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in vivo oriented modeling with consideration of intracellular crowding*

Noriko Hiroi, Keisuke Iba, Akito Tabira, Takahiro Okuhara, Takeshi Kubojima, Takumi Hiraiwa, Tetsuya J Kobayashi, Kotaro Oka and Akira Funahashi

***Abstract*— *In vivo* reaction space is constrained by complex structures which are made of entwined cytoskeletons and organelles; this create the difference between *in vivo* and *in vitro* in respect of molecular mobility, and it may affect reaction processes. Our motivation is to reveal the background mechanisms of the properties of molecular behaviors *in vivo* by numerical approach. For this object, we reassembled a pseudo-intracellular environment in 3D lattice space, and executed Monte Carlo simulation. By changing the relative amount of non-reactive obstacles in the simulation space, we tested the effect of the level of crowdedness to the molecular mobility and reaction processes. Our results showed that molecules demonstrated anomalous diffusion correlating to the restriction level of the reaction space. Reaction processes also showed distinct characteristics, that is increase of reaction rate at the beginning of reactions, with the decrease of the reaction rate at later time frame of reactions. Our results suggested that the anomalous behaviors at singe molecule level *in vivo* could bring an essential difference to the reaction processes and the results.**

I. Introduction

In vivo reaction space is very different from *in vitro* reaction space because of their crowdedness and inhomogeneity [1]. The conditions of reaction space in classical numerical models are assumed ideal conditions, which differ from the actual conditions of *in vivo* biochemical reactions [2]. Recently, Aoki *et.al.* have revealed the differences of reaction processes between *in vivo* and *in vitro*, and also the effect of crowding which affects reaction processes by experiments [3]. Although classical models can compute *in vivo* reactions with sufficient approximation in specific conditions, such approximation does not stand as a general approach. A new method is desired to apply various biological phenomena occurring in non-ideal conditions.

We showed in our previous works that the characteristics of *in vivo* structures with fractal dimension, and the level of anomaly of molecular diffusion in cytoplasmic region, which is considered as a typical *in vivo* environment [4]. In the paper, we also showed that parameter values may not change essential behaviors of *in vivo* molecules (Supplemental Figures of [4]). We also reconstructed an artificial *in vivo* reaction environment to test the realistic size distribution and the diffusion constant of non-reactive obstacles (NROs) *in vivo* [5]. Finally we suggested the significance of the ratio between the surface and its volume of environmental obstacles in physiological environment, and the simple rule could define the size, shape and density of environmental obstacles [6]. By those 3 papers, we showed that there is a specific range of relative amount of NRO to enhance the mean square displacement (MSD) of molecules.

The missing point among our former studies for *in vivo* oriented modeling is the investigation of reaction processes, such as a catalytic process of a substrate with an enzyme to produce a specific product. This kind of test has important meaning, for example to confirm if one of the characteristics of *in vivo* diffusion, which is slow-exhaustion [4], could have realistic effects to the dynamics of the reaction substrate and the product.

Here, we examined Michaelis-Menten Model, which is a popular simulation method for enzymatic reaction, with Monte Carlo simulation. The results showed that the characteristics which was observed in the molecular diffusions were reflected to the results of molecular reaction.

II. Experimental Procedures

A. Michaelis-Menten kinetics by Monte Carlo simulation in crowded reaction space

We assembled intracellular environment with lattices in a 3 dimensional space, and executed Monte Carlo simulations [Fig. 1].

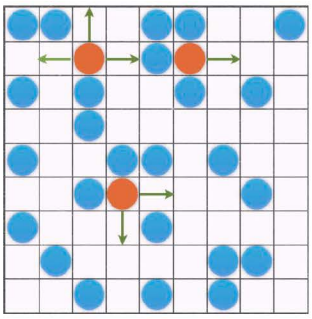

Figure 1. A schematic figure of a Reaction space. Red solid-filled circles: reactants, blue solid-filled circles: non-reactive obstacles, green arrows: mobile direction.

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N. H., T. O., T. K., K. O. and A. F. are with Department of Biosciences

N. H., T. O., T. K., K. O. and A. F. are with Department of Biosciences and Informatics, Keio University, Yokohama, 223-8522 Japan (corresponding author to provide phone: +81-45-566-1584; fax: +81-45-566-1789; e-mail: hiroi@bio.keio.ac.jp).

K. I. and A. T. were with Department of Biosciences and Informatics, Keio University, Yokohama, 223-8522 Japan.

T. J. K. is with Institute of Industrial Science, the University of Tokyo, Meguro Ward, 153-8505 Japan.

By changing the relative amount of non-reactive obstacles in the simulation space, we tested the effect of the crowdedness to the molecular diffusion and the reaction processes.

The reaction we modeled is as follows;

$$E + S \rightleftarrows ES \rightarrow E + P \quad (1)$$

We defined that reactions occur when the reactants exist on the same lattice. We verified the reaction processes and the results of Michaelis-Menten kinetics by the simulation of this model.

We additionally simulated the diffusion processes of single molecule for the verification of the condition of our reaction space.

B. *Detailed definition of Reaction Space*

We enabled three types of reaction spaces to simulate: limited Cube, periodic Cube, and Sphere [Fig. 2]. Also, molecules and obstacles are available to set randomly or manually in these reaction spaces.

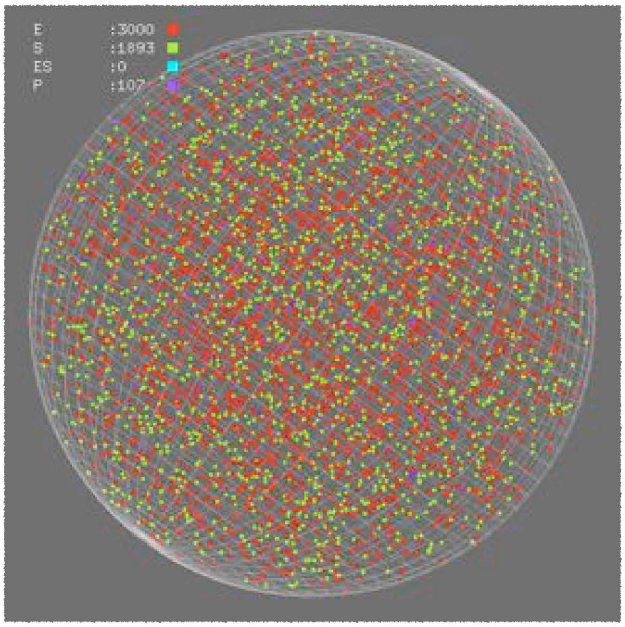

Figure 2. An example of graphical presentation of executing Michaelis-Menten kinetics in sphere space. We visualized simulation processes to help visceral understanding of users. In this figure, Enzymes are represented with red dots, substrates are represented with green dots, reaction intermediates are represented with light blue dots, and the products are represented with purple dots in a latticed-sphere reaction space.

C. *Definition of Reaction processes*

Our simulator requires the order of the reaction space to be set. Once started the simulation, simulator chooses a lattice among neighbors randomly as the next position for a mobile molecules. Then the reaction or movement is determined for each molecules by following rules.

1. movement
   If neighbor lattice is empty, a non-degradable molecule moves to the neighbor lattice.
2. stay
   If a non-reactive obstacle exists on the neighbor lattice, a molecule stays the same lattice. This means our model is equivalent with a blind ant model in this case.
3. reaction
   3-1. binding reaction: If a reactive species exists on the neighbor lattice, binding reaction occurs with a fixed probability [Fig. 3A].

   3-2. degradation: If neighbor lattice is empty, degradable species divides into the original and the neighbor lattices with a fixed probability [Fig. 3B].

Figure 3 Binding reaction (A) and degradation (B) in our definition. A. binding reaction. Two reactants bind in one of the lattices which they had been in the previous step, and transform into one new reactant. B. degradation. One reactant may occupy two lattices, which include the original lattice and the next lattice of the original lattice, in the next step, and will start to behave as independent two reactants, respectively.

We performed each simulation 100, 000 times and showed the average among 100,000 times simulation in Figure 4, 5 and 6.

III. Results

We adopted a cubic lattice as our reaction space. The boundary condition was defined as periodic in the following tests. By changing the relative amount of NRO in the simulation space, we executed single molecular Monte Carlo simulation.

A. *Verification of the molecular behaviors in pseudo-in vivo space*

We evaluated our pseudo-*in vivo* reaction space with MSD of mobile molecules [Fig. 4].

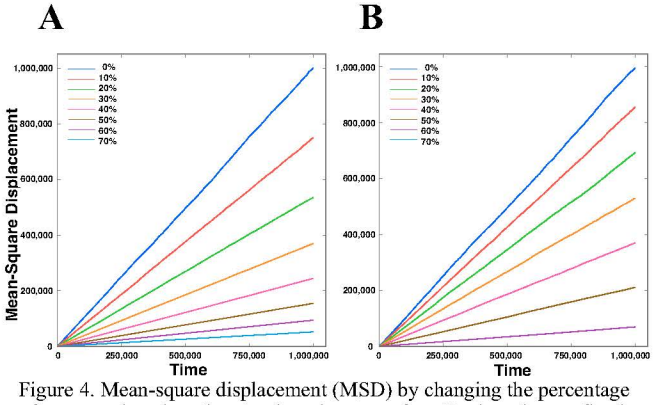

Figure 4. Mean-square displacement (MSD) by changing the percentage of non-reactive obstacles. A. obstacles move free. B. obstacles are fixed. The standard deviations are ±0.0276 ~ ±88.9 during the simulation time.

Figure 4 shows that the increase of relative amount of non-reactive obstacles makes the molecular mobility lower. We confirmed that if non-reactive obstacles move freely, the molecular behaviors show the same characteristics with the behaviors of concentrated reactants in ideal environment [Fig. 4A]. Molecular mobility is higher with 10-50% of

fixed obstacles than free obstacles, but this result is reversed with over 50% of obstacles in the reaction space [Fig. 4B].

B. *Verification of the reaction processes*

Based on the above results, we fixed the non-reactive obstacles to execute Michaelis-Menten kinetics by changing the relative amount of non-reactive obstacles [Fig. 5]. Figure 5 shows that the crowdedness makes difference in reaction processes. The difference between non-crowded [Fig. 5A] and crowded [Fig. 5B] condition was appeared clearly at the beginning of the reaction. The reaction rate was facilitated at the beginning of the reaction. It is also visible the difference after 300 steps of the simulation. The reaction after 300 steps reaches asymptotical steady state with crowded condition although the reactions under non-crowded condition have not stopped.

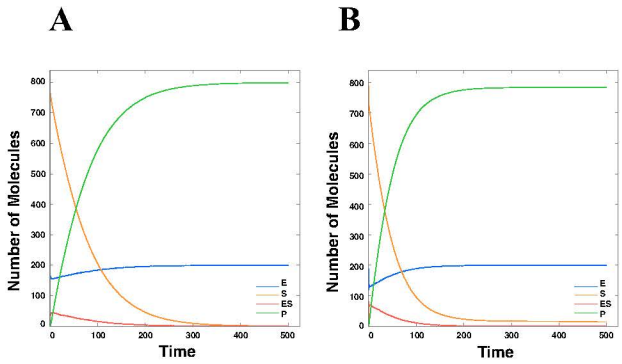

Figure 5. Time course of the concentrations of molecules of Michaelis-Menten Reaction. A. Non-reactive obstacles are not involved in the reaction space. B. 50% of the reaction space is occupied by non-reactive obstacles. The standard deviations are ±2.25 ~ ±40.6 during the simulation time.

We checked the dynamics the concentration of substrates and products [Fig. 6]. Figure 6 shows that the crowdedness accelerates enzymatic reaction in the early process of the reaction, while it decreases in the late steps. Figure 6 also shows that the crowdedness accelerates the production processes in the early steps of the reaction, while it decreases the rate in the later steps.

IV. Discussions

We exhibited here that the characteristics of single molecular behaviors including diffusion are reflected in the result of biochemical reactions. In this paper, first we checked the environmental condition which affects the molecular diffusion [Fig. 4].

The molecular diffusion *in vivo* environment has a distinct characteristics, that is quick-response and slow exhaustion [4], and our simulation showed the first part of the characteristics when the environment includes less amount of non-reactive obstacles than percolation threshold of 3 dimensional space [7].

Afterwards, we demonstrated that how the same environment affects the process of Michaelis-Menten type, enzymatic reaction. To test the reaction process, we defined the biochemical reaction process as we could describe it in the style of Monte Carlo simulation [see *C. Definition of reaction processes*]. By simulating the defined reaction

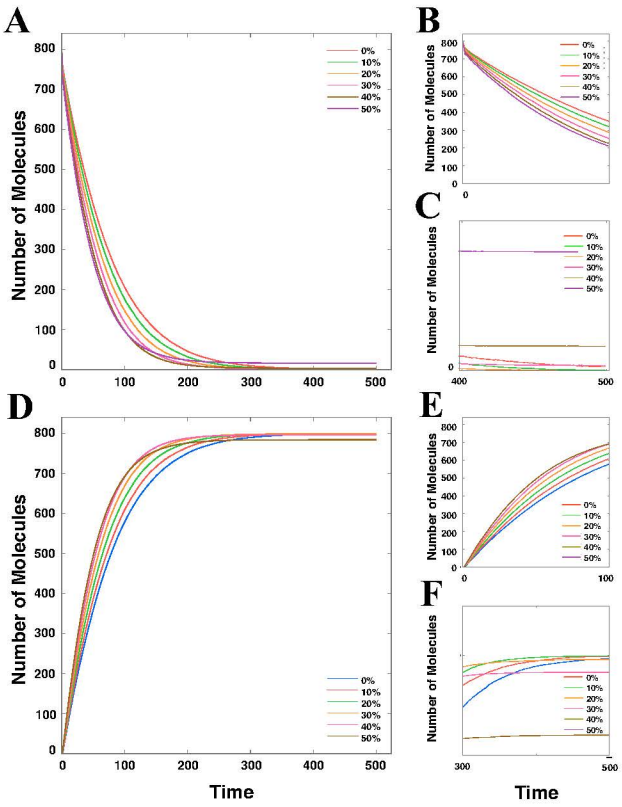

Figure 6. Time course of the concentrations of A ~ C. substrate, and D ~ F. product. Simulations were executed by changing the relative amount of non-reactive obstacles. B and C are the parts of A. B shows the result of the early time frame, C shows its later time frame. E and F are the parts of D. E shows the result of the early time frame, F shows its later time frame. The standard deviations are ± 3.50~ ± 38.8.

processes, we found that the same characteristics with the diffusion process appeared in the reaction results. The same characteristics is the quick-response [Fig. 6E] and slow exhaustion [Fig. 6C]. In our preliminary investigation, these two prime characteristics appear independently from the ratio between enzyme and substrate in Michaelis-Menten type reactions [Fig. 7].

In the simulation of diffusion process, the meaning of slow exhaustion was obscured. Now it is clear that the substrate are much remained when the non-reactive obstacles occupied the reaction space nearly 40%, which is close to the realistic *in vivo* environment [4].

The interesting point is that these reactions at *in vivo* like environment show asymptotical equivalent phase after long reaction time [Fig. 6]. We need to prove if the apparent characteristics is mathematically true or not; and if it would be proved, it may be one of the essential properties of an *in vivo* reaction.

We have paid much attention to percolation theory as the cause of anomalous behavior of intracellular molecules. It will be necessary to consider two more candidates of anomalous behaviors of *in vivo* molecules; that are viscoelasticity which is produced between mobile molecules and environmental structures [8], and the combination of diffusing and trapped time of molecules [9].

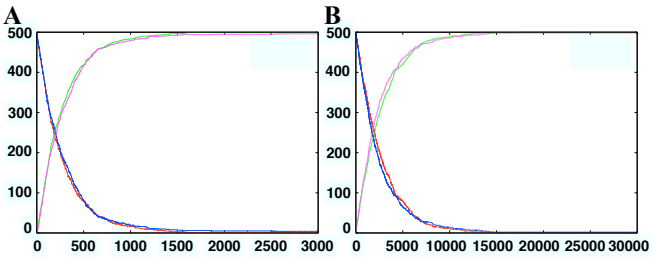

Figure 7. The effect of changing the ratio between enzyme and substrate. A. The amount of enzyme is 10 times of the amount of substrate. B. The amount of substrate is 10 times of the amount of enzyme. Red lines; substrate in free 3D space. Green lines; product in free 3D space. Blue lines; substrate in the space with 40% of NRO. Pink lines; product in the space with 40% of NRO. In both conditions, Reactions start quicker in the space with 40% of NRO than the reactions in free space, also substrates are remained after the reactions had achieved a steady state.

Briefly, our first experiments with biological materials suggested a simple rule to construct *in vivo* like structures and interior complexes of molecular behaviors, which consists of both diffusing and confined characters [Fig. 8].

We will confirm the *in vivo* behaviors of molecules by further biological experiments and will try to define the general model as an *in vivo* oriented model with appreciating the structure of the reaction space and the inhomogeneous behaviors of individual molecules.

V. Conclusions

Our simulation results revealed that molecular behaviors and reaction processes are affected with the relative amount of non-reactive obstacles in a reaction space. Especially, the crowdedness yielded high reactivity between substrates and enzymes at early stage of reaction processes, while it conserves substrates at the late steps of reaction processes. This means the crowdedness affects the process and the result of intracellular reactions, and the effect appears as quick-response and slow-exhaustion.

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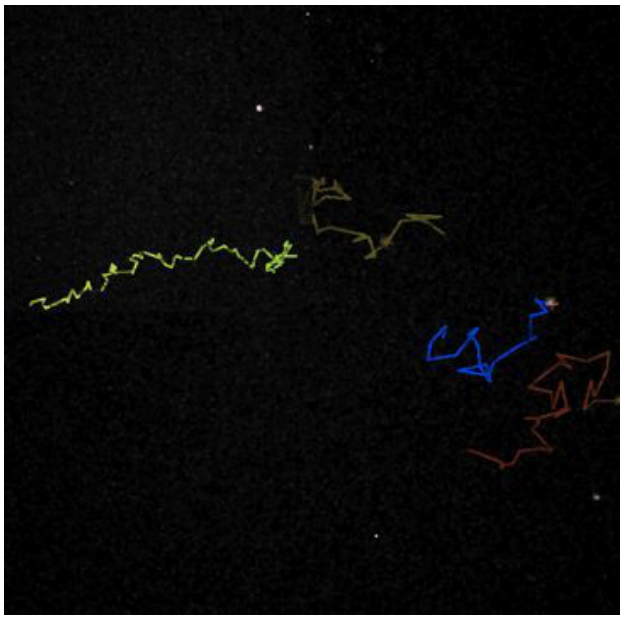

Figure 8. Particle tracking in cytoplasmic sol. The 4 tracked particles are green fluorescent proteins (GFP) in cell free system. GFPs showed nealy-random walking and sometimes they showed more confined behaviors (the right end of green track and the left end of yellow track). The tracks were analysed by SpotTracker [10].

References

[1] Medalia, O. et al. Macromolecular architecture in eukaryotic cells visualized by cryoelectron tomography. Science, Vol. 298, No. 5596, pp. 1209–1213.

[2] N. Hiroi and A. Funahashi. Kinetics of Dimension Restricted Condition (Chapter 14 of Introduction to Systems Biology). Humana Press, NJ USA, 2007

[3] Aoki, K. et al. “Processive phosphorylation of ERK MAP kinase in mammalian cells. PNAS, Vol.108, No. 31, pp. 12675-12680, 2011.

[4] Hiroi N, Lu J, Iba K, Tabira A, Yamashita S, Okada Y, Flamm C, Oka K, Kohler G and Funahashi A. "Physiological environment induce quick response - slow exhaustion reactions" Frontiers in Systems Physiology, Vol 2, Article 50. 2011

[5] Hiroi, N., Klann, M., Iba, K, Ciechomski, PH., Yamashita, S., Tabira, A., Okuhara, T., Kubojima, T., Okada, Y., Oka, K., Mange, R., Unger, M., Funahashi, A. and Koeppl, H. "From Microscopy Data to *in silico* Environments for *in vivo* Oriented Simulations" EURASIP Journal on Bioinformatics and Systems Biology. 2012:7.

[6] Hiroi, N., Okuhara, T., Kubojima, T., Iba, K., Tabira, A., Yamashita, S., Okada, Y., Kobayashi, TJ. and Funahashi. A. "Physiological inracellular crowdedness is defined by perimeter to area ratio of subcellular compartments" Frontiers in Fractal Physiology, Vol 3, Article 293. 2012.

[7] Stauffer, D., Adler, J. and Aharony, A. “Universality at the three-dimensional percolation threshold.” J. Phys. A., Math. Gen. 27, L475-L480 , 1994.

[8] Stephanie C. Weber, S.C., Spakowitz, A.J., and Theriot, J.A. “Bacterial Chromosomal Loci Move Subdiffusively through a Viscoelastic Cytoplasm.” Pysical Review Letters, vol 104, 238102, 2010.

[9] Weigel, A.V., Simon, B., Tamkun, M.M., and Krapf, D. “Ergodic and nonergodic processes coexist in the plasma membrane as observed by single-molecule tracking.” Proc Natl Acad Sci U S A. vol 108, no 16, pp. 6438–6443, 2011.

[10] Sage, D., Neumann, F.R., Hediger, F., Gasser, S.M., and Unser, M. "Automatic Tracking of Individual Fluorescence Particles: Application to the Study of Chromosome Dynamics," IEEE Transactions on Image Processing, vol. 14, no. 9, pp. 1372-1383, 2005.