is an inhibitor protein of NF-κB. IκB binding to NF-κB prevents it from translocation to the nucleus.

9 As in the ERK model, there was no spatial discrimination between the cytoplasm and the nucleus in the simulation, and therefore, the simulation was temporal but not spatio-temporal 4D.

10 It should be noted that a simulation study with basically the same mechanism was reported earlier without specifying any transcription factor (Goodwin B.C., Adv.Enzyme Reg., 3(1965), 425).

Utilization 3: Show Comprehensive Mechanism

Scientists try to explain phenomena with minimum mechanisms. This attempt is generally and typically seen in physics. Although this philosophy might not always be true for biology, biologists also try to explain phenomena by as few mechanisms as possible. In Utilization 3, a simulation is aimed at showing that multiple phenomena have the same mechanistic origin (Category 1). Here I show our research as an example[11].

Stress granules (SGs) are small non-membranous cytoplasmic aggregates of mRNAs and related proteins (sub- to several micrometers in size), and assembled in response to environmental stresses such as heat shock and hypoxia. Experimental observations reported the following:

1) Self-aggregating protein TIA-1 is required for the assembly of SGs,
2) SGs are assembled with a 5 min delay after the application of a stress stimulus followed by a peak in the number of SGs at 30 min (~30SGs/cell),
3) SGs are assembled at perinuclear region in the cytoplasm by arsenite application (Figure 3).

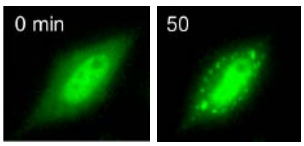

Figure 3 SGs (green puncta at perinuclear region at 50 min) assembled by arsenite.

We searched for comprehensive mechanism for these observations. First we hypothesized that SGs were assembled by the self-aggregation of TIA-1 as shown in Figure 4A. There was no other pathway in our simulation. Second, we assumed enlargement of SGs by their fusion. Third, large SGs were assumed to be transported on microtubules.

Since there are only countable SGs (~30) in a cell and traditional simulation by differential equations using concentration was quite unsuitable, we ran a stochastic particle simulation[12]. We replicated observations 1)-3) by this minimal mechanism (Figures 4B and C). In addition, SG size was predicted to follow γ distribution, which was experimentally validated (Figure 4D). This strongly suggested the consecutive occurrence of independent random processes in SG assembly.

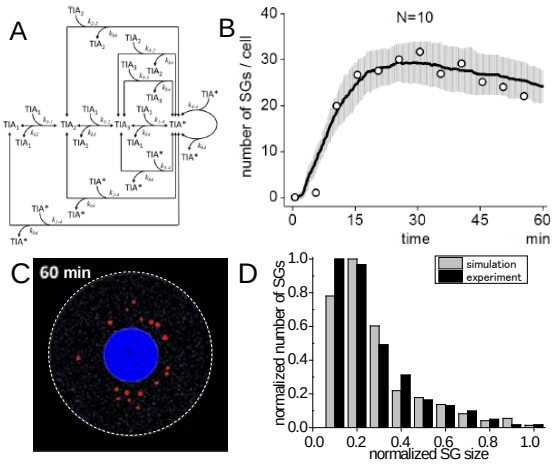

Figure 4 A minimal model of SG assembly (A) and its simulation results (B-D). (B) time course of the number of SGs (open circles: experiment; black line with SD shown by gray area: simulation), (C) spatial distribution of SGs (red puncta), and (D) SG size distribution by simulation (gray bars) and experiments (black bars).

Simulation plays important role in Utilization 3 as in 2. In addition, a prediction was made by the simulation in this example. Prediction is the most important role of simulation.

11 Ohshima, D., et al., PLoS Comp.Biol., 11(2015), e1004326.
12 Ichikawa, K., et al., Physical Biol., 7(2010), 046010.

Utilization 4: Reveal Unknown Essential Mechanisms

In this utilization, cell simulation was used for the elucidation of unexpected essential mechanisms required for a known phenomenon (Category 2). Here, I show our study as an example, in which a predicted mechanism was experimentally validated[13].

It is commonly agreed that many cancer patients can survive if metastasis is blocked. In the initial step of metastasis, membrane protein MT1-MMP, which degrades the extracellular matrix (ECM) and opens a space into which cancer cells can migrate, is heavily expressed. The following were reported observations:

1) TIMP-2 regulates the activity of MT1-MMP, and MMP-2 also degrades ECM (Figure 5A),
2) MT1-MMP is expressed at invadopodia, which are tiny protrusions on the cell surface with the size smaller than 1μm (orange puncta in Figure 5B left),
3) ECM was degraded within about 1 hour in *in vitro* experiments.

Pathway simulation according to observation 1) replicated observed ECM degradation (thin red curve in Figure 5C).

This simulation, however, did not include observation 2), a spatial aspect of the experiment. Therefore, we ran a spatio-temporal 4D simulation (Figure 5B right). The simulation results were contrary to our expectation in that, ECM was not degraded (blue curve in Figure 5C). This was quite curious because 4D simulation, which was a much better representation of the experiments, turned out to be inconsistent with them. This strongly suggested that something essential was missing. After an extensive analysis, a very narrow activity period of MT1-MMP (~4 s) was found. Then we hypothesized that the repetitive insertion of MT1-MMP was required. In fact, simulations with a turnover of MT1-MMP degraded ECM (thick red curve in Figure 5C). The interval of the insertion was predicted to be <70 s. This prediction was validated by our FRAP experiment. Later, another group reported the same validation by direct measurement of vesicle life time[14]. Such quick turnover of MT1-MMP was totally unexpected.

Cell simulation cannot be run without experimental data. Similarly, one cannot know everything by experiments. This is a typical example of the fruitful collaboration between experimentation and simulation aimed at elucidation of essential mechanisms.

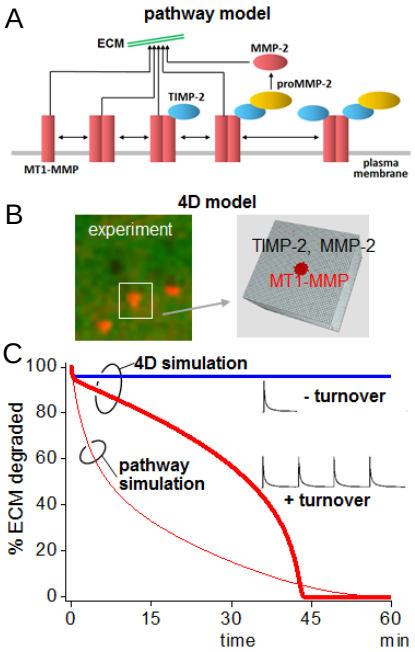

Figure 5 Degradation of ECM by MT1-MMP.

13 Hoshino, D., et al., PLoS Comp.Biol., 8(2012), e1002479; Watanabe, A., et al., PLoS Comp.Biol., 9(2013), e1003086.

14 Hagedorn, E.J., et al., J.Cell Biol., 204(2014) 1209.

Utilization 5: Predict Unknown Mechanism

Novel phenomena are found by experiments, and researchers elucidate underlying mechanisms. However, there might exist additional unknown mechanisms regulating the phenomena. Simulation can reveal such mechanisms (Category 2).

An example here is NF-κB in our study[15]. Although more than 60 simulation studies on NF-κB were published, there have been no 4D simulations before our studies. So we constructed a 3D spherical cell model, in which spherical nucleus was located at the center, and the change in the oscillation pattern of NF-κB (frequency, persistency, etc.) relative to the change in spatial parameters was investigated (Figure 6A). The pathway used for the 4D simulation was exactly the same as that for the reported pathway simulation. Unexpectedly, however, the simulation result was largely different (red and black curves in Figure 6B for pathway and 4D simulations, respectively). This strongly suggested that spatial parameters regulated the oscillation pattern. After replicating the experimental oscillation pattern by 4D simulation, we investigated five spatial parameters: 1) nuclear to cytoplasmic volume ratio, 2) diffusion coefficient, 3) nuclear membrane transport, 4) locus of protein translation, and 5) shape of the nucleus. We found that all spatial parameters 1)-4) but not 5) regulated the oscillation pattern of nuclear NF-κB. Figure 6B shows a summary for 2) and 3), in which green and orange lines indicate parameters regulating persistency and frequency, respectively. Zig-zag lines indicate diffusion process. Thus, novel mechanisms regulating NF-κB oscillation were found by the simulation (Category 2).

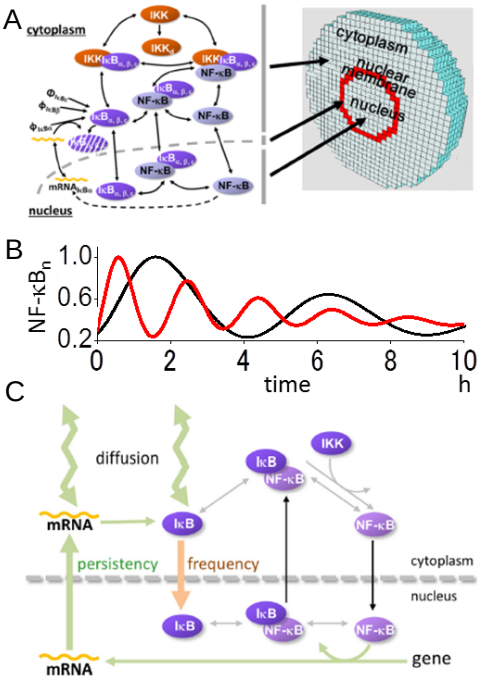

Figure 6 A model of 4D simulation for the oscillation of nuclear NF-κB (A), importance of 4D simulation (B), and summary of simulation results (C).

Larger nuclear to cytoplasmic volume ratio is well documented in malignant cancers. Mitochondrial crowding around the nucleus due to hypoxia was reported recently, which would reduce the effective diffusion coefficient. In addition, alteration in transport via the nuclear membrane by aging was reported. Results summarized in Figure 6C show novel target regulating NF-κB in the nucleus. One advantage of simulation is that any parameters in the model such as diffusion coefficient can be changed, which is not easily achieved by experiments.

15 Ohshima, D., et al., PLoS ONE, 7 (2012), e46911; Ohshima, D., et al., PLoS ONE, 9 (2014), e109895; Ohshima, D., et al., PLoS ONE, 10 (2015), e0127633.

Utilization 6: Predict Unknown Phenomenon

Here, I introduce an example of a simulation predicting an unknown phenomenon (Category 3). This utilization of simulation might not appear to be an obvious choice because in a case where a phenomenon and mechanism are well defined, nothing new happens by simulation. In several cases, however, mechanisms leading to unknown phenomenon are hiding in the model.

Signal transduction between neurons in a brain is modifiable, which is thought to be the basis for learning and memory (synaptic plasticity[16]). In the synaptic transmission, transmitter molecules are released from the presynaptic terminal by action potentials of various frequencies. High and low frequencies lead to long-term potentiation (LTP) and depression (LTD) of transmission efficacy, respectively (black continuous line in Figure 7A), and it is implicitly thought that at much higher frequency, LTP would occur too (black broken curve in Figure 7A).

Molecular bases of synaptic transmission are well documented, where the binding of transmitter molecules to receptors (orange ellipse in Figure 7B) leads to the activation of kinases (red rectangle) and phosphatase (blue rectangle) through a common regulatory molecule (green rectangle)[17]. In the simulation, it was assumed that LTP or LTD occur depending on whether the activity of the kinase (CaMKII) is higher or lower than that of phosphatase (CaN). Simulation replicated the occurrences of LTD and LTP at low and high frequency region (red curve in Figure 7A). It was surprising, however, to find that at a much higher frequency region, LTD occurred again. Since high-frequency LTD was not known previously, we validated this novel phenomenon by experiments. In addition, experiments strongly suggested that the mechanisms shared the common pathway as for known LTD and LTP.

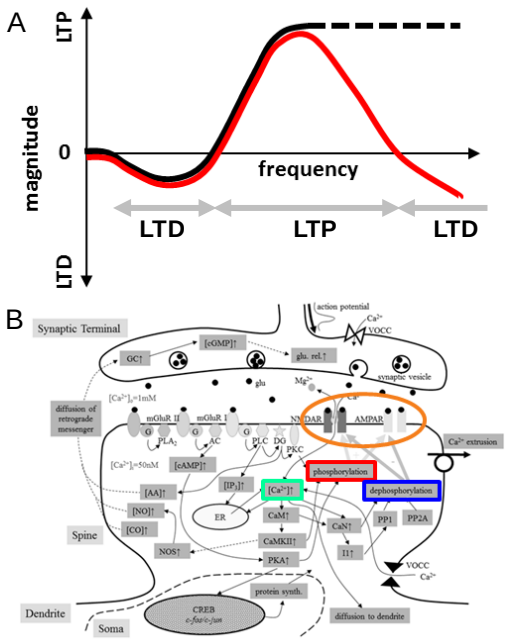

Figure 7 Molecular mechanism of synaptic plasticity (B) and a prediction of unknown phenomenon by simulation (A).

Thus, a phenomenon of high frequency LTD was predicted by the simulation and validated by experiment. This prediction, however, was not made intentionally. Intentional prediction of novel phenomenon is not easy. This is a good example showing a detailed analysis of a model has a chance of finding a novel phenomenon without enlargement and expansion of a pathway model.

16 Synaptic plasticity refers to a phenomenon where transient transmission through a synapse resulted in a long-term modification (strengthening or weakening) of synaptic transmission efficacy.

17 Ichikawa, K., et al., Neurocomp., 70(2007), 2055.

Utilization 7: Identifying Most Effective Druggable Target (Systems Pharmacology)

Utilizations 1-6 show us applications of simulation in basic molecular cell biology. In Utilizations 7 and 8, I introduce applications in drug development. An example here is using simulations aimed at finding the most effective druggable target in the NF-κB pathway for chronic obstructive pulmonary disease[18]. Oscillation of nuclear NF-κB is already introduced in Utilizations 2 and 5. Nuclear NF-κB regulates transcription. The problem is determination of the most effective suppressor point in the pathway for the blocking NF-κB activity in the nucleus.

In experiments, inhibitors are applied to each target one-by-one to investigate the effect on the inhibition of a target. This is a time consuming and expensive experiment. More importantly, not all targets can be appropriately inhibited. There is a problem in the quantitation, too. In the simulation, on the other hand, Sensitivity Analysis is used, in which a parameter, IKK concentration for example, is selected, and two simulations are run: one is for its base value ($[IKK]_1$) and another is for slightly perturbed value ($[IKK]_2$). Then, the change in an index for the NF-κB oscillation is compared. An example in which time integration of nuclear NF-κB ($S_{NF\text{-}\kappa Bn}$) is taken as the index is shown in Figure 8A (hatched area)[19]. Sensitivity S for parameter $[IKK]$ is calculated by

$$S = \frac{\Delta P_C / P_C}{\Delta P_S / P_S} = \frac{\left(S_{NF-\kappa B_{n2}} - S_{NF-\kappa B_{n1}}\right) / S_{NF-\kappa B_{n1}}}{\left([IKK]_2 - [IKK]_1\right) / [IKK]_1} \qquad 1)$$

Larger S indicates larger sensitivity, because S is larger when fractional change in P_C ($\Delta P_C/P_C$) is larger by the same fractional change in P_S ($\Delta P_S/P_S$). By calculating S for other parameters, one obtains comparison in S for parameters as shown in Figure 8B. Since $[IKK]$ gives the largest S in this example, it is concluded that IKK is the most effective druggable target to regulate nuclear NF-κB activity.

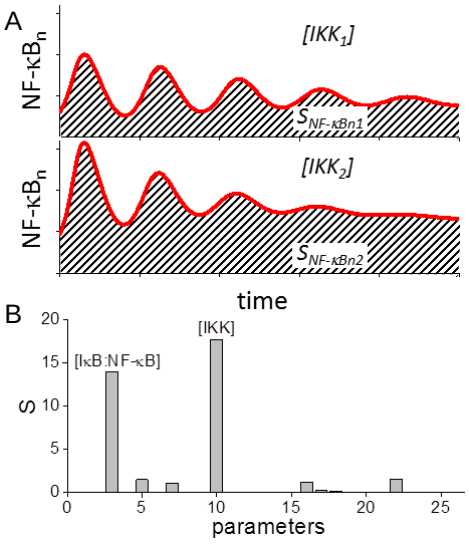

Figure 8 Sensitivity of NF-κB pathway.

This method requires many simulations with changing parameters. However, there are many advantages to simulations: first, any parameters can be tested; second, quantitative comparison is realized; third, comparison between parameters of different dimensions is available, because Eq.1) is non-dimensionalized.

[18] Cucurull-Sanchez, L., et al., Drug Discovery Today, 17 (2012), 665.

[19] In the paper by Cucurull-Sanchez, et al., they used different index from that shown here.

Utilization 8: Testing Effects of Putative Drugs (Systems Pharmacology)

This utilization is similar to Utilization 7, but it directly tests the effect of putative drugs by incorporating them into the pathway with varying K_D values for the target.

A study shown here focused putative inhibitors in NF-κB pathway[20]. Those were inhibitors for IKK and NF-κB translocation to the nucleus and the known proteasome inhibitor bortezomib (red ellipsoids in Figure 9). This type of simulation is easy to run if there is a starting pathway. In addition, further realistic simulation can be run if pharmacokinetic data are available.

Simulations also showed oscillation of nuclear NF-κB as in Utilizations 2, 5, and 7, which was reduced or even enhanced by the addition of these putative drugs. Although the concept and the model were simple, simulation results were beyond our expectation as shown below:

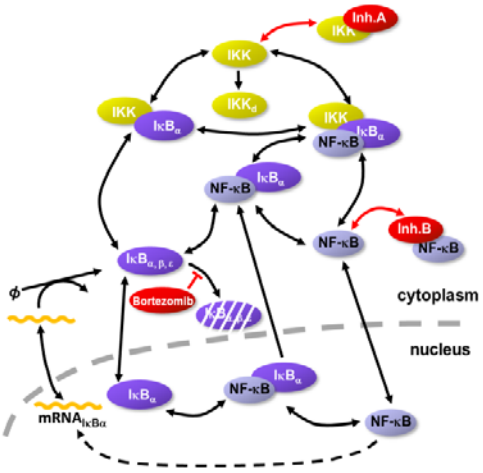

Figure 9 Testing effects of putative drugs.

1) While the low dose of Inh.A, which inhibited IKK, slightly decreased the peak concentration of nuclear NF-κB, persistency of oscillation was greatly enhanced,
2) If Inh.A was applied by three-fold higher concentration, oscillation was almost abolished,
3) Inh.B, which inhibited NF-κB, behaved conversely, where low dose resulted in the reduction in the oscillation, and higher dose increased the persistency.
4) Simulations with pharmacokinetic consideration showed increased persistency by the application of Inh.A and bortezomib, and large reduction in the oscillation by Inh.B.

Simulation results showing enhanced oscillation by the addition of inhibitors as in 1), 3), and 4) were counter intuitive. There was no analysis for these results in the paper. However, these results suggested that we should be very careful about the addition of drugs, because the result could be the opposite from what we were expecting.

Although Utilization 8 is a simple and direct application of simulation, one can expect to obtain more data that were not obtained by Sensitivity Analyses shown in Utilization 7. Thus, Utilization 8 provides advantages that are not obtained by Sensitivity Analysis.

[20] Sung, M.-H., et al., Mol.Pharmacol., 66(2004), 70.

Conclusions

When you try to introduce simulation study in your molecular cell biology research for the first time, you may face to some hurdles. These include finding and selecting simulation software and how and from where the simulation should begin. These are practical questions. More importantly, how much can be obtained by the simulation may be unclear. The aim of this e-booklet was to answer these questions by illustrating how cell simulations have been utilized. Although the utilizations shown in this e-booklet are not comprehensive, I hope they help you in getting started with simulation studies.

However, there still remain uncertainties. For example, it may be difficult to determine what aspect of your research is suitable for simulation studies, or to which of Utilizations 1-8 your study corresponds, and by which part of your study you can obtain impressive simulation result. These uncertainties are largely due to the indirect relationship between the end and the means in the simulation as a tool. This situation is quite different from tools used in wet experiments, where the end and means are directly related, such as utilization of immunoprecipitation if one wants to know protein-protein interactions. Cell simulation, however, can be utilized in many ways as shown in this e-booklet[21]. Among them, Utilization 1 is easiest to be introduced in your study. It is the first step to drawing a pathway about your research and running a simulation.

There are many review articles on pathway simulation. However, a document focusing on the utilization of simulation had until now not been located to the best of our knowledge. After several trials, I categorized the situations the study is facing, and examples for four categories were shown in this e-booklet. Cell simulation is a growing field of research in molecular cell biology. Therefore, the categorization and utilization shown in this e-booklet will be modified and expanded based on the advancement in research.

e-booklets in other topics will be published hereafter. Experience and know-how play important roles in the cell simulation. If you have questions or difficulties in cell simulation, contact us (contact@tc-simulations.com). We will access your pathway and cell simulations based on our long-term experience.

[21] In fact, simulation is more than a tool.