We aim to create a Virtual Physiological Human (VPH), which will integrate the hierarchical modular structures that consist of molecules, cells, tissues, organs, and bodies. It provides an intelligence framework for biomedical research, promoting computer-aided systems for drug design/development; disease diagnosis, prevention, and treatment; and care/welfare. In this symposium, we would like to define the Virtual Physiological Human, and present its roadmap through intensive discussion.

The Third BMIRC International Symposium for Virtual Physiological Human

March 5-6, 2015, lizuka, Japan

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Hsuan-Cheng Huang, National Yang-Ming University
Ueng-Cheng Yang, National Yang-Ming University
Tobias Bollenbach, Institute of Science and Technology Austria
Satoru Miyano, The University of Tokyo
Yoshihiro Usuda, Ajinomoto Co., Inc
Yousuke Nishio, Ajinomoto Co., Inc
Hirotsada Mori, Nara Advanced Institute of Science and Technology (NAIST)
Hirotshi Matsuno, Yamaguchi University
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Biomedical Informatics R&D Center (BMIRC) at Kyushu Institute of Technology (Kyutech)
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March 5 - 6, 2015
Nogami President Hotel, Iizuka, Fukuoka, Japan
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The proceedings of the Third BMIRC International
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Message

The medical expenditure increases every year and is readily expected to increase, considering transitions toward an aging society. Therefore, it is critically important to attain biomedical innovation to reduce medical expenses while achieving high-quality medical care.

To solve such a global problem, we aim to create a Virtual Physiological Human (VPH), which will integrate the hierarchical modular structures that consist of molecules, cells, tissues, organs, and bodies. It provides an intelligence framework for biomedical research, promoting computer-aided systems for drug design/development; disease diagnosis, prevention, and treatment; and care/welfare. In this symposium, we would like to define the Virtual Physiological Human, and present a roadmap through intensive discussion.

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Registration
For registration or participation, send an e-mail to the office (kurata@bio.kyutech.ac.jp). Free registration fee.

Symposium Dinner
The symposium dinner is held on March 5, 2015 at Ikeno-Okuen

Venue
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Ikeno-Okuen, Fukuoka, Japan on March 5 2015

Nogami President Hotel, Fukuoka, Japan on March 6 2015
Schedule

March 5 (Thursday) at Ikeno-Okuen Garden

Session 1  Virtual Cells  (Chair: Mariko Okada-Hatakeyama)
9:10-9:40  Hiroyuki Kurata  Kyushu Institute of Technology (Kyutech)
9:40-10:10  Yoshihiro Usuda  (Invited speakers)
            Ajinomoto Co., Inc.
10:10-10:40  Hiroshi Matsuno  (Invited speakers)
            Yamaguchi University

Session 2  Advanced Systems Biology  (Chair: Hiroyuki Kurata)
10:50-11:30  Tobias Bollenbach  (Invited speakers)
            Institute of Science and Technology Austria
11:30-12:00  Mariko Okada- Hatakeyama  RIKEN

12:00-13:00  Lunch

Session 3  Metabolic and Genetic Network Analysis
            (Chair: Hiroshi Matsuno)
13:00-13:40  Johnjoe Mcfadden  (Invited speakers)
            University of Surrey
13:40-14:10  Hirotada Mori  (Invited speakers)
            Nara Advanced Institute of Science and Technology (NAIST)
14:10-14:40  Atsushi Mochizuki  (Invited speakers)
            RIKEN

14:40-16:00  Japanese Tea Party
Session 4  Biomedical Informatics (Chair: Hiroto Saigo)
16:00-16:30  Hsuan-Cheng Huang  (Invited speakers)
               National Yang-Ming University
16:30-17:00  Ueng-Cheng Yang  (Invited speakers)
               National Yang-Ming University
17:00-17:40  Satoru Miyano  (Invited speakers)
               The University of Tokyo

18:00-20:00  Symposium Dinner

March 6 (Friday) at Nogami President Hotel

Session 5  Computational Systems Biology (Chair: Hiroyuki Kurata)
9:00-9:20  Kazuhiro Maeda  Kyutech
9:20-9:50  Hseuh-Fen Juan  National Taiwan University
9:50-10:20  Fumiko Matsuzaki  Kyushu University
10:20-10:50  Katsuya Nagayama  Kyutech

10:50-11:10  Coffee break

Session 6  Genome Biology (Chair: Atsushi Mochizuki)
11:10-11:40  Huai-Kuang Tsai  Academia Sinica
11:40-12:10  Hiroto Saigo  Kyutech

12:10-13:30  Lunch

Session 7  Systems Biology & Perspectives (Chair: Hirotada Mori)
13:30-14:10  Tobias Bollenbach  (Invited speakers)
               Institute of Science and Technology Austria
14:10-14:50  Johnjoe Mcfadden  (Invited speakers)
               University of Surrey

Session 8  Poster presentation
15:30-16:30  Poster presentation
TABLE OF CONTENTS

**Oral presentation**

Synthesis and decomposition approach for rational design of a biochemical network

Hiroyuki Kurata .......................................................... 13

Metabolic Engineering of Amino Acid Producing Microbes Based on Dynamic Metabolic Simulation

Yousuke Nishio and Yoshihiro Usuda ........................................ 15

Modeling of Glycogen Utilization in *E. coli*

Hiroshi Matsuno and Zhongyuan Tian ........................................ 16

The underlying mechanisms of drug interactions

Tobias Bollenbach .............................................................. 17

Analog to Digital Conversion in Signal-Transcription Networks

Mariko Okada-Hatakeyama .................................................... 18

Carbon fixation and a mixed diet for the intracellular tuberculosis bacillus


*E. coli* systems Biology: from the comprehensive experimental side

Hirotada Mori ...................................................................... 20

Sensitivity of chemical reaction networks: A structural approach

Atsushi Mochizuki and Bernold Fiedler ....................................... 21

Phylostratification of Human Cellular Networks

Hsuan-Cheng Huang .................................................................. 22
Bioinformatic Analysis on Disease Candidate Genes and Mechanisms
Ueng-Cheng Yang

Understanding Cancer Heterogeneity with Supercomputers
Satoru Miyano

Toward a quantitative in silico model for the *E. coli* ammonium assimilation system
Kazuhiro Maeda, Fred C. Boogerd, Frank J. Bruggeman, Hans V. Westerhoff and Hiroyuki Kurata

Genome-wide analysis of enhancer RNA in gene regulation across 12 mouse tissues
Huai-Kuang Tsai

Absolute quantification of all human metabolic enzymes and metabolic systems analysis
Fumiko Matsuzaki, Masaki Matsumoto, Kiyotaka Oshikawa and Keiichi I. Nakayama

Dynamic Response Network of Ectopic ATP Synthase Blockade
Hsueh-Fen Juan

Particle simulation of tissue formation and angiogenesis toward constructive systems biology
Katsuya Nagayama

Towards predicting the epistasis in genome wide association study
Kento Kodama and Hiroto Saigo
**Poster presentation**

**cEM: a tool for integrating heterogeneous biological data into metabolic flux distribution**
Md. Bahadur Badsha and Hiroyuki Kurata

**Automatic construction of calculable metabolic networks from public database**
Cuncun Chen, Daisuke Koishi, Noorlin Mohd Ali, Hiroyuki Kurata

**Stochastic Simulation of Dynamical Behavior in Gene Regulatory Network**
A.B.M. Shamim Ul Hasan and Hiroyuki Kurata

**Dynamic sensitivity analysis of ErbB signaling system**
Hiroyuki Masunaga, Ryunosuke Itasaki and Hiroyuki Kurata

**Functional membrane for influenza virus sensors**
Hirotaka Matsumasa, Takeshi Goto and Kazuya Uezu

**Modeling and simulation for the effect of oxygen level on the main metabolism in Escherichia coli**
Yu Matsuoka and Hiroyuki Kurata

**Genetic Modification Flux (GMF): A tool to predict flux distribution of mutants**
Noorlin Mohd Ali, Koishi Daisuke, Kentaro Inoue and Kurata Hiroyuki

**The analysis of reaction kinetics for dynamic modeling of metabolic systems**
Yurie Sugimoto and Hiroyuki Kurata

**Automatic Registration of Phalange Regions in CR Images Based on Genetic Algorithm**
Shota Kajihara, Seiichi Murakami and Hyoungseop Kim
Automatic Detection of Lung Nodules Based on Statistical Features in Volume of Interest on Temporal Subtraction Images........................................41
Shuji TANAKA, Yuriko YOSHINO, Hyoungseop KIM, Joo Kooi TAN,
Seij ISHIKAWA, Seiichi MURAKAMI, Takatoshi AOKI,
Rie TACHIBANA, Yasushi HIRANO and Shoji KIDO

Particle Simulation on Cancer Growth and Angiogenesis - Modeling of stromal cells .................................................................42
Yuikio Nagamizu, Youhei Tajima, Katsuya Nagayama, Ichiro Miura and Sakae Saito

System analysis for oscillation and switch-like response of NF-kappaB in B cell..................................................................................43
Kentaro Inoue, Hisaaki Shinohara and Mariko Okada-Hatakeyama

Identification of novel anti-mycobacterial inhibitors through in silico screening ..............................................................................44
Hironori Kanetaka, Yuji Koseki, Tomohiro Umei, James C. Sacchettini and Shunsuke Aoki

Discovering novel active compounds against Mycobacterium thymidine monophosphate kinase with pharmacophore-based in silico screening ......45
Yuji Koseki, Hironori Kanetaka, Hélène Munier-Lehmann and Shunsuke Aoki

Numerical Simulation of Alveolar Bone Regeneration and Angiogenesis -Implementing the Stress Factor........................................46
Akihiro Kitamoto, Katsuya Nagayama and Masato Matsuo

The relationship of epileptic form activities and carbachol-induced β oscillation in rat hippocampal slices........................................47
Toyohiro Sawada and Kiyohisa Natsume

A study on signal transmission system realized by biochemical reactions for molecular robot ........................................................48
Ryosuke Suzukawa and Takashi Nakakuki
Modeling and Simulation on Cancer Cell Migration and Adhesion .......... 49
    Ayane Ito, Katsuya Nagayama, Reiko Minamikawa-Tachino
    and Kiyoshi Ogura

Particle Simulation on Formation of Epidermal Skin - Formation
Mechanism of Basal Layer ................................................................. 50
    Takeshi Kurihara and Katsuya Nagayama

2D Numerical Simulation of Liver Cell Proliferation with Angiogenesis
- Hepatic Lobule Formation - ................................................................. 51
    Hiroto Tanaka, Katsuya Nagayama, Nana Shirakigawa
    and Hiroyuki Ijima

Toward a quantitative in silico model for the E. coli ammonium assimilation
system .......................................................................................................... 52
    Kazuhiro Maeda, Fred C. Boogerd, Frank J. Bruggeman,
    Hans V. Westerhoff and Hiroyuki Kurata

Mining discriminative patterns from graph data with multiple labels....... 53
    Zheng Shao and Hiroto Saigo

Estimating the number of SNPs in interaction by kernel methods .......... 54
    Kento Kodama and Hiroto Saigo

Mining n-way SNP interactions with statistical metric pruning .......... 55
    Takuro Yamaguchi and Hiroto Saigo

Importance of averagings in EEG engineering ...................................... 56
    M.Yamada, Y.Uchida, S.Okamoto, H.Nagaoka, M.Muraki, R.Ueda,
    K.Kondo, T.Ozaki, H.Tsutaka, K.Matsumoto, T.Yamamoto and
    T.Yamazaki

Novel optical approaches for monitoring of green fluorescence from biological
samples under newly designed artificial light ............................................. 57
    Ayaka Hara and Tomonori Kawano
Induction of superoxide generation by application of ferrous ion to human methemoglobin ........................................................................................................... 58
  Shota Kondo, Makoto Kimura and Tomonori Kawano

Plant enzymes as immune-therapeutic tools for cell-targeted lethal control. ................................................................................................................................. 59
  Makoto Kimura, Yosuke Umemoto and Tomonori Kawano

Reflectivity measurement as nondestructive optical monitoring of vegetable and fruit nutrients: Towards development of product identification system linked to nutritional data .......................................................................................................................... 60
  Yuki Murata, Makoto Kimura, Ayaka Hara, Hiroshi Takaichi, Junichiro Iwase, Diego Comparini and Tomonori Kawano

Evaluation of DNA-targeted photolytic actions of ultraviolet light sources ................................................................................................................................. 61
  Yuki Hara, Asuka Kikuchi and Tomonori Kawano

Practical procedure for simulation of lethal toxicity curves based on limited number of experimental data points: Model demonstration using green paramecium cells exposed to toxic metals ................................................................................................................................. 62
  Hiroshi Takaichi and Tomonori Kawano

Perturbation-Response relation and network structure in biochemical reaction system ................................................................................................................................. 64
  Takashi Okada

Integrated dynamic model for the main metabolism of *Escherichia coli* ................................................................................................................................. 65
  Nusrat Jahan, Yu Matsuoka, Hiroyuki Kurata

Index ..................................................................................................................................... 67
Oral Presentation
Synthesis and decomposition approach for rational design of a biochemical network

Hiroyuki Kurata\textsuperscript{1,2}

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The goals of synthetic and systems biology are to understand the mechanisms of how biochemical networks generate particular cellular functions in response to environmental stresses or genetic changes and to rationally design these molecular processes to meet an engineering purpose. It is difficult to understand the entire biochemical network within a cell because it is too large and complicated. An alternative method would be to decompose the whole network into subnetworks in terms of topology or regulatory architecture and to build their associated mathematical models. Analogous to engineering systems, biochemical networks can be decomposed into hierarchical modules consisting of biomolecules, elementary networks/modes, and their combined networks. Biomolecules are assembled to form elementary networks, which are called network motifs or building blocks, with basic functions such as ultrasensitivity, adaptation, oscillation, and bistability. Elementary networks are further assembled to form functional combined networks to generate a variety of biological functions. Combined networks can produce additional, synergistic, or emergent functions. This synthetic approach is analogous to the standard strategy of engineering systems with a scalable, hierarchical modular structure, where a set of off-the-shelf parts with operation specifications can be combined.

In this report, such decomposition and synthesis approach is applied to a central metabolic system with gene regulatory networks. We found the intrinsic elementary modes out of a huge number of them and that the TCA cycle is coupled with glycolysis for complete digestion of carbon sources. To take intermediate metabolites from the TCA cycle as materials required for synthesis of some compounds, the pathway from PEP to OAA is essentially required. The glucose PTS and ammonia assimilation module responsible for uptaking environmental carbon and nitrogen sources are regulated in a more complex manner and using more genes than any other metabolic reactions, suggesting that the uptake systems are critically important for metabolic systems. In addition, we show that the complicated ammonia assimilation system consists of just three elementary networks and they are rationally assembled.
in the form of control block diagrams, providing a robust property to a change in environmental ammonia concentration. Out of a huge number of combinations, the existing network structure evolves. In the same manner, the complex PTS involving many gene expressions can be identified as the incoherent feedforward loops with autoregulation. We built the mathematical models of those uptake systems to reveal the mechanism of how they provide a robust property to changes in environmental glucose and ammonia.

References
• Koichi Masaki, Kazuhiro Maeda, Hiroyuki Kurata, Biological design principles of complex feedback modules in the *E. coli* ammonia assimilation system. *Artificial Life*, 18:53-90, 2012.
Metabolic Engineering of Amino Acid Producing Microbes Based on Dynamic Metabolic Simulation

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Primary metabolites are important industrial products, which can be produced by fermentation process. Among amino acids used as seasoning, feed additives, and fine chemicals and mainly produced by bacteria such as Escherichia coli, glutamate is produced all over the world in the greatest amount, over two million tons. Thus, fermentation process of primary metabolites is of industrial importance and has been one of promising target for metabolic engineering. Dynamic simulation of cell metabolism is becoming a useful method for bioprocess development. Our motivation is to construct a practical simulation system which can describe the transient metabolic and regulatory states of amino acid production process and facilitate identification of novel factors involved in the metabolic regulation.

The metabolic part of the model included PTS, glycolysis, pentose-phosphate pathway, TCA cycle, glyoxylate shunt, and anaplerotic pathways and regulatory part including regulation by transcription factors, CRP, Mlc, Cra, PdhR, and IclR. Metabolic fluxes measured by a $^{13}$C-substrate method were used as in vivo enzyme activities for parameter adjustment. The manual parameter adjustment based on batch-cultivation of a wild-type strain and a model glutamate producing strain, MG1655 Δ$sucA$, achieved a certain level of simulation results. Furthermore, systematic sensitivity analysis was performed and revealed that the results corresponded with previous knowledge obtained from experimental data. It also allowed us to predicted the possibility that accumulation of 3-phosphoglycerate in the cell would regulate the carbon flux into the TCA cycle and lead to an increase in the yield of glutamate production. We validated this hypothesis through a fermentation experiment involving a MG1655 Δ$sucA$ strain, in which the phosphoglycerate kinase gene had been amplified to cause accumulation of 3-phosphoglycerate. The observed increase in glutamate production verified the biologically meaningful predictive power of our dynamic metabolic simulation model.
Modeling of Glycogen Utilization in *E. coli*

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A living thing absorbs and excretes materials with environment to sustaining its life by metabolism, in which glucose metabolic pathway occupies a central position. However, in studies of central metabolic pathway, glycogen metabolic pathways have always been unseen. Glycogen is intracellular carbohydrate storage and donor, and its efficient metabolism could cause many types of glycogen storage diseases and even cancers.

A hybrid functional Petri net (HFPN) model of glucose-glycogen centered metabolic pathways was built up by combining an ordinary differential equation model of glycolysis, a mass theory balance-based PTS model, and a top-down strategy model of glycogen metabolism by fitting kinetic coefficients to experimental data of glycogen and G6P.

Modeling of regulatory mechanisms was carried out based on thousands of research publications. By filtering and integrating them, an HPr and EIIA\(^{\text{Glc}}\) centered regulatory circuit over glucose-glycogen metabolism was selected, which goes across as many as five cellular levels; gene expression, metabolism, molecules localization, materials flux, and protein levels.

By integrating HPr localization, HPr::GlgP complex formation, an HFPN model of PTS control and gene expression, glucose-glycogen metabolism and HPr and EIIA\(^{\text{Glc}}\) centered regulatory circuit is constructed, with which a secret of an *E. coli* in controlling glucose-glycogen metabolic pathway was unveiled.

In this work, via systematic study of G6P entry part of the central metabolic pathway, glycogen was evaluated as important source, along with the process of whole proliferating time course data together with regulatory mechanism of HPr and EIIA\(^{\text{Glc}}\).
The underlying mechanisms of drug interactions

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When drugs are combined, their individual effects may be amplified or weakened. Such drug interactions are crucial for treatment efficacy but their underlying causes remain largely unknown. We investigated the underlying causes of antibiotic interactions by measuring individual and joint effects of these drugs on growth of genome-wide Escherichia coli gene deletion strains. Drug interactions turn out to be highly robust to genetic perturbation. This robustness is encapsulated in a general principle of bacterial growth which enables the quantitative prediction of mutant growth rates under drug combinations. Rare violations of this principle exposed recurring cellular functions controlling drug interactions; in particular, we found that polysaccharide and ATP synthesis control multiple different drug interactions. Small molecule adjuvants targeting these functions reshape drug interactions in predictable ways. These results suggest that seemingly unrelated drug interactions have similar underlying mechanisms and offer a new strategy for the design of multidrug combinations.
Analog to Digital Conversion in Signal-Transcription Networks

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Mammalian signal transduction pathways have developed to sense, sort and transfer a variety of extracellular information to transcription factors in the nucleus to regulate gene expression. Interestingly, signaling pathways often control these processes in a nonlinear fashion and, in some cases, analogous graded doses of extracellular stimuli such as growth factors and antigens promote digital activation of transcription factors. Such digitally activated transcription factors may be more easily recognized by their association partners in a noisy cellular environment. Time and space-resolved context-dependent network topology plays an essential function to realize these types of responses.

In this talk, digital activation mechanisms of c-Fos and NF-κB transcription factors, which play important roles in cellular commitment, will be described. Based on wet-lab experiments and mathematical modeling, we showed that prolonged ERK activation along with c-fos transcription and c-Fos protein stabilization by ERK forms a coherent feedforward AND gate for full activation of c-Fos in breast cancer cells [1]. In this system, duration of ERK activity is a critical factor to determine the c-Fos response, and the ERK kinetics are determined at the level of membrane receptor activation. On the other hand, NF-κB activity is controlled by two positive feedback loops within the signaling pathway, from TAK1 to IKK and from IKK to IKK, to produce a switch-like activation of NF-κB [2]. These feedback loops contribute to determine the threshold for NF-κB-mediated B cell proliferation, suggesting that the mechanism is important for B cell lineage commitment.

Our studies suggest that cellular complexity might arise from combinatorial regulation of binary states of transcription factors.

Carbon fixation and a mixed diet for the intracellular tuberculosis bacillus

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Novel control strategies are urgently required to combat the emergence of multi- and extensively-resistant *M. tuberculosis* (Mtb) strains yet fundamental questions, such as the identity of the nutrients obtained from the host, remain unknown. We previously constructed a genome-scale metabolic model of Mtb and used 13C-Metabolic Flux Analysis (13C-MFA) to identify a novel metabolic pathway, the Glyoxylate-Anaplerotic-Succinyl CoA synthetase (GAS) pathway (Figure 3), operating in slow-growing cells grown in vitro.

MFA cannot be applied to dynamic systems so to extend our investigation into the interaction of Mtb with the host macrophage cell we fed macrophages 13C-labelled labelled glucose, infected them with Mtb, and used CG-MS to determine the isotopomer distribution in amino acids extracted from the macrophage and Mtb fractions. We developed another new tool, 13C-Flux Spectral Analysis Analysis (13C-FSA), to analyse 13C labelling data. 13C-FSA and identify likely nutrients that the pathogen is obtaining from its host cell and metabolic pathway utilization. 13C-FSA and confirmatory experiments demonstrated that Mtb imports a range of nutrients from its host cell including C1, C2 and C3 sources and also fixes carbon dioxide via a GAS-like metabolic pathway. The finding that intracellular *M. tuberculosis* has access to diverse carbon sources within a macrophage has significant implications for the development of therapeutics targeting intracellular bacteria and drug-screening protocols.

This work was supported by a grant from the Wellcome Trust, grant reference number 088677.
Since the completion of the genome project of E. coli K-12 in the beginning of 1997, we have long been focusing on the systems approaches based on the comprehensive quantitative measurements. To achieve this, we started to construct two types of plasmid clone libraries of the predicted ORFs, so-called ASKA ORF libraries. Both are His-tagged clones at N-terminus with or without eGFP fusion at the C-terminus. All of the DNA fragments were amplified by PCR from the W3110 or MG1655 chromosomes based on the 1997's version of the annotation. Once the libraries had been established, the new gates for the new world was opened, 1) transcriptome using cDNA type microarray analysis, 2) proteome by the pull-down assay by His-tagged proteins, 3) physiome by protein localization visualized by GFP fluorescence of each of the clones.

Before 2000, E. coli had been thought as a difficult organism to generate recombinants by homologous recombination because of the short lives of the linear exogenous DNA molecules to compare with Saccharomyces cerevisiae or Bucillus subtilis. In 2000, however, Lambda RED recombinase technology developed by Datzenko et al. broke through this difficulty comparable level to other model organisms. Then, we started to make single knockout strain library, Keio collection.

Those experimental resources could activate E. coli bacterium in the systems biology field and this situation has been producing other new requirements for the new purposes, such as genetic interaction and population dynamics.

To perform these, we have developed the new tools and resources listed below.

1) two sets of single gene knockout strain libraries to make double knockout strain by conjugation.
2) tools to control host cell sexuality to transfer the deletion region of the donor cell into the recipient cell.
3) new plasmid clone expression library transmittable into the recipient cell by conjugation.
4) single gene knockout library with bar-code for population analysis by deep-sequencing.
5) single gene knockout with bar-code complemented by plasmid clone.

I will introduce these new resources and their applications, also I will show some pilot test results.
Sensitivity of chemical reaction networks: A structural approach.

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In biological cells, chemical reaction pathways lead to complex network systems like metabolic networks. One experimental approach to the dynamics of such systems examines their sensitivity: each enzyme mediating a reaction in the system is increased/decreased or knocked out separately, and the responses in the concentrations of chemicals or their fluxes are observed. In this study, we present a mathematical method, named structural sensitivity analysis, to determine the sensitivity of reaction systems from information on the network alone. We investigate how the sensitivity responses of chemicals in a reaction network depend on the structure of the network, and on the position of the perturbed reaction in the network. We establish and prove some general rules which relate the sensitivity response to the structure of the underlying network. We describe a hierarchical pattern in the flux response which is governed by branchings in the network. We apply our method to several hypothetical and real life chemical reaction networks, including the metabolic network of the E. coli TCA cycle.
Phylostratification of Human Cellular Networks

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Molecular networks, such as the protein-protein interaction (PPI) network, offer a conceptual framework for better understanding the functional organization of cells. However, the intricacy of network complexity complicates comprehensive analysis. Here, we adopted a phylogenetic stratification method combined with force-directed graph simulation to decompose the human PPI network in a multi-dimensional manner. This network model enabled us to associate the network topological properties with evolutionary and biological implications. First, we found that ancient proteins occupy the core of the network, whereas young proteins tend to reside on the periphery. Second, the presence of age homophily suggests a possible selection pressure may have acted on the duplication and divergence process during the PPI network evolution. Lastly, functional analysis revealed that each age group possesses high specificity of enriched biological processes and pathway engagements, which could correspond to their evolutionary roles in eukaryotic cells. More interestingly, the network landscape closely coincides with the subcellular localization of proteins. Together, these findings suggest the potential of using conceptual frameworks to mimic the true functional organization in a living cell.
Bioinformatic Analysis on Disease Candidate Genes and Mechanisms

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In our cancer research, we have been using K computer (10 PFLOPS) of RIKEN and Shirokane Supercomputer Series of Human Genome Center. The project called “Post K computer” started recently in order to co-design the “Post K computer” by applications. This project is also involved with cancer big data analysis. In this talk, we will first present highly parallel software applications developed in the project “HPCI Strategic Programs for Innovative Research Field 1 “Supercomputational Life Science” (2011-2015) and the Grand Challenge Project for Life Science “Next-Generation Integrated Simulation of Living Matters (2006-2012). The first series of applications include various gene network estimation software applications (http://sign.hgc.jp/signbn/, http://sign.hgc.jp/signl1/, http://sign.hgc.jp/signssm/, http://www.csrp.riken.jp/application_e.html) on both supercomputers. The second series include a series of software applications and pipelines for cancer genome analysis (exome, whole genome, RNA sequence) on these supercomputers. By using these software applications, we are currently making challenges to understand cancer intertumor heterogeneity on network-level from cancer big data and intertumor heterogeneity from multi-regional analysis of a single colorectal cancer. Also by modeling cancer evolution and by simulation by the above supercomputers for a huge variations of parameters in models, some insights are obtained about how cancer acquires heterogeneity.
Ammonium supports the fastest growth rate and is therefore considered the preferred nitrogen source for *Escherichia coli* [1]. The bacterium adds ammonium to α-ketoglutarate an intermediate of TCA cycle, for synthesizing glutamate and glutamine. ~88% of cellular nitrogen is derived from α-amino group of glutamate whereas the remainder is derived from the amide group of glutamine [2].

We propose a new kinetic model of *E. coli* ammonium assimilation network, centered on the regulation of glutamine synthetase (GS) and an ammonium transporter AmtB. Our model employs realistic kinetic parameter values and can quantitatively reproduce both metabolome dynamics upon ammonium upshift (Yuan et al., Mol Syst Biol, 2009) and experimental data regarding cell growth, ammonium assimilation flux and intracellular ammonium concentration under low ammonium conditions (Kim et al., Mol Syst Biol, 2012). Our modeling is achieved by the following novel ideas. (i) Incorporating the diffusion resistance enables the model to explain both Yuan’s and Kim’s data, because we found that Yuan’s experimental data cannot be explained without taking into account ammonia/ammonium diffusion resistance in media. (ii) The parameter estimation problem is formulated as a constrained optimization problem, which minimizes changes in kinetic parameter values from their literature values and thus greatly enhances development of a realistic model. We revealed rational and integrative regulations of GS and AmtB, which limits futile cycling of ammonium.
Enhancers and enhancer RNAs (eRNAs) play a crucial role in enhancer-regulated transcription in a cell- and temporal-specific manner. However, the precise participation of eRNAs in regulatory systems remains unclear. To examine the role of eRNA in the enhancer-promoter relationship, we provide an in silico analysis that globally investigates genome-wide data across 12 mouse tissues. We show the positive correlations between the expression level of eRNAs and enhancer target genes is consistent across tissues. We then show that the expression level of genes targeted by transcribing enhancers is significantly higher than genes targeted by non-transcribing enhancers. Our results imply the presence of eRNA indicates a state of enhancer that further increases gene expression, distinguishing it from an enhancer state not-transcribing eRNAs. The two states of enhancers may be under tissue-specific controls, as we discovered the same eRNA-transcribing enhancer may not transcribe eRNA in other tissues. These effects of eRNAs on expression and tissue-specific control is similar to ncRNA activation (ncRNA-a). We further found a large number of eRNAs contain particular regions in which sequences and secondary structures are similar to microRNAs, which also possess gene activation potential. These results thus show that eRNAs may be involved in the positive control of the expression of the target genes, possibly in a microRNA-like ncRNA-a manner.
Ten years after completion of the Human Genome Project, fundamental principles behind living organisms remain poorly understood. Given that proteins serve as direct functional devices for biochemical reactions, comprehensive quantification of proteins is an essential step for advanced systems biology. We have developed a new technology termed in vitro proteome–assisted multiple reaction monitoring (MRM) for protein absolute quantification (iMPAQT) to measure the absolute quantity of all human proteins. With the use of iMPAQT, we have now measured the absolute quantity of almost all metabolic enzymes in human cells and uncovered the quantity for each node of human metabolic networks. In addition, we developed a new computational method to integrate the absolute quantity of metabolic enzymes, as well as those of metabolites, experimentally available fluxes and metabolic network structure. We can now estimate each flux, calculate sensitivities for fluxes and metabolite concentrations with respect to each enzyme concentration and simulate metabolite concentrations under some perturbations. We expect that further development of this approach to lead to comprehensive understanding of how the metabolic network system is regulated and open up a new branch of biology.
Dynamic Response Network of Ectopic ATP Synthase Blockade

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Ectopic expression of F1Fo-ATP synthase on the plasma membrane has previously been described in several different cancer cell types and may serve as a tumor marker, but its role remains unclear. In this study, we demonstrate the presence of ectopic ATP synthase on the plasma membrane of lung adenocarcinoma cells. Using homology modeling and docking simulation, we successfully discover the ATP synthase inhibitor citreoviridin. Moreover, we found that ATP synthase inhibitor citreoviridin can induce cell cycle arrest and inhibit the proliferation and anchorage-independent growth of lung cancer cells. We performed temporal proteomics and phosphoproteomics to elucidate the dynamic changes of ecto-ATP synthase blockade in cells and xenograft model. Based on the protein expression profiles and mathematical modeling, we propose a response network implying citreoviridin induces an unfolded protein response (UPR) with phosphorylation of a protein synthesis regulator, eukaryotic translation initiation factor 2α (eIF2α) leading to inhibition on cell growth. Furthermore, citreoviridin-enhanced eIF2α phosphorylation could be reversed by knockdown of PKR-like ER kinase (PERK) and antioxidant N-acetylcyesteine, indicating reactive oxygen species (ROS) boost UPR under citreoviridin treatment. Elevation of UPR and ROS generates a positive feedback loop and inhibits cell proliferation in a convergent way. These findings reveal the molecular role and the therapeutic potential of inhibiting ectopic ATP synthase in targeting lung cancer cells.
Particle simulation of tissue formation and angiogenesis
toward constructive systems biology

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In recent years, research on the formation of biological tissue has been attracting attention, but they are often difficult to observe. Numerical analysis is expected to visualize and predict such phenomena, but modeling is not easy. We propose a particle model to simulate complex biological phenomena. In our particle model, the computational domain is divided into particles, and 3D tissue formation including vascular network is simulated by pursuing the particles motions, deformations and transitions directly.

Our particle model has features such as,
1. The model can handle changes such as cell division and tissue growth.
2. Inter-particle force is represented by two forces. One is the force to keep the particle volume constant and another is spring force to express elastic body.
3. Multiple types of particles (such as pieces of tissue, blood vessels) can be used, and they can express complex changes.

Applications of particle model will be exhibited in the poster cession as follows.
4. 2D Numerical Simulation of Liver Cell Proliferation with Angiogenesis - Hepatic Lobule Formation - Hiroto Tanaka, et al.
5. Particle Simulation on Formation of Epidermal Skin - Formation Mechanism of Basal Layer - Takeshi Kurihara, et al.

The simulated results could visualize and predict the formation of biological tissue in the body. In future, the models will be expected as tools for health diagnosis and prediction of therapeutic effect.
Towards predicting the epistasis in genome wide association study

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Despite the accumulation of quantitative trait loci (QTL) data for many complex human diseases, most of current approaches that attempted to relate genotypes to phenotype had achieved only limited success, and genetic factors of many common diseases are yet remained to be elucidated. One of the reasons that makes this problem complex is the existence of genetic interaction, or epistasis. Due to excessive amount of computation required for searching the combinatorial interaction space, existing approaches could not fully incorporate high-order genetic interactions in their model, but limited themselves to detecting lower-order genetic interactions.
Poster Presentation
The integration of heterogeneous biological data and model building have become essential activities in biological research as technological advancements continue to empower the measurement of biological data of increasing diversity and scale. But the challenge becomes how to integrate this data. Also, the major human diseases like as diabetes, obesity, high blood pressure, cardiovascular disease and cancer are involved in failure of human metabolic systems. Therefore, analysis of metabolism is an important biological process, but these are complex and highly interconnected each others. Metabolic network maps are represented by a complex chain of chemical reactions and are highly associated between genes, proteins and enzymes; consequently mathematical and/or computational approaches are necessary for integration of them. Two possible approaches for constraint-based analysis, namely, optimization-based and pathway-based, which are the necessary for integrating heterogeneous biological data into metabolic network. Pathway-based analysis is the most widely used and more advantageous than optimization-based analysis, because it generally employs without the specifying the cellular objective function.

Elementary modes (EMs) and extreme pathways (Expas) are the pathway-based analysis, which are the potentially effective in integrating transcriptome or proteome data into metabolic network analyses and maintain the steady state level. The computation of all EMs/Expas in the large-scale metabolic model is seriously hampered by the problem of combinatorial explosion, because the computational time increases exponentially with an increase of network sizes. Another major problem is that many organisms still does not have provide any specific objective biological function for estimating the EM coefficients to predict the flux distribution relate to the optimum physiological states and EMs can be described by different scalar products or many possible vectors of each EM, but the predicted flux distributions must be independent of them.

To address such aforementioned problems, in this study, we present a fast and efficient algorithm, called complementary EM (cEM) analysis, to reduce the number of EMs/Expas and which is useful for integration of heterogeneous biological data into a metabolic flux distribution. To demonstrate the feasibility of cEM analysis, we compared it with EM/Expa analysis by using a simulation study with an artificial metabolic network model and real metabolic network analysis by two medium-scale metabolic network model of E. coli and a genome scale model for head and neck cancer cells. Application of cEM analysis to Genetic Modification of Flux (GMF) accurately predicts the flux distributions of genetic mutants under particular conditions. Use of cEMs analysis, to plans a genetic engineering strategy for genome-scale metabolic network model for producing useful compounds.
Automatic construction of calculable metabolic networks from public database

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Metabolic system is a complex system composed of gene regulation and a large number of enzymatic reactions. Metabolic system produces energy, amino acids and nucleic acid to support life and is highly regulated in response to change in various environments. When abnormality occurs in our metabolic systems, it may cause a variety of diseases, such as diabetes, obesity, high blood pressure.

At present, large-scale metabolic networks are being constructed. There are many public databases that store many network maps for each human organ with different diseases, such as HMA (Human Metabolic Atlas) database and so on. We extracted a variety of information on experimental conditions, reactions, and metabolites and compartments from literatures, HMA database and Recon X. All data files are prepared in the same manner to perform Elementary Mode (EM) analysis. For the purpose of data calculability, those files are tested by Genetic Modification of Flux (GMF), an algorithm that predicts the flux distribution of gene knockout mutants.

The HMA database is written in the SBML format, while the GMF application software requires the Excel format. To solve this inconsistency, we make the conversion program including three Java libraries (JSBML, JDBC, Apache POI). These data are prepared in the excel format that can be used as an input file for many application programs. We also make the web conversion program version. You can upload any SBML format file, the web conversion program can convert it into Excel format file automatically.
Stochastic Simulation of Dynamical Behavior in Gene Regulatory Network
A.B.M. Shamim Ul Hasan¹ & Hiroyuki Kurata ¹, ²

Abstract

Stochasticity in gene regulatory network has become increasingly distinguished in the current thinking of system biology. Experimentalists and modelers, are showed by an increasing number of theoretical, computational, and experimental tools. These ways and means have been proven successful in each strand of biology, including neural, and genetic networks. Gene expression that occur in stochastic diffusion of substances is a complex that a lot of biochemical processes in the cell involve low molecule numbers or rare interactions and consequently give rise to stochastic fluctuations. Now a days the system biology is becoming the successful in spite of existing in a stochastic environment and in spite of the probabilistic nature of the biochemical reactions. So it is important to know variety of bistable in gene network. Here the aim of investigation of the research work is to study the phenomena of bistable in stochastic behavior using Gillespie Algorithm.

Key words: stochastic simulation, Gillespie algorithm, bistable, noise.

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Dynamic sensitivity analysis of ErbB signaling system

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ErbB signaling plays an important role in the regulation of cell proliferation, survival, metastasis, and invasion into various tumors. We employ the Birtwistle and Okada’s dynamic model that describes how stimulation of all four ErbB receptors with epidermal growth factor (EGF) and heregulin (HRG) activates two critical downstream proteins, extracellular-signal-regulated kinase (ERK) and Akt. To understand the behavior of this model, we performed the dynamic sensitivity (DS) with the direct method (DM) under different initial ligand (EGF and HRG) concentrations.

The dynamic sensitivity is an important measure to estimate the robustness and to find critically important kinetic parameters. We simulated the time-varying of protein concentrations or their activities and the dynamic sensitivity of the activities of ERK (ERK*) and Akt (Akt*) with respect to kinetic parameters at different sets of the initial concentrations of EGF and HRG. To measure the DS time-course of Akt and ERK with respect to kinetic parameters, the integrated and maximum DS values are defined. They are highly correlated. The integrated DS values show a high correlation between Akt and ERK except specific kinetic parameters. The integrated DS values of some parameters greatly depend on the initial EGF and HRG concentrations. The robustness of Akt and ERK with respect to perturbations to intracellular kinetic parameters is enhanced at high initial concentrations of EGF and HRG.
Functional membrane for influenza virus sensors

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In this study, we focused on this specific interaction between hemagglutinin protein on influenza virus and sialic acid to develop the effective material for virus sensor. The concept of virus sensor system is shown in Fig.1. Our object is to prepare the functional membrane immobilizing the sialic acid for influenza virus detection by radiation-induced polymerization. Furthermore, the high speed and high sensitive virus detection system will be developed for the mitigation of pandemic to human and livestock.

Preparation procedure of functional membrane for virus sensor is shown in Fig.2. An epoxy-group-containing monomer, glycidyl methacrylate (GMA) was grafted onto a porous nonwoven fabric membrane made of polyethylene, by radiation-induced graft polymerization. The sialic acid as the recognition site of the hemagglutinin protein on the influenza virus was immobilized by the reaction with N-acetylneuraminic acid (NANA) after introducing the coupled monomer of iminodiacetic acid (IDA) and 1-ethyl-3-carboxylic acid (EDC) on GMA polymer brush. Lanthanum ion as a signal substance was adsorbed on the sialic acid.

The conversion rate of IDA-EDC was over 30%. After the reaction with NANA, the amount of sialic acid immobilized on the membrane corresponds to 67% of GMA molecules. The density of sialic acid in the membrane was very high, and it is expected that the membrane could show the cluster effect to interact with influenza virus.
Modeling and simulation for the effect of oxygen level on the main metabolism in *Escherichia coli*

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*Escherichia coli* contains several metabolic regulation mechanisms to cope with the changes in culture environment and/or genetic perturbations. These mechanisms consist of several levels such as gene level via transcription factors and enzyme level via intermediate metabolite. It is quite important to understand these mechanisms for the interpretation of the fermentation data. Among various types of metabolic regulations, catabolite regulation and oxygen level regulation are by far important from the practical application point of view. Although several attempts have been made for the modeling of catabolite regulation, the modeling approach for oxygen level regulation is limited. Therefore, the modeling of oxygen level regulation as well as catabolite regulation is strongly desired.

Under limited oxygen level, the two transcription factors such as Fnr (fumarate nitrate reduction) and Arc (anoxic respiration control) system play essential roles for the metabolic regulation, where the direct oxygen sensor Fnr regulates the expressions of metabolic pathway genes under anaerobic condition, while ArcA/B regulates them under both microaerobic and anaerobic conditions. In the present investigation, we considered a mathematical model which can describe the transition from aerobiosis to anaerobiosis by taking into account the roles of transcription factors such as ArcA/B and Fnr as well as fermentative pathways such as lactate dehydrogenase and alcohol dehydrogenase etc.
Genetic Modification Flux (GMF): A tool to predict flux distribution of mutants

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Systems biology approach is ultimately aimed to design the complex cellular behavior and interaction for comprehensive engineering purposes. The study on gene modifications for micro-organisms is essential for the production of target compounds.

We present Genetic Modication Flux (GMF); a MATLAB tool that predicts flux distribution of gene deletion and with under-expressed/over-expressed mutant conditions. GMF is an elementary mode analysis (EMA) based method, combining two algorithms; modified CEF (mCEF) and Enzyme Control Flux (ECF). mCEF estimates the enzyme activity rate (relative gene expression) based on the experimental value. The algorithm is further integrates the estimated enzyme activity into EMA, which is used in ECF program. ECF estimates the correlation between relative enzyme activity profiles, and predicts the flux distribution of mutants. Using an input file represented in a metabolic network, user is able to estimate the flux distribution of mutants and/or relative gene expression.
The analysis of reaction kinetics for dynamic modeling of metabolic systems

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Dynamic modeling of metabolic systems is a method to show cellular processes dynamically by representing time variations of metabolites as a state equations. General method is calculating the flux using the rate equations represented by Michaelis-Menten equation. To do that, it is necessary to decide on the rate equation and the most of the parameters used in that to determine the flux about each enzyme. Enzymes originally have a very complex reaction mechanism, so the rate laws have a variety of formats. However, we can't often obtain the necessary information to determine the accurate rate laws let alone what the parameters values to use. In addition, for the purpose of modeling, it is said that it's more important to describe the appropriate reaction rate than to worry excessively about mechanistic details.

We therefore researched about the generalized rate laws proposed for modeling presently and then compared to the rate equations that are used in existing dynamic models of the metabolic system. We eventually determine the rate equations suitable for the metabolic system model and aim to build dynamic models.
Rheumatoid arthritis (RA) and osteoporosis are known as the main disease of the bone. Early detection and early treatment are important for these diseases. However, some problems such as mass screening on data sets and mis-diagnosis are still remained in visual screening. In order to solve these problems and reduce the burden to physicians, needs of an automatic diagnosis system capable of performing quantitative analysis is anticipated. In this research, we carry out the development of a registration method of phalange regions from CR images of the hand to perform a quantitative evaluation of RA and osteoporosis. The proposed method is carried out registration using normalized mutual information (NMI). Furthermore, we explored optimum solution by genetic algorithm (GA) [1].

First, the centroid of phalange regions are calculated on segmented phalanges regions automatically. Next, the calculated centroids are positioned between previous image and current one as global registration. Finally, local registration is performed by using the NMI which is evaluation index. The NMI is scale that represented degree of similarity between images. So as to maximize degree of similarity, we performed rigid registration. In order to register, translation, rotation and scaling parameters should be obtained. But, computational times are required to best matched position on registration. To overcome this problem, we used GA. GA is the learning algorithm that engineered as to mimic process of evolution of creature. The advantage of the GA is efficient detection of approximate solution of optimized solution in a short time. We use the GA to speed up on computation time without having to debase for accuracy of approximate solution of optimized solution.

In this research, we performed 84 sets of phalange regions on 3 normal cases using our proposed method. And as compared with the results calculated for all combination, good result with NMI of 0.616 was obtained. Moreover, the computation time was increased from 141.8[s] to 57.5[s]. However, there are some problems such as the accuracy of registration depends on the accuracy of segmentation and it still remains as future work.

Reference
Automatic Detection of Lung Nodules Based on Statistical Features in Volume of Interest on Temporal Subtraction Images

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In recent years, to diagnose the lesion of patients, chest MDCT (Multi Detector-row Computed Tomography) images are used in medical fields. On the other hand, it makes burden to the radiologists which are caused by many slice images that needed to interpret. To solve this problem, many researchers developed the CAD (Computer Aided Diagnosis) systems for reducing the burden of radiologists. One of the CAD systems, a temporal subtraction technique which is subtracted from previous image to current one is introduced as powerful tools in medical fields to diagnose the lesions [1]. Radiologist can easily detect lesions and shorten the diagnosis time on image by compare the two images. It is because the subtraction image can enhance the temporal changes, such as shaped of new lesions and/or the temporal changes in existing abnormalities by removing most of the normal background structures. However, subtraction artifacts which are caused by miss-registration are still remained on temporal subtraction images, and it is recognized as lesions.

In this paper, to determine the obtained regions from temporal subtraction images whether the lesions or subtraction artifacts, we have developed a new detection method for lung nodules that are or less 20[mm] from the temporal subtraction of thoracic MDCT images. At first, we create a density histogram from temporal subtraction image, and first candidate regions are detected by multiple threshold processing based on area of one. Next, we reduce false positive from the first candidate regions by using spiral scanning filter based on current MDCT image. Region of remaining candidate nodule are segmented by Voronoi-based segmentation. Then, we calculate features such as density, shapes and textures from segmented regions (totally 28 features). Finally, we identify lung nodules and subtraction artifacts by using AdaBoost classifier based on those features. As a result, we obtained TP:96.9[%](31/32) and FP:5.91[/scan] respectively.

Reference

Particle Simulation on Cancer Growth and Angiogenesis
- Modeling of stromal cells -

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While a tumor is small, cancer increases only by the surrounding nutriment, but when a tumor grows, to get the nutrition to maintain it more, a cancer cell makes capillary proliferation. Therefore a cancer cell proliferates while taking out vascular growth factor such as VEGF. Details of proliferative process of the cancer cell which considered such proliferation of a blood vessel and nutritional situation are still unclear.

The purpose of this study is to elucidate cancer growth phenomenon and to support the development of treatments. This paper suggests the development of a simulator that can quickly express cancer growth. Cancer growth is observed difficulty because it takes a lot of time to grow. In this study, we analyze cancer growth and angiogenesis using a particle model. A particle model is an analysis method. We treat particles as cancer cells or blood vessels; the phenomenon is expressed by the interaction of the particles.

The paper suggests particle simulation of cancer growth and angiogenesis, which is based on an image. We analyze cancer and stromal cells growth and angiogenesis by the simulation to predict the cancer growth. We also pursue interaction of cancer and stromal cells.
System analysis for oscillation and switch-like response of NF-kappaB in B cell

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Mathematical modeling is a powerful tool to identify molecular mechanisms and to predict the dynamical behaviors of biological systems. In signaling networks, extra-cellular information is transmitted to intra-cellular signaling pathways followed by activation of transcription factors that control gene expression. Among them, the transcription factor nuclear factor-kappa B (NF-kappaB) plays important roles in proliferation, differentiation and apoptosis of variety of cells, therefore it is important to understand and control the regulatory mechanism of this particular transcription factor. In particular, in response to tumor necrosis factor (TNF), two major dynamics features are reported for NF-kappaB activation; oscillation and switch-like response. We recently found similar behaviors in B cell development upon B cell receptor (BCR) engagement.

Previously, we constructed a model to explain switch-like response of NF-kappaB activity in BCR signaling and identified a positive feedback loop as a mechanism of this behavior. Here we further constructed the expanded model to explain an interplay between the switch-like response and oscillatory behavior of NF-kappaB.

Simulation in the model well reproduced both oscillation and switch-like response of NF-kappaB activity shown in BCR-activated B cells, and showed consistent results to several genetic mutations of B cell. Furthermore, in some molecular parameter conditions, sensitivity analysis and bifurcation analysis showed coupling and decoupling of oscillation and switch-like responses.

Our study proposes the first model for NF-kappaB signaling pathway which integrates both switch-like response and oscillatory behavior of NF-kappaB, which is necessary for B cell activation and differentiation, respectively. The model provides valuable insights for understanding the regulatory mechanisms of NF-kappaB signaling for cell determination.
Identification of novel anti-mycobacterial inhibitors through in silico screening

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Keywords: Antibiotics, Enoyl-acyl-carrier protein reductase (InhA), Fragment library, in silico structure based drug screening, Matched molecular pair (MMP), Mycobacterium

Tuberculosis (TB) is global chronic infection and there are lots of health concerns represented by emergence of multidrug-resistant TB (MDR-TB) and co-infection with human immunodeficiency virus (HIV). System of bacterial cell wall synthesis is one of potential targets, and isoniazid (INH) which has inhibitory effect against mycobacterium InhA is a major drug in TB treatment [1,2]. Lately, in the research and development of medicinal drugs, the information of bioisosteric and nonbioisosteric transformation is useful for medicinal chemists to modify lead compounds. Of such approaches, matched molecular pair (MMP) analysis can describe the relation of substructure and biological property, and the analysis leads to comprehension of chemical pharmacology and protein-ligand interactions [3].

In this study, by combination of the MMP and docking simulation approaches, we tried to discover novel InhA inhibitors alternative to a compound (KES4) we previously identified [4]. 461,383 compounds in the virtual chemical library were cut at all exocyclic single bonds, and single-, double-, triple-cut [5] unique MMPs were generated. After that, 10 candidate compounds associated with KES4 were identified through the docking simulation with M. tuberculosis InhA structure. We then evaluated the antibacterial effects of these compounds on growth of mycobacteria M. smegmatis as a model bacteria of M. tuberculosis, and all of 10 compounds showed inhibitory effects on growth of M. smegmatis. In addition to this, 6 of 10 compounds have lower experimental IC₅₀ value than 50 μM and do not have any toxicity against enterobacteria (E. coli BL21 and JM 109) and mammalian cells (MDCK and SH-SY5Y cells).

We found various potential anti tuberculosis agents with exchanged molecular substituents from the prototype compound (KES4) through in silico approaches, and these structural and experimental data exhibited reasonable correlations. Moreover, protein-ligand interaction data in this study have potential to improve chemical and biological properties in TB drug design.

Discovering novel active compounds against *Mycobacterium* thymidine monophosphate kinase with pharmacophore-based *in silico* screening

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Tuberculosis (TB, the causative agent being *M. tuberculosis*) remains the most common and foremost infectious disease-affecting humans in terms of both morbidity and mortality. The increasing prevalence of drug-resistant TB (e.g., multi-drug resistant TB: MDR-TB) which is resistant to effective multiple antibiotics, presents a major global threat. Therefore, the discovery of novel chemical compounds with anti-mycobacterial activity is important due to a lack of effective drugs.

The *M. tuberculosis* thymidine monophosphate kinase (mtTMPK) is a potential target for the treatment of TB. In this study, using a three-step pharmacophore-based *in silico* screening approach specifically targeting mtTMPK, we identified 5 compounds (KTP1-KTP5) as potential chemical agents against TB. We then experimentally tested whether these compounds could serve as antibiotics with *in vitro* assays using the model *Mycobacterium* strain *M. smegmatis*. We found that one of the selected compounds (KTP3) completely inhibited the growth of *M. smegmatis*. In addition, two KTP3 analogs (KTPS1 and KTPS2) showed strong inhibitory effects on *M. smegmatis* growth. The most potent inhibitor (KTPS1) does not have any toxic effects on model intestinal bacteria and several mammalian cells. Moreover, we also confirmed that two of these compounds (KTPS1 and KTPS2) directly inhibit the enzymatic activity of mtTMPK.

Our results strongly suggest that the structural and biological information on these chemical compounds are likely useful for future research for the development of novel anti-TB drugs. Furthermore, our screening methodology could contribute to the further identification of novel hit compounds for other candidate medicinal drug.
Numerical Simulation of Alveolar Bone Regeneration and Angiogenesis -Implementing the Stress Factor-

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Summary

In recent years, bone regeneration therapy has been noted in implant treatment. Blood vessels are required to supply the calcium for bone growth, and the transport of calcium is carried out continuously between bone and blood vessels. While the blood vessels supply the calcium, bone is formed along the collagen fibers. In this way, bone and blood vessels grow by interacting each other. However, there is little numerical research that focuses on both angiogenesis and bone regeneration. Therefore, the interaction of bone regeneration and angiogenesis is examined by numerical analysis in this study.

Bone regeneration and angiogenesis were simulated by particle model. In particle model, the interaction among particles, such as movement and transport can be considered. Therefore, particle model can easily analyze the complex phenomena.

Bone regeneration analysis in 90 days was carried out. Blood vessels were assumed to extend toward the region of blood clots. As a result with Ca transport, it was confirmed that the distribution of Ca was diffused steadily from blood vessels and alveolar bone was formed. In this analysis, the factor of bone regeneration is the concentration of Ca, which is given by blood vessels, but not stress factor.

Bone regeneration up to about 30 days does not need stress factor because the bone density is too small to transmitting stress factors. From 30 days to 90 days, however, the bone regenerates strongly enough to transmit them. In previous studies, the stress has been found to contribute to the strength and shape of bone. Accordingly, the effect of stress factor is examined by simulation. The stress was modeled by using particle, and accuracy of the stress model was confirmed.

Then, the stress factor was implemented to the simulation. It was confirmed that the stress factor made the structure of trabecular bone.

We will do the coupled analysis which includes angiogenesis and the stress factor. Then, we will confirm the accuracy of the coupled analysis model, and reach the practical model such as simulator of bone regeneration.
A hippocampus in a brain has the functional rhythms, $\theta$, $\beta$, and $\gamma$ rhythms, which are related to memory processing. The rhythm can be also induced in rat hippocampal slices with the application of the cholinergic agent carbachol. A hippocampus also has the pathological epileptic discharges. The discharges lead to the seizure of the subject. The discharges are induced by the synchronized hippocampal neurons. They are also induced in the slices with the application of a $\text{GABA}_A$ receptor antagonist. $\text{GABA}_A$ receptor antagonists picrotoxin (PTX), bicuculline (BIC), and SR-95531 (GABAzine) induces the epileptic discharges. In the present study, we studied whether $\beta$ oscillation induced by carbachol has a protection effect against the epileptic discharges. The data were obtained from hippocampal slices (450-μm thick) of male Wistar rats. The recording glass electrode ($< 2 \text{M} \Omega$) was placed in the CA3 stratum pyramidale to record the epileptic discharges and $\beta$ oscillation. The epileptic discharges were induced with the application of $\text{GABA}_A$ receptor antagonists. They were induced with PTX at the concentration above 0.2 μM. The application of BIC and GABAzine also induced the epileptic discharges. These results suggest that disinhibition can induce the epileptic ischarges. But the epileptic discharges were not induced in case that the slices were pre-treated by carbachol and $\beta$ oscillations were induced. The results of the application of BIC and GABAzine to carbachol-induced $\beta$ oscillation were the same. Epileptic discharges emerged after only carbachol was washed out, and only $\text{GABA}_A$ receptor antagonist was applied. These results suggest that $\beta$ oscillation was not affected with the application of PTX, BIC, and GABAzine at the concentration which can induce the epileptic discharges. Based on the obtained results, we will propose the protection system against the epileptic discharges’ inducing in a hippocampus using deep brain stimulation. This technology can lead to the computer-aided prevention system for the epileptic seizure.
A study on signal transmission system realized by biochemical reactions for molecular robot

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Cellular processes such as proliferation, differentiation and apoptosis are rigorously controlled by intracellular signal transduction systems that are biochemical reaction networks with proteins and genes. An extracellular stimulus as a reference input is transmitted toward target genes by means of chains of activations of signaling proteins in signal transduction pathways in which the information in the flow is a concentration change of an activated protein. It is amazing that the information-communication system is reasonably reliable and robust against environmental change and disturbances in a cell. Recently, technology for controlling a biochemical actuator based on a sensor signal that is also a physical value of a molecule is needed to realize a motion control of molecular robot.

In this paper, we consider how to realize a signal transmission system in a biochemical reaction network for molecular robotics. To this end, we begin with a discussion regarding functions of negative feedback control comparing with one in an engineering system. Since a negative feedback is implemented with an inhibition reaction in a signal transduction system instead of a negative gain or an error signal in mechatronics, some fundamental properties of the closed loop system such as equilibrium state and oscillation are investigated. Next, we analyze some example models to clarify the effect of negative feedback control in a case study. Since the models are highly nonlinear and it is difficult to analytically evaluate the model, we calculate the input to output properties by applying various kinds of input signal. Then, we demonstrate that the model is a possible candidate for a signal transmission system in molecular robot.
Modeling and Simulation on Cancer Cell Migration and Adhesion

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Three-dimensional (3D) cell cultures have developed as environments, where cells mimic their functions in living tissues. The 3D cultures enable cancer cells to form multicellular spheroid while migrating and adhering. The processes are recorded as time-lapse live images under an optical microscope. Precise estimation of the growth inhibition of cancer spheroids based on those images is necessary for development of anti-cancer drugs.

We analyzed the relationship between the culture time and distance of cell migration, and between the culture time and occurrence probabilities of cell adhesion from the recorded images. The migration of cancer spheroids is observed as random motion. We propose a migration model and an adhesion model with distance distribution and occurrence probabilities obtained from those images, respectively.

The simulation on the number of spheroids for seven days provides the almost same number of spheroids in the initially recorded image and in the finally recorded image. The preliminary work shows the usefulness of the cell migration and adhesion model. We will improve this work in order to support tests \textit{in vitro} effectively by the simulation \textit{in silico}, so that \textit{in vitro} tests will enhance basic biological research as well as anti-cancer drugs development.
In recent years, researches on skin disease and cosmetics have been increasing because the expectation by society or women has been rising. For example, it is expected to develop disease treatment and anti-aging therapy preventing aging from internal of our body. Besides, women are interested in beautiful skin and cosmetics because of worries about spot or freckle. Men are also interested in them in recent years.

Among skin tissue, epidermis is at the most outside part of skin. The epidermis consists of four layers of various differentiation states. Especially, basal layer, which is at the bottom of epidermis, is formed by unevenness. There is a feature that unevenness is larger near spot. Because of relevance like this, understanding formation mechanism of basal layer is expected from the field of cosmetics. However, it is difficult to observe formation of basal layer. Furthermore, it is impossible to observe cells one by one as a movement in epidermis. For these reasons, formation mechanism of basal layer is not made clear yet.

Therefore, we simulate skin formation including basal layer, using particle method. The particle method, in which each particle follows designated algorithms, can simulate three-dimensional skin formation with proliferation and cornification of basal cells. We analyze a process of long time skin formation by this model and make formation mechanism of basal layer clear.
Recently, viral hepatitis patients have been increasing. Therefore, the reconstruction of liver based on tissue engineering attracts attention. This research aims to create a numerical analysis method for the establishment of practical regenerative medicine technology based on tissue engineering. This research proposes an analysis model using the particle method as the first step in this project. The analysis object is a hepatic lobule. A purpose of this analysis is to elucidate the process, mechanism, and condition of cell growth in the micro-scale. This research uses parameters which were obtained by experiments using rats about diffusivity, oxygen concentration and oxygen consumption rate of a cell.

At first, this research compares simulation results with theoretical solutions about oxygen diffusion. Furthermore, this research creates an analysis model of cell growth and cell death. Restriction of cell survival area by oxygen depletion is the most serious problem in liver cell proliferation technology development. It is necessary to extend the cell survival area by angiogenesis. Therefore, this research constructs a model of angiogenesis. Finally, this research got results which blood vessels formed from portal veins to a central vein, repeating branching and connecting. This is why a hepatic lobule was filled with liver cells. This research is going to analyze more extensive range of an organ, using the analysis results in the micro-scale.
Toward a quantitative in silico model for the E. coli ammonium assimilation system

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Ammonium supports the fastest growth rate and is therefore considered the preferred nitrogen source for Escherichia coli [1]. The bacterium adds ammonium to α-ketoglutarate an intermediate of TCA cycle, for synthesizing glutamate and glutamine. ~88% of cellular nitrogen is derived from α-amino group of glutamate whereas the remainder is derived from the amide group of glutamine [2].

We propose a new kinetic model of E. coli ammonium assimilation network, centered on the regulation of glutamine synthetase (GS) and an ammonium transporter AmtB. Our model employs realistic kinetic parameter values and can quantitatively reproduce both metabolome dynamics upon ammonium upshift (Yuan et al., Mol Syst Biol, 2009) and experimental data regarding cell growth, ammonium assimilation flux and intracellular ammonium concentration under low ammonium conditions (Kim et al., Mol Syst Biol, 2012). Our modeling is achieved by the following novel ideas. (i) Incorporating the diffusion resistance enables the model to explain both Yuan’s and Kim’s data, because we found that Yuan’s experimental data cannot be explained without taking into account ammonia/ammonium diffusion resistance in media. (ii) The parameter estimation problem is formulated as a constrained optimization problem, which minimizes changes in kinetic parameter values from their literature values and thus greatly enhances development of a realistic model. We revealed rational and integrative regulations of GS and AmtB, which limits futile cycling of ammonium.
Mining discriminative patterns from graph data with multiple labels

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Graph data are becoming increasingly common in machine learning and data mining, and its application field pervades to bioinformatics, natural language processing and social network analysis. Accordingly, methods to extract patterns from graph data; graph mining has been studied and developed rapidly these years. Since the number of patterns in graph data is huge, a central issue in graph mining community is how to efficiently collect informative patterns suitable for subsequent tasks such as classification or regression. In this paper we consider mining discriminative subgraphs from graph data with multiple labels. Given a set of graphs, labels attached to graph data comprise a target response matrix, and whose structure is taken into account to our algorithm. It efficiently collects subgraph patterns corresponding to a few major eigenvectors of the response matrix, compared to existing approaches that need to collect subgraph patterns separately from each target response vector. In extensive computational experiments based on both synthetic and real-world data, we verify the effectiveness of the proposed method.
Estimating the number of SNPs in interaction by kernel methods

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The risk of common diseases is likely determined by the complex interplay between environmental and genetic factors, including single nucleotide polymorphisms (SNPs). However, genome wide association studies (GWAS) to date have not been able to discover associations between diseases and SNPs for many of common diseases. One of keys to associate SNPs to diseases is believed to employ a model that considers genetic interactions. In general, however, the number of true interactions is unavailable for each disease. We propose a kernel-based method to predict the degree of SNP interactions. Our computational experiments in both simulated and real data show the effectiveness of the approach.
Mining n-way SNP interactions with statistical metric pruning

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In human genetics, despite the accumulation of genome wide association data, much of the relationship between genotypes and phenotypes are yet to be elucidated. One of the keys to fill this gap is believed to be incorporation of interaction effects among genes. However, consideration of all possible gene interactions is computationally prohibitive. In this work, we propose a method for enumerating all gene combinations satisfying user-defined significance level. Our method can efficiently prune the search space without sacrificing the completeness of the search.
Importance of averagings in EEG engineering

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Averaging is one of the basic but key technologies for EEG (electroencephalogram) engineering. This study will demonstrate the importance of the averaging through the following three experiments.

In Experiment 1, EEGs during 990 trials were averaged for both the actual and silent speeches. There was the difference in motor potentials between the two tasks, while not that in Bereitschaftspotential.

In Experiment 2, EEGs for female and male face photographs were averaged, both of 213 trials. For 6 male subjects, amplitudes of the ground averages for male faces were higher than those for female faces.

In Experiment 3, English words were presented whose Japanese meanings high school student could recall or not. EEGs were averaged for both the words which they could recall (“could recall”) and could not (“not recall”), both of 288 trials. We found the difference between “could recall” and “not recall” in the frontal cortex.

Thus, because the averaging yielded some interesting findings, it was concluded that the averaging is important for EEG engineering.
Novel optical approaches for monitoring of green fluorescence from biological samples under newly designed artificial light

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Today, fluorescence (FL) analyses is routinely performed in the medical and biological laboratories, by focusing on the expression of green fluorescent protein (GFP)-fused proteins, localization of FL-labelled antibodies, and outcomes of the events of interest using various FL proves such as FITC-conjugated phalloidin targeting actin filament and fluorescein labelled chemical probes for various metal ions, nitric oxide, and reactive oxygen species. In general, observation of FL from biological samples by optic means requires dark room or shielded compartments so that excitation by specific light sources and emitting light from FL active component can be readily distinguished from the background light. However, by this way, diagnosis by eyes under the visible light condition and FL measurements in dark compartments cannot be performed simultaneously.

In this study, we attempted to configure the optical techniques inspired after the satellite-based technology for remote sensing global flora, known as Fraunhofer line discrimination method by which a dark line found in the solar spectrum is used as the base for measurement of chlorophyll FL (discrimination of FL signal hindered in the reflection spectra) in the open space instead of using dark compartments. Here, model experiments were performed for discrimination of FL signal emitted from plant roots (roots of lettuce; Lactuca sativa L., cv. Lollo Rossa) loaded with FITC (excitation wavelength: 492 nm, fluorescence wavelength: 518 nm) under newly designed artificial white light with pseudo-dark line at 520 nm. Additionally, exciting light at 470 nm was supplemented for enhanced FL yield. By monitoring the reflection spectra using Hand-Held 2 Portable Spectroradiometer (ASD Inc., CO, USA), the signal for green FL due to FITC positioned at the pseudo-dark line could be detected. Pseudo-dark line-based FL discrimination was also confirmed by an imaging technique, by mounting a band-pass filter which transmits only the wavelength around 520 nm matching the pseudo-dark lines onto a handy digital camera.
Induction of superoxide generation by application of ferrous ion to human methemoglobin

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Currently various studies have been promoted for development of blood substitutes, such as approaches for utilizing hemoglobin solution. Hemoglobin (Hb) is commonly known to be readily denatured into methemoglobin (metHb) which lacks the capacity for binding to O₂, once Hb is isolated from the cells and exposed to O₂ dissolved in in aqueous solution. Upon single-electron (1e⁻) reduction of metHb, the resultant deoxyhemoglobin (deoxyHb) with high affinity for binding to O₂ may participate in the circulation of O₂ if introduced in the vascular system. In this presentation, we would like to point out the hidden dangers in such approaches. If metHb-derived deoxyHb is circulated, binding of dissolved O₂ may results in accumulation of oxyhemoglobin (oxyHb). Then, oxyHb is likely being denatured into metHb, concomitantly releasing superoxide anion radicals (O₂⁻). Here, we attempted to testify the danger of the use of cell-free Hb being circulated in the vascular system, by monitoring the generation of O₂⁻ in the metHb solution supplemented with 1e⁻ reducing agents. In the model experiments, generation of O₂⁻ was detected with O₂⁻-specific chemiluminescence of Cypridina luciferin analog (CLA) after addition of ferrous ion (Fe²⁺) to met Hb solution, since Fe²⁺ reportedly regenerates ferrous intermediates of heme proteins from ferric heme proteins, thus, deoxyHb can be generated from met Hb. We observed that deoxyHb/oxyHb formed in the presence of Fe²⁺ and O₂ results in robust and long-lasting long-lasting O₂⁻ generation.
Plant enzymes as immune-therapeutic tools for cell-targeted lethal control.

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Plant peroxidases (POXs) are enzymes known to remove hydrogen peroxide (H$_2$O$_2$) which is a member of reactive oxygen species (ROS). However, POXs show alternative catalytic actions producing superoxide anion radical (O$_2^-$) which is a highly reactive member of ROS, depending on the condition. Our earlier works have been identified that redox states of prosthetic heme group in POXs are responsible for releasing O$_2^-$. Once the native form of POX with heme at ferric state (Fe$^{III}$) is converted to the reduced form (Fe$^{II}$) by single electron (e$^-$) oxidation in the presence of an e$^-$ donor, interaction of ferrous POX (Fe$^{II}$) results in formation of O$_2^-$-bound form of POX intermediate known as Compound III. It is noteworthy that decomposition of Compound III into native ferric POX accompanies the generation of O$_2^-$, thus cytotoxic.

To date one of most effective inducer of O$_2^-$ in plant POX system is indole-3-acetoc acid (IAA) which is a natural plant growth regulator. Based on the view that formation of enzyme-substrate complexes such as [POX-IAA-O$_2$] results in release of O$_2^-$, medical application of POX-labelled antibodies and IAA has been proposed as a novel O$_2^-$-generating system for cancer cell-targeted and controlled cell death induction, by designing the POX-conjugated immuno-labelling of cancer-related molecules or expression of recombinant horseradish peroxidase (HRP) in mammalian cells. Here, we propose the combination of three candidate plant POXs, namely, HRP, soybean peroxidase (SBP) and rice cell wall-bound peroxidase (RCP), and two O$_2^-$-generating stimuli, namely, IAA and free ferrous ion (Fe$^{2+}$) as the model system for transient or continuous generation of O$_2^-$, thus applicable for cancer cell-targeted and controlled cell death induction. Generation of O$_2^-$ by HRP, SBP and RCP in responses to addition of IAA and Fe$^{2+}$ were assessed using O$_2^-$-specific chemiluminescence probe.
As in the case of nation-wide Livestock Identification System (LIS) in Japan and Australia, identification codes should be allocated to all agricultural products and each product code are expected to be linked with safety and/or nutritional data. In case of LIS, individual animals are subjected to sampling of the small meat portions for various testing. In contrast, fresh vegetables or fruits are hardly subjected to such semi-destructive sampling protocols since majority of markets and consumers accept the intact and fresh vegetables. Therefore, nondestructive and rapid protocols for monitoring of nutritional quality in fresh vegetables and/or fruits are highly required.

In the present study, post-harvest tomatoes were used as a model material. Tomatoes contain nutritional components chiefly, vitamin C, carotenoids in the form of lycopene. In general, measurement of pigments by optic means requires dark room or shielded compartment. However, simple and nondestructive protocols allowing pigments measurements in the agricultural and/or ecological fields under the open environment are highly desired. In this study, we attempted to reconfigure the optical techniques inspired after the satellite-based remote sensing technology, by employing the handheld sensing protocols for monitoring of chlorophyll fluorescence (CFL). The solar spectrum recorded in the terrestrial surface inevitably shows the signature of specific light absorption by atmospheric composites such as O₂, thus dark lines (Fraunhofer lines) are observed. Such dark lines can be used as “open air dark room” for monitoring of CFL based on the dark line discrimination principle. Therefore, by monitoring of reflectivity spectra under solar light, presence of CFL can be readily elucidated as demonstrated using tomatoes. Since reading of CFL is sensitive to the presence of other nutritional pigments, therefore, changes in CFL can be clues for estimating the contents of various pigments.
Evaluation of DNA-targeted photolytic actions of ultraviolet light sources

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Ultraviolet (UV) rays are commonly employed for disinfection of medical equipment, by expecting the lethal impact of UV rays against contaminating microbes chiefly bacteria. We assumed that the target molecules in biochemical compositions of micro-organisms include nucleic acids, namely DNA and RNA, thus, by selecting the appropriate light sources, we can expect that not only bacteria but also contamination by viral nucleic acids can be eliminated. In the present study, photolysis of small-sized (177 bp) double-stranded (ds) DNA fragment was allowed by exposing to UV rays differed in wavelength, using conventional UV lamps (peaking at 254 nm and 365 nm) and light emitting diodes (LEDs, peaking at 265, 280, 310, 325, and 340 nm). Then, DNA left in the sample solution was detected and quantified through the hyper-fast polymerase-chain reactions (Hyper-PCR, Trust Medical Co., Ltd., Japan). Among light sources examined 265 nm LED, matching the absorption maxima of thymine, showed the most active DNA degradation suggesting that AT-base pairs are more sensitive to UVs compared to GC-base pairs. As expected, fluorescence analyses with two ds-DNA hexamers, namely CGCGCG and ATATAT showed that ATATAT was highly sensitive to UV-dependent photolysis.
Practical procedure for simulation of lethal toxicity curves based on limited number of experimental data points: Model demonstration using green paramecium cells exposed to toxic metals

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Background
Humans and other members of ecological systems are surrounded by a large number of chemicals, among which negligible portion may be toxic to organisms. Therefore, from the environment-centric point of view, importance of accurate eco-toxicological analyses is increasing day-by-day. Eco-toxicity of chemicals can be scored by several parameters and LC$_{50}$ and LD$_{50}$ values are examples of most commonly employed measures of the acute-toxicity in animals and other organisms. In general LC$_{50}$/LD$_{50}$ values for a given chemical can be graphically determined upon obtaining a curve for concentration/dose-dependent increase in lethality in individuals and/or cells. In cases, only limited number of data points are available, it would be highly difficult for obtaining the eco-toxicological scores such as LC$_{50}$, especially when the chemicals of interest are precious and rare, and therefore, the number of experiments needs to be limited, or size of animal testing is required to be minimized by considering the animal welfare.

In the present study, we attempted to perform simulation of eco-toxicological nature of given chemicals based on limited data from model experiments (3-5 points) performed with green paramecia (*Paramecium bursaria*) exposed to toxic metals, showing apparently incomplete curves for toxicity response.

Experimental and Simulating Procedures
The equations tested here were of Michaelis-Menten’s (MME) and Hill’s (HE).

(1A) Firstly, we attempted to perform the curve-fit by employing an equation derived from MME, $L=L_{max}*[T]/(L_{m}+[T])$; where, $L$ is lethal rate, $L_{max}$ is the maximal lethality, $[T]$ is concentration of the given toxic agent, and $L_{m}$ is the concentration of toxic agent at which half of lethality is manifested, thus equivalent to LC$_{50}$.

(1B) Although the value for $L_{max}$ is trivial (no value higher than 100% lethality is possible), $L_{m}$ value must be determined through analysis of experimental data. By analogy to kinetic analyses of enzyme reactions, Lineweaver-Burk plots (LBP) were performed in order to elucidate the $L_{m}$ values. In the preliminary model tests, normal-sized experimental data were used for performing the LBP, so that the $L_{m}$ values can be successfully determined.
(1C) However, this approach failed to simulate the toxicity curve based on MME-type equation after substitution of the L_m in the equation with the experimentally determined values (MME-type equation has tendency to under-estimate the toxicity of toxic agent in the range of concentrations higher than L_m value), therefore the modification of the equation is obviously required.

(2A) MME-type equation, \( L = L_{\text{max}} \cdot \frac{[T]}{([L_m]+[T])^n} \), can be transformed into HE, \( L = \frac{1}{1+(L_m/[T])^n} \), simply by substituting the exponential coefficient \( n \) with 1. Note that Hill’s equation allows fitting of the curves by modulating the exponential coefficient \( n \) other than 1.

(2B) It is obvious to us that LBP can be applicable for obtaining L_m for HE too, since HE can be transformed as follows: \( L^{-1} = 1 + (L_m[T])^n \). Here, by assuming \( L^{-1} = y \) and \([T]^4 = x\), then \( y = 1 + (L_m x)^n \). Thus, \( L_m = -1/x \) (where \( y = 0, L_m > 0 \)). This is what LBP of enzyme kinetic analysis graphically tells us.

(2C) Using the Hill’s equation, we attempted to perform simulations of toxic curves from a limited number of data points. Then, it become highly difficult to determine the L_m value based on LBP. Instead, L_m values were randomly selected and used for simulation of the toxicity curves with HE while the exponential coefficient \( n \) was set as 1. Then, residual sum of squares (RSS) were determined from the difference between the measured values and simulated values. By plotting the RSS against the range of L_m selected, the optimal L_m value giving the minimal RSS was graphically determined.

(2D) Simulation with HE was allowed by introducing the graphically determined L_m. However, in order to enhance the curve-fitting, appropriate \( n \) other than 1 must be introduced in HE. Here, values for \( n \) were randomly selected and used for simulation of the toxicity curves with HE. Again, RSS were determined from the difference between the measured values and simulated values. By plotting the RSS against the range of \( n \) selected, the optimal value for \( n \) giving the minimal RSS was graphically determined.

**Results and Discussion**

By using HE with graphically determined values for L_m and \( n \), eco-toxicological curves were shown to be nicely simulated. In case of *Paramecium bursaria* exposed to Ni^{2+}, HE can be expressed as \( L = \frac{1}{1+(20/[T])^{4.3}} \) since L_m and \( n \) were graphically determined to be 20 \( \mu \)M and 4.3, respectively. This equation nicely predicted the rate of cell death. By plotting the simulated lethality against experimentally determined lethality, linear relationship \( (y = 1.021x - 0.0579) \) with \( R^2 = 0.9923 \) was obtained. Similarly, toxicities of various metal ions namely, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{+}, Cu^{2+}, and Zn^{2+}, were assessed and the recoded lethal curves were compared with model simulation using HE and finally all data showed that this approach successfully predicted the toxicological natures of the chemicals of interest.
Perturbation-Response relation and network structure in biochemical reaction system

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In living cells, chemical reaction pathways of metabolic reactions form a complex network. To elucidate the dynamics of metabolism experimentally, one of standard approaches is to examine the sensitivity to perturbations of reaction rates parameters, which corresponds to enzyme concentrations, through knockout experiments. In this work, we construct a mathematical framework to predict the sensitivity of a biochemical system from the network structure alone.

We find that there is a transitivity property among the flux sensitivities. The effect of a perturbation is generally localized in some part of the network, and we also find that there is a topological condition that determines the extent to which the effect can spread in the network. Our results depend on the network structure alone, and we propose a strategy that would be useful to elucidate a true metabolic network from existing database information.
Mathematical models are widely used and precious tools for the researcher to reconstruct and predict complex cellular system for our understanding of the living cells. A common type of mathematical model is the differential equation model, which particularly suitable to investigate the dynamic behavior of the metabolic networks and molecular interactions. Both sciences (for example, biochemistry) and engineering (for example, metabolic engineering) angle, it is deeply essential to recognize the whole metabolic regulation mechanism of bacterial cells, like as *Escherichia coli* (*E.coli*).

However, different kind of models has been suggested for analyzing the dynamic behavior of the cells and most of them concentrate on the definite metabolic pathways. It has been studied and proposed the kinetic equation for the glycolysis pathway, the pentose phosphate (PP) pathway, the tricarboxylicacid (TCA) cycle for *E.coli*. These models do not focus on enzyme activity and transcription factor (TF) activity. In the current study, we integrated Kadir and Kotte model and built a large-scale metabolic and regulatory model of the central metabolism in *E. coli*, where we included the glycolysis pathway, the PP pathway, the TCA cycle, anaplerotic enzymes, and the glyoxylate shunt. For transcriptional regulation, we take in cyclic AMP receptor protein (Crp), catabolite repressor/activator (Cra) protein, pyruvate dehydrogenase complex repressor (PdhR) protein and acetate operon repressor (IclR) protein. The total 61 differential equation present in our integrated model, which include biomass, concentration of extracellular carbon sources (glucose and acetate), concentrations of metabolites, concentrations and phosphorylation states of enzymes and PTS proteins, binding states of transcription factors.
A dynamic model, which simulates the main metabolic pathways such as glycolysis, TCA cycle, PP pathway and the anapleorotic pathways of *E.coli* has been proposed. This model contains transcriptional and enzymatic regulation and focused on the combination of the genetic and metabolic layers, which completed by TF–metabolite interactions. For batch culture, we investigated the effects of the knockout of Ppc, Pyk, Pgi gene on the metabolism. For continuous culture, we investigated the deviation of the predicted steady-state flux from experimental data for pyk, pgi, zwf, pck mutant at a dilution rate (D) =0.1 and ppc mutant at D=0.2. Overall, we use around 18 reactions in every mutant strain for flux measurements, which contain the major intracellular reactions in glycolysis, TCA cycle and PP pathway. For both batch and continuous culture, we compare simulated results with experimental data. The comparison result shows reasonably good predictability of the simulation result.
Index

A
A.B.M. Shamim Ul Hasan .......................... 34
Shunsuke Aoki ................................. 44, 45
Takatoshi AOKI ................................. 41

B
Md. Bahadur Badsha ................................. 32
Bernold Fiedler ................................. 21

C
Cuncun Chen ................................. 33

D
Dany J V Beste ................................. 19
Diego Comparini ................................. 56

F
Frank J. Bruggeman ................................. 25, 52
Fred C. Boogerd ................................. 25, 52

G
Takeshi Goto ................................. 36

H
Ayaka Hara ................................. 53, 56
Yuki Hara ................................. 57
Hans V. Westerhoff ................................. 25, 52
Hélène Munier-Lehmann ................................. 45
Huai-Kuang Tsai ................................. 26
Hsuan-Cheng Huang ................................. 20
Hsueh-Fen Juan ................................. 28
Yasushi HIRANO ................................. 41
Hyoungseop Kim ................................. 40, 41

I
Hiroyuki Ijima ................................. 47
Seiji Ishikawa ................................. 40, 41
Ryunosuke Itasaki ................................. 35
Ayane Ito ................................. 49
Kentaro Inoue ................................. 38, 43
Junichiro Iwase ................................. 56

J
James C. Sacchettini ................................. 44
Jane L Ward ................................. 19
Johnjoe McFadden ................................. 19
Joo Kooi Tan ................................. 40, 41

K
Shota Kajihara ................................. 40
Hironori Kanetaka ................................. 44, 45
Katharina Nöhl ................................. 19
Tomonori Kawano ................................. 53, 54, 56, 57, 58
Asuka Kikuchi ................................. 57
Akihiro Kitamoto ................................. 46
Shoji KIDO ................................. 41
Makoto Kimura ................................. 54, 55, 56
Hiroyuki Kurata ................................. 1, 13, 25, 32, 33, 34, 35, 37, 38, 39, 52, 65
Takeshi Kurihara ................................. 50
Daisuke Koishi ................................. 33, 38
Yuji Koseki ................................. 44, 45
Kento Kodama ................................. 30, 54
K.Kondo ................................. 52
Shota Kondo ................................. 54

M
Kazuhiro Maeda ................................. 25, 52
Hiroyuki Masunaga ................................. 35
Yu Matsuoka ................................. 37, 65
Fumiko Matsuzaki ................................. 27
Masato Matsuo ................................. 46
Hiroshi Matsuno ................................. 16
Hirotaka Matsumasa ................................. 36
Masaki Matsumoto ................................. 27
Michael H Beale ................................. 19
Ichiro Miura ................................. 42
Reiko Minamikawa-Tachino ................................. 49
Please be aware that the venues are different on March 5th and 6th.
5th is at Ikeno-Okuen Garden in Tagawa City, and 6th is at Nogami President hotel in Iizuka City.

March 5, 9:20～17:30
Ikeno-Okuen Garden/池のおく園
◆西鉄バス 関の山・中村美術館前バス停下車 徒歩1分
西鉄天神バスセンターから80分
・後藤寺・伊田行き（★直行★篠栗北 筑豊烏尾トンネル [特急]）
・後藤寺行き（篠栗北・篠栗 [特急]）
新飯塚駅バス停から20分
・後藤寺行き
後藤寺バスセンターから6分
・天神行き
・博多駅行き

March 6, 9:00～16:30
NOGAMI PRESIDENT HOTEL
◆JR福北ゆたか線 新飯塚駅下車 徒歩5分

3/5は、のがみプレジデントホテル池のおく園
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