P1-01

Computational approaches to drug-receptor binding kinetics

Osamu Ichihara[1]
osamu.ichihara@schrodinger.com

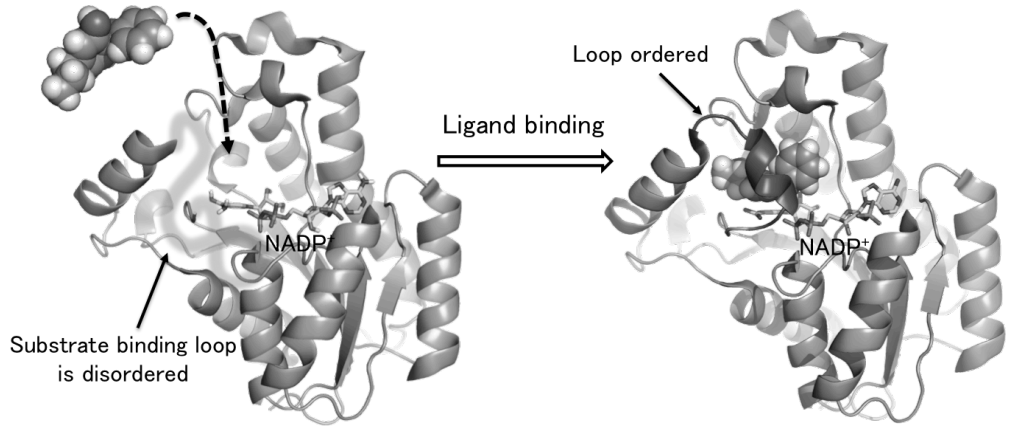
[1] Schrödinger K.K., 17F Marunouchi Trust Tower North, 1-8-1 Marunouchi, Chiyoda-ku, Tokyo 100-0005, Japan

Keywords: Binding kinetics, Residence time, Solvation, Water molecules, Association rate, Dissociation rate

It has been proposed by Pearlstein *et al.* that desolvation and resolvation of the binding sites of proteins can be a key determinant of ligand binding kinetics.[1] They have shown that, for a set of inhibitors of p38 MAP kinase,[2] calculated desolvation and resolvation costs during ligand binding are qualitatively consistent with observed on and off rates respectively.

In this work, application of the hypothesis was further extended to rationalizing the binding kinetics (residence time) of the inhibitors of *Staphylococcus aureus* enoyl-ACP reductase (saFabI), where more complex two-step binding mechanism is suggested to be in operation.[3] It has been proposed that, on ligand binding, saFabI may undergo a directional structural change, i.e. ordering of the substrate-binding loop to form an α-helical structure, and thereby locks the inhibitor into the cavity and increases its residence time.[3]

We have calculated a set of thermodynamic parameters associated with the solvation properties around the ligands bound to saFabI using molecular dynamics simulations (WaterMap).[4] The observed rate of ligand dissociation (residence time) are shown to be in excellent correlation with the parameters calculated. Moreover, the formulation of the parameters strongly supports the expected two-step binding mechanism.



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P1-02

Exploration of Protein-Ligand Complex Configuration in The Equilibrium State

Miwa Sato[1*]
sato-miwa@mki.co.jp

Yoshiki Namiuchi[2]
yoshiki.namiuchi@riken.jp

Mitsugu Araki[3*]
mitsugu.araki@riken.jp

[1] Mitsui Knowledge Industry Co., Ltd. Biomedical Dept. 2-5-1 Atago Minato-ku, Tokyo 105-6215, Japan
[2] RIKEN HPCI Program for Computational Life Sciences, 7-1-16 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan
[3] RIKEN AICS, 1-6-5 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan
* corresponding authors

Keywords: Binding Free Energy Calculation, Protein modeling, Ligand docking, Extended ensemble MD simulation

In drug discovery research, prediction of the most stable protein–ligand binding structure is one of the key issues. To effectively and precisely calculate the binding free energies by using MP-CAFEE method [1], which is based on the Jarzynski equality, it is essential to set the accurate protein-ligand complex configuration in thermodynamic equilibrium state. But it is a challenging task, if there is no protein-ligand crystal structure, or the ligand is de novo designed and is not yet synthesized. Starting from a result of docking based studies, we explore the possibility to precisely calculate the equilibrium state configuration of a protein-ligand complex (in this case, CDK2-CS12 complex, whose crystal structure is registered in CSAR). For this purpose, we use three molecular dynamics (MD) methods, long-time MD simulation, Temperature Replica Exchange Method (T-REMD), and Hamiltonian Replica Exchange Method (H-REMD).

After the small-molecule compound (CSAR ID: CS12) was docked into the ATP-binding site of the CDK2 kinase by use of GLIDE SP mode (Schrodinger, LLC, NY), two kinds of docking poses (gscore rank 1st and 3rd), in which overall or partial chemical structure is flipped with respect to that observed in the crystal structure, were selected as initial structures for the MD simulations. These binding modes did not drastically changed during 1μs conventional MD simulations. In contrast, among trajectories obtained by T-REMD or H-REMD, we observed several kinds of binding poses distinct from the initial structures, including binding modes similar to the crystal structure (Ligand RMSD<0.183nm between the poses and crystal structure). Although a molecular size of CS12 is relatively small (MW=206.3) compared with general drug candidates, our study might suggest that T-REMD and H-REMD have a potential to explore the protein-ligand complex configuration. Based on these results, we are next planning to construct a prediction method of the most stable binding pose among the poses sampled by these extended ensemble MD simulations.

Acknowledgements: This research used computational resources of the K computer provided by the RIKEN Advanced Institute for Computational Science through the HPCI System Research project (Project ID:hp140042&140208). This work was supported in part by MEXT SPIRE Field 1 and K supercomputer-based drug discovery project by Biogrid pharma consortium.

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P1-03

Analysis of the SLP-76 ligand recognition mechanism of Mona/gads by molecular dynamics simulation

Yoshiyuki Uemura[1]
uemura-yoshiyuki-td@ynu.jp

Kazuyoshi Ueda[1]
k-ueda@ynu.ac.jp

[1] Graduate school of Engineering Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Kanagawa 240-8501, Japan

Keywords: Molecular Dynamics simulation, Ligand docking,

Intracellular signal transduction is an important mechanism for the growth and the differentiation of the immuno competent cell (T cell, etc.) in vivo.[1] Mona/gads and SLP-76 are known as the member proteins of such signal transduction pathway in cell. Meanwhile, SH3 is a domain which is known as one of the most abundant protein interaction modules in nature and can binds Pro-rich peptide strongly. Mona/gads also includes a SH3 domain in its structure. However, SH3 domain in Mona/gads does not recognize the Pro-rich peptide, instead, it recognizes the RxxK motif included in SLP-76.[2] The mechanism of these different binding, nature has not been cleared yet. In this work, we tried to elucidate the binding mode and recognition mechanism between Mona/gads and SLP-76 complex using the molecular dynamics simulation method.

We performed 10 ns MD simulation on the system of Mona/gads and SLP-76 complex. From the trajectories, we calculated the interaction energy between them to elucidate the modes of the binding. Molecular dynamics calculation was performed using the package of GROMACS. The force field of protein is AMBER03, and TIP3P model was used for water. The peptide sequence of PAPSIDRSTKPPL is used as a binding motif instead of whole SLP-76 peptide. The results show that the interaction between SLP-76 and Mona/gads was found to be divided into two parts; One is the part where the coulombic electrostatic interaction works mainly, and the other is the part where the van der Waals interaction works mainly. The ratio of these interactions is about 2:1. On the other hand, the residues of Pro1,Ser4,Asp6,Ser8,Pro12 of Mona/gads were found not to involved in the interaction.

We next prepared some mutants of SLP-76, which is substituted one of the residues of SLP-76 to Ala, to investigate the importance of the specific residue. The calculations were also performed with 10ns MD simulation. The results show that the hydrophobic residues in SLP-76 affect the interaction between Mona/gads and SLP-76. On the other hand, charged groups in SLP-76 affect the interaction through the conformation change. In other words, coulomb interactions of the charged residues play an important role to maintain the conformation of SLP-76.

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P1-04

Designed Autonomic Motion in Novel Organized Self-oscillating Gels

DO Junghee junghee-do@aist.go.jp
MUKAI Masaru
TOMINAGA Kenichi
ARIMURA Takashi takashi-arimura@aist.go.jp

Institute of Advanced Industrial Science and Technology(AIST), AIST Tsukuba Central 5-2, Tsukuba, Ibaraki 305-8565, Japan

Keywords: Ferroin, Self-oscillation, Polymer gel, Belousov-Zhabotinsky reaction, LCST

Self-oscillating polymer gel represent an intriguing class of stimuli-responsive materials, which an embedded system of chemistry can elicit responsive forms of mechanics in dissipative/autonomous manner. The self-oscillating properties of the Belousov-Zhabotinsky (BZ) gels result from the incorporation of the BZ reaction catalysts within the polymer that can cause periodic oscillations of chemical composition.[1] The self-oscillating BZ gels containing ferroin complex[2], modified to provide a flexible chemistry for photo-induced polymerization and the amplitude of swelling-deswelling has been characterized.

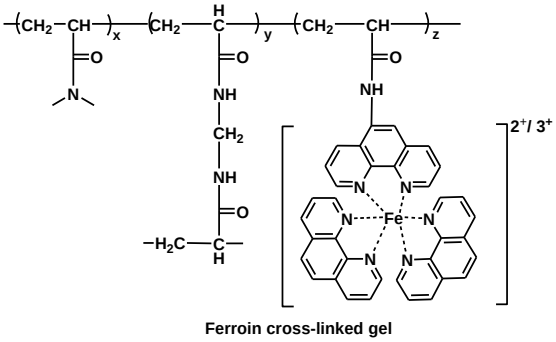

Fig. 1 Chemical structure of a ferroin cross-linked gel.

The photopolymerization was carried out using a UV light source (>300nm, SUPERCURE-204S) at 20 ℃. The behavior of NIPAAm hydrogel at the lower critical solution temperature (LCST) was analyzed. NIPAAm hydrogels by polymerization via UV exposure showed stunning swelling and deswelling behavior with change, inducing the volume phase transition at 29 ℃ lower than that of NIPAAm hydrogel (32 ℃) by thermal synthesis.[3] As shown in the Figure 2, the homogeneity hydrogel was induced the photo-induced polymerization for 30 min. Reported below, synthesized a new a self-oscillating ferroin-copolymerized N-isopropylacrylamide (NIPAAm) cross-linked gel by the photo-induced polymerization (Figure 1). The autonomous motion of the ferroin cross-linked gel on the groove was took place by the BZ oscillating reaction driven from ferroin catalyst.

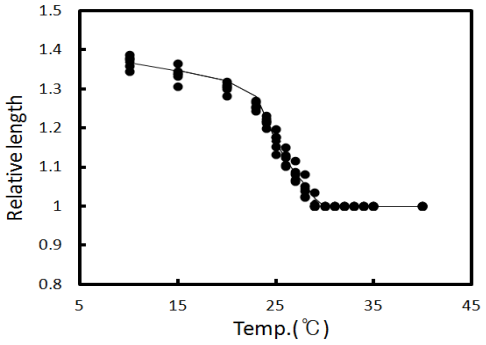

Fig. 2 Equilibrium swelling ratio of the NIPAAm as a function of temperature.

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P1-05

Incorporation of Water Displacement Effect into Scoring Functions for Protein-ligand Docking

Shota Uehara[1] uehara@eniac.scitec.kobe-u.ac.jp

Shigenori Tanaka[1] tanaka2@kobe-u.ac.jp

[1] Department of Computational Science, Graduate School of System Informatics, Kobe University, 1-1, Rokkodai, Nada-ku, Kobe, Hyogo, 657-8501, Japan

Keywords: Protein-ligand docking, Scoring function, Grid inhomogeneous solvation theory(GIST)

In structure-based drug design (SBDD), an accurate scoring function and an efficient optimization algorithm are urgently required for successful virtual screening via docking [1]. In the last years, a number of scoring functions have been developed to achieve accurate estimation of binding affinity between protein and ligand, but the correlations between the experimentally determined binding energies and the calculated scores are still not satisfactory [2]. Most of these scoring functions are formulated based on interatomic potentials, and give relevant estimations of interaction energy between protein and ligand. However, docking scoring functions often miss various thermodynamic effects upon protein-ligand binding, such as water displacement effect.

The binding of a ligand to a protein leads to displacement of water molecules from the protein's pocket, and this displacement process significantly contributes to the overall thermodynamics of protein-ligand binding. For example, the waters in the protein hydrophobic regions which cannot make appropriate hydrogen bonds are energetically unfavorable, and the displacement of such waters into bulk region earns a large contribution in thermodynamics. In earlier works, important contributions of active site waters were revealed using explicit solvation simulations with Water Map [3], GIST [4] and other methods [5,6]. In this work, we incorporate the water displacement effect into docking scoring function, to realize accurate and efficient docking simulation.

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P1-06

Multiple binding poses of a fragment-like molecule to a receptor and their affinities using metadynamics and multiple trajectories multistate Bennett acceptance ratio

Yoshiaki Tanida
tanida.yoshiaki@jp.fujitsu.com

Azuma Matsuura
matsuura.azuma@jp.fujitsu.com

Fujitsu Laboratories Ltd., Atsugi, Kanagawa 243-0197, Japan

Keywords: free energy, multiple poses, metadynamics, alchemical transformation

Current “state-of-the-art” free energy calculations based on alchemical transformation for ligand binding have been widely accepted in computational ligand design and optimization [1]. Here, the prediction of the binding poses of a ligand in the active site of a target receptor is crucial for the use of quantitative evaluation of binding free energy.

In this work, we demonstrate the alchemical free energy calculations coupled with exploring metastable structures using metadynamics. Theophylline/RNA complex (PDB code 1O15 [2]) is used. Several metastable structures in the binding site can be found using the well-tempered metadynamics in three collective variables. In alchemical free energy calculations, to restrict the conformation sampling of theophylline molecule to a finite volume within the binding site, we use the harmonic potentials restraining the translational and rotational motions of the molecule relative to RNA [3]. The multistate Bennett acceptance ratio (MBAR) [4] is employed in evaluating free energies. Multiple trajectories are also used for reducing the statistical error. After computing the free energy difference for each metastable structure, we estimate the binding free energy using the summation of the partition function. Our obtained value agrees well with the experimental one.

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P1-07

Evaluation of protein-ligand binding affinity by replica exchange thermodynamic integration

Osamu Takarada[1] osamu-takarada@ds-pharma.co.jp
Osamu Ichikawa[1] osamu-ichikawa@ds-pharma.co.jp
Kazushi Fujimoto[2] k-fuji@apchem.nagoya-u.ac.jp
Kazuto Yamazaki[1] kazuto-yamazaki@ds-pharma.co.jp
Atsushi Yamada[2] ayamada@apchem.nagoya-u.ao.jp
Tomoko Mizuguchi[2] mizuguchi.tomoko@apchem.nagoya-u.ac.jp
Susumu Okazaki[2] okazaki@apchem.nagoya-u.ac.jp

[1] Sumitomo Dainippon Pharma Co., Ltd., 3-1-98, Kasugade-naka, Konohana-ku, Osaka 554-0022, Japan
[2] Department of Applied Chemistry, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8603, Japan

Keywords: Molecular dynamics simulation, Protein-ligand binding free energy, Replica exchange thermodynamic integration (RETI), Induced-fit binding

The free energy calculation of protein-ligand binding is becoming an essential process for drug discovery. But it is not always enough accuracy for any proteins. Especially, the free energy calculation for induced-fit binding is known to have low accuracy [1]. In order to expand the versatility of the free energy calculations, we applied replica exchange thermodynamic integration (RETI) to the protein-ligand binding and discussed the efficacy of RETI for induced-fit binding.

We selected 9 protein-ligand complexes, which composed of 3 target proteins (Bcl-xL: B cell lymphoma-extra large, MBP: maltose-binding protein, GR: glucocorticoid receptor) and 3 ligands for each protein, as evaluation data of RETI. All complexes are known to be formed by induced-fit binding mechanism, and each of them has respectively different binding affinities. Firstly, we performed MD simulation at high temperatures in order to produce the diverse conformations for each protein. And the obtained conformations were individually assigned to the temperature replicas from 310K to 400K so that the neighboring replicas had similar conformations. Then, we executed the replica exchange simulation and calculated the free energies of protein-ligand binding from 310K replicas by the thermodynamic integration.

The calculated free energy values of 9 complexes by RETI were generally different from the experimental values. However, the differences between the calculated and experimental values in RETI tended to be smaller than the differences in TI. It means that RETI can be effective for improvement the accuracy of the free energy calculation. It was considered that most of these improvements come from more precise simulation of apo-form dynamics. On the other hand, it was suggested that RETI could be also effective in the evaluation of induced fit binding, because some complexes showed higher binding affinity values by RETI than TI.

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P1-08

An Experimental “Twist” to Conformer Generation

Scott A. Gothe[1]
gothe@ccdc.cam.ac.uk

Ilenia Giangreco[2]
giangreco@ccdc.cam.ac.uk

[1] Cambridge Crystallographic Data Centre Inc., 174 Frelinghuysen Road, Piscataway, NJ, 08854-8076, USA
[2] Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, United Kingdom

Keywords: Molecular Modeling, Knowledge-based Methods, Structure-Based Design

Understanding a molecule’s available “conformational landscape” is of central importance in the success of modern Structure-Based Drug Design methods. A number of approaches have been developed by the scientific community to map this landscape, utilizing either theoretical calculations of knowledge-based methods. Many of these methods potentially suffer from oversampling of areas of geometric space inaccessible to the molecule, or undersampling likely conformations due to energetic considerations.

Utilizing information derived from over 750000 small-molecule crystal structures in the Cambridge Crystallographic Database, we have developed a new, rapid, knowledge-based method for enumerating and selecting the conformational profiles for molecules of interest. This presentation will describe the science, methodology, validation and utilization of this experimentally-informed technique.

P1-09

Molecular Dynamics Simulation of Shiga Toxin, III

Kazumi Omata[1]
komata@hosp.ncgm.go.jp

Hisashi Okumura[2,3]
hokumura@ims.ac.jp

Yoshiharu Mori[2]
ymori@ims.ac.jp

Kiyotaka Nishikawa[4]
knishika@mail.doshisha.ac.jp

[1] Medical Sociology Section, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku, Tokyo 162-0052, Japan
[2] Institute for Molecular Science, 38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585, Japan
[3] The Graduate University for Advanced Studies, 38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585, Japan
[4] Faculty of Life and Medical Sciences, Doshisha University, 1-3 Miyakotani, Tatara, Kyotanabe, Kyoto 610-0394, Japan

Keywords: Shiga toxin, Ligand docking, Molecular dynamics, O157

Shiga toxin 1 B-subunit (Stx1B) is a pentamer protein produced by Escherichia coli O157:H7 [1]. The number of residues is 5 x 69 = 345. Its shape is relatively flat, and unlike other proteins, it does not have a distinct binding pocket; the number of binding sites is three per monomer, i.e. the total number is 5 x 3 = 15, and they are distributed on its surface. Using these binding sites, Stx1B recognizes and binds receptors on a cell surface, and intrudes by endocytosis into the cell, accompanying the catalytic A-subunit. Nishikawa and collaborators have developed a ligand (MMAtet) by using a peptide library technique [2], which binds Stx1B. This ligand is a branched peptide oligomer designed such that it covers the greater part of the Stx1B surface. Stx1B-MMAtet complexes cannot be crystallized, so their static structure is still unknown. As such, the present study has investigated the docking of Stx1B and the ligand by use of molecular dynamics simulations [3]. We have obtained the following results: (1) the ligand recognized and docked on the three classes of binding sites in Stx1B, in agreement with the deduction based on the experiments; (2) the Stx1B-ligand binding occurs inhomogeneously on the Stx1B surface, implying that in fact more than one ligand bind to one Stx1B in actual situations; (3) it remained unclear why the ligand binds strongly one of the three binding-site classes which consists of arginine or tryptophan residues. In the meeting, quantitative analysis concerning the above results will be presented in detail.

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P1-10

Molecular simulations on specific interactions between curcumin derivatives and amyloid-β peptide

Shintarou Ota[1], Hiromi Ishimura[1], Ryushi Kadoya[1], Takeru Murakawa[1], Sergiy Shulga[2] and Noriyuki Kurita[1]

kurita@cs.tut.ac.jp

[1]Department of Computer Science and Engineering, Toyohashi University of Technology
[2]Institute for Food Biotechnology and Genomics, National Academy of Sciences of Ukraine

Keywords: curcumin; inhibitor; amyloid-β; fragment molecular orbital; protein-ligand docking

Alzheimer's disease (AD) is developed in relation to the accumulation of amyloid-β (Aβ) peptides in a brain. Aβ peptides are produced from the cleavage of amyloid precursor protein (APP) by both β- and γ-secretases. Among the produced Aβ peptides species, Aβ40 and Aβ42 have a large population, and the amount of Aβ42 is larger than Aβ40 in the brains of the AD patients. To suppress the production of Aβ peptides, it is effective to inhibit the APP cleavage process by the secretases. However, the secretases also play key roles in production of other vital proteins, so secretase inhibitors have an increased side effect risk. Therefore, it has been desired to develop novel compounds which bind specifically to the cleavage site of APP and inhibit the γ-secretase attacks to APP.

To propose new agents for suppressing the generation of Aβ peptides, we [1] investigated the interactions between a short APP peptide and derivatives of curcumin, a constituent of turmeric, which is a spice used in Indian cuisine. We performed molecular simulations based on protein-ligand docking as well as *ab initio* fragment molecular orbital (FMO) methods and investigated the specific interactions between APP and curcumin I, II and III. The results simulated reveals that curcumin I binds to APP in a way as it covers over the γ-secretase cleavage site of APP, although the binding of curcumin I with APP is not so strong. It is thus expected that curcumin I can suppress the cleavage of APP by γ-secretase.

In the present study, in order to enhance the binding between APP and curcumin I, we proposed novel curcumin derivatives by introducing some groups into curcumin I and investigated the specific interactions between these derivatives and APP. The results reveal that the introduction of CH_3 groups into the central part of curcumin I significantly enhances the binding between APP and curcumin I. It is thus expected that the curcumin I derivative covers over the γ-secretase cleavage site of APP to be a potent inhibitor for the production of Aβ40 and Aβ42 peptides. The details of our molecular simulations and the results simulated will be shown in the conference.

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P1-11

Fragment Molecular Orbital Calculations on the Interactions of Enzyme Lipases and Organic Compounds:Elucidation of the Enantioselectivity and Reactivity of Lipases in Organic Synthesis

Yoichiro Yagi [1]
yagi@sp.ous.ac.jp

Takatomo Kimura[2]
kimura@konankako.co.jp

Makoto Kamezawa[2]
makoto.kamezawa@konankako.co.jp

Yoshinobu Naoshima[1]
naoshima@sp.ous.ac.jp

[1] Research Institute of Natural Sciences, Okayama University of Science, 1-1 Ridai-cho, Kita-ku, Okayama 700-0005, Japan
[2] Konan Chemical Industry Co., Ltd., 5-12 Nakagawa-cho, Takatsuki-shi, Osaka 569-0066, Japan

Keywords: FMO calculation, lipase, enantioselectivity, organic synthesis, azulene

Over the last several years we have been focusing our attention on the biomolecular computational simulations of the complexes of lipases with organic compounds to elucidate the enantioselectivity and reactivity of the enzyme in organic synthesis. We have recently shown that both molecular dynamics (MD) and fragment molecular orbital (FMO) calculations are useful means for the prediction of the enantioselectivity of *Burkholderia cepacia* lipase (BCL) [1, 2] and *Candida antarctica* lipase typeB (CALB) [1] toward a variety of aliphatic and aromatic alcohol esters. This presentation describes the FMO calculations on the interactions of *Candida antarctica* lipase typeA (CALA) and the enantiomers of trifluoroazulene alcohol esters. Azulene and its derivatives are composed of a characteristic fused five-seven bicyclic aromatic ring system and are typical nonbenzenoid aromatic hydrocarbons. Azulenes are of interest because of the expected reaction specificities and physicochemical and pharmaceutical properties. [3]

CALA-azulene alcohol esters complexes, like BCL- and CALB-aliphatic and aromatic alcohol esters ones, in the presence of TIP3P water molecules were subjected to MD calculations over a period of 2000ps with ABMER11. After MD calculations, surrounding water molecules were removed and the FMO computations of the resulting CALA-azulene complexes were carried out at FMO2-MP2/6-31G level using ABINIT-MP/BioStation program.

We found that for 3-chloro-and 3-bromotrifluoroazulene alcohol derivatives having high enantioselectivity, each fast reacting (*S*)-enantiomer strongly interacts with a definite amino acid residue in CALA, that is, with ASP95; those in BCL and CALB were HIS286 and THR40, respectively. Our FMO computations also indicated that for an azulene alcohol ester possessing azulenothiophene ring with low enantioselectivity, both (*R*)- and (*S*)-enantiomers show interaction with several identical amino acid residues including ASP95. We have reached a conclusion that particular amino acid residues in three different lipases, ASP95 in CALA, HIS286 in BCL, and THR40 in CALB, can play an important role in the chiral recognition of substrate enantiomers through the individual lipase-catalyzed biotransformations.

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P1-12

Designing Noise-immune Gates

Tetsuya J. Kobayashi[1]
tetsuya@mail.crmind.net

[1] Institute of Industrial Science, University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 108-8639, Japan

Keywords: Bayesian inference, optimal decoding, molecular gates

Intracellular reactions are intrinsically noisy because of the small number of molecules and of the extrinsic fluctuation of environment and other factors [1]. Nonetheless, a variety of biological systems and functions operate robustly even though they are implemented by noisy reactions [2]. This fact suggests that intracellular systems and networks are designed so as to be immune to the intrinsic noise from the molecular components. Such design may be employed to artificially design intracellular reactions networks to conduct various functions. However, little is clarified on the design principle of such noise-immune systems. In our previous work, we derived a intracellular network structure that can robustly sense the environmental change via noisy receptor by employing Bayesian inference approach.

In this work, I extend our previous work for designing noise-immune gates that operate robustly even with noise in the input signals to be computed. The properties of this gates are compared with previously proposed noise-immune gates based on logical stochastic resonance.

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P1-13

Protein–ligand binding pathways revealed by coarse-grained molecular dynamics simulations

Tatsuki Negami
negami@bi.a.u-tokyo.ac.jp

Kentaro Shimizu
shimizu@bi.a.u-tokyo.ac.jp

Tohru Terada
tterada@iu.a.u-tokyo.ac.jp

Department of Biotechnology and Agricultural Bioinformatics Research Unit,
Graduate School of Agricultural and Life Sciences, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Keywords: coarse-grained molecular dynamics simulation, protein–ligand binding, ligand-binding pathway

Clarifying the mechanism of protein–ligand interactions is one of the most important research subjects in computational chemistry and biology. However, most of the research efforts have been devoted to predicting docked structures. The process of the ligand binding remains to be clarified. Molecular dynamics (MD) simulation is a straightforward way to study the ligand-binding process. However, reproducing the ligand-binding process in an all-atom MD simulation is still difficult because it requires a very long time simulation. For this reason, we have explored the possibility of the coarse-grained simulation.

Recently, we have shown that the ligand-binding processes can be reproduced in coarse-grained (CG) MD simulations with the MARTINI force field [1]. In this study, we performed CGMD simulations for two protein–ligand pairs that have different physicochemical and structural properties. To make a complete comparison between protein–ligand pairs with different properties, we classified all the protein–ligand complex structures in PDB into groups according to these properties, and chose two representative protein–ligand pairs from each group. For each pair, 1–5 microsecond CGMD simulations were performed 50–100 times with different initial ligand placement. For all the protein–ligand pairs, the ligand molecules entered into the correct ligand-binding pockets. To obtain the details of the ligand binding pathways, we calculated the flow of the ligand molecules on the protein surfaces. The results demonstrated that the CG ligand molecules entered the ligand-binding pockets through specific pathways. Based on these results, we will discuss the determinant of the ligand-binding pathway in relation to the physicochemical and structural properties of the ligand and the protein surface.

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P1-14

Elucidation of transition state for the interactions between ERK2 and inhibitors using SPR

Sou Yamaguchi[1] **Satoru Nagatoishi**[1,2]
6711877632@mail.ecc.u-tokyo.ac.jp nagatoishi@bioeng.t.u-tokyo.ac.jp

Kouhei Tsumoto[1,2,3]
tsumoto@bioeng.t.u-tokyo.ac.jp

[1] Department of Bioengineering, School of engineering, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
[2] Drug Discovery Initiative DDI, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
[3] Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Keywords: Protein-small compound interaction, Transition state, Thermodynamic analysis

To develop drug discovery of small compounds, it is important to elucidate how small compounds interact with protein. The purpose of this study is elucidating the process of protein-small compound interactions and proposing to the design of small-molecule drugs using the process of small-molecule bindings.

To date, protein-small compound interactions have been evaluated in term of their final state where a small compound is bound to protein. However, it is difficult to design compounds which can bind to target protein specifically, because many binding sites of protein are preserved among proteins [1]. This study focuses on the process of the interaction. Since it is assumed that a small compound forms some intermediate states with various amino acids and finally binds to the energy-stable site of protein, the process is possibly differ among proteins. It has been reported that the *in silico* studies have also proposed the possibilities, which is important for efficiency of drugs, and specificity of compound to target protein. Here, the binding kinetic analysis was carried out to elucidate the binding process of the compound to protein. In this study, complexed energy transition in the binding process [2] is simplified as two state reaction and the state where a compound is in process is regarded as transition state. From the analysis of thermodynamic parameters in transition state, the transition state is examined at amino acid level and the relationship between transition state and binding process is discussed. This allows us to verify the possibility of obtaining an important indicator for drug design.

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P1-15

Tautomeric reactions of DNA base pairs induced by H and OH radicals: DFT calculations in water

Naoko Okutsu[1] okutsu@klab.cs.tut.ac.jp **Eisuke Shimizu**[1] **Victor I Danilov**[2] **Sergiy Shulga**[2] **Noriyuki Kurita**[1] kurita@cs.tut.ac.jp

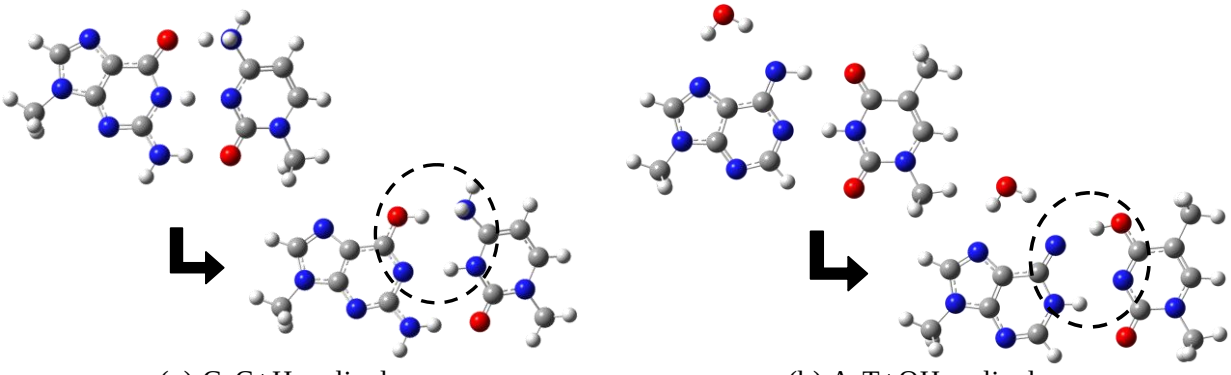
[1] Toyohashi University of Technology, Toyohashi, Aichi, 441-8580, Japan
[2] National Academy of Sciences of Ukraine, Kyiv, Ukraine

Keywords: DFT, Electronic states, Molecular orbital calculation, DNA base pair, Radical,

Genetic information recorded in DNA can be damaged indirectly by radiations. Their interactions to the water molecules in living organs produce the ionization of water molecules, leading to highly reactive H and OH radicals. These radicals react with organic cellular components, especially DNA and lipids, so that DNA is damaged in its structure and electronic properties seriously. OH radicals can reach an active site of DNA duplex and remove a hydrogen ion from the sugar moiety or the nucleobase of DNA. As a result, the native double-helical structure of DNA is changed, leading to errors in the genetic information stored in DNA [1]. It is thus important to understand the change in DNA structure induced by the radical attack for revealing the indirect effect of radicals on DNA.

To elucidate the effect of radicals, we have investigated the attacking mechanism of OH-radical to the G-C and A-T base pairs, using the density functional theory (DFT) calculations [2]. The effect of solvation on the mechanism was also revealed by performing the same DFT calculations under the continuum solvation approximation. The results elucidated that the hydrogen atom of NH_2 group of G or A base is abstracted by the OH radical. The solvation around the base pair stabilizes the dehydrogenated structures significantly, indicating the acceleration of the attacking reaction by OH-radical to the base pairs in water.

In the present study, we searched for the transition states of the attacking mechanism between the base-pair and OH/H radicals in water, using the DFT calculations. The results elucidate that OH radical can cause tautomeric reaction in A-T base pair, while H radical affects significantly on G-C base pair to cause the tautomeric reaction into G*-C* form, as shown below. The details of the tautomeric reaction paths and their energetics will be described at the meeting.



(a) G-C+H-radical (b) A-T+OH-radical

Figure Chang in structure during tautomeric reaction for (a) G-C+H-radical and (b) A-T+OH-radical

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P1-16

Specific interactions between amyloid-β peptides in three-fold symmetric hexamer: *ab initio* molecular simulations

Hiromi Ishimura **Shintarou Ota**

Ryushi Kadoya **Noriyuki Kurita**
kurita@cs.tut.ac.jp

Depart. Computer Science & Engineering, Toyohashi University of Technology

Keywords: amyloid-β; fragment molecular orbital; protein-protein interaction; aggregation

Alzheimer's disease (AD) is most common symptoms of dementia, and the number of patients increases remarkably all over the world, and the development of the cure and therapeutic drugs is an urgent research theme now. The cohesion of amyloid-β peptides (Aβs) in the brain is related to the onset of AD. Therefore small molecules inhibiting the Aβ cohesion are expected to be useful as AD therapeutic drugs. A diagrammatical view of the cohesion process was elucidated by the solution NMR analysis. However, it is not possible to elucidate the process at an atomic level by only the experimental analysis. This fact is a bottleneck in developing effective inhibitors for Aβ cohesion. To solve the problem, many classical molecular dynamics (MD) simulations have been performed to analyze the tertiary structures of Aβ monomer, dimer and fibrils at an atomic level. For example, MD simulations were performed for the pair of Aβ-fibrils [1] to predict the growth mechanism of Aβ fibrils. In addition, MD simulations were carried out for the Aβ-fibrils with a three-fold symmetric conformation, whose structure was obtained from the senile plaques of the AD patient [2]. However, since a molecular force field was used in these MD studies, the relative stability among the MD snapshots cannot be analyzed accurately. To predict the most stable conformation properly, *ab initio* molecular orbital (MO) calculations are necessary to be done for many conformations of Aβ fibrils.

In the present study, we performed *ab initio* fragment MO (FMO) calculation for the three-fold symmetric structure of Aβ-fibrils obtained by the solid-state NMR [2]. Based on the results of the FMO calculation, we investigated the specific interactions between Aβ monomers in the Aβ-fibrils and elucidated which amino acid residues of Aβ monomers are important for stabilizing the Aβ-fibril structure with a three-fold symmetry. The results will be useful for proposing novel inhibitors to Aβ aggregation.

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P1-17

Acceleration of Protein-ligand Binding Energy Calculation Using Fragment Molecular Orbital Method

Takao Otsuka[1] totsuka@riken.jp
Noriaki Okiomoto[1] okimoto@riken.jp
Makoto Taiji[1] taiji@riken.jp

[1] RIKEN Quantitative Biology Center (QBiC), 1-6-5 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan

Keywords: electronic structure calculation, fragment molecular orbital method, protein-ligand binding energy

Computational prediction of protein-ligand binding affinities is so important for rational drug design. Especially computational methods using molecular docking and/or molecular dynamics simulation are central methods for evaluation of protein-ligand binding affinity, and are expected to improve the efficiency of the drug development in the near future [1].

Recently first-principles (or ab initio) electronic structure calculations are also used in this field to evaluate accurate protein-ligand interactions. However, the electronic structure calculations are often not available for the large molecular system because of its high computational costs. One of the most popular calculation methods of electronic structure for large biomolecular system is the fragment molecular orbital (FMO) method proposed by Kitaura and co-workers [2]. Nowadays many studies on protein-ligand interactions by FMO method have been reported [3].

In this study we report our recent proposed acceleration scheme for the binding energy calculations of protein-ligand systems by using FMO method, which is based on a multilayer FMO method focusing on the protein-ligand interaction distance [4]. Our acceleration scheme reduces computational costs, while maintaining accuracy in the evaluation of binding energy.

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P1-18

Specific interactions between *M.tuberculosis* CYP130 and econazole derivatives: Molecular docking and *ab initio* FMO calculations

Ryushi Kadoya[1], Ryosuke Takeda[1], Mitsuki Fujimori[1], Hiromi Ishimura[1], Pharit Kamsri[2], Pijittra Meewong[2], Pornpan Pungpo[2] and Noriyuki Kurita[1]

kurita@cs.tut.ac.jp

[1] Department of Computer Science and Engineering, Toyohashi University of Technology, Tenpaku-cho, Toyohashi, Aichi, 441-8580, Japan

[2] Department of Chemistry, Faculty of Science, Ubon Ratchathani University, 85 Sthollmark Rd., Warinchamrap, Ubon Ratchathani 34190, Thailand

Keywords: Tuberculosis, Econazole, Cytochrome P450, CYP, Molecular docking, Fragment molecular orbital, Protein-ligand interaction, Molecular mechanics.

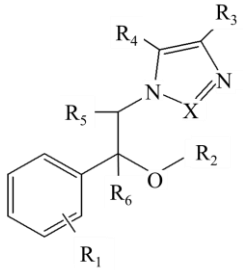
Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). In 2013, 9 million people were infected with TB, and 1.5 million were died from it. This high rate of TB deaths was mainly caused from the strong drug-resistant of TB as well as the concurrent illness of TB and HIV. Among the commercially available drugs for TB, an anti-tubercular rifampicin induces the expression of drug-metabolizing cytochrome P450 (CYP) enzymes, which reduce the blood level of the anti-tubercular as well as the anti-HIV agents. As a result, the effects of these agents are decreased by the simultaneous usage of rifampicin. It is thus needed to inhibit the expression of CYP enzymes under the existence of rifampicin.

The previous experiments [1,2] elucidated that azole antifungal agents can coordinate to the Fe ion of HEME and inhibit the CYP function. For example, econazole was found to bind to CYP and inhibit the growth of *M. tuberculosis*. In addition, various econazole derivatives such as nitroimidazole and 1*H*-1,2,3-triazole were synthesized and their anti-TB functions were analyzed.

In the present study, to propose novel agents with large binding affinity with CYP130, we investigated the specific interactions between CYP130 and econazole as well as some azole compounds at atomic and electronic levels, using molecular simulations based on protein-ligand docking, classical molecular mechanics (MM) and *ab initio* fragment molecular orbital (FMO) methods. The FMO results elucidate which residues of CYP130 and which parts of the ligands are important for the binding between CYP130 and the ligands. Based on the results, we designed novel agents listed in Table1 and investigated their binding energies and specific interactions with CYP130. The results will be shown in the poster.

Table1 Our proposed novel agents for CYP130

Ligand	R_1	R_2	R_3	R_4	R_5	R_6	X
Econazole	2,4-diCl-Phenyl	CH_2-4-Cl-Phenyl	H	H	H	H	CH
Eco-COH	2,4-diCl-Phenyl	CH_2-4-Cl-Phenyl	H	COH	H	H	CH
Eco-OH	2,4-diCl-Phenyl	CH_2-4-Cl-Phenyl	H	OH	H	H	CH
C-36	4-Phenyl	H	NO_2	H	H	H	C-OCH_3
C-36-CN	4-Phenyl	H	NO_2	H	CN	H	C-OCH_3
C-36-NO	4-Phenyl	H	NO_2	H	NO	H	C-OCH_3
C-36-O	4-Phenyl	H	NO_2	H	O	H	C-OCH_3
C-36-OH	4-Phenyl	H	NO_2	H	H	OH	C-OCH_3



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P1-19

Changes in conformation and electronic states of CAP dimer induced by ligand-binding: molecular dynamics and FMO simulations

Kanako Shimamura, Ittetsu Kobayashi, Yuki Matsushita, Noriyuki Kurita*

Toyohashi University of Technology, *E-mail: kurita@cs.tut.ac.jp

Keywords: catabolite activator protein (CAP), MD, fragment MO, transcription, cAMP

1. Introduction

Catabolite activator protein (CAP) plays an important role in the transcription mechanism of genetic information from DNA to RNA. CAP is activated by the binding of cyclic adenosine monophosphate (cAMP) as ligand, and CAP+cAMP complex binds specifically to near the transcription domain of DNA. As a result, RNA polymerase can bind specifically to the binding site to start the transcription. In the present study, we investigated the change in structure of CAP-dimer+DNA complex in water induced by the cAMP binding, using classical molecular dynamics (MD) simulations. In addition, we elucidated the specific interactions between CAP-dimer, DNA and cAMP at an electronic level, using *ab initio* fragment molecular orbital (FMO) calculations.

2. Details of molecular simulations

We obtained the structure of CAP-dimer+cAMP+DNA complex (PDB ID: 1ZRC) from PDB, and the defect of amino acid residues of CAP was complemented. Because PDB structure has no information of H atoms, we add H atoms as well as counter ions around DNA backbones. The structure was optimized in water by classical molecular mechanics (MM) calculation, and MD simulations were carried out for 100 ns under the conditions of temperature 300K and pressure 1 atom. For some MD snapshots, electronic states were evaluated by *ab initio* FMO method, in order to elucidate the change in specific interactions.

3. Results and discussion

To elucidate the structural change, we first analyzed the Root Mean Square Distance (RMSD) between the initial structure and the MD snapshot for the Cα atoms of CAP residues. RMSD became the largest 3.57 Å at 10.6 ns, and it became larger than 3.0 Å at several snapshots, indicating significant structural change of CAP-dimer during the MD simulation. In particular, at 70.7 ns, the CAP-dimer conformation deforms significantly from the initial one, and the displacement of the residues around Pro160 of CAP monomer-1 was found to be remarkably large as shown in Figure 1. Since these residues are in the DNA binding domain of CAP, the interactions between CAP and DNA are expected to be changed significantly. The details of structural change and electronic states of CAP-dimer+DNA complex evaluated by FMO will be present at the meeting.

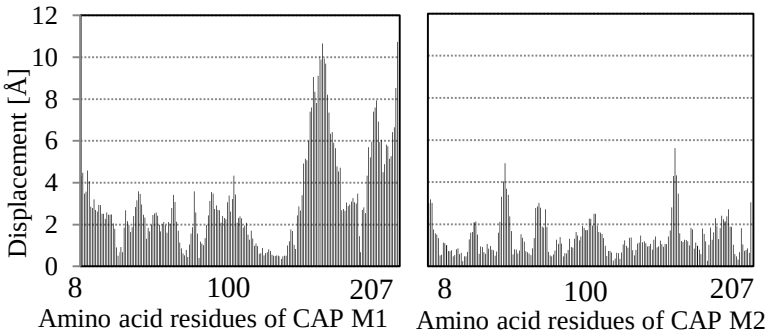

Figure 1 Displacement of Cα between the initial and 70.7 ns

P1-20

Molecular dynamics study of conformation change in calmodulin

Hiromitsu Shimoyama[1]
shimoyamah@pharm.kitasato-u.ac.jp

Mayuko Takeda-Shitaka[1]
shitakam@pharm.kitasato-u.ac.jp

[1] Faculty of Pharmacy, Kitasato University, 9-1, Shirokane 5-Chome Minato-ku, Tokyo 108-8642, Japan

Keywords: calmodulin, conformation change, conformational fluctuation

Calmodulin (CaM) is a calcium ion binding protein which mediates many essential biological processes such as fertilization, contraction, cell differentiation and proliferation[1, 2]. The information of transient Ca^{2+} is converted into a variety of biochemical changes by intracellular proteins[1, 3]. Some of these proteins can bind to Ca^{2+} by itself, however, others requires intermediate Ca^{2+}-binding proteins such as CaM.

CaM is 148 amino acids long protein which forms approximately 7-8 α-helices in solution. The N- and C-terminal domains (NTD, CTD) are mutually connected by long linker-region, then, the overall CaM's structure is coarsely a symmetrical dumbbell-like shape. The ion binding to CaM causes conformation change[4, 5].

In order to understand the conformation change, structural fluctuations around holo-state[6] was studied by molecular dynamics simulation using GROMACS[7].

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P1-21

Development of hydration structure prediction method around the protein with 3D-RISM theory

Shunsuke Chiba[1]
pl10027@st2.pharm.kitasato-u.ac.jp

Yasuomi Kiyota[1]
kiyotay@pharm.kitasato-u.ac.jp

Mayuko Takeda-Shitaka[1]
shitakam@pharm.kitasato-u.ac.jp

[1] School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

Keywords: Hydration structure, 3D-RISM method, Monte Carlo Sampling

Water plays important roles in protein function. In particular, in the process of molecular recognition, it is necessary to consider the hydration and dehydration. Determination of hydration structure is a useful approach to analyze this influence. For predicting the hydration structure, there are many theoretical methods like MD simulation, but these methods have complex processes because of higher entropy of water. 3D-RISM (3-Dimensional Reference Interaction Site Model) theory, the integral equation theory of liquids, can calculate the hydration structure as a 3-dimensional distribution function (3D-DF), directly [1,2]. The 3D-DF, however, is difficult to image the molecular shape in hydration structure [3]. Therefore, we developed a sampling method for protein hydration structures based on the 3D-DF with Inversed-transformed Monte-Carlo simulation. This method can reproduce the conformation of hydration structure accurately.

Our method gives higher prior conformation of water based on the 3-dimensional DF obtained from 3D-RISM calculation. The position where the peak of water tends to appear would be replaced to explicit water molecule in accordance with the Inversed-transformed Monte-Carlo method. The 3-dimensional DF is the probability density function, then the peak height indicates the degree of appearance and it can be used as the weight in the Monte-Carlo calculation.

In order to evaluate the prediction ability of our method, we applied our method to 112 globular-protein structures which had been solved by X-ray diffraction as monomer. For comparison of the predicted and experimental hydration structures, we calculate the RMSDs between them. One of the results from accurate samples is shown in Figure 1. It was displayed that the method can reproduce experimental hydration structure. Most predicted hydration structures demonstrate small RMSDs whose range is roughly from 1.5 Å to 2.5 Å.

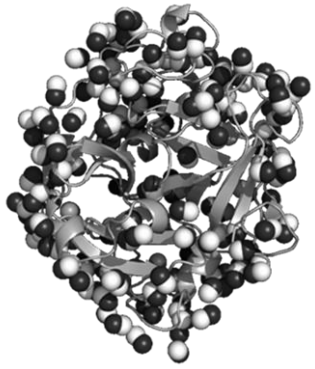

Figure 1. A result of predicted hydration structure around protein, Serine Protease (PDB ID: 1ARB). The color code is assigned to predicted (black) and X-ray (white) water molecules.

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P1-22

Molecular dynamics simulation of a protein-ligand binding process with conformational changes

Takashi Mitsui[1,2]
tmitsui@jp.fujitsu.com

Hajime Sugiyama[2]
sugiyama.hajime@jp.fujitsu.com

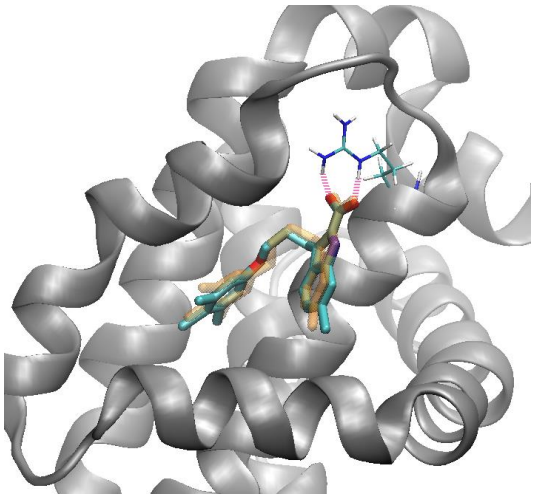
Hideaki Fujitani[1]
fjtani@lsbm.org

[1] Laboratory for System Biology and Medicine, Research Center for Advanced Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan
[2] Bio-IT R&D Office, Research & Development Division, Next-generation Healthcare Innovation Center, Fujitsu Limited, 1-17-25 Shin-kamata, Ohta-ku, Tokyo 226-8503, Japan

Keywords: Molecular dynamics, Ligand docking

Understanding the physical process of protein-ligand binding has significant importance in structure-based drug design [1, 2]. In order to capture the binding processes of a protein-protein interaction inhibitor and its receptor, we performed 600 unguided molecular dynamics simulations of 300 ns length for the system of Mcl-1 and its ligand on K computer.

The initial structure of Mcl-1 was obtained from the crystal structure of PDB code 4HW4 which is a complex structure with a peptide ligand. The ligand was obtained from PDB code 4HW2, and placed by a distance of 10Å from the binding groove of Mcl-1. Bound structures in the groove were observed in 473 trajectories of 600 MD simulations, and the binding poses within RMSD of 1 Å from the X-ray cocrystal structure were observed in 25 trajectories. One of typical ligand binding processes found in this study was as follows: a indole ring approached to the binding groove, then salt bridges were formed between a carboxy group of the ligand and a side chain of Arg263 in Mcl-1, finally the ligand changed its conformation and 4-chloro-3,5-dimethylphenoxy group of the ligand bound to the deep P2 pocket in the binding groove.



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P1-23

Binding Affinity Prediction of Protein-Protein Complexes Based on MM/3D-RISM Method

Narumi Sai[1]
pl10017@st2.pharm.kitasato-u.ac.jp

Yasuomi Kiyota[1]
kiyotay@pharm.kitasato-u.ac.jp

Mayuko Takeda-Shitaka[1]
shitakam@pharm.kitasato-u.ac.jp

[1] School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

Keywords: Protein-protein interaction, Binding free energy, MM/3D-RISM method, Protein structure prediction

Protein-protein interactions (PPIs) play important roles in biological processes. In order to understand the binding mechanism of PPIs, quantitative information about protein-protein binding affinity is essential. Therefore, computational methods to predict binding affinity are valuable. In the present work, we developed a system for predicting binding free energies of protein-protein complexes using structure-based approach. In this system, Template Based Modeling (TBM) method is used for preparing protein-protein complex structures of various mutants, and MM/3D-RISM method is used for binding free energy calculation. 3D-RISM theory is a powerful tool for studying solvation thermodynamics and properties of a solvation structure.

Figure 1 shows the prediction scheme of our system. First, based on a template structure of a protein-protein complex, monomer and complex model structures of various mutants are constructed by TBM method. Next, binding free energies of the various mutants are calculated by MM/3D-RISM method based on the obtained model structures. Evaluation of this system shows that the predicted energies are in good agreement with the experimental data. MM/3D-RISM method improves the accuracy of binding free energy prediction.

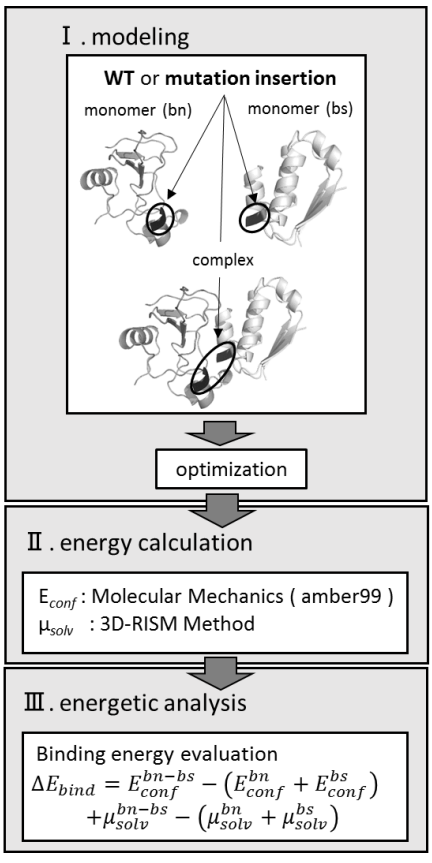

Figure 1. Prediction scheme. Barnase(bn)-barstar(bs) complex is shown as an example.

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P1-24

Novel insights into drug hypersensitivity by long-term molecular dynamics simulations.

Takahiro MURAI[1]
u879782i@ecs.osaka-u.ac.jp

Norihito KAWASHITA[1,2]
kawashita@gen-info.osaka-u.ac.jp

Yu-Shi TIAN[3]
yushi-tian@ist.osaka-u.ac.jp

Tatsuya TAKAGI[1,2]
ttakagi@phs.osaka-u.ac.jp

[1] Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
[2] Research of Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan
[3] Graduate School of Information Science and Technology, Osaka University, 1-5 Yamadaoka, Suita, Osaka 565-0871, Japan

Keywords: drug hypersensitivity, HLA, abacavir, MD simulation

The discovery that several drug hypersensitivity reactions (DHRs) are associated with specific human leukocyte antigen (HLA) alleles attracted increasing interest [1, 2]. However, the underlying mechanisms of these HLA-induced DHRs, especially those of drug-induced immediate activation of T cell clones (TCCs) remain unclear. Recently a novel hypothesis, the partial detachment between HLA molecule and self-peptide(s), is proposed to explain this unresolved mechanism [3]. Here in order to validate this hypothesis long-term molecular dynamics (MD) simulations were performed.

We focused on HLA-B*57:01-restricted abacavir (ABC) hypersensitivity, one of the most famous DHRs. Firstly the models of four self-peptides were constructed and next complexes of these self-peptides and the HLA-B*57:01-peptide were generated. Subsequently we performed 500ns MD simulations of all these complexes.

One of the simulation results showed that the distance between the specific residues of HLA-B*57:01 and those of self-peptide(s), which are implicated in ABC binding, was changed to be larger during the period of simulations. A detailed analysis also revealed that the distance between α1 and α2 helix of HLA-B*57:01 became large enough to allow the entrance of ABC into the peptide binding cleft. These results implied that some self-peptides are partially dissociated from HLA-B*57:01, which enables some ligands to accommodate and stabilize the peptide binding cleft. Our findings provided novel insights into ABC-induced immediate activation of TCCs and these findings might also be applied to other DHRs, such as HLA-B*58:01-restricted allopurinol hypersensitivity reactions.

This work was supported by the TSUBAME grand-challenge program and the numerical calculations were carried out on the TSUBAME2.5 supercomputer in Tokyo Institute of Technology.

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P1-25

Comprehensive Analysis of Protein-Ligand Interactions in Estrogen Receptor α Using Fragment Molecular Orbital Method

Satoshi Anzaki[1] anzaki@eniac.scitec.kobe-u.ac.jp
Chiduru Watanabe[2] chiduru.watanabe@riken.jp
Yoshio Okiyama[2] yoshio.okiyama@riken.jp
Teruki Honma[2] honma.teruki@riken.jp
Kaori Fukuzawa[3] fukuzawa.kaori@nihon-u.ac.jp
Shigenori Tanaka[1] tanaka2@kobe-u.ac.jp

[1] Department of Computational Science, Graduate School of System Informatics, Kobe University, 1-1, Rokkodai, Nada-ku, Kobe, Hyogo 657-8501, Japan
[2] RIKEN Center for Life Science Technologies, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
[3] School of Dentistry at Matsudo, Nihon University, 2-870-1 Sakaecho-Nishi, Matsudo, Chiba, 271-8571, Japan

Keywords: Estrogen receptor α (ERα), Fragment molecular orbital (FMO) method, Visualized cluster analysis (VISCANA)

Estrogen receptors play important roles in genesis of reproductive functions and multiplications of cells. Estrogen receptor α (ERα) binds either agonists which have the same functions as estrogens or antagonists which do not. In a previous study[1], we have analyzed a positional difference of helix12 between agonist complexes and antagonist complexes.

In this research, we calculate protein-ligand interactions with fragment molecular orbital (FMO) method[2] comprehensively after preparing computational models using 22 PDB entries with IC_{50} values under the standardized condition (residue complementation, hydrogen addition, energy minimization). Moreover, using FMO method, we obtain the inter-fragment interaction energy (IFIE) based on quantum chemical calculations which can incorporate electron correlation energy[3]. We study the correlation of the IFIE with IC_{50} and the binding specificities of ligands with visualized cluster analysis (VISCANA)[4] for drug design.

Acknowledgment: This work is supported by the FMO Drug Design Consortium (FMODD) and the "Construction of platform of FMO-based drug discovery using HPCI system" project (hp150160) using K computer.

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P1-26

Analysis of Interaction between Glucagon-like Peptide-1 and Lipid Bilayers by Molecular Dynamics Study

Sakiko Mori[1] s086232@toyaku.ac.jp
Yamada Hironao[1] s077112@toyaku.ac.jp
Yoh Noguchi[1] s086181@toyaku.ac.jp
Takeshi Miyakawa[1] takeshi@toyaku.ac.jp
Ryota Morikawa[1] morikawa@toyaku.ac.jp
Takuya Watanabe[1] watanabe@toyaku.ac.jp
Masako Takasu[1] takasu@toyaku.ac.jp

[1] School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Horinouchi, Hachioji, Tokyo 192-0392, Japan

Keywords: Molecular dynamics, Peptide-bilayer interaction, Glucagon-like peptide-1, Phospholipid membrane

Glucagon-like peptide-1 (GLP-1), a 30-amino acid peptide hormone, promotes insulin secretion to lower plasma glucose levels in pancreas. When GLP-1 binds to GLP-1 receptor, which is a class B G protein-coupled receptor (GPCR), GLP-1 shows single-stranded α-helical structure [1]. On the other hand, GLP-1 is highly flexible and the α-helicity is small in physiological saline [2]. It is interesting to study how α-helical structure of GLP-1 is formed in the circulating blood. Two-step ligand transportation model [3] is a scheme to transport a ligand, which is in α-helical state, to the receptor. In this model, a ligand binds to the cell membrane before binding to class B GPCR, and diffuses along the membrane surface. Similarly, lipid micelles in the circulating blood may help the ligand to form α-helix.

We have carried out molecular dynamics (MD) simulations of GLP-1 with dodecylphosphocoline (DPC) micelle to investigate the interaction between GLP-1 and micelle [4]. From the results of the simulations, among all the residues of GLP-1, Arg^{30}, a C-terminal residue of GLP-1, has extremely high tendency to make hydrogen bonds with oxygen atoms of DPC lipids. The binding state of GLP-1 and micelle were stabilized when Arg^{30} made hydrogen bonds with DPC lipids. Since the hydrophobic amino acids of GLP-1 are squeezed into a space in the lipid head groups of DPC micelle, GLP-1 stuck along the micelle surface and kept the α-helical structure.

We have also performed MD simulations of GLP-1 with phospholipid bilayer in physiological saline to investigate the interaction between GLP-1 and bilayer. We found that GLP-1 also bound to the bilayer. The details of the results will be discussed.

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P1-27

Ligand Binding Analysis of p38 MAP Kinase with the Fragment Molecular Orbital Method

Yinglei Sheng[1*], Keiya Maruyama[1], Hirofumi Watanabe[2], Yoshio Okiyama[3], Chiduru Watanabe[3], Teruki Honma[3], Kaori Fukuzawa[4], Shigenori Tanaka[1*]

1 Department of Computational Science, Graduate School of System Informatics, Kobe University, 1-1, Rokkodai, Nada-ku, Kobe, Hyogo 657-8501, Japan
2 Education Center on Computational Science and Engineering, 7-1-48, Minatojima-Minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan
3 RIKEN Systems and Structural Biology Center, Tsurumi, Yokohama 230-0045, Japan
4 School of Dentistry at Matsudo, Nihon University, 2-870-1, Sakaecho-nishi, Matsudo, Chiba, 271-8587, Japan
**email: sheng@eniac.scitec.kobe-u.ac.jp, tanaka2@kobe-u.ac.jp*

Keywords: p38 MAP kinase, Interaction energy analysis, Ligand binding analysis, Fragment Molecular Orbital (FMO) method, IFIE (Inter-Fragment Interaction Energy), Structure preparation

p38 mitogen-activated protein (MAP) kinase[1] plays an important role in the functional expression of various cellular processes which cause cell aging and autoimmune diseases, and is activated by phosphorylation with external stress such as heat, osmotic pressure and ultraviolet rays. In the present study, we investigate the calculated results of the IFIE[2,3] (Inter-Fragment Interaction Energy) between p38 MAP kinase and various binding inhibitors using Fragment Molecular Orbital[4] (FMO) method, as one of the activities of "FMO Drug Design Consortium" which was established last year. The p38 MAP kinase possesses more than 85 ligand binding structures in PDB with the activity data on IC_{50}. Moreover, p38 MAP kinase is the key for the database of interaction analysis among the kinase family.[5] This time, the structure preparations before carrying out the FMO-MP2/6-31G* calculations on the K computer (hp150160 for industrial use: "Construction of platform of FMO-based drug discovery using HPCI system") were performed carefully. Structural optimizations for the added hydrogen atoms were performed by using MOE [6] with classical force fields (Amber10:EHT and MMFF94x etc.) under a realistic situation as much as possible on chemical basis: protonated state, bond order of ligands, complement of missing residues and consideration of crystal water.

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P1-28

Molecular dynamics simulation of unbinding pathway

Teruhisa S. Komatsu [1] **Noriaki Okimoto**[1]
teruhisa.komatsu@riken.jp okimoto@gsc.riken.jp

Makoto Taiji[1]
taiji@riken.jp

[1] QBiC, RIKEN, 1-6-5 Minatojima-minami, Chuo-ku, Kobe, Hyogo 650-0047, Japan

Keywords: molecular dynamics simulation, protein-ligand binding affinity

In order to develop a new drug design or to improve the tentative drugs for the target receptor, the precise estimation of the binding free energies for the various ligands is essential. Aiming at the application to drug improvements, rather high precision (around the order of thermal energy k_BT=2.5kJ/mol) for the binding free energies should be established. Atomistic (classical mechanical) forcefields optimized for organic molecules have been developed[1-2], and methods to calculate binding free energies by molecular dynamics simulations are proposed[3-7]. Nevertheless, it seems that the blind use of the result from the molecular dynamics simulation is not yet guaranteed, and there is a room for further investigations to clarify the difficulties and for developing more efficient schemes.

Here we try to simulate unbinding process of protein-ligand system. The typical time scale of such process is order of second. It is far beyond to reach directly from all-atom molecular dynamics simulation by the state of the art computer architecture. Thus a kind of biased sampling method should be employed. We try molecular dynamics simulation of unbinding pathway by following PaCS-MD method [8] and its variants and present preliminary results and discuss its application to free energy calculations.

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P1-29

State Transition Analysis of H-Ras with PaCS-MD Simulation

Yuta Saito[1] saito@eniac.scitec.kobe-u.ac.jp
Yuta Hano[1] hano@eciac.scitec.kobe-u.ac.jp
Kazuhiro J. Fujimoto[2] k-fujimoto@hokuriku-u.ac.jp
Takashi Kumasaka[3] kumasaka@spring8.or.jp
Shigeyuki Matsumoto[4] smat@med.kobe-u.ac.jp
Tohru Kataoka[4] kataoka@people.kobe-u.ac.jp
Fumi Shima[4] sfumi@med.kobe-u.ac.jp
Shigenori Tanaka[1] tanaka2@kobe-u.ac.jp

[1] Department of Computational Science, Graduate School of System Informatics, Kobe University, 1-1, Rokkodai, Nada-ku, Kobe, Hyogo 657-8501, Japan
[2] Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanagawa-machi, Kanazawa 920-1181, Japan
[3] Protein Crystal Analysis Division,Japan Synchrotron Radiation Research Institute (JASRI/SPring-8),1-1-1 Kouto, Sayo, Hyogo 678-5198 Japan
[4] Division of Molecular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicin, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017,Japan

Keywords: Ras protein, State transition, Hydrogen bond network, Information entropy, Parallel cascade selection molecular dynamics (PaCS-MD)

Small GTPases Ras (H, K-, and N-Ras), the products of the *ras* proto-oncogenes function as guanine nucleotide-dependent molecular switches regulating cell growth, development and apoptosis by cycling between GTP-bound (Ras-GTP) and GDP-bound (Ras-GDP) forms. Ras are frequently activated in a wide variety of human cancers, making them some of the most promising targets for anti-cancer drug development. Ras-GTP adopts two inter-converting conformational states, state 1 and state 2, corresponding to inactive and active forms, respectively [1,2]. Since the state 1 conformation possesses the drug-accessible surface pockets between the two switch regions, named Switch I (32-38) and Switch II (60-65), the information on the transition mechanism between the states is essential for the efficient development of Ras-specific inhibitors. We investigated herein the state transition mechanism of H-Ras-GTP using molecular dynamics [3,4].

First, we performed PaCS-MD simulation which was proposed as a molecular simulation method to yield conformational transition in short time [5]. As a result, we found a conformational transition between two states.

Second, we analyzed the relationship between the hydrogen bond network composed of tightly bound water around Ras protein and the state transition using informational entropy. From these analyses, we have found the hydration waters and hydrogen bonds which are associated with the state transition.

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P1-30

Electronic-Structure Data Mining for Optimization of Anti-Tuberculosis Drugs

Manabu Sugimoto[1,2,3] sugimoto@kumamoto-u.ac.jp
Takayuki Kurogi[1] 112t1710@st.kumamoto-u.ac.jp

[1] Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan
[2] Institute for Molecular Science, National Institutes for Natural Sciences (NINS), 38 Nishigo-Naka, Myodaiji, Okazaki 444-8585, Japan
[3] Research Center for Advanced Science and Technology (RCAST), University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan

Keywords: Electronic structure, Data mining, Drug, Tuberculosis

Chemoinformatics tools based on electronic-structure calculations are developed and applied to investigate important electronic characteristics of anti-tuberculosis drugs. Herein we focus on pyridinamides (Fig. 1) which have been extensively studied. The electronic descriptors are molecular orbital energy, excitation energy, dipole moment, ionization energy, electron affinity, oscillator strength, and transition dipole moment. The electronic structure calculations were carried out by using the density functional theory (DFT) method with the CAM-B3LYP functional. The 6-31G(d,p) basis set was used in all the calculations. Molecular structures were fully optimized without any constraints. The spin-singlet excited states were calculated by using the time-dependent DFT (TDDFT) method. In this calculation, the low-lying 30 excited states were calculated.

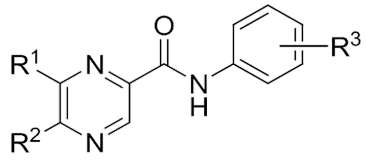

Fig. 1. Pyrimidine derivatives

We carried out the data-mining analysis in which exhaustive search of observing correlation between each of the calculated electronic descriptors and experimentally measured biological activity data. Firstly we applied singlet regression analysis using various mathematical functions. No good correlations were observed in this analysis. Therefore, we tried to apply the linear multiple regression analysis with respect to the electronic descriptors. We were successful to obtain a function reasonably reproducing the experimental IC_{50} values for inhibitory activity related to membrane affinity of pyramidines. In this analysis, it was found that the molecular orbital localized on the benzene moiety should be an important factor, suggesting that its chemical modification would be effective to improve the pharmacological action.

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P1-31

Sonification of Ligand-Protein Docking Structures: Quantification of 3D Topological Similarity

Takafumi Inoue[1]
112t1710@st.kumamoto-u.ac.jp

Manabu Sugimoto[1,2,3,4]
sugimoto@kumamoto-u.ac.jp

[1] Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan
[2] Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan
[3] Institute for Molecular Science, National Institutes for Natural Sciences (NINS), 38 Nishigo-Naka, Myodaiji, Okazaki 444-8585, Japan
[4] Research Center for Advanced Science and Technology (RCAST), University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan

Keywords: Chemoinformatics, Sonification, Protein, Ligand, Docking, Similarity

In Structure-based drug design (SBDD), one of the important issues is to specify topological characteristics of docking structures. The information is essential to understand structure-function relationship in various biological phenomena. Herein we propose a new chemoinformatics method by which three-dimensional topology is represented by sonification. This is a technology of expressing information by "sound". By expressing the docking structure by sound detected at a binding site of a ligand, the topological information essentially becomes one dimension. This provides an advantage for description and comparison with other ligands.

Sonification is carried out by defining a unique sound for each amino acid. Its intensity is modulated to reflect the distance from the target ligand. By implementing the Fourier Transform, the synthesized sound is converted into the frequency spectrum. Spectral comparison with a reference compound provides information about how much the local environment is similar with each other.

In this presentation, we report an application of the presently developed sofinication method to the 2VT4 protein [1] which is one of the G protein-coupled receptors. We evaluate similarity in local environment in some docked structure with the ligands of P32, NA, Sog, and D10 given in [1]. For there ligands, there are 4, 4, 12, and 2 equivalent docking sites in the 2VT4 protein. It is shown that our sonification method is successful to distinguish similarity and difference of the ligands and the binding sites.

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P1-32

Interation analysis of cell adhesion inhibitor by fragment molecular oribital method.

Norihito KAWASHITA[1,2] kawashita@gen-info.osaka-u.ac.jp
Yu-Shi TIAN[3] yushi-tian@ist.osaka-u.ac.jp
Tatsuya TAKAGI[1,2] ttakagi@phs.osaka-u.ac.jp

[1] Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
[2] Research of Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan
[3] Graduate School of Information Science and Technology, Osaka University, 1-5 Yamadaoka, Suita, Osaka 565-0871, Japan

Keywords: Cell adhesion, Fragment molecular orbital (FMO) method, Inter-fragment interaction energy (IFIE)

FimH is a cell adhesion factor in adherent-invasive *Escherichia coli* (AIEC) and uropathogenic *Escherichia coli* (UPEC), and it is triggerd cell adhesion by the interaction with mannose in their host cell. Therefore, compounds inhibiting the interaction become a drug candidate for these infections and some mannose derivatives are reported. [1, 2] For the drug design of these inhibitors, we calculated inter-fragment interaction energy (IFIE) by fragment molecular orbital (FMO) method [3] and determined the strength of a correlation by compared with the experimental value.

In this study, twelve complexes of the FimH and mannose derivatives were used for FMO calculation. The complexed were prepared by MOE (CCG Inc.). One crystallization water molecule was included in the structures. For FMO calculation, ABINIT-MP6.0+ was used for software and the calculation level was MP2/6-31g*. Then we calculated the correlation between IFIE and the enthalpy change of ligands. The experimental data for comparison with the calculated value were used from the results of Wallens *et al.* [1]

From the result of the calculations, correlation between IFIE of twelve complexes and enthalpy change ΔH was low and correlation coefficient *R* was 0.51. However, when we removed three outliers and then recalculated the correlation coefficient only using 9 complexes, the coefficient *R* was improved 0.90. It was good correlation between IFIE and the enthalpy change. The results suggested that slight differences of complex structures affected IFIE.

This work is a part of Construction of platform of FMO-based drug discovery using HPCI system and this research used computational resources of the K computer provided by the RIKEN Advanced Institute for Computational Science through the HPCI System Research project (Project ID: hp150160).

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P2-01

SBDD project database management: sharing the underlying wisdom behind the data using MOE

Howard Feldman[2], Akitoshi Okada[1], Ryoichi Kataoka[1], Junichi Goto[1], Paul Labute[2]
okada@rsi.co.jp

[1] Ryoka Systems Inc., Science & Technology Systems Division, Tokyo Skytree East Tower, 1-1-2 Oshiage, Sumida-ku, Tokyo 131-0045, Japan
[2] Chemical Computing Group Inc., 1010 Sherbrooke St. West, Suite #910, Montreal QC, Canada H3A 2R7

Keywords: Data organization, Data Collection, Project data management, SBDD

In recent years, Structure based drug design (SBDD) research has become more specialized allowing it to be divided into a research area and perhaps more streamlined. This allows SBDD research to be carried out more efficiently. However, it can also be noted that the large amount of data obtained from one particular research area maybe looked at very differently by the people from other research area. This difference in perspective may be very meaningful in the SBDD research. However, as the research area divides, it becomes more difficult to share the information among the research group. Moreover, the data needs to be pre-processed in a meaningful way (standardized) so that when any people from any research area looks at the data, the data is meaningful at the same level of standard.

Molecular Operating Environment[1] (MOE) employs database in its system and also carries many functions that can perform the necessary pre-processing of the structural data (*e.g.* superposition, hydrogen addition etc.). In the latest version of MOE (MOE 2014.09), new function named MOE Project was implemented that gathers and pre-processes all sorts of SBDD-related data into one database. Below diagram outlines the data accumulation MOE performs (Figure 1). The output is a single database where all the data is stored.

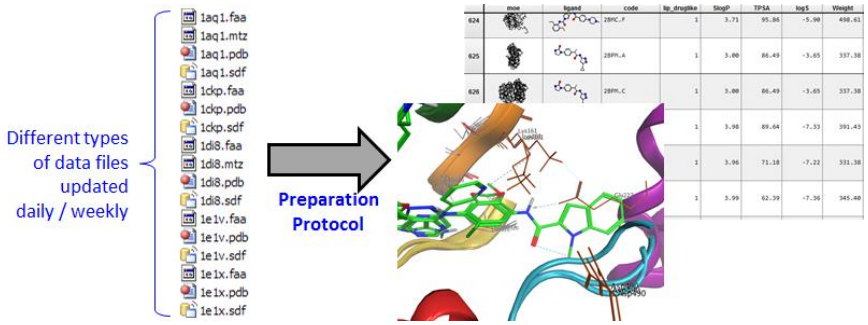

Figure 1. Automation process MOE project enables in project data accumulation The resulting database contains crystal structures and physical/experimental properties that are gathered from different types of data.

MOE Project allows sharing the latest and standardized data within the research group very easily. Data accumulation becomes automated, allowing one to gather the data rapidly. The pre-processing of the data is also carried out as the data is accumulated. The resulting database can be searched by relevant keywords or values so the necessary data can be easily retrieved. The “data” now becomes “information” allowing the research group to come together and step forward to decision making with the same level of information provided.

[1] Molecular Operating Environment (MOE), 2014.09; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2014**.

P2-02

Target-based Drug Repositioning Using Large-scale Chemical-protein Interactome Data

Ryusuke Sawada[1]
rsawada@bioreg.kyushu-u.ac.jp

Hiroaki Iwata[1]
iwata@bioreg.kyushu-u.ac.jp

Yoshihiro Yamanishi[1,2]
yamanishi@bioreg.kyushu-u.ac.jp

[1] Division of System Cohort, Multi-scale Research Center for Medical Science, Medical Institute of Bioreg-ulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan
[2] Institute for Advanced Study, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka, Fukuoka 812-8581, Japan

Keywords: drug reposting, drug target protein, drug indication, compound-protein interaction

In recent years, the development of new drugs based on conventional drug discovery processes has become increasingly difficult [1], as reflected by a decrease in the number of newly approved drugs worldwide. Drug repositioning is the identification of new drug indications for known drugs, and it has been recognized as a useful strategy for drug discovery [2], because with drug repositioning, some of the information on known drugs can be reused. Most drugs may interact not only with the therapeutic target proteins but also with additional proteins (hereafter referred to as off-targets), resulting in unexpected side effects [3]. Drug side effects derived from off-targets are undesired, but they may occasionally be beneficial for new therapeutic indications. Therefore, there is a strong incentive to develop computational methods to predict both drug targets and drug indications for systematic drug repositioning [4].

In this study, we developed novel computational methods to predict potential drug targets and new drug indications for systematic drug repositioning using large-scale chemical-protein interactome data. We explored the target space of drugs (including primary targets and off-targets) based on chemical structure similarity and phenotypic effect similarity by making optimal use of millions of compound-protein interactions (mainly in ChEMBL [5]). Based on the target profiles of drugs, we constructed statistical models to predict new drug indications for a wide range of diseases with various molecular features of diseases (e.g., disease-causing genes, diagnostic markers, disease-related pathways, and environmental factors). The proposed method outperformed previous methods in terms of interpretability, applicability, and accuracy. Finally, we conducted a comprehensive prediction of the drug-target-disease association network for 8,270 drugs and 1,401 diseases, and showed biologically and clinically meaningful examples of newly predicted drug targets and drug indications. The predictive model is useful to understand the mechanisms of the predicted drug indications.

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P2-03

A New Ligand-based Virtual Screening Method that uses an Exhaustive Shape-based Ligand Alignment

Hideyuki Sato[1] hsato@eyesopen.com
Paul C.D. Hawkins[2] phawkins@eyesopen.com
Robert W. Tolbert[2] bob@eyesopen.com

[1] OpenEye Japan Co. Ltd., AIOS Toranomon 904, 1-6-12 Nishishimbashi, Minato-ku, Tokyo 105-0003, Japan
[2] OpenEye Scientific Software Inc., 9 Bisbee Court, St.D, Santa Fe, NM, 87508, USA

Keywords: virtual screening, in silico screening, ligand-based virtual screening, shape-based alignment, shape-based pose prediction

Ligand-based Virtual Screening (LBVS) is a widely used computational method in drug discovery for identifying hit compounds against a target molecule of interest. LBVS techniques use the 3D as well as 2D structure of ligands to search molecule databases. We developed ROCS[1], which uses shape and chemical features of mo ring molecules. ROCS aligns a target molecule against a query molecule, init of mass (CoM) of both molecules, and optimizes the overlap using a smooth Ga shape and chemical similarity between the molecules can be calculated, whic nd rank the target molecules. ROCS performs very fast virtual screenings a l databases. Many successes using this LBVS method in drug discovery have b

We have developed a new ligand-based virtual screening method; subROCS. The new method uses an exhaustive molecular alignment method based on shape and chemical features of ligand molecules. subROCS starts finding the best overlay of a target molecule against a query molecule from many starting points, not only from the CoM used in standard ROCS. These points are based on all heavy atoms of the input molecules, in order to exhaustively explore the best overlay between the molecules. Using the Directory of Useful Decoys, Enhanced (DUD-E), which consists of 102 drug targets, [2] we present both virtual screening performance of subROCS against this standard virtual screening database; and its pose prediction performance improved by the exhaustive shape-based ligand alignment.

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P2-04

Rapid Technique for New Scaffold Generation

Tim Cheeseright[1] tim@cresset-group.com
Marina Takahashi[2] m_takahashi@hulinks.co.jp
Sumie Tajima[2] tajima@hulinks.co.jp
Giovanna Tedesco[1] giovanna@cresset-group.com
Susana Tomásio[1] susana@cresset-group.com
Paolo Tosco[1] paolo@cresset-group.com
Mark Mackey[1] mark@cresset-group.com

[1] Cresset, New Cambridge House, Bassingbourn Road, Litlington, Cambridgeshire, SG8 0SS, UK
[2] HULINKS Inc., 5-14 Nihonbashi Hakozaki-cho, Chuo-ku, Tokyo, 103-0015, Japan

Keywords: Drug design, Scaffold hopping, Bioisostere

Scaffold hopping remains a central task in medicinal chemistry for generating and protecting intellectual property. We have previously presented a technique for rapidly generating reasonable yet novel scaffold replacements using molecular fields which has been extended to include R-group replacement. The approach uses a database of molecule fragments or available reagents to suggest replacements that maintain the shape and electrostatic character of a known active molecule.

However, for both of scaffold hopping and R-group replacement, activity is not the only requirement for any suggested replacement. To be useful they must be synthetically accessible and must fall within the window of acceptable physicochemical properties for the project. The task of ranking scaffold hops or bioisosteric replacements is thus one of multi-parameter optimisation, where several often-competing requirements have to be considered simultaneously.

In this poster we suggest methods to address these issues with reference to case studies of both scaffold hops and R-group replacements[1]. Synthetic accessibility can be handled by tying the bioisostere search to addressable chemistry space, utilising the chemist's knowledge of what synthetic routes are feasible to guide the search. The best guide of novelty in a scaffold-hopping situation is the experience of the user: what other scaffolds are known in the literature or in patents? To this end, we suggest a clear and minimal user interface to allow rapid triage of large result lists. Finally, assessing results in the light of physicochemical and predicted ADMET requirements can be achieved through a configurable radar plot giving clear visual feedback on how close any suggested replacement is to the ideal.

[1] http://www.cresset-group.com/spark

P2-05

Predicting Regioselectivity and Lability of Cytochrome P450 Metabolism using Quantum Mechanical Simulations

Jonathan Tyzack[1]
jon@optibrium.com

Sumie Tajima[2]
tajima@hulinks.co.jp

Marina Takahashi[2]
m_takahashi@hulinks.co.jp

Peter Hunt[1]
peter@optibrium.com

Matthew Segall[1]
matt@optibrium.com

Nick Foster[1]
nick@optibrium.com

[1] Optibrium Ltd., 7221 Cambridge Research Park, Beach Drive, Cambridge, CB25 9TL, UK
[2] Hulinks Inc., 5-14 Nihonbashi Hakozaki-cho, Chuo-ku, Tokyo 103-0015, Japan

Keywords: Predicting P450 metabolism, Quantum mechanical simulation, Quantitative estimate

Many computational methods have been developed that predict the regioselectivity of metabolism by drug metabolising isoforms of the Cytochrome P450 class of enzymes (P450) [1-5]. Here we describe recent developments to a method for predicting P450 metabolism that combines quantum mechanical (QM) simulations to estimate the reactivity of potential sites of metabolism on a compound with a ligand-based approach to account for the effects of orientation and steric constraints due to the binding pockets of different P450 isoforms. These new developments include modelling reaction pathways for epoxidation and developing models for an extended range of P450 isoforms. The resulting models achieve accuracies of 85-90% on independent test sets.

While valuable, predicting the relative proportion of metabolite formation at different sites on a compound is only a partial solution to designing more stable compounds. The advantage of a quantum mechanical approach is that it provides a quantitative estimate of the reactivity of each site, from which additional information can be derived regarding the vulnerability of each site to metabolism in absolute terms. One such measurement is the site lability, as calculated by StarDrop™ [6], which is a measure of the efficiency of the product formation step. This is an important factor influencing the rate of metabolism and we will illustrate how this provides valuable guidance regarding the potential to redesign compounds to overcome issues due to rapid P450 metabolism.

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[6] StarDrop, Optibrium Ltd, Cambridge, UK, www.optibrium.com/stardrop

P2-06

Ligand-Guided Approach to Validate Computational Hydrophobic Binding Pocket Models of Lipid GPCRs

Misa Sayama[1]
4884487850@mail.ecc.u-tokyo.ac.jp

Tomohiko Ohwada[1]
Ohwada@mol.f.u-tokyo.ac.jp

Takatsugu Hirokawa[2]
t-hirokawa@aist.go.jp

Sho Nakamura[1], **Sejin Jung**[1], **Masaya Ikubo**[1], **Yuko Otani**[1]

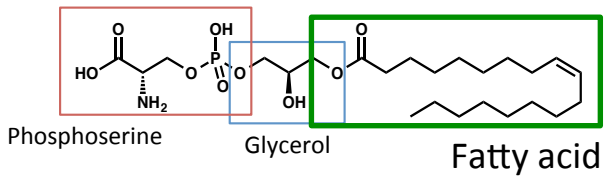
[1] Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[2] National Institute of Advanced Industrial Science and Technology (AIST), 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan

Keywords: G-protein-coupled receptor, lysophosphatidylserine, structure-activity relationship, ligand docking

Several G-protein-coupled receptors (GPCRs) have been reported to be activated by lipid or lipid-derived molecules. Lysophosphatidylserine (LysoPS) has been proposed to be one of such lipid ligands of GPCRs.[1,2] LysoPS is an amphipathic molecule, generated by enzymatic hydrolysis of phosphatidylserine, which is a component of membrane bilayers. In order to understand how lipid ligands activate GPCRs, we have synthesized various LysoPS analogues.[3] This project is also of interest in terms of medicinal chemistry because LysoPS and its receptors have been proposed to be involved in regulation of the immune system.

We found that the fatty acid moiety of LysoPS contributes significantly to receptor activation potency and subtype-selectivity[3], however their molecular basis is unknown. In order to consider significance of the fatty acid moiety in the viewpoint of receptor-ligand interactions, we constructed binding models of some of the LysoPS analogues containing non-lipid fatty acid surrogates and homology models of LysoPS receptors. Robustness of these binding models was confirmed by using molecular dynamics simulation and then we designed and synthesized compounds that are predicted to be active according to the models. Our models are consistent with the significant roles of the fatty acid terminal.

LysoPS (18:1): Endogeneous pan-agonist



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P2-07

Fragment-Based Drug Discovery Applied to the Antimicrobial Target CapF from *Staphylococcus aureus*

Takeru Chigira[1]
8120661875@mail.ecc.u-tokyo.ac.jp

Koichiro Nakano[2]
koi.nakano@so.taisho.co.jp

Takamitsu Miyafusa[3,4]
t-miyafusa@aist.go.jp

Satoru Nagatoishi[5]
nagatoishi@bioeng.t.u-tokyo.ac.jp

Jose Caaveiro[5]
jose@bioeng.t.u-tokyo.ac.jp

Kouhei Tsumoto[1,3,5]
tsumoto@bioeng.t.u-tokyo.ac.jp

[1] Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
[2] Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[3] Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[4] Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology, Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan
[5] Department of Bioengineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

Keywords: Fragment-based drug discovery, Structure-activity relationship, Calorimetry

The capsular polysaccharide (CP) surrounding *Staphylococcus aureus* (*S. aureus*) is an important pathogenic factor during infections caused by this gram-positive bacterium [1]. The bi-functional enzyme CapF, catalyzing two key reactions for the synthesis of a CP precursor, is a suitable target to develop novel antibacterial agents [2]. A fragment-based screen was performed to identify novel CapF inhibitors. A hit compound of the family of natural tropolones inactivates the first reaction catalyzed by the enzyme at the Zn^{2+} binding pocket of its cupin domain. Calorimetry and X-ray crystallography were employed to clarify the mechanism of inhibition. The novel inhibitor binds exothermically ($\Delta H° < 0$) to the Zn^{2+} ion using two coordination bonds, blocking access of the substrate to the active site, and suggesting that the hit compound binds to CapF employing specific interactions [3]. Based on structure-activity relationship analysis from other natural tropolones and our own structural data, the compound is amenable to optimization (to increase affinity and specificity) using rational structural-based ligand design methodologies, such as computer-aided drug discovery and lead optimization methodologies for the design of antibiotic agents. We suggest that extension of the ring at the C3-position of the tropolone ring will yield compounds of increasing potency. In summary, we propose tropolones as candidates to develop novel antibacterial agents.

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P2-08

Analyses of Fragment-Subsite Interactions in Protein-Ligand Complexes

Noriyuki Yamaotsu[1] yamaotsun@pharm.kitasato-u.ac.jp
Shuichi Hirono[1] hironos@pharm.kitasato-u.ac.jp

[1] School of Pharmacy, Kitasato University
5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

Keywords: Fragment, Ligand, Binding site

We analyzed interactions between fragments of ligands and subsites of the binding site on proteins. In order to obtain fragment-subsite pairs, we used the database of 3D-structures of protein-ligand complexes (PDBbind v.2013) [1-3]. The core set of PDBbind was chosen from 38,918 complexes in PDB according to known binding data, resolutions of X-ray data, clustering of protein sequence similarities and sampling of binding constants. The core set was composed of 195 protein-ligand complexes in 65 protein clusters and thus its diversity was considered to be suitable for this study. The ligands in the database were divided into fragments using Fragmenter of JChem 6.3.0 (ChemAxon Ltd., Budapest, Hungary, 2014). The RingChainRecap.xml configuration file in Fragmenter was used as a rule for the fragmentation. This rule cut non-ring single bonds starting from a ring atom, and thus a molecule split into the ring systems and the connecting chains. When cutting makes the single atoms, they will be attached to the rings. A set of residues within 4Å of each fragment was defined as the fragment-subsite using SYBYL-X 2.1.1 (Certara, L.P., Princeton, NJ, USA, 2013). Finally, 644 fragment-subsite pairs were obtained as the fragment-subsite set. For comparison, we computed subsite- and fragment-similarities. The similarities of subsites were calculated on the basis of their 3D coordinates and physicochemical properties using our SUPERPOSE_SITE program [4]. The chemical similarities of the fragments were calculated as Tanimoto similarities between 64-bit fingerprints of 3D distances of atom pairs using Canvas 2.4 in Small-molecule Drug Discovery Suite 2015-2 (Schrödinger, LLC., New York, NY, USA, 2015). R 3.1.3 was used as a statistical software (The R Foundation for Statistical Computing, 2015) [5].

We found that the subsite-similarities correlated well with fragment-similarities. The Pearson correlation coefficient was very good and the Spearman rank correlation coefficient was moderate when larger subsites were used (n= 2766, r_p = 0.900, r_s = 0.564). Using largest subsites, the both coefficients were very good (n= 620, r_p = 0.869, r_s = 0.910). These results implied that similar fragments bind to similar subsites independent of types of proteins.

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P2-09

Prediction of Residues with Key Interactions with Ligands Based on Receptor Environmental Properties

Daisuke Takaya[1]
daisuke.takaya@riken.jp

Teruki Honma[1]
honma.teruki@riken.jp

[1] RIKEN Center for Life Science Technologies, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan.

Keywords: SBDD, Protein function prediction, Amino Acid Property, Machine learning

In SBDD, protein-ligand docking is a powerful method to identify its inhibitors. Generally, docking programs for that purpose worked rapidly because of the force-field-based empirical scoring functions. They give a great advantage for *in-silico* screening to dock huge amount of molecules in commercially available compound databases (e.g. ZINC[1]) to a drug discovery target protein. However, because the scoring functions employed many approximations and limited training data, the prediction performance is still not enough to find all potential inhibitors in the upper rank every time. Moreover, a correlation coefficient between the experimental value (e.g. IC50, Ki) and the calculated values by the docking methods is not so high. For these reasons, the highly scored compounds by the scoring functions have to be narrowed down by rescoring or filtering using other kinds of properties such as interaction fingerprint. For example, mutation analysis of ligand binding involved residues, interaction fingerprints of docked structures, similarity to the known inhibitors or medicinal chemist's visual inspections for scaffolds of docked compounds were widely used for this purpose. From this point of view, we focused on residues involving in key interaction(s) such as H-bonds, ionic bonds, tight hydrophobic contact with ligands. If a prediction method for importance measure of the binding site residues is given beforehand, we could use the rank for the do rescoring and filtering. In this study, we newly set a purpose to develop prediction methods to quantify the importance of residues with the key interaction(s) around the pockets.

Firstly, experimental data such as X-ray crystal structures were extracted from Protein Data Bank[2] and a definition of the residues with key interactions was introduced to distinguish positive and negative data. Secondly, probe molecules to estimate protein ligand interactions were generated around the binding pockets and surrounding amino acid properties were calculated for the residues to obtain descriptors. Using the descriptors, machine learning methods were applied to construct prediction models and estimated the performance of the constructed models. In the future, we will try to construct prediction models to quantitatively predict importance of the ligand binding.

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P2-10

Adverse effect prediction by random forests model using polypharmacological profile of a small compound

Tomohiro Sato[1] tomohiro.sato@riken.jp
Teruki Honma[1] honma.teruki@riken.jp

[1] Center for Life Science Technologies, RIKEN, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa, 230-0045, Japan

Keywords: adverse effect, polypharmacology, machine learning, random forests

Recently, polypharmacology caught the attention to investigate both efficacy and side-effects of drugs. With increasing data of 3D structure of proteins and biological activities of small compounds, several efforts to predict adverse drug reactions (ADRs) considering polypharmacology were reported.[1,2] In this study, a novel method to predict ADRs based on machine leaning of polypharmacological profile of a small molecule was developed. Employing random forests, machine learning models for 326 proteins in ChEMBL database were built, and used to predict pharmacological profile of a compound. Then, the pharmacological profile was used as the explanatory variables to build the learning models for 129 ADRs registered in SIDER2 drug side effect database (Figure 1). In the cross validation experiments about 129 ADRs, the prediction based on polypharmacology profile showed ROC value of 0.826 on average, and successfully exceed those predicted using conventional molecular fingerprints (Figure 2). Furthermore, leave-cluster-out validation showed that the proposed method could maintain higher prediction accuracy than the conventional prediction models directly using molecular fingerprint, even when compounds in training set and test set had less structural similarity.

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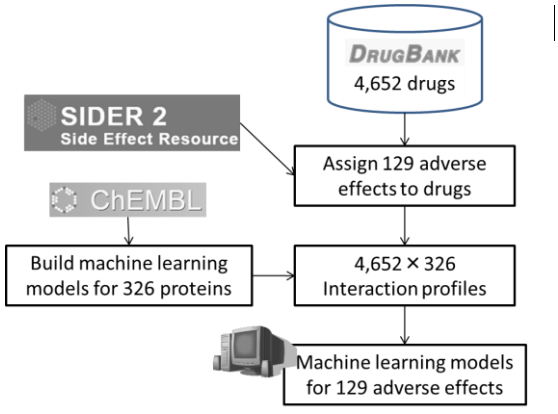

Figure 1. Procedure to build the machine learning models for 129 ADRs.

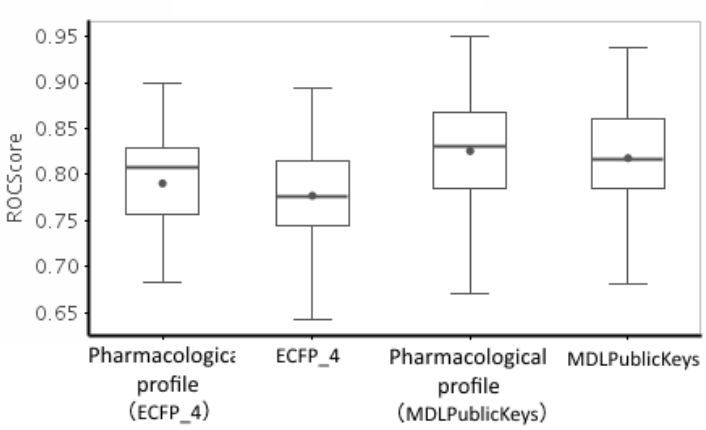

Figure 2. Box plot showing ROC values of the machine learning models for 129 ADRs

P2-11

Pharmacophore based search for novel STING activators as vaccine adjuvants

Yuko Tsuchiya [1]
tsuchiya@protein.osaka-u.ac.jp

Philip Prathipati [2]
philip@nibiohn.go.jp

Kenji Mizuguchi [2]
kenji@nibiohn.go.jp

[1] Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[2] National Institutes of Biomedical Innovation, Health and Nutrition, 7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan

Keywords: Pharmacophore modeling, molecular dynamics simulations, vaccine adjuvants

STING (stimulator of interferon gene) is an essential adaptor protein in innate immunity. It senses cytosolic DNA and induces IFN-β production when a cell is infected. A better understanding of the downstream signaling mechanism, triggered by ligand binding, is essential for the development of anti-cancer drugs or vaccine adjuvants.

In this study, we attempt to find novel STING activators based on structural bioinformatics analyses and pharmacophore modeling, in combination with the screening of candidate compounds by molecular dynamics simulations.

P2-12

Fragment screening of WNK1 inhibitor

Nae Saito[1]
nae-saito@mol.f.u-tokyo.ac.jp

Yukio Tada[2]
tada.y.af@m.titech.ac.jp

Takayoshi Okabe[1]
tokabe@mol.f.u-tokyo.ac.jp

Tetsuo Nagano[1]
tlong@mol.f.u-tokyo.ac.jp

[1] Drug Discovery Initiative, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[2] Interdisciplinary Graduate School of Sience, Tokyo Institute of Technology, 3-3-6 Shibaura, Minato-ku, Tokyo 108-1123, Japan

Keywords: Fragment screening, WNK1, SPR

Pseudohypoaldosteronism type II has been known as a rare autosomal dominant disorder allegedly triggered by overexpression of WNK [with no lysine(K)]1 or mutations in WNK4. Thus far, there have been only a few reports regarding the specific inhibitor for WNK1 or WNK4. We therefore explored the lead compounds for WNK1 from a mixed fragment-based drug discovery whereby a competition experiment using surface plasmon resonance (SPR) allows for selecting better lead compounds in the early stages.

The 9,000 fragments were examined in order to select hit compounds by the mobility shift assay, by which an inhibitory activity for phosphorylation of WNK1 is assessed. Using SPR, we then evaluated binding abilities of the hit compounds against WNK1. A competition assay by SPR was carried out using the candidate compounds as well.

The WNK1 possesses characteristics where, due to the lack of normal lysine residue in subdomain II, Lys45 in subdomain I shows the activity instead, and there are two cavities associated with inhibition activity in WNK1 in the ATP binding sites and back pocket just behind. Given these unique points, we selected the lead compounds using both SPR and docking study.

P2-13

Prediction of Protein-ligand Binding Affinities Using Molecular Mechanics and Quantum Mechanics Calculations

Noriaki Okimoto[1] okimoto@riken.jp
Takao Otsuka[1] totsuka@riken.jp
Yoshinori Hirano [1] hirano@riken.jp
Makoto Taiji[1] taiji@riken.jp

[1] QBiC, RIKEN, 1-6-5 Minatojima-minami, Chuo-ku, Kobe, Hyogo 650-0047, Japan

Keywords: affinity prediction, molecular docking, molecular mechanics calculation, quantum chemical calculation

Accurate computation of the protein-ligand binding affinity is of significant importance for lead optimization in drug development research. Various methods have been proposed to predict the protein-ligand binding affinity. There are more accurate free energy calculation techniques based on molecular dynamics (MD) simulations. These include linear interaction energy methods, the molecular mechanics/Poisson-Boltzmann and surface area (MM-PB/SA) method, alchemical free energy calculations, and various other methods. However, these methods employing molecular dynamics simulations are limited by the use of classical molecular mechanics (MM) force fields, because the preparation of appropriate parameters for organic compounds is difficult.

Protein-ligand systems exhibit various non-bonded interactions, such as π-stacking, charge transfer, polarization effects, and dispersion, as well as the usual and weak hydrogen bonds, as evidenced by structural studies[1]. These non-bonded interactions cannot be understood with sufficient accuracy by using classical MM force fields. On the other hand, application of quantum mechanical (QM) calculations considering such non-bonded interactions can significantly improve the accuracy of the MM force fields.

In this study, we tried to predict binding affinities of protein-ligand systems using approaches by combining MM and QM calculations.

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P2-14

Computational analysis of a conformational epitope of a broadly neutralizing antibody in influenza A virus hemagglutinin

Manabu Igarashi[1] igarashi@czc.hokudai.ac.jp
Masakazu Sekijima[2] sekijima@gsic.titech.ac.jp
Nobuaki Yasuo[2] yasuo.n.aa@m.titech.ac.jp
Takashi Abe[3] takaabe@ie.niigata-u.ac.jp
Teruaki Watabe[4] twatabe-mi@umin.ac.jp
Hiroaki Tokiwa[5] tokiwa@rikkyo.ac.jp
Takatsugu Hirokawa[6] t-hirokawa@aist.go.jp
Ayato Takada[1] atakada@czc.hokudai.ac.jp

[1] Hokkaido University Research Center for Zoonosis Control, Sapporo, Hokkaido, Japan
[2] Global Scientific Information and Computing Center, Tokyo Institute of Technology, Meguro-ku, Tokyo, Japan
[3] Department of Information Engineering, Niigata University, Niigata, Japan
[4] Center of Medical Information Science, Kochi Medical School, Kochi University, Kochi, Japan
[5] Department of Chemistry, Rikkyo University, Toshima-ku, Tokyo, Japan
[6] Molecular Profiling Research Center for Drug Discovery (molprof), National Institute of Advanced Industrial Science and Technology (AIST), Koto-ku, Tokyo, Japan

Keywords: Molecular dynamics, Influenza virus, Antibody, Protein modeling

Background
The hemagglutinin (HA) of influenza A viruses is classified into 16 subtypes (H1-H16). It is generally known that HA-specific antibodies have little cross-neutralizing activity against multiple HA subtypes. Recently, however, several broadly neutralizing antibodies were reported and have attracted attention due to their potential application to therapeutics and vaccine design. We have previously reported a cross-reactive antibody, designated S139/1, which neutralizes H1, H2, H3, H13, and H16 subtypes and its crystal structure in complex with the HA of the A/Victoria/3/1975 (H3N2) strain. However, detailed structural basis of its cross-neutralizing activity still remain to be elucidated. In this study, we characterized the S139/1 recognition sites on different HAs using computational structural biology methods.

Materials and Methods
The HAs from eight strains (subtypes H1, H2, H3, H6, H9, and H13) were analyzed. The structure models of the HA-S139/1 complex were constructed by homology modeling. Using the structures as starting points, we performed molecular dynamics (MD) simulations, and then calculated binding free energies (ΔG) between S139/1 and each HA.

Results and Discussions
The ΔG values of the strains neutralized by S139/1 were lower than the other strains tested. We next investigated the contribution of individual residues on each HA to the interaction with S139/1 and found that amino acids at positions 98, 136, 153, 156, 158, 159, 193, 194, 196, and 226 (H3 numbering) on HA strongly contributed to S139/1 binding as for the strains neutralized by S139/1. Principal component analysis emphasized that the residues at positions 158 and 193 were the most important for S139/1 binding. Indeed, amino acid substitutions at these two positions were experimentally observed in the mutant viruses escaping from neutralization by S139/1. Thus, our computational methods identified the amino acid residues critical for the cross-neutralizing activity of S139/1.

P2-15

A Comparative QSAR Analysis and Molecular Docking Studies of Thiophene Analogues as Protein Tyrosine Phosphatase 1B (PTP1B) Inhibitors: A Rational Approach to Antidiabetic Drug Design

Shabana Bibi[1] dazzling.grin@gmail.com
Katsumi Sakata[1] ksakata@maebashi-it.ac.jp
Saima Kalsoom[2] genious_chemist@yahoo.com

[1] Department of Environment and Life Engineering, Maebashi Institute of Technology, 460-1 Kamisadori-machi, Maebashi, Gunma 371-0816, Japan
[2] Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan

Keywords: Diabetes mellitus, PTP1B inhibitor, QSAR, Molecular docking

In this study, we focused to develop the *In silico* methods to identify potent inhibitor from PTP-1B target focused library. After the evaluation of drug-likeness profiles and toxicity risk assessment of the target focused library, an attempt was made to develop a quantitative structure–activity relationship (2D- and 3D-QSAR) and molecular docking studies on a series of thiophene analogues acting as protein tyrosine phosphatase 1B (PTP1B) inhibitors. 2D-QSAR was performed using multiple linear regression (MLR), principal component regression (PCR) and partial least squares regression (PLS) methods. Among these three methods, the PLS method has comes out with a very promising result (r^2 = 0.9063 and q^2 = 0.8412) as compared to other two methods. According to the model generated by PLS method; antidiabetic activity of thiophene analogues were influenced by physiochemical descriptor (molecular volume and dipole moment), alignment independent descriptor (T_2_S_4 signifies number of double bonded atoms separated from sulphur atoms by four bond distance) and semi-empirical descriptors, (i) SK Most Hydrophobic Hydrophilic Distance signifies distance between most hydrophobic and hydrophilic point on the vdW surface (by Kellogg method using Slogp), and (ii) XA Most Hydrophobic signifies the most hydrophobic value on the vdW surface (by Audry method using Xlogp). These help in understanding the effect of substituent at different position of PTP1B inhibitors. 3D-QSAR study was performed using k-nearest neighbor molecular field analysis (kNN-MFA) approach for both electrostatic and steric fields. The molecular field analysis was applied for the generation of steric and electrostatic descriptors based on aligned structures. The steric and electrostatic field effects are discussed in the light of contribution plot generated. Finally, molecular docking analysis was carried out to better understand the interactions between PTP-1B target and inhibitors. Hydrophobic and hydrogen bond interactions lead to identification of key residues involve in the binding sites of PTP1B protein. The present study will be helpful in further optimization and MD simulation studies and proposed model can be employed to design new derivatives of thiophene with specific PTP1B inhibitory activity.

P2-16

VISCO: visualization and evaluation tool for amino acid sequence conservation of ligand-binding sites

Nobuaki Yasuo[1]
yasuo.n.aa@m.titech.ac.jp

Masakazu Sekijima[1, 2]
sekijima@gsic.titech.ac.jp

[1] Department of computer science, Tokyo Institute of Technology, Meguro, Tokyo 152–8550, Japan
[2] Global Scientific Information and Computing Center, Tokyo Institute of Technology, Meguro, Tokyo 152–8550, Japan

Keywords: amino acid sequence conservation, ligand-binding site, visualization, structure-based drug design (SBDD)

Drug resistance is one of the difficulties for developing antiviral or antimicrobial drugs[1]. We developed a new application named VISCO to quantitatively evaluate the sequence conservation of ligand-binding sites in order to overcome the drug resistance issue[2]. VISCO integrates information pertaining to protein structures, ligand-binding sites, and amino acid sequences. Moreover, VISCO can calculate ConservationScore to find versatile, inappropriate target sites. These data are visualized onto protein structures via Jmol or PyMOL interface. These visualization and comparison can facilitate structure-based drug design (SBDD)[3].

Furthermore, we conducted an experiment for validation of visualization and calculation of ConservationScore of four viral proteins and an experiment to visualize the differences among proteins of the human β adrenergic receptor family. This application is available at http://www.bio.gsic.titech.ac.jp/visco.html .

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P2-17

Accelerating drug discovery for neglected tropical diseases through free energy perturbation calculations on the Tsubame supercomputer

Suguru Roy Kimura[1,2] roy.kimura@schrodinger.com
Kazuki Ohno[2,3] ohno@catalyst.jp.net
Masakazu Sekijima[2,4] sekijima@gsic.titech.ac.jp
Daniel Ken Inaoka[5] danielken@m.u-tokyo.ac.jp
Kiyoshi Kita[5] kitak@m.u-tokyo.ac.jp

[1] Schrodinger KK, Marunouchi Trust Tower North 17F, 1-8-1 Marunouchi, Chiyoda-ku, Tokyo 100-0005, Japan
[2] Tokyo Institute of Technology, Academy of Computational Life Sciences, 2-12-1 O-okayama, Meguro-ku, Tokyo 152-8552, Japan
[3] Catalyst Inc, Resona Kudan Building 5F KS Floor, 1-5-6 Kudan Minami, Chiyoda-ku, Tokyo 102-0074, Japan
[4] Tokyo Institute of Technology, Global Scientific Information and Computing Center, 2-12-1 O-okayama, Meguroku, Tokyo 152-8550, Japan
[5] Tokyo University, Department of Biomedical Chemistry, 7-3-1 Hongo, Bunkyu-ku, Tokyo 113-0033, Japan

Keywords: Docking, Binding free energy prediction, Free energy perturbation, Drug design, Lead optimization

Free energy perturbation (FEP) is a rigorous physics-based computational method that enables the prediction of small-molecule binding affinities towards their enzyme or receptor targets. Although FEP has been applied to protein and ligand binding affinity predictions for over 30 years and is considered the “Gold Standard” in accuracy, its extreme computational cost has prevented it from enjoying widespread use. However in the last few years, through the development of novel computational acceleration algorithms and by taking advantage of general purpose Graphics Processing Units (GPUs), FEP is now becoming a practical tool that is increasingly used in drug discovery research.

We recently ported such an FEP implementation [1] to the Tsubame 2.5 supercomputer at Tokyo Institute of Technology consisting of over 4000 GPUs. Our preliminary benchmarks on Tsubame demonstrated calculation rates of approximately 150 to 200 predictions per day using 400 GPUs, and prediction errors within 1.2 kcal/mol root mean square error, consistent with prior published results [1]. Such accuracy enables distinguishing between compounds that exhibit at least a 10-fold activity difference prior to synthesis of those compounds. Although this level of error is still larger than the expected errors of experimental assays (which is often estimated to be 2 to 3-fold), we believe that the effective use of FEP should enable discovery of potent inhibitors at significantly shorter time frames and lower costs compared to those involved in traditional medicinal chemistry team efforts..

As a preliminary validation on Tsubame, we performed binding affinity predictions for a series of recently discovered promising compounds [2] that inhibit dihydroorotate dehydrogenase (DHOD) from *Trypanosoma cruzi*, a protozoan causing Chagas’ disease. Co-crystal structures of many of them were determined [2] prior to prediction and were used in the calculations. The remaining compounds were manually docked into the protein crystal structures. We observed accuracy consistent with previously published validations. Through this effort, we aim to accelerate the discovery of potent inhibitors for a wide class of tropical disease-causing targets.

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P2-18

Protein-ligand Binding Prediction using Supervised Molecular Dynamics Simulation

Atsushi Ogahara[1] ogahara-atsushi-mk@cb.k.u-tokyo.ac.jp
Chie Motono[2] c-motono@aist.go.jp
Masahiro Gomi[3] gomi-masahiro@mki.co.jp
Miwa Sato[3] satou-miwa@aist.go.jp
Takatsugu Hirokawa[2] t-hirokawa@aist.go.jp

[1] Graduate School of Frontier Sciences, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa-shi, Chiba-ken 277-8561, Japan
[2] Molecular Profiling Research Center for Drug Discovery, National Institute of Advanced Industrial Science and Technology, 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan
[3] Mitsui Knowledge Industry CO., LTD., 2-5-1 Atago, Minato-ku, Tokyo 105-6215, Japan

Keywords: Computer-aided drug design, Structure based drug design

The study of interaction between a chemical and its target protein using molecular dynamics (MD) simulation is of importance for drug discovery. The technique allows us to visualize the protein-ligand binding pathways as well as the binding structures.

Shan et al. has succeeded to provide a full dynamical description of protein-ligand bindng[1]. It is necessary to carry out a classical MD simulation in a microsecond time scale, and usually requires special-purpose computational system for MD simulation.

Sabbadin and Moro has succeeded exploring ligand-protein recognition pathway in a nanosecond time scale using supervised MD (suMD) simulation[2]. The method allows, however, acceleration of ligand binding simulations only to membrane proteins.

To overcome this limitation, we implemented the suMD method which can be applied to soluble proteins. From the starting position of a ligand to the neighbourhood of its binding site, the 3D shortest path is searched[3] and a suMD is carried out guided by the shortest path. After ligand's reaching the neighborhood of a binding site, the ligand is subjected to a usual suMD without the shortest path.

In our preliminary evaluation of this approach on chk1-H5K system the ligand H5K reached near the center of the mass of binding site residues within 32 nanoseconds. The results showed that the combination of the 3D shortest path search and suMD is helpful to explore the ligand binding event in applicable time scale, though resulting binding pose of the ligand is different from the corresponding X-ray crystal structure.

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P2-19

In silico identification of novel inhibitors of S6K1 kinase by SBDD and LBDD approach

Shunta Sasaki[2] sh.sasaki@teijin.co.jp
Hitomi Yuki[1] yuki@gsc.riken.jp
Hideaki Niwa[1] niwa@gsc.riken.jp
Junko Mikuni[1] mikuni@gsc.riken.jp
Naoko Ogawa[1] naoko.ogawa.aj@riken.jp
Akiko Tanaka[1] aktanaka@riken.jp
Shigeyuki Yokoyama[3] yokoyama@riken.jp
Teruki Honma[1*] honma@gsc.riken.jp

[1] RIKEN Center for Life Science Technologies, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan
[2] Teijin Institute for Bio-medical Research, Teijin Pharma Ltd., 4-3-2, Asahigaoka, Hino-shi, Tokyo 191-8512, Japan
[3] RIKEN Structural Biology Laboratory, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan

Keywords: S6K1, kinase, in silico screening, SBDD, LBDD

S6K1 is the member of the serine-threonine kinase family and phosphorylates 70S ribosomal protein S6. Because S6K1 plays important roles in metabolism, cell growth, and overexpresses at renal cell carcinoma, S6K1 is expected to be a potential new target of anticancer agent. To find novel scaffolds as S6K1 kinase inhibitors, we demonstrated two strategies of in silico screening and inhibition assay of S6K1 kinase supported with X-ray crystal structure analysis.

At the first round of in silico screening, we selected the screening compound sets by structure-based drug design (SBDD) and ligand-based drug design (LBDD). We demonstrated an efficient in silico screening based on k-PALLAS [1], which is the semi-automatic optimization system of docking conditions developed by Honma and co-workers at RIKEN. In parallel with the docking simulations, 3D similarity searches were performed using the shape-comparison program ROCS (Rapid Overlay of Chemical Structures), followed by the kinase mobility shift assay. Known S6K1 ligands and S6K1 X-ray ligands were used as queries for the 3D similarity searches. In the second round of in silico screening, 2D similarity searches were performed by using the 23 hits with IC_{50} < 10 μM as queries, identified in the first round in silico screening. Compound library which were used for the first and second rounds, are supplied by Open Innovation Center for Drug Discovery at the University of Tokyo.

After the kinase mobility shift assay, five and four compounds with IC_{50} <= 500 nM, were obtained from the first and second rounds respectively. Furthermore, we succeeded in getting five X-ray complex structures of S6K1 and the hit compounds. From docking simulation approach with k-PALLAS, we acquired the novel scaffold of S6K inhibitors which are different from known S6K1 ligands so far.

In this poster session, we will discuss our in silico approach to identify of new scaffolds of S6K1 inhibitors.

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P2-20

Computational approaches in structural based drug design

Yoshinori Hirano[1] hirano@riken.jp
Noriaki Okimoto[1] okimoto@riken.jp
Takao Otsuka[1] totsuka@riken.jp
Makoto Taiji[1] taiji@riken.jp

[1] Laboratory for Computational Molecular Design, Computational Biology Research Core, Quantitative Biology Center (QBiC), RIKEN, 1-6-5, Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan

Keywords: Molecular Modeling, Drug Design, Molecular Screening

Structure-based drug design (SBDD) has become the preferred approach in the field of drug discovery. Among the various structure-based computational methodologies adopted for compound screening, the principal one is molecular docking.

Proteins fluctuate dynamically under physiologic conditions, and this flexibility is essential for ligand binding. Apparent conformational changes upon ligand binding have been observed in several pharmacologically relevant proteins. Although the importance of the effects on protein dynamics and solvent water molecules is widely recognized, most methods for SBDD do not fully consider related effects. Thus far, there have been considerable efforts to incorporate these effects on protein flexibility and solvent water molecules into molecular docking. Ensemble docking, which uses multiple receptor conformations, is one of an approach to consider protein flexibility during molecular docking. However, if conformational change on ligand binding is very large, conformational selection is extremely difficult.

In this study, we focus on the incorporation of protein flexibility by combining molecular docking and molecular dynamics (MD) simulation.

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P2-21

Open Innovation Approach to Computer-Aided Inhibitor Identification

Shuntaro Chiba[1]
chiba.s.ac@m.titech.ac.jp

Kazuyoshi Ikeda[2]
ikeda@level-five.jp

Takashi Ishida[3,1]
ishida@cs.titech.ac.jp

Kazuki Ohno[4,1]
ohno@catalyst.jp.net

Masakazu Sekijima[5,1]
sekijima@gsic.titech.ac.jp

[1] Education Academy of Computational Life Sciences, Tokyo Institute of Technology, 4259, Nagatsutacho, Midori-ku, Kanagawa 226-8501, Japan
[2] Level Five Co. Ltd., Shiodome Shibarikyu Bldg., 1-2-3, Kaigan, Minato-ku, Tokyo 105-0022, Japan
[3] Department of Computer Science, Tokyo Institute of Technology, 2-12-1, Ookayama, Meguro-ku, Tokyo 152-8550, Japan
[4] Catalyst Inc., Risona Kudan Bldg., 1-5-6, Kudan-Minami, Chiyoda-ku, Tokyo 102-0074, Japan
[5] Global Scientific Information and Computing Center, Tokyo Institute of Technology, 2-12-1, Ookayama, Meguro-ku, Tokyo 152-8550, Japan

Keywords: Computer-aided drug discovery, Inhibitor identification

It is known that chance of finding potent inhibitors by computer-aided methods varies depending on a selected targeted biomolecule.[1] We assumed that the more methods we applied to the target biomolecule, the more inhibitors could be found. In the present study we conducted the open contest where many individuals and organizations participated in order to suggest compounds IDs searched from a large compound library. As a result, the compounds suggested by all participants were diverse. The compounds' inhibition activity was experimentally assessed and compounds with high activity were found to be diverse, which indicated that having such an open innovation contest to find various potent inhibitors is valuable.

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P2-22

Consideration of Protonate States in Protein-Ligand Complex for Drug Design Based on Fragment Molecular Orbital Method

Chiduru Watanabe[1] chiduru.watanabe@riken.jp
Teruki Honma[1] honma.teruki@riken.jp

[1] RIKEN Center for Life Science Technologies, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

Keywords: Protonate state, MTH1, Fragment molecular orbital (FMO) method

Protonate states of amino acid residues (e.g. Asp, Glu, Lys) on ligand binding pocket are critically important information to structure-based drug design. Hydrogen atoms including protons on the amino acid residues, however, are not determined in general PDB entries with resolution ca. 1.0Å ~ 3.0Å. For example, we have generally treated Asp and Glu as deprotonate states at physiological condition (pH 7.4). There are concerns that ligand binding mode depends on wrong protonate states of amino acid residues in protein. If we use protein structure with wrong protonate states, drug design makes misleading by its incorrect ligand binding mode. Therefore, we have to predict these protonate and tautomer states by pKa calculations of ligand and the visual inspection of X-ray crystal structures and its electron densities. In addition, we must confirm whether that the predicted protonate stats of complex are energetically appropriate. Thus, we will attempt to estimate validity of the protonate states on protein by quantum chemical calculations based on fragment molecular orbital (FMO) method [1].

The MutT Homolog 1 (MTH1) protein, which belongs to the Nudix family of phosphohydrolases, is reported to be related with cancers caused by the accumulation of oxidized nucleotides in DNA. The hydrogen bond network among the substrate and the aspartic acids of Asp119 and Asp120 in MTH1 plays a key role in substrate binding. Svesson et al. [2] have reported that the protonate/deprotonate states of two aspartic acids would depend on 3D property of a ligand on the basis of atomic distance between the aspartic acids and the ligand. In this study, we examined the protonate/deprotonate states of Asp119 and Asp120 in MTH1 and tautomer states of ligands (e.g. substrate and inhibitors). We performed FMO calculations at MP2/6-31G* level of several protonate states of the aspartic acids and tautomer stets of ligand. The protonate states were analyzed by inter-fragment interaction energies (IFIEs) between protein and ligand. In the poster session, the relationship of IFIE data and the binding mode candidates will be discussed.

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P2-23

Construction of research platform in FMO drug design consortium (FMODD)

Yoshio Okiyama[1] yoshio.okiyama@riken.jp
Chiduru Watanabe[1] chiduru.watanabe@riken.jp
Kaori Fukuzawa[2] fukuzawa.kaori@nihon-u.ac.jp
Shigenori Tanaka[3] tanaka2@kobe-u.ac.jp
Teruki Honma[1] honma.teruki@riken.jp

[1] RIKEN Center for Life Science Technologies, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
[2] School of Dentistry at Matsudo, Nihon University, 2-870-1 Sakaecho-Nishi, Matsudo, Chiba, 271-8571, Japan
[3] Department of Computational Science, Graduate School of System Informatics, Kobe University, 1-1, Rokkodai, Nada-ku, Kobe, Hyogo 657-8501, Japan

Keywords: Fragment molecular orbital (FMO) calculation, Drug design, Consortium, HPCI

Fragment molecular orbital (FMO) method, which can accurately describe interaction energies and chemical reactions in biomolecules based on quantum mechanics, has been thought to be a promising tool for *in silico* drug design in the next generation and increasingly applied to several realistic models of bio-systems [1,2]. Despite these successful examples, it seems to be not usable at ease yet for many researchers except the developers and around.

Now, in order to promote the application and confirmation of FMO still more in the field of drug design, FMO drug design consortium, abbreviated to FMODD, was established in November 2014 as an open community for developing and spreading practical techniques to master FMO-based analyses with industrial-government-academic cooperation. In this consortium, we will share calculated data with its computational know-how through applying FMO method to several practical themes such as

- constructing a database of inter-fragment interaction energies (IFIE) between several proteins and ligands and screening these ligands with using cluster techniques analyzing the IFIE patterns in a large scale,
- refining molecular coordinates obtained by X-ray crystal structure analysis in cooperation with FMO electron density, and
- simulating dynamics of chemical reaction between proteins and their substrates,

by utilizing HPCI resources like a massively parallel supercomputer, K-computer. Additionally, we plan to not only develop new computational methodologies of FMO, but also form a user-friendly interface supporting to prepare structures, configure calculations, submit jobs, and so on for more accessibility. We will show the progress of our activities in the session.

Acknowledgment

This work is performed as one of the activities of the FMO drug design consortium (FMODD) and supported by the “Construction of platform of FMO-based drug discovery using HPCI system” project (hp150160) using K computer.

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P3-01

Whole Cell Migration Modeling reveals How Cells Sense the Electric Field Gradient even in Noisy Environment

Masato Yasui[1] masato.yasui@riken.jp
Satomi Matsuoka[1] s.matsuoka@riken.jp
Masahiro Ueda[2] masahiroueda@fbs.osaka-u.ac.jp

[1] Quantitative Biology Center, RIKEN, OLABB, 6-2-3, Huruedai, Suita-shi, Osaka, 565-0874, Japan
[2] Graduate School of Science, Osaka University, 1-1, Machikaneyama, Toyonaka, Osaka, 560-0043, Japan

Keywords: Whole cell modeling, Cell migration, Electro-taxis, *Dictyostelium Discoideum*

Cell moves toward the attractant molecules or electrode, which is called “taxis.” Taxis are used in a wide range of biological processes including morphogenesis, immunity, neuronal patterning, and wound healing. *Dictyostelium discoideum* cells shows a chemotactic response to cyclic adenosine 3’,5’-monophosphate (cAMP) [1]. Cells can sense the 0.0033 nM/um cAMP gradient (0.5 nM) [2], which imply that cells have the ability to determine the gradient even if the before and after difference of cAMP binding to the receptors is smaller than the noise of that. How cells sense the gradient even in noisy environment? In our research, to answer this question, we perturbed phosphatidylinositol lipid system, which determines the direction of the cell migration, by using genetic engineering. We measured the time-series change of phosphatidylinositol lipid system, cell migration and taxis of the perturbed cells by live imaging. Then, we modeled the phosphatidylinositol lipid system and cell migration from the quantitative data. Curiously, our model expected that cells sense the attractant gradient in noisy environment better than without noise. The result of experimental verification also supported our expectation of the model. In this poster, we will present the mechanism how cells sense the gradient even in noisy environment.

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P3-02

Computational Analysis of PM 2.5 by Text Mining

Riho Miyaki[1]
puttyo@kwansei.ac.jp

Hiroyuki Toh[1]
tohhir@kwansei.ac.jp

[1] Bioscience, School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda, Hyogo, 6691337, Japan

Keywords: Text mining, PM2.5

Recent development of omics technologies has introduced the flood of big data in the molecular life sciences. Associated with such trends, biological literatures have emerged as a rich resource of knowledge. The overall perspective of the biological literatures is called "Bibliome", and the knowledge extraction from the literatures by text mining has become one of the important methods for data analysis in bioinformatics. Actually, there are many applications of text mining to the bibliome. For example, protein-protein interaction network [1] and the association of genes methylated in diseases [2] have been investigated by text mining.
In this study, we apply the text mining approach to the analysis of PM2.5. PM2.5 is a small particle (under 2.5 μm in diameter) and it has a risk that it causes respiratory illness and cardiovascular disease [3]. We performed keyword search in PubMed [4] using PM2.5 as a query. 3375 literatures were detected at 20.8.2015. Then, all the abstracts were downloaded as a set of text data. Then, the set was divided into a unit corresponding to each literature. Next, the text data was preprocessed as follow. The title, the author information, and the other data rather than the main body of the abstract were removed from each abstract. Furthermore, the characters except for alphabetical texts and the title of the sections in the abstract were removed if any. Finally, the stemming was performed with SnowballC package of R [5]. The, the preprocessed text data were transformed into a term-document matrix with tm package of R. The term-document matrix was analyzed with several R packages for multivariate analysis. Based on the result, we will discuss the association of elements related to PM2.5.

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P3-03

Inferring the mode-of-action of bioactive compounds using large-scale transcriptome data in LINCS toward drug repositioning

Michio Iwata[1] m-iwata@bioreg.kyushu-u.ac.jp
Ryusuke Sawada[1] rsawada@bioreg.kyushu-u.ac.jp
Hiroaki Iwata[1] iwata@bioreg.kyushu-u.ac.jp
Yoshihiro Yamanishi[1,2] yamanishi@bioreg.kyushu-u.ac.jp

[1] Division of System Cohort, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan
[2] Institute for Advanced Study, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan

Keywords: Drug repositioning, Transcriptome data, Mechanism of action, Bioactive compound, Drug target

Identifying the mode-of-action of bioactive compounds (including drugs) is a major challenge in chemical systems biology. In fact, there are many drugs whose mechanisms of actions remain unknown. Most drugs are chemical compounds that interact with therapeutic target proteins implicated in a disease of interest. However, drug molecules may interact not only with the primary target proteins but also with additional proteins (hereafter referred to as off-targets), resulting in unexpected side effects. Discovering target and off-target effects of drug candidate compounds is crucial to address the efficacy and safety of compounds in drug development. Side effects derived from the off-target interactions are basically undesired, but they may occasionally be beneficial for new therapeutic indications. Therefore, understanding the complex responses of the human body to the treatments of bioactive compounds is a vital important issue in medical and pharmaceutical research. A promising unbiased approach is genome-wide gene expression profiling of the transcriptional response to chemical perturbations of human cell lines.

In this study, we developed a novel method to infer the mode-of-action of bioactive compounds by using large-scale chemically perturbed transcriptome data, which enables to identify potential target pathways, target proteins, and therapeutic indications of each bioactive compound. We analyzed chemically induced changes in global patterns of gene expression profiles in response to the treatments of 20122 bioactive compounds (including most approved drugs) for 68 human cell lines derived from the Library of Integrated Network-based Cellular Signatures. Our pathway enrichment analyses of up-regulated and down-regulated genes revealed a set of target pathways that were activated and inactivated by the chemical perturbations, respectively. We explored the target proteins of compounds (including primary targets and off-targets) with the gene expression similarity by making the best use of millions of compound-protein interactions. The cross-validation experiments show that our target prediction method outperforms previous methods in terms of accuracy, interpretability, and applicability, and the predictive performance does not depend on compound chemical structures. Based on the target proteins and pathways, we finally performed a comprehensive prediction of new drug indications for a wide range of diseases defined in International Classification of Diseases. The proposed method is expected to be useful for understanding the mode-of-action of bioactive compounds and for predicting new drug indications toward drug repositioning.

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P3-04

Exploration of Target Molecules Affecting the Conversion of iPS Cells into Cancer Stem Cells Using Chemical Compound Library

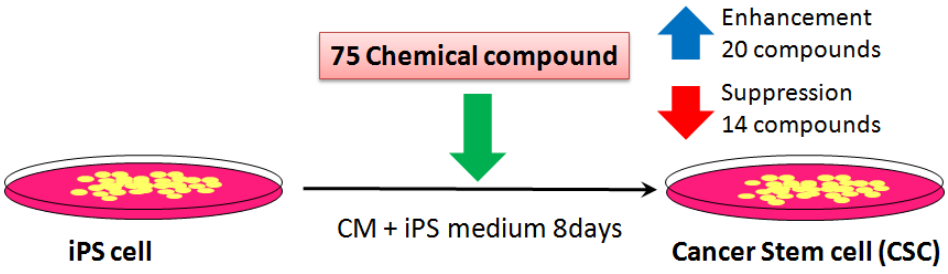
Takayuki Kudoh[1] kudoh@okayama-u.ac.jp
Tomonari Kasai[1] tkasai@okayama-u.ac.jp
Saki Sasada[1] pxa57en3@s.okayama-u.ac.jp
Kenta Hoshikawa[1] kenta-star@lion.ocn.ne.jp
Takuma Matsumoto[1] pvxn4t5n@s.okayama-u.ac.jp
Anna Sanchez Calle[1] annasc.nit@gmail.com
Arun Vaidyanath[1] arunvnath@okayama-u.ac.jp
Masaharu Seno[1] mseno@okayama-u.ac.jp

[1] Graduate School of Natural Science and Technology, Okayama University, 3-1-1Tsushima-Naka, Kita-ku, Okayama 700-8530, Japan

Keywords: Cancer Stem Cell (CSC), iPS, Tumorigenesis

Cancer stem cells (CSCs) are considered as the principal cause of the origin and recurrence of tumor and promising source of curative information for cancer [1]. However, the developmental processes of CSC are yet to be elucidated, because there are few appropriate model systems for replicating the events of tumorigenesis. We succeeded in converting iPS cells into CSCs by culturing iPS cells in the conditioned media of cancer cell lines [2]. This process is considered to be a good model of spontaneous tumorigenesis. Proper analysis of the process would provide us the underlying mechanism of conversion of normal undifferentiated progenitor cells into CSCs.

In this study, we assessed 75 chemical compounds, which are considered to regulate cellular proliferation, differentiation and development, for the ability to exhibit the effects on the conversion of normal iPS cells into CSCs by adding each compound in the culture media. The effect of these chemical compounds on the conversion of iPS cells into CSCs was evaluated after 8 days of exposure to each chemical compound by the relative number of spheroids and the GFP expression compared with the control experiment performed without the chemical compounds [3]. We successfully identified 20 compounds significantly enhanced and 14 compounds suppressed the conversion of iPS cells into CSCs. Searching the target molecules of these compounds through Pubchem database [4], we identified the candidate target proteins, which should be related with the conversion.



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P3-05

A database for protein-ligand complexes in enzymatic reactions

Yoichi Murakami[1]
yoichi@nibiohn.go.jp

Satoshi Omori[2]
omori@sb.ecei.tohoku.ac.jp

Kengo Kinoshita[2,3,4]
kengo@ecei.tohoku.ac.jp

[1] Center for Drug Design Research, National Institutes of Biomedical Innovation, Health and Nutrition, Japan
[2] Graduate School of Information Sciences, Tohoku University, Japan
[3] Institute of Development, Aging and Cancer, Tohoku University, Japan
[4] Tohoku Medical Megabank Organization, Japan

Keywords: Database, Protein-ligand interactions, Protein modeling, enzymatic reaction

NLDB (Natural Ligand DataBase; URL: http://nldb.hgc.jp) is a database of automatically corrected and predicted 3D protein-ligand interactions in the enzymatic reactions of various metabolic pathways [1]. Information about these non-covalent interactions is important, not only for studying the molecular functions of specific proteins but also for enzyme-targeted drug discovery. Therefore, in order to complement the structural information about the reactions, we predict 3D protein-ligand interactions using state-of-the-art programs if their structures are unknown, and then construct a database of the 3D interactions in various reactions. NLDB produces three different types of data resources; the *natural* complexes are experimentally determined protein-ligand complex structures in the PDB [2], the *analog* complexes are predicted based on known protein structures in a complex with a similar ligand [3,4], and the *ab initio* complexes are predicted by docking a ligand to predicted [5] or high confidence ligand-binding sites of a protein. Furthermore, the database has a flexible search function, based on various types of keywords, and an enrichment analysis function based on a set of KEGG compound IDs. NLDB will be a valuable resource for experimental biologists studying protein-ligand interactions in specific reactions, and for theoretical researchers wishing to undertake more precise simulations of interactions for drug discovery purposes.

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P3-06

Epigenetic Study between Normal and Cancer Cell Using NGS data from Bile Duct Cancer Patients

Bharata Kalbuaji[*]
kalbuaji.b.aa@m.titech.ac.jp

Konagaya Akihiko[*]
kona@dis.titech.ac.jp

[*]Department of Computational Intelligence and Systems Science
Interdisciplinary graduate school of Science and Engineering
Tokyo Institute of Technology

Keywords: RNA sequencing, bile duct cancer, gene regulatory network

Next Generation Sequencing (NGS) is the latest method for DNA or RNA sequencing. NGS can sequence many type of sample depend on the purpose, for example RNA-seq for sequencing mRNA to measure the gene expression level, Exome-seq for sequencing the protein coding region of the DNA, and BS-Seq for measuring the level of methylation. NGS is widely used for cancer research now because it gives a complete picture of human genome.

This research use publicly available database from Bile Duct Cancer patients from NCBI GEO with accession number GSE63420. The data consist of 7 pair normal-cancer cell for RNA-seq and 8 pair normal-cancer cell for Exome-seq. The objective of this research is to understand the cause of differentially expressed (DE) genes in cancer cell.

The method being used to investigate the cause of DE genes is gene regulatory network (GRN). GRN will be used to visualize the interaction of target gene with its transcription factor (TF). The correlation of TF and target gene will be calculated to see whether the DE genes are caused by the TF or not.

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P3-07

Development of the Structural Feature Analysis System based on the Motif Combination Pattern of Proteins

Sota Azuma
azuma @ mbi.cs.tut.ac.jp

Hiroaki Kato
kato @ cs.tut.ac.jp

Department of Computer Science and Engineering, Toyohashi University of Technology,
1-1 Hibarigaoka, Tempaku-cho, Toyohashi, Aichi 441-8580 Japan

Keywords: protein motif, combination pattern, structural feature, co-occurrence relationship, calcium-binding protein, zinc protease, PROSITE

It is well known that the amino acid sequence of a protein is closely related to its structure and function. This is especially true for particular structural features called motifs, and they are considered to be well-reserved sites in the genomic sequences. It is known that some proteins contain several types of motif. For example, S100 proteins have two different types of calcium-binding sites: a low affinity one with a special structure and a normal EF-hand type high affinity site [1]. In the present work, the authors have focused the co-occurrence relationship between the known motif patterns, and developed the structural feature analysis tools for proteins.

We have prepared the target dataset taken from NCBI RefSeq (Rel 61) which contained 35,665 entries (the total number of residues: 19,864,982) for human proteins. For 1,308 motif patterns that are available on the PROSITE database (Rel 20.87) [2], 310 patterns were found in the target dataset [3] which hit from 10 to 1,000 protein entries. They were used to make the motif–protein matrix diagram. The fraction function, $s = c / min(a, b)$, was employed as the measurement of the co-occurrence relationship. Where there are a hits in motif A, b hits in motif B, and c hits common to both A and B. The sequence distance for a pair of motifs was also calculated. For example, (*a*) 377 proteins hit in the EF-hand motif (PS00018) search, (*b*) 27 proteins in S100_CABP motif (PS00303), and (*c*) 23 proteins were also common to both EF-hand and S100. It was confirmed that S100 motif pattern spans the region of the EF-hand high affinity site.

In the matrix diagram, the zinc protease motif [4] was often co-occurred with other motif patterns. For example, the zinc protease (PS00142) and cysteine switch (PS00546) have a characteristic co-occurrence pattern. A cysteine switch motif has been found in the certain type of zinc proteases, such as matrix metalloproteinases (MMP) [5]. In the sequence pattern, zinc protease motif was always followed by the cysteine switch motif with the gap of about 120 amino acids. Structural arrangement for a pair of motifs was also identified using the corresponding PDB files. These results show the potential applicability of the present approach for structural feature analysis of proteins.

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P3-08

The integration of biological data and its application to drug discovery

Yi-An Chen[1]
chenyian@nibiohn.go.jp

Lokesh P. Tripathi[1]
lokesh@nibiohn.go.jp

Kenji Mizuguchi[1]
kenji@nibiohn.go.jp

[1] National Institutes of Biomedical Innovation, Health and Nutrition, 7-6-8 Saito-asagi, Ibaraki city, Osaka, 567-0085, Japan.

Keywords: Data warehouse, Drug discovery, Proteomics, Systems biology, Bioinformatics

Effective analysis of complex biological data is a key and challenging objective in systems biology and drug discovery. Thus, bioinformatics tools and approaches that can translate the vast amounts of biological data into actionable research are essential to new discoveries. Biological data leveraged from different sources provide deeper insights into gene function underlying the biological processes under study. Therefore, biological data integration coupled with analysis and visualisation of different data types are necessary to expand our understanding of biological systems for knowledge discovery. We have previously developed TargetMine, an integrated data warehouse optimised for target discovery and prioritisation of candidate genes. Here we describe new developments of TargetMine, including newer data types and data models and a host of interactive data analysis tools to enhance the scope of TargetMine as a broad-based data analysis and knowledge discovery platform.

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P3-09

A comprehensive genomic analysis reveals the genetic landscape of mitochondrial respiratory chain complex deficiencies

Masakazu Kohda [1] mkoda@saitama-med.ac.jp

Kei Murayama [2] kmuraya@mri.biglobe.ne.jp

Akira Ohtake [3] akira_oh@saitama-med.ac.jp

Yasushi Okazaki [1,4] okazaki@saitama-med.ac.jp

[1] Division of Translational Research, Research Center for Genomic Medicine, Saitama Medical University
[2] Department of Metabolism, Chiba Children's Hospital
[3] Department of Pediatrics, Saitama Medical University
[4] Division of Functional Genomics & Systems Medicine, Research Center for Genomic Medicine, Saitama Medical University

Keywords: Mendelian genetic disorders, Metablic disorders, Mitochondria

We present the first report of a comprehensive genomic analysis of a cohort of patients with mitochondrial respiratory chain deficiency. Using high-throughput sequencing and chromosomal aberration analysis, we identified 3 mitochondria-related genes as novel disease causative genes, and 37 novel mutations in genes linked to mitochondrial disorders including recently reported NDUFB11, COQ4, GTPBP3 genes. We also identified 2 non-mitochondria-related disease genes (MECP2, and TNNI3) and 3 chromosomal aberrations (6q24.3-q25.1, 22q11.21, and 17p12) in this cohort.

Using comprehensive genomic analyses, we achieved firm genetic diagnoses in 49 of 142 patients (34.5%), which is higher than the general diagnosis rate of approximately 25%. Comprehensive genomic analyses also enabled us to identify closely related disorders such as idiopathic cardiomyopathy and Rett syndrome. Identification of closely related disorders is important for patient care and application of an efficient drug repositioning. We demonstrated that extensive clinical heterogeneity exists in this disorder, and comprehensive genomic analyses could reveal its clinical and genetic heterogeneity for better understanding of this complex disorder.

P3-10

Classification of Mitochondrial DNA with using deep learning

Yuhei Kaneshita
k-yuhei@bpe.es.osaka-u.ac.jp

Kanako Sugiyama
k.sugiyama@bpe.es.osaka-u.ac.jp

Satoshi Asatani
s.asatani@bpe.es.osaka-u.ac.jp

Hirohiko Niioka
niioka@bpe.es.osaka-u.ac.jp

Jun Miyake
Jun_miyake@bpe.es.osaka-u.ac.jp

Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama-cho, Toyonaka, Osaka, 560-8531, Japan

Keywords: Deep Learning, Auto Encoder, Mitochondrial DNA, DNA analysis

A mechanical learning method "deep learning", proposed by Hinton, opened a promissing way to artificial intelligence [1]. Deep learning extended its application range for example speech recognition [2], generic object detection [3] and molecular biology. Analysis on alternative DNA splicing was also proposed [4].

The application to the field of information processing of biological system could give a breakthrough on biology and medicine. We should like to examine "deep learning" method in comparison with PCA. In this research, we focused on mitochondrial DNAs.

There are many researches about human classification of haplogroups. Many indexes were used, for example blood type, virus, Y-chromosome. Mitochondrial DNA (mtDNA) is one of the most popular substance for the analysis. It is regarded as a key factor to study human evolution [5]. Some gene regions in mtDNA have been selected as research indexes. There are many factors to consider in these approaches as the evolution rates are not uniform among sequences.

We tried whole sequence mtDNA-based classification (The number of bases:16560, The number of data:375) with deep learning. We plotted the results compressed by deep auto encoder in 2 or 3 dimension. The program of deep learning and a computer system with GPU ware designed in our laboratory [6].

Spatial dispersion and correlation distances were obtained in 2D to 4D space by the encoder. The dimension compression results suggest that mtDNA sequences should be classified in some groups. The meanings of the spacial dispersion and the correlation distances are discussed with reference to the divergence of human haplogroups.

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P3-11

Application of Deep Learning for Analysis of Biological Information

Satoshi Asatani
s.asatani@bpe.es.osaka-u.ac.jp

Shoichiro Fukushima
syou_fukushima@sml.me.es.osaka-u.ac.jp

Hirohiko Niioka
niioka@bpe.es.osaka-u.ac.jp

Jun Miyake
jun_miyake@bpe.es.osaka-u.ac.jp

Graduate School of Engineering Science, Osaka University,
1-3 Machikaneyama-cho, Toyonaka, Osaka,560-8531, Japan

Keywords: Deep Learning, Biological network analysis,

Deep Learning (DL) that is a kind of Neural Network gives us useful considerations for sequence data and representation of big data. DL architectures have been developed for computer vision and voice recognition, and produced a successful result. In recent years, DL begun to be used for Bioinformatics [1] as an analyzing method that uses many big databases. DL can use not only extracting the features and tendency of big data but also making models of prediction. In previous research, Brendan J Frey predicted the sequence specificities of DNA- and RNA-binding proteins by using DL [2].

Recent DL techniques include many practical tricks (dropout [3], rectified linear function [4], momentum [5], etc.) that make it easier and quickly to train the networks. Some of these tricks are not understood completely though these bring successful result. We should research the cause of good effect.

We developed a new framework of DL and practical tricks for the application to Biology. To satisfy the huge steps of calculation, a parallel computing system is desired with GPU. A computer system was prepared in our laboratory with an Intel Core i7 CPU and GTX980Ti GPU operated under 5.63TFLOPS.

The system was used for the analysis of cells and DNAs. A convolutional neural network structure was prepared because it has a high precision for cell identification. Human / mammalian cells appeared in microscopy images were sharply classified into distinctive two kinds as: 2-digits (A = 00, T = 01, G = 10, C = 11) or 4-digits (A = 1000, T = 0100, G = 0010, C = 0001). The latter set gave stable results. The divergence of mitochondrial DNA on Deep Autoencoder compression gave suggestive results on the divergence of human haploid group.

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P3-12

Classification of C2C12 cells before and after differentiation with using deep learning

Hirohiko Niioka niioka@bpe.es.osaka-u.ac.jp
Satoshi Asatani s.asatani@bpe.es.osaka-u.ac.jp
Aina Yoshimura aina.3am@bpe.es.osaka-u.ac.jp
Jun Miyake jun_miyake@bpe.es.osaka-u.ac.jp

Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama-cho, Toyonaka, Osaka, 560-8531, Japan

Keywords: cell differentiation, deep learning, image processing

Regenerative medicine industry was accelerated by development of iPS cells that will be useful for tissue/organ regeneration as well as drug discovery. To this date, well-skilled humans have to check the cellular differentiations. However, to realize the industry, numerous numbers of cells are necessary and must be treated correctly, rapidly and automatically. Especially for tissue/organ regeneration, non-invasive method is required for transplant and the cells should not be stained by fluorescent markers. We utilized deep learning (DL) technology to classify the level of differentiation of cells with bright-field images of cellular morphologies.

DL is emerging in image recognition field [1,2]. In 2012 DL got a lot of attention because DL won the first prize in a competition (ImageNet Large Scale Visual Recognition Challenge (ILSVRC)) with a huge lead. Recently, it is starting to apply DL for recognition of cellular conditions [3,4].

In this study C2C12 cells (mouse myoblast) were used as samples and the cells were cultured in glass base dishes. The differentiation of C2C12 myoblasts into myotubes was induced by changing the culture medium (defined as day 0). In the differentiation cellular morphology changed from round shape to elongated tubular shape due to fusion of cells (day 0 to 5). We developed DL program based on Convolutional Neural Networks (CNN) [2,5] and self-build PCs for the classification of the differentiating cells.

It was possible to classify C2C12 before and after differentiation. The features generated by the DL showed both round and elongated shape cellular images. We expect that DL will classify differentiation of other cell species, cellular species e.g. cancer cells and so on.

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P3-13

Analysis of serum microRNA expression and cytokine production profiles for understanding febrile episodes after vaccination

Yayoi Natsume-Kitatani[1] natsume@nibiohn.go.jp
Koji Ozasa[1] ozasa_183cm@nibiohn.go.jp
Tetsuo Nakayama[2] tetsuo-n@lisci.kitasato-u.ac.jp
Ken J. Ishii[1,3] kenishii@biken.osaka-u.ac.jp
Kenji Mizuguchi[1] kenji@nibiohn.go.jp

[1] National Institutes of Biomedical Innovation, Health and Nutrition
7-6-8 Asagi Saito Ibaraki-city Osaka 567-0085, JAPAN
[2] Kitasato Institute for Life Sciences, Kitasato University,
Shirokane 5-9-1, Minato-ku, Tokyo 108-8641, Japan.
[3] Immunology Frontier Research Center, Research Building 6F, Osaka University,
3-1 Yamada-oka, Suita-city, Osaka 565-0871, Japan

Keywords: vaccination, microRNA, WGCNA

Vaccination for children is performed world wide to protect from infectious diseases. One of the major side effects of vaccination is a fever, the molecular basis of which remains to be elucidated. Here, we report the results of microRNA microarray analysis and cytokine multiplex assays for serum samples collected after simultaneous inoculation of 33 children (fever(+) = 24, fever(-) = 9). We performed weighted gene co-expression network analysis (WGCNA) [1,2] to link the microRNA expression and cytokine production. The result showed that microRNAs that were up-regulated in fever(+) children were divided into two modules and each of them was significantly (module-trait relationship p-value = 0.002) correlated with cytokine production. The result of transcription factor binding sites (TFBS) search [3] in microRNA promoter regions implied that the combination of activated transcription factors (TFs) differed in fever(+) and fever(-) children, and that TFs related with cell proliferation were activated in fever(+) children.

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P4-01

The drug discovery and the development in academia: the identification and the development of positive allosteric modulators of PGI_2 receptor for the treatment of pulmonary arterial hypertension

Hiroyuki Miyachi[1] miyachi@pharm.okayama-u.ac.jp
Kohki Yamamoto[1] psdq45rm@s.okayama-u.ac.jp
Toshifumi Suzuki[2] tsfm.suzuki@gmail.com
Riyo Imamura[3] riyo-ima@mol.f.u-tokyo.ac.jp
Takayoshi Okabe[3] tokabe@mol.f.u-tokyo.ac.jp
Tetsuo Nagano[3] tlong@mol.f.u-tokyo.ac.jp

1 Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University. 1-1-1, Tsushima-Naka, Kita-ku, Okayama 700-8530, Japan
2 Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
3 Drug Discovery Initiative, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Keywords: Pulmonary arterial hypertension, positive allosteric modulator, HTS screen

Pulmonary arterial hypertension (PAH), is a generic term for conditions that allow an increase in pulmonary arterial pressure. Rise of long-term pulmonary artery pressure causes right heart failure is a rare and incurable disease of life prognosis average three years. Only continuous infusion therapy of PGI_2 derivative is the choice for patients placed in class III or IV PAH. However, the need for equipment mounting, adaptation method, side effects, decrease QOL of the patients. PGI_2 production amount in PAH patients is reduced to half of the healthy individuals. The authors speculated that if it is possible to increase the sensitivity of the PGI_2 receptor (IP) additionally, it is considered possible to utilize the remaining PGI_2 for the treatment of late stage PAH. Therefore, the authors focused on the search to identify positive allosteric modulators of PGI_2 receptor (IPPAM).

In contrast to the competitive compounds (orthosteric modulators), allosteric modulators interact with binding sites that are topographically distinct from the binding site of the endogenous ligand. Positive allosteric modulators do not activate receptors on their own, and in the presence of the endogenous ligand, allosteric modulators enhance the natural physiological activity of the receptor. Consequently, allosteric modulators can exert their effects while preserving normal physiological signaling patterns.

The authors performed HTS screen of 140,000 University Tokyo (UT) compound libraries, and found only *one hit compound*, i.e., a tetrazole derivative.

In this poster presentation, the authors would like to disclose the design, synthesis, *in vitro* IPPAM activity and structure-activity relationship (SAR) of a series of tetrazole derivatives.

The synthetic route to the optically active tetrazole derivatives and the stereochemistry-IPPAM activity relationship of a series are also discussed.

P4-02

The site of CYP3A4 metabolism prediction of tolterodine using MD and its application to compound design

Atsuko Sato[1] atsuko.sato@kyowa-kirin.co.jp
Hitomi Yuki[2] hitomi.yuki@riken.jp
Chiduru Watanabe[2] chiduru.watanabe@riken.jp
Jun-ichi Saito[1] jun.saito@kyowa-kirin.co.jp
Teruki Homma[2] honma.teruki@riken.jp

[1] Kyowa Hakko Kirin Co.,Ltd., Research Core Function Laboratories, Research functions Unit, R &D Division, 1188, Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka, 411-8731, Japan
[2] RIKEN Center for Life Science Technologies, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

Keywords: Cytochrome P450 (CYP), tolterodine, Sites of metabolism (SOM), Molecular dynamics simulation, Multiple initial structures

CYP3A4 contributes to more than 50% of clinical drug metabolism. Therefore, optimization of drug candidates considering the CYP3A4 metabolism is critically important. It is enormously challenging to precisely predict CYP3A4-ligand complex due to variable conformational possibilities, with its numerous binding substrates. Our purpose of this research is to establish methods for metabolically stable drug design. In our previous study, we have examined the prediction for sites of metabolism (SOM) on carbamazepine (CBZ), which included docking and molecular dynamics simulation from multiple initial structures [1].

In this presentation, we constructed a complex model of CYP3A4 and tolterodine, which was a more flexible substrate than CBZ, by applying a similar method (**Figure 1**). With this process, we compared clustering of docking poses by PLIF (Protein Ligand Interaction Fingerprint) and RMSD for efficient selection of MD initial structures. Following the MD simulation, we calculated the binding energy of tolterodine and its accessibility to the heme iron by each pose in the simulation and estimated the binding pose of tolterodine in CYP3A4. Based on this model, some compound designs to improve metabolic stability would be proposed.

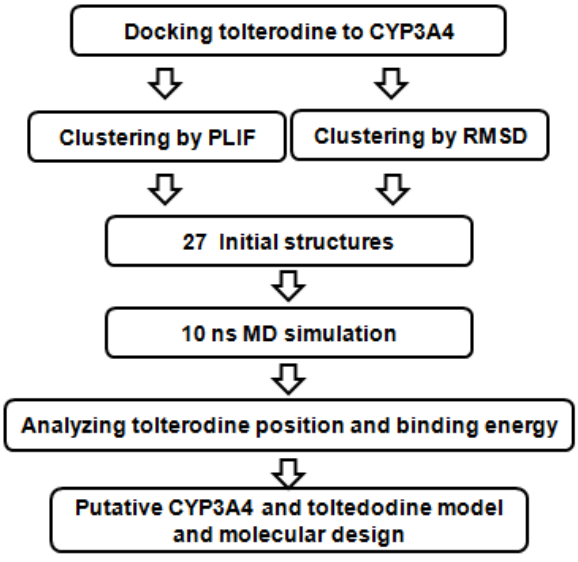

Figure 1. Workflow of MD simulations using multiple initial structures

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P4-03

Utilization of UV spectrum prediction for identification of drug metabolites

Kaori Murakoshi[1]
murakoshi.kaori@mc.mt-pharma.co.jp

Hideaki Sato[1]
sato.hideaki@ma.mt-pharma.co.jp

Hidefumi Kaji[1]
kaji.hidefumi@mh.mt-pharma.co.jp

[1] DMPK Research Laboratories, Mitsubishi Tanabe Pharma Corporation, 2-2-50 Kawagishi, Toda-shi, Saitama 335-8505, Japan

Keywords: UV spectrum prediction, Metabolite identification, Estimation of metabolite amount

Prediction of metabolic pathways in animals and humans in the preclinical stage is extremely important to develop drug candidates with good DMPK profiles. So we obtain the drug metabolism information using various biological samples such as plasma, urine and the reaction mixtures obtained from in vitro metabolic studies. From the point of view of drug-drug interaction and toxicity, it is necessary to estimate the amount of metabolites in human and animal samples. When the authentic sample of metabolite is not available, we generally estimate its amount by comparison with the UV spectrum of the parent compound. In general, the UV spectra of metabolites such as hydroxide and desmethyl form are hardly different from those of the parent. On the other hand, in the case where the conjugated system is changed or the original structure is separated in two by hydrolysis, the UV spectra of metabolites are significantly changed. As a result, it is difficult to estimate the amount of metabolites. Therefore, we thought that if it is possible to predict the UV spectrum from the estimated structure of the metabolite, we can estimate the amount of metabolites with good precision. After several trials with commercially available software to predict UV spectrum, we concluded that it is difficult to completely predict the λmax and absorbance of various types of compounds and their metabolites. However, SCIGRESS V2 (FUJITSU) is very useful for the prediction of a significant change of the UV spectrum pattern. The UV spectra of compound A are shown in Figure 1, and those of its metabolite compound B are shown in Figure 2. Both predicted spectra indicated suitable similarity to the observed spectra. In this study, we report how we can utilize the software to elucidate the structure of metabolites.

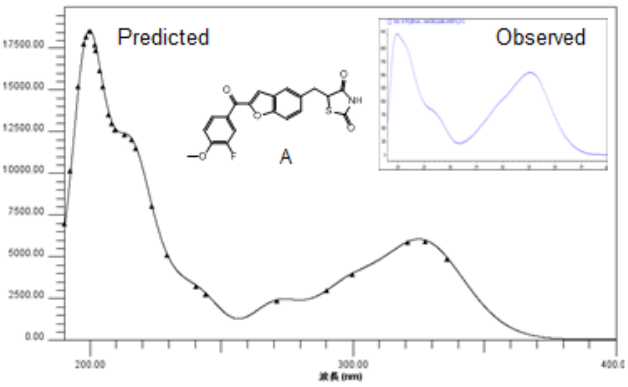

Figure 1. UV spectra of compound A

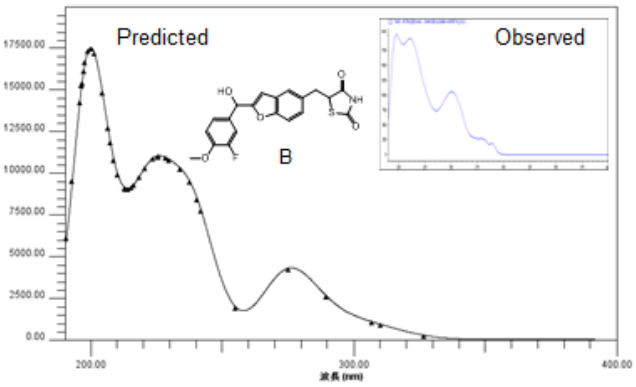

Figure 2. UV spectra of compound B

P4-04

Comparison of various three-dimensional culture plates and monolayer culture plates for CYP metabolic activity and induction with cryopreserved human hepatocytes

Orie Terai[1] orie.terai@scas.co.jp
Takeshi Izukawa[1] takeshi.izukawa@scas.co.jp
Kotaro Okajima[1] k.okajima@scas.co.jp
Yasushi Tsutsumi[1] yasushi.tsutsumi@scas.co.jp
Chiaki Ueda[1] c.nishiyama.ueda@scas.co.jp
Yoshihisa Sogame[1] yoshihisa.sogame@scas.co.jp
Kazunori Yanagi[1] kazunori.yanagi@scas.co.jp

[1] Sumika Chemical Analysis Service, Ltd., 1-135, Kasugade-Naka 3-chome, Konohana-ku, Osaka 554-0022, Japan

Keywords: CYP enzyme activity, CYP induction, 3D culture

[Introduction and Purpose]

Hepatocytes form three-dimensional (3D) structure *in vivo*. Recently, various 3D culture plates for hepatocytes have come onto the market. These plates have a structure such as scaffolds, polymer gels, hydrophobic coating and so on,; which support the cell-cell interactions and 3D-conformation of hepatocytes so to offer *in vivo*-like microenvironment. However, there are no comparable studies among 2D and 3D culture plates for CYP metabolic activity and induction.

We tested the CYP metabolic activity and induction of hepatocytes on various 3D culture plates. Here we report the results of comparable study to evaluate features of commercial 3D culture plates.

[Cell Culture]

Cryopreserved human hepatocytes were cultured in humidified atmosphere containing 5% CO_2 at 37 ^{o}C. The CYP metabolic activities were measured at 3, 7, 10, 14, 17, and 21 days after seeding cells. On CYP induction assay, CYP mRNA expression levels were measured at day6.

[CYP Metabolic Activity Analysis]

On each day for CYP metabolic activity assay, culture medium was aspirated and replaced with KHB buffer including a cocktail of probe substrate for CYPs. After incubation, the supernatant was collected and pretreated to give an injection sample for the LC/MS/MS system.

[CYP Induction Assay]

Human hepatocytes were cultured with CYP inducer or DMSO for 2days. Total RNA of the cells was extracted and then reverse transcribed. The expression levels of CYP1A2, 2B6 and 3A4 mRNA were evaluated by Real-Time PCR.

[Results and Discussion]

We confirmed that the cryopreserved human hepatocytes formed 3D structure at 1 day after seeding cells. CYP metabolic activities in 2D monolayer culture plates were dramatically reduced at 7 to 14 days after seeding cells. However, the activities in some 3D culture plates were maintained. CYP induction in 3D culture plates was almost equal to 2D monolayer culture plates.

P4-05

Construction of web services to evolve drug like properties of ligands

Naoki Arai[1]
arai.n.ai@m.titech.ac.jp

Shunsuke Yoshikawa[2]
yoshikawa@bi.cs.titech.ac.jp

Nobuaki Yasuo[2]
yasuo.n.aa@m.titech.ac.jp

Yusuke Nakashima[2]
nakashima.y.ac@m.titech.ac.jp

Ryunosuke Yoshino[3]
yoshino.r.aa@m.titech.ac.jp

Masakazu Sekijima[2, 3]
sekijima@gsic.titech.ac.jp

[1] Department of Computer Science, School of Engineering, Tokyo Institute of Technology, 2-12-1, Ohokayama, Meguro-ku, Tokyo, 152-8550, Japan
[2] Department of Computer Science, Graduate School of Information Science and Engineering, Tokyo Institute of Technology, 2-12-1, Ohokayama, Meguro-ku, Tokyo, 152-8550, Japan
[3] Global Scientific Information and Computing Center, Tokyo Institute of Technology, 2-12-1, Ohokayama, Meguro-ku, Tokyo, 152-8550, Japan

Keywords: Drug discovery, Virtual compounds library, Web server

In Structure Based Drug Discovery (SBDD), molecular docking is widely used to search hit compounds. After obtaining of them, their properties are optimized for lead compound.

In this study, we attempted to evolve properties of ligands by applying synthetic rules involved in the improving Hydrogen Bonding Donor (HBD), Hydrogen Bonding Acceptor (HBA) and stability in multiple stages. In addition, we implement it as web services. After the user find hit compounds, our services make it possible for user to obtain virtual libraries of drug-like compounds than hit compounds while checking used virtual chemical reactions interactively.

In response to the compound data input, our services simulate virtual chemical reactions accordingly to generate digital data of new chemical compounds, thereby compiling virtual compound libraries. By imparting purposes to the virtual chemical reactions, the server completes the construction of libraries including compounds with properties as desired.

[1] http://www.rdkit.org/
[2] https://www.djangoproject.com/

P4-06

Evaluation of Applicability of Human iPS cell-derived Hepatocyte to Pharmacokinetic Studies

Seiichi Ishida **Shinichiro Horiuchi** **Takashi Kubo** **Yukie Kuroda**
ishida@nihs.go.jp

Riho Ishida **Shoko Uchida** **Su-Ryang Kim** **Yuko Sekino**

Division of Pharmacology, National Institute of Health Science,
1-18-1 Kamiyoga, Setagaya-ku Tkyo 1588501, Japan

Keywords: iPS cell-derived hepatocyte, pharmacokinetics, functional evaluation

Pharmacokinetic studies of drug candidates conducted at early stage of drug development are important for securing their safety in human administration. Today, basic data are provided from experiments using the human primary hepatocytes. However, problems are inherited in them, such as differences among lots based on donor variations and limited supply of every lots. To circumvent these problems, hepatocytes derived from human iPS cells (hiPSC-hepatocytes) are expected as an alternative cell source.

We have been evaluating their drug metabolizing enzyme activities and induction abilities by chemical inducers, and the results were reported at last year's meeting. In this meeting, we will report results obtained from a new version of commercially available hiPSC-hepatocytes. For evaluating hiPSC-hepatocytes, we measured metabolic activities of CYP1A2, CYP2D6, CYP2C9, CYP2C19 and CYP3A4 by LC-MS/MS (Sumika Chemical Analysis Service, Ltd.), and expressions of CYP1A1, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, CYP3A7, GSTA2, UGT1A1, ABCB1, ABCC2, PXR, CAR, AhR, CEPBα, and HNF4α by qPCR (Life Technologies) in hiPSC-hepatocytes from three vendors (Takara Bio Europe AB, Cellular Dynamics International, ReproCELL). We also evaluated induction of CYP1A2, CYP2B6, and CYP3A4 by typical inducers (omeprazole, phenobarbital and rifampicin, respectively) in mRNA level.

CYP enzyme activities in hiPSC-hepatocytes from two venders showed close to HepaRG cells, which was used as a control hepatocyte cell line. Induction of CYP1A2 and CYP3A4 was observed in hiPSC-hepatocytes from one vender. Preliminary results for the improvement of CYP expressions and inducibility will be also presented.

P4-07

High-Performance Prediction for Estrogenic Compounds based on the Tox21 10K Compound Library

Yuki Asako
y121007@std.my-pharm.ac.jp

Yoshihiro Uesawa
uesawa@my-pharm.ac.jp

Meiji Pharmaceutical University, 2-12-11, Noshio, Kiyose, Tokyo 204-8588, Japan

Keywords: Random forest, Estrogen receptor, Tox21data challenge 2014

Introduction: Compounds related with estrogen receptor (ER) activation can disrupt human endocrine processes, severely affecting human reproduction and growth. Our prediction model for ER-ligand binding domain activation compounds performed well in the Tox21 data challenge 2014 [1,2], a competition organized by the National Institute of Health. In this study, we review the model construction process to improve the prediction performance.
Methods: Datasets published in the Tox21 data challenge 2014 and based on the Tox21 10K compound library [3] were used in the present analysis. Several properties were computed using the MOE, Marvin, and Dragon software packages. The random forest method [4] included in the JMP Pro, SAS statistical software was adopted for the model construction. The optimum model was selected from a lot of random forest models with numerous combinations of the hyperparameters. Prediction abilities of the models were validated with area under ROC (ROC_AUC) in the external test set and the final evaluation set.
Results and discussion: Overfitting was observed in the model submitted in the competition. We were able to improve the prediction model's discrimination ability for estrogenic compounds by investigating the optimal combination of hyperparameters used in the random forest algorithm. Furthermore, the model analyses revealed that certain structural and physicochemical properties such as the number of phenolic hydroxyl groups contributed to the interactions between ER and the compounds.
Conclusion: In this study, an optimized random forest model is presented for the prediction of ER activation compounds. This method is expected to be useful for the screening of toxic compounds.

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P4-08

Structural Characterization of Compounds with PPARγ Activation based on the Tox21 10K Compound Library

Masaya Aoki
y121002@std.my-pharm.ac.jp

Yoshihiro Uesawa
uesawa@my-pharm.ac.jp

Meiji Pharmaceutical University, 2-12-11, Noshio, Kiyose, Tokyo 204-8588, Japan

Keywords: Random forest, PPARγ, Tox21data challenge 2014

Introduction: Peroxisome Proliferator-Activated Receptor-γ (PPARγ) shows bioactive functions such as adiponectin production and inflammatory cytokine production inhibition. PPARγ activation compounds are valuable antidiabetics and analgesics [1]. In this study, discrimination models were constructed based on information for chemical structures included in the Tox21 10K library [2]. Pattern regression was performed using the random forest algorithm [3] to calculate the importance index for descriptors.
Methods: Datasets published in the Tox21 data challenge 2014 [4] were used in the present analysis. Structural and physicochemical properties were computed using the MOE software package. The random forest method included in the JMP Pro, SAS statistical software was adopted for the model construction. The optimum model was selected from a lot of random forest models with numerous combinations of hyperparameters. Prediction abilities of the models were validated with area under ROC (ROC_AUC) in the external test set and the final evaluation set.
Results and Discussion: The discrimination model produced an excellent ROC_AUC value in the final evaluation set that was used for ranking the competitors in the Tox21 data challenge 2014. Thus, our improved model was equally ranked among the top 10 in the competition, based on its prediction ability. Hereafter, the physicochemical properties of the active compounds will be investigated based on the importance of the descriptors used in the model construction.
Conclusion: The results of the current study are expected to contribute to drug design and development using PPARγ activation compounds.

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P4-09

Current Status of Population Pharmacokinetic Studies in Drug Development and Their Usefulness in Medical Practice

Megumi Watanabe-Uchida[1] **Mamoru Narukawa**[1]

watanabem@pharm.kitasato-u.ac.jp narukawam@pharm.kitasato-u.ac.jp

[1] Department of Clinical Medicine (Pharmaceutical Medicine), Graduate School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

Keywords: Population pharmacokinetics (PPK), Package Insert (PI), Interview Form (IF)

With the publication of the ICH-E7 guideline, the guidance document on population pharmacokinetics (PPK) by the FDA, and the guideline on clinical pharmacokinetic studies in Japan, PPK analysis has been recognized widely in drug development. Also, a section to describe the result of PPK analysis was added in the interview form (IF) preparation manual in 2008, which is a positive movement toward utilizing PPK analysis results in medical practice. In this study, we investigated the recent situation of utilizing PPK analysis results in new drug application (NDA) and other documents in Japan and the US and the usefulness of the provided information in medical practice.

Data on PPK analysis results were collected from package insert (PI), IF, review report and common technical document for Japan, and label and clinical pharmacology review by the FDA for the US, for new molecular entities approved between Jan 2012 and Dec 2014. As for the usefulness of the provided information, degree of contribution to the pharmacokinetic parameter estimation of an individual patient in medical practice was classified into 6 based on the information of pharmacokinetic parameters and covariates in the PI, IF and label.

The proportion of documents that contain information of PPK analysis results for new molecular entities approved in 2012, 2013 and 2014 were 43.6% (17/39), 51.9% (14/27) and 61.0% (25/41) in label, 14.8% (9/61), 21.9% (7/32) and 13.3% (6/45) in PI, and 52.5% (32/61), 53.1% (17/32) and 46.7% (21/45) in IF, respectively. As for the usefulness of the provided information, the information of PPK parameters and cause of variation was provided in 92.9% of the label, 50.0% of the PI and 68.6% in the IF.

When the information was compared between label and PI, and between clinical pharmacology review and review report, the situation of utilizing PPK analysis results still hasn't progressed in Japan. PPK analysis is often used to examine the change of pharmacokinetic profiles in patients with hepatic or/and renal impairment in the US, whereas standard pharmacokinetic studies or stratified analysis are usually used in Japan. This is considered as one of the factors to differentiate the situation in the US and Japan. Result of the examination of usefulness of the provided information suggested that in some cases the information was not sufficient to precisely estimate patient pharmacokinetic parameters.

P5-01

Pseudorotaxane formation methods targeting on nucleic acids

Kazumitsu Onizuka **Takuya Miyashita**
onizuka@tagen.tohoku.ac.jp

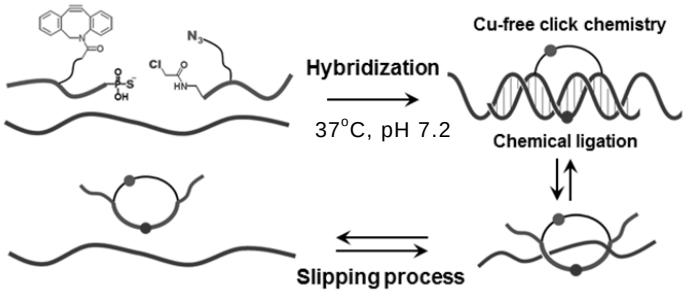
Takuya Amemiya **Fumi Nagatsugi**
nagatugi@tagen.tohoku.ac.jp

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai, Miyagi, 980-8577, Japan

Keywords: Nucleic acids, Pseudorotaxane, Template reaction, Molecular robotics

Rotaxane is a molecular architecture consisting of a dumbbell shaped molecule which is threaded through a molecular ring. In the nucleic acid chemistry, unique mechanically interlocked molecular architectures such as catenane and rotaxane have been constructed by taking advantage of base pairing. A variety of methods to construct interlocked molecular architectures have been developed for DNA nanotechnology[1] and DNA topological labeling[2]. However, these methods either require a toxic chemical reagent or an enzyme to form topological structures; thus, it is difficult to construct them inside the cell.

Currently, we are investigating novel methods to form topological DNA/RNA architectures that neither require a toxic chemical reagent nor an enzyme. Here we report a method to form a pseudorotaxane architecture using only a pair of reactive oligodeoxyribonucleotides (ODNs), which we designed and synthesized, and then performed the pseudorotaxane formation reaction with both DNA and RNA oligonucleotides. The reaction proceeded smoothly without any extra reagents at 37 °C and pH 7.2, leading to the formation of a stable complex on a denaturing polyacrylamide gel[3]. Interestingly, the pseudorotaxane was formed with the cyclized ODN reversibly by the slipping process. This new pseudorotaxane formation represents a promising method for developing new DNA nanotechnologies and antisense oligonucleotides.



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P5-02

Modularity analysis of a cascade of DNA circuits and its application to DNA feedback regulator for molecular robotics

Takashi Nakakuki[1]
nakakuki@ces.kyutech.ac.jp

[1] Department of Systems Design and Informatics, Kyushu Institute of Technology, 680-4 Kawazu, Iizuka, Fukuoka 820-8502, Japan

Keywords: Molecular robotics, Regulator, Feedback control, Retroactivity, Stability

In this study, we consider a kind of regulation problem for molecular robotics, realized by DNA reactions. The control objective is to regulate the concentration of a target DNA strand to a desired level using practical DNA circuits. It is a challenging problem in the architecture of the molecular robot because of the positiveness, modularity, and finiteness problems. A DNA comparator-based controller with DNA amplifiers is proposed, and it is shown to successfully achieve the control objective. The property and stability of the system are evaluated in terms of retroactivity and the Lyapunov stability theory for a positive second-order system. To our knowledge, this study is the first to realize a regulator in a practical DNA reaction system.

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P5-03

Structural Instability Conditions of Boolean Networks

Shun-ichi Azuma[1] **Takahiro Yoshida**
sazuma@i.kyoto-u.ac.jp

Toshiharu Sugie[1]
sugie@i.kyoto-u.ac.jp

[1] Graduate School of Informatics, Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan

Keywords: Boolean network, structural instability

Any networked control system is specified by the combination of the network structure and the dynamics of the components. Meanwhile, one may encounter a situation where the former is known but the latter is (almost) unknown. In such a case, we cannot apply any method using the full information of the target system [1]. In this presentation, we introduce our recent results on the structural instability, that is, the instability with unknown component dynamics, for a class of networked control systems. In particular, we consider here Boolean networks and present a series of (necessary and) sufficient conditions for the structural instability.

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P5-04

Evaluation of drug-induced CB2 cannabinoid receptor activity in the CNS using the ERK1/2 phosphorylation pathway of microglial cells

Yukari Shigemoto-Mogami[1], Kaoru Sato[1], Kazue Hoshikawa[1], Ruri Kikura-Hanajiri[2],
shigemot@nihs.go.jp
Takashi Hakamatsuka[2], Yuko Sekino[1]

1: Division of Pharmacology, National Institute of Health Science, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. 2: Division of Pharmacognosy, National Institute of Health Science, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan.

Keywords: microglial cell, CB2 receptor, ERK1/2 phosphorylation

Herbal products containing synthetic cannabinoids have caused serious social problems. If the compounds found in the products have CB1/CB2 receptor agonist activity, they should be controlled as “Designated Substances” under the Pharmaceutical and Medical Device Act in japan, because of their psychoactive and intoxicating effects. Although it has been considered that only CB1 receptors are expressed in the central nervous system (CNS), recent studies shows that CB2 receptors are also expressed and causing modulation of neural functions. In the CNS, microglial cells are main cells expressing CB2 receptors. CB2 receptors are $G_{i/o}$-protein-coupled and have been reported to activate the extracellular signal-regulated kinase (ERK) pathway, which is one of mitogen activated protein kinases (MAPK). So, we examined whether the stimulation of CB2 receptor induced ERK1/2 phosphorylation in microglia using anti-phosphorylated ERK1/2 antibody and anti-total ERK antibody, in order to develop a risk detection system of herbal products.

First, we analyzed by Western Blotting. We confirmed that the CB2 receptor is highly expressed in primary cultured rat microglia. CB2 receptor selective agonists JWH015 and HU308 induced ERK1/2 phosphorylation of microglial cells at the 10 minutes after treatment with a concentration-dependent manner. In addition, its phosphorylation was inhibited by selective CB2 receptor antagonist SR144528, whereas selective CB1 antagonist AM251 had no effect. The analysis by high-throughput ELISA method confirmed the similar results. These results demonstrate that selective stimulation of CB2 receptor induced ERK1/2 phosphorylation in microglial cells. The microglial ERK1/2 phosphorylation pathway through the CB2 receptors might be a useful tool for the prediction of the potential health damage caused by the herbal products acting through CB2 receptors in the CNS.

P5-05

Prediction for chemical-induced hepatomegaly from chemical structure by machine learning methods

Kaori Ambe[1] ambek@phar.nagoya-cu.ac.jp
Sorami Tamura[1] c072037@ed.nagoya-cu.ac.jp
Masahiro Tohkin[1] tohkin@phar.nagoya-cu.ac.jp

[1] Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

Keywords: Hepatomegaly, Machine learning methods, QSAR, Toxicological data

Hepatomegaly, such as hepatocellular hypertrophy or the increased relative liver weight, is hepatic responses to chemicals and is generally observed at non-clinical toxicological study. Since the chemical structures of causative chemicals show a wide variety, the comprehensive understanding of the toxicological significance of hepatomegaly has not been elucidated [1]. So, we have developed the predicting methods for hepatomegaly based on the structure of the chemicals.

In this study, at first, we made the toxicological database from the risk assessment reports of pesticides, food additives, and veterinary medicinal products that were published by Food Safety Commission of Japan [2]. Our database consists of the toxicological information from 28-day rat repeated-dose toxicological study and the chemical data which is linked to PubChem [3]. Then, we constructed the prediction Quantitative Structure Active Relationship (QSAR) model for the hepatomegaly based on chemical substance's descriptors by machine learning methods [4] (known as deep learning, support vector machine, random forest), by using our toxicological database. To improve the performance of the prediction model, variable selection [5] and applicability domain [6] were determined. As a result, the deep learning shows the highest performance hepatomegaly prediction QSAR model among all methods, which had the nearly 80% prediction accuracy. Then, the reliability of models was assessed by Y-randomization test [7]. In this research, we have developed the practical QSAR model of the hepatomegaly for chemicals in a wide range with a high degree of accuracy by using highly reliable large-scale toxicological data.

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P5-06

Regulation of Dynamics of Oil-Droplets and Molecular Assemblies at Electrified Interfaces

Takamasa Sagara[1]
sagara@nagasaki-u.ac.jp

Tetsuro Morooka[1]
bb52315201@cc.nagasaki-u.ac.jp

Hironobu Tahara[1]
h-tahara@nagasaki-u.ac.jp

[1] Graduate School of Engineering, Nagasaki University, Bunkyo, Nagasaki 852-8521, Japan

Keywords: Au electrode/aqueous solution interface, Adsorption-desorption, phase change

Control over the dynamic self-assembling, reshaping, and movements of water-immiscible liquid droplets and molecular assemblies at a solid electrode/water interface can be made by the change of electrode potential. The potential regulates interfacial electric potential distribution, surface electron density and chemical activity, and solvent molecular orientation in proximity to the surface. Through these changes, we may realize a highly controllable dynamics of droplets and molecular assemblies with large amplitudes and short time constants. As an example, a potential-dependent wetting/dewetting transition of hexadecane (HD) macro-droplet on mercury was previously reported [1]. Such a dynamics may be applicable to both the trigger and driving force of a prototype molecular robot in mesoscopic and even macroscopic scales in future.

We herein describe in detail the dynamics of HD ranging from its monolayer to a μm-size droplet on a Au(111) electrode in water and discuss the roles played by coexistent surfactants.

On a Au(111) electrode surface in 0.05 M $KClO_4$ solution, a monolayer-equivalent amount of liquid HD splits into many tiny droplets (<50 μmϕ) because of the interfacial tension balance. Upon addition of an anionic surfactant, sodium dodecyl sulfate, the potential-dependent phase changes of adsorbed dodecyl sulfate ion (DS^-) became dominant, albeit showing difference of interfacial capacitance in the ad-micelle potential region. To reveal the micro-droplet reshaping from the monolayer amount HD, a zwitterionic surfactant dye Di10ASP-PS as a fluorescent probe was added in HD. The fluorescence intensity (FI) obtained for a linear cyclic potential sweep in the coexistence with DS^- was closely in line with the change of contact angle of HD droplet (1 μL) on Au(111) electrode (Fig. 1-a), indicating that the change of FI can track the small HD droplet reshaping. The two-stage decreases of FI at positive potentials were interpreted as the gradual spreading of HD micro-droplets, followed by the phase transition of DS^- from hemi-micellar to interdigitated bilayer state (Fig. 1-b), even in the presence of HD. We are under study of the fine control methods with a goal to achieve an ameba-like motion in mind.

This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas “Molecular Robotics” (No. 24104005) from MEXT, Japan.

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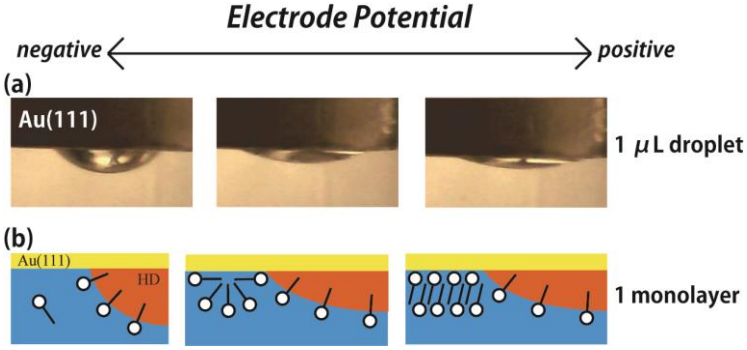

Fig. 1. HD + DS^- adlayer on Au(111) electrode: (a) droplet reshaping, (b) monolayer-level model.

P5-07

Dynamical Properties of Chemical Reaction Networks by using Strand Displacement reactions

yasuhiro SUZUKI

Graduate School of information science, Nagoya University, Furocho Chikusa Nagoya, 4648603, Japan

Keywords: molecular robotics, strand displacement reaction, chemical reaction networks, dissipative of information equation

Chemical Reaction Networks, CRNs have been implemented by using strand displacement reactions of DNA. In such reactions, intermediate substances play key roles [1];

$$
\begin{cases}
1 & \begin{cases} X_1 + L_1 \underset{q_{\max}}{\overset{q_1}{\rightleftharpoons}} H_1 + B_1 \\ X_2 + H_1 \xrightarrow{q_{\max}} O_1 \\ O_1 + T_1 \xrightarrow{q_{\max}} 2X_2 \end{cases} \\
2 & \begin{cases} X_1 + G_2 \xrightarrow{q_2} O_2 \\ O_2 + T_2 \xrightarrow{q_{\max}} 2X_1 \end{cases} \\
3 & \begin{cases} X_2 + G_3 \xrightarrow{q_3} O_3 \end{cases}
\end{cases}
,
$$

e.g., in implementing the Lotka-Volterra (LV) reaction, the amplification of the substance X_i is implemented as $X_i + G_i \rightarrow O_i$, $O_i + T_i \rightarrow 2X_i$; where G_i and T_i are auxiliary substances and they are prepared before the reaction starts (G_i is called “Gate” and T_i, “Transfer”, respectively), while O_i is not prepared and it is produced and consumed in the reactions between the input substance X_i and these auxiliary substances; we call such intermediate substances *intermediators*.

Although intermediators are not used in the formal LV chemical reaction system; their mathematical characteristics are different; in the formal LV, there are two equilibriums and in the LV with *intermediators*, there are infinite number of equilibriums that include equilibriums of the formal LV.

It has been known that one of the characteristics of dynamical system of the formal LV model (2 species) is periodic, and there are no attractors, and the orbits of oscillations are given by the initial concentration. On the other hand, we found that in the LV with intermediators (implemented by using strand displacement reactions [1]), even if the initial concentrations are different, every orbit is attracted near to the equilibrium space.

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P5-08

DNA strand exchange reaction activated by cationic comb-type copolymers modified with ureido groups

Naohiko Shimada[1]
nshimada@bio.titech.ac.jp

Wei Song[2]
songwei@ms.ifoc.kyushu-u.ac.jp

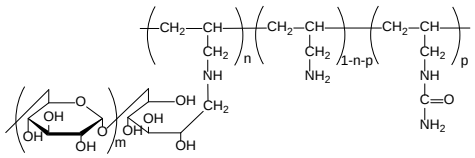
Atsushi Maruyama[1]
amaruyama@bio.titech.ac.jp

[1] School and Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama-shi, Kanagawa Pref. 226-8501, Japan
[2] Departments in Graduate School of Engineering, Kyushu University,744, Motooka, Nishi-ku, Fukuoka, 819-0395, Japan

Keywords: molecular robotics, cationic polymers, DNA strand exchange reaction

Strand exchange reactions (SERs) of nucleic acids are essential for genetic recombination. A variety of proteins induce strand exchange reactions *in vivo*. SER has been utilized in biotechnological applications. There are many examples for DNA nanomachines driven by SER.[1-4] Recently, DNA logic gates or circuits employing SER have been also built.[5,6] Nanomachines or logic gates work properly because of sequence specific SER. However, there is an issue with the response speed of the action. Acceleration of SER is required for the rapid response of the nanomachines or the logic gates.

We have reported that cationic comb-type copolymers composed of a polycation backbone and hydrophilic graft chains such as dextran and poly(ethylene glycol) greatly accelerate the SER *in vitro*[7]. To elucidate structure/function relationships involved in the SER accelerating activity of the copolymer, we modified the copolymer with ureido groups, poly(allylamine-*co*-allylurea)-graft-dextran (PAU-*g*-Dex, Figure 1). The melting temperature of dsDNA in the presence of the copolymers with ureido groups decreased with increasing ureido content, suggesting that the ureido groups have a destabilizing effect on the DNA duplex. Copolymers modified with ureido groups (about 50 mol%) accelerated a SER more strongly than unmodified copolymers. Given our previous observation that modification of the copolymer with guanidino groups resulted in enhanced SER accelerating activity, we propose that incorporation of chaotropic functional groups into the copolymer structure results in an increase in SER accelerating activity.



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P5-09

Formation of two-dimensional crystalline DNA origami lattices on lipid membranes

Yuki Suzuki[1]
ysuzuki79@kuchem.kyoto-u.ac.jp

Masayuki Endo[2]
endo@kuchem.kyoto-u.ac.jp

Hiroshi Sugiyama[1,2]
hs@kuchem.kyoto-u.ac.jp

[1] Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan
[2] Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Yoshida-ushinomiyacho, Sakyo-ku, Kyoto 606-8501, Japan

Keywords: DNA nanotechnology, DNA origami, Self-assembly, Atomic force microscopy

Self-assembly is a ubiquitous approach to the design and fabrication of novel supramolecular architectures. Here, we report a strategy termed ‘lipid-bilayer-assisted self-assembly’ that is used to assemble DNA origami nanostructures into two-dimensional lattices. DNA origami structures are electrostatically adsorbed onto a mica-supported zwitterionic lipid bilayer in the presence of divalent cations. We demonstrate that the bilayer-adsorbed origami units are mobile on the surface and self-assembled into large micrometer-sized lattices in their lateral dimensions. Using high-speed atomic force microscopy imaging (HS-AFM), a variety of dynamic processes involved in the formation of the lattice, such as fusion, reorganization and defect filling, are successfully visualized. The surface modifiability of the assembled lattice is also demonstrated by in situ decoration with streptavidin molecules. Our approach provides a new strategy for preparing versatile scaffolds for nanofabrication and paves the way for organizing functional nanodevices in a micrometer space.

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P5-10

Comparison of the NMDA receptor expression and the extent of excitotoxicity in human induced pluripotent stem cell (hiPSC)-derived neurons.

Kanako Takahashi[1] ktakahashi@nihs.go.jp
Yukari Shigemoto-Mogami[1] shigemot@nihs.go.jp
Hideo Shimizu[1] hshimizu@nihs.go.jp
Kaori Chujyo[1] chu-773@nihs.go.jp
Kazue Hoshikawa[1] hoshikawa@nihs.go.jp
Yohei Okada[2] yohei@aichi-med-u.ac.jp
Hideyuki Okano[3] hidokano@a2.keio.jp
Yuko Sekino[1] yukos@nihs.go.jp
Kaoru Sato[1] kasato@nihs.go.jp

1 Division of Pharmacology, National Institute of Health Sciences, Setagaya-ku, Tokyo 158-8501, Japan
2 Department of Neurology, School of Medicine, Aichi Medical University, Nagakute Aichi 480-1195, Japan
3 Department of Physiology, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160-8582, Japan

Keywords: Human induced pluripotent stem cell-derived neurons, Excitotoxicity, L-glutamate, NMDA, Toxicity evaluation system

Neurons differentiated from human induced pluripotent stem cells (hiPSC) neurons are expected to improve predictability of the non-clinical CNS evaluation in the drug development. 'Excitotoxicity' is a neuron-specific cell damage, which is caused by excessive stimulation of L-glutamate receptors (L-GluR). Ca^{2+} influx via NMDA type of L-GluR (NMDAR) is an initial step of excitotoxicity.

In this study, the functional expression of NMDAR and excitotoxicity of three kinds of hiPSC neurons, neurons differentiated from 253G1 neurosphere (253G1-EB), iCell® neurons (iNeurons), and ReproNeuro™ (ReproDA), were investigated. The NMDAR expression was studied by measurements of Ca^{2+} increases induced by brief bath applications of L-Glu, (100 μM) with Ca^{2+}imaging method using fura-2 AM, and the excitotoxicity was studied by propidium iodide (PI)-calcein staining, lactate dehydrogenase (LDH) release, MTT reduction and MAP2 staining. In cultured rodent neurons, a L-Glu treatment (100 μM) causes severe neuronal damage 24 hrs after. We used the treatment to study excitotoxicity of hiPSC neurons.

Ca^{2+} imaging revealed that 253G1-EB started to response to L-Glu at 10 days after differentiation, while they did not response to NMDA (100 μM) at 40 days after differentiation. We did not observe the excitotoxicity in 253G1-EB. Almost of all iNeurons showed rapid increase of Ca^{2+} responses to L-Glu at 1 day *in vitro* (DIV). AP5, a NMDAR antagonist, did not suppress the L-Glu-induced Ca^{2+} increase, but in some of iNeurons, AP5 suppressed the L-Glu-induced Ca^{2+} increase, namely that functional NMDAR expression was not stable in iNeuons. In accordance with this, the reproducibility of excitotoxicity was also unstable in iNeurons. ReproDA started to response to L-Glu at 7 DIV and most of cells with neuron-like shape were AP5-responsive at 28 DIV. However, the number of neurons damaged by L-Glu was small.

In summary, we found that hiPS-neuron, which had the functional NMDAR expression, failed to reproduce severe excitotoxicity that was observed in cultured rodent neurons.

P5-11

Accelerated construction of matched molecular pairs-based dataset on PG-Strom architecture

Satoshi Takahashi[1]
takahashi@itmol.com

Kohei Kaigai[2]
kaigai@ak.jp.nec.com

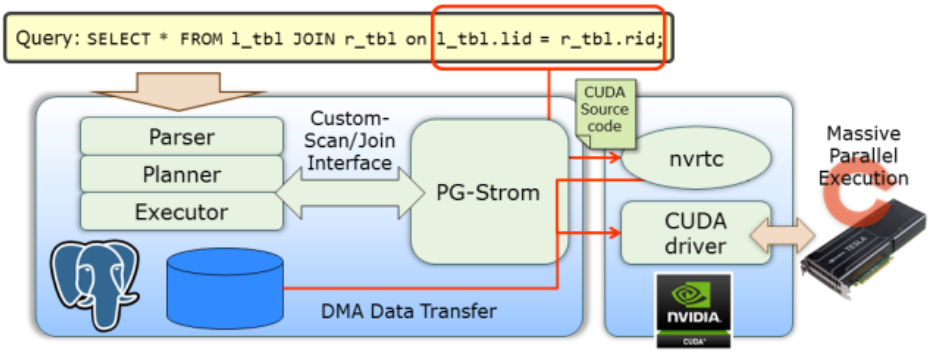
Takanori Ohgaru[3]
ohgaru.takanori@mb.mt-pharma.co.jp

Atsushi Yoshimori[1]
yoshimori@itmol.com

[1] Institute for Theoretical Medicine, Inc., 4259-3 Nagatsuta-cho, Midori-ku, Yokohama 226-8510, Japan
[2] NEC Business Creation Division, NEC Corporation, 1753, Shimonumabe, Nakahara-ku, Kawasaki, 211-8666, Japan
[3] Chemistry Research Laboratories, Mitsubishi Tanabe Pharma Corporation, 1000, Kamoshida-cho, Aoba-ku, Yokohama 227-0033, Japan

Keywords: Matched molecular pair (MMP), GPU acceleration, Query acceleration engine

PG-Strom is an extension of PostgreSQL, designed to off-load several CPU intensive query workloads (scan, join, aggregation and sorting) to GPGPU [1, 2]. It works as a query acceleration engine, and provides cost-effective solution for processing massive amount of records. Its characteristics well fits usual workloads of data manipulation in chemoinformatics.



Matched molecular pair analysis (MMPA) has widely used in chemoinformatics area for extracting promising chemical transformations from massive chemical data [3]. Matched Molecular pair (MMP) is a pair of molecules that have only a structural change at a single site. Systematic calculations of activity changes on the basis of MMPs provide rules that affect activities of interest. However, the systematic calculations are time consuming process.

In this study, we attempted to construct MMPs-based dataset from ChEMBL [4] by use of standard SQL queries on PG-Strom architecture. The construction time of the dataset was observed to be significantly accelerated with PG-Strom. We will discuss about the relationship between the SQL queries and PG-Strom architecture and its applications to chemoinformatics.

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P5-12

Control of DNA Nanostructure Formation by Using a G-Rich-DNA Binding Artificial Peptide and a Protease

Kenji Usui
kusui@center.konan-u.ac.jp

Masayuki Shimooka
m1561004@s.konan-u.ac.jp

Arisa Okada
m1261001@center.konan-u.ac.jp

Faculty of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, Kobe, Hyogo 650-0047, Japan

Keywords: Peptide, Peptide nucleic acid(PNA), Protease, G-quadruplex, G-wire

Recent research has revealed that DNA secondary structures regulate a variety of cellular events. Guanine-rich (G-rich) DNA sequences can fold into a secondary structure, the G-quadruplex structure, and this structure regulates cellular events such as transcription and telomerase elongation, which play roles in various serious diseases and cellular aging [1,2]. In addition, Guanine-rich DNA can fold into a supramolecular structure called the G-wire. The G-wire possesses promising characteristics as a functional element for various applications in nanotechnology and electronics. Therefore, many ligands, which can alter the stability of G-quadruplex structures by binding to G-rich DNAs, have been investigated. However, the next-generation ligands should have a greater degree of functionality including on-off-switching. In this study, we attempted to construct a system to regulate G-quadruplex structure forming with on-off-switching module depending on a particular environment (a particular protease activity in this study) by using a peptide (a small protein) conjugated with peptide nucleic acid (PNA) [3]. Then we attempted to apply this system to a nanotechnological field.

At First, a peptide conjugated with nucleic acid bases was designed. It consisted of two parts. One part was composed of guanine PNA-rich sequences for induction of DNA to form DNA-PNA hybrid G-quadruplex structures. The other part was a switching module depending on activity of a particular protease as the environment. Thus, this system would induce DNA to form G-quadruplex structures when the protease would not exist, and once the protease emerging, the peptide would be digested and simultaneously loose the induction ability resulting in collapse of a DNA/PNA hybrid G-quadruplex structure. After synthesis of the peptides by Fmoc chemistry, we checked the G-quadruplex structure induction by CD spectroscopy, electrophoresis, UV-melting and fluorescence spectroscopy. Then, we demonstrated the switch function depending on the protease activity by UV-melting or fluorescence spectroscopy. Finally, the switching DNA nanosturacure (G-wire <--> particles) by the protease was demonstrated. Throughout this study, we established a control system for forming DNA G-wire structures depending on protease activity using designed small proteins. It is obvious that these small proteins would be able to one of the promising tools for regulation of nanowire structural formation on various applications including an electronic circuit toward nano and nanobio technology.

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P5-13

Object Tracking of Multi-crossing Behaviors on Microtubule Gliding Assay

Bulibuli Mahemuti [1] bulbul.aa@m.titech.ac.jp
Daisuke Inoue[2] daisukeinoue@mail.sci.hokudai.ac.jp
Akira Kakugo [2 3] kakugo@sci.hokudai.ac.jp
Akihiko Konagaya [1] kona@dis.titech.ac.jp

[1]Departement of Computational Intelligence and Systems Science,
Interdisciplinary graduate school of Science and Engineering, Tokyo Institute of Technology
[2]Faculty of Science, Hokkaido University
[3]Graduate School of Science, Department of Chemistry, Hokkaido University

Keywords: Microtubule gliding assay, image processing, object tracking

Object tracking is one of important issues in bio-imaging necessary to elucidate the dynamics of molecules from video data. In microtubule (MTs) gliding assays, object tracking becomes non-trivial due to the occurrences of compound objects such as crossing and snuggling of MTs as well as sudden appearance and disappearance of MTs. There are several alternative methods for MTs tracking. Most of the methods have focused on the movements of MTs heads or several selected points on a MTs. However, these methods ignore the length and shape information of MTs which turn out to be the most important factors in the study of MT dynamics. Continuation of previous work [1], we investigate MT dynamics based on its morphological information. We also develop easy and useful workflow of decomposing and tracking. With this algorithm, single MTs, crossing MTs and multi-crossing MTs can be decomposed and tracked correctly.

We proposed a new method for measuring the swarm behavior of MTs with gliding assay videos [2]. This method enables us to estimate the degree of swarming with regards to of the density fluctuation score. An of the density fluctuation score is obtained from the information entropy of estimated MTs numbers distributed over a picture frame. Aggregation scores help us to estimate how MTs swarm behavior is different from random movement.

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P5-14

Going Beyond Real-Time with a 3-D Live controlled Simulation Environment for Microtubule Swarm Dynamics

Greg Gutmann[1] gutmann.g.aa@m.titech.ac.jp
Daisuke Inoue[2] daisukeinoue@mail.sci.hokudai.ac.jp
Akira Kakugo[2] kakugo@sci.hokudai.ac.jp
Akihiko Konagaya[1] kona@dis.titech.ac.jp

[1]Departement of Computational Intelligence and Systems Science,
Tokyo Institute of Technology,Yokohama 226-8502, Japan
[2]Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan

Keywords: Microtubule gliding assay, GPGPU, Simulation, Microtubule Dynamics

Microtubule dynamics is now an area of research interest. With the goal of being able to control the dynamics for use in molecular motors. However gaining a clear understanding from experiments alone can prove difficult. As a solution to this we have developed a 3-D live controlled simulation environment for microtubule swarm dynamics, which is able to run beyond real-time speeds[1]. The simulation is able to deal with very large test cases while maintaining the desired performance, speed, needed for easy 3-D viewing. This was accomplished by using GPU cards, for both general purpose computing and graphical rendering; optimized parallel CUDA algorithms for calculations and DirectX for rendering. With the use of these technologies the simulation is capable of simulating tens of thousands microtubules, while allowing for live control by the user. These include DNA linker interactions between microtubules that can be induced or broken up by the user, a microtubule flow[2] option and collision interactions handled by a Lennard-Jones potential. The individual parameters of each of these can be adjusted live for easy tuning. Custom microtubule placements can also be set up prior to running.

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P5-15

Simulating Dynamic Behaviors in Molecular Arrays

Teijiro Isokawa[1]
isokawa@eng.u-hyogo.ac.jp

Ferdinand Peper[2]
peper@nict.go.jp

Anirban Bandyopadhyay[3]
Anirban.BANDYOPADHYAY@nims.go.jp

Nobuyuki Matsui[1]
matsui@eng.u-hyogo.ac.jp

[1] Graduate School of Engineering, University of Hyogo, 2167 Shosha, Himeji, Hyogo, 671-2280, Japan
[2] Brain Networks & Communication Laboratory, National Institute of Information and Communications Technology, 1-4 Yamadaoka, Suita, Osaka,565-0871, Japan
[3] Surface Characterization Group, Nano Characterization Unit, Advanced Key Technologies Division, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki, 3-5-0044, Japan

Keywords: Molecular array, DDQ, Universal computation

The building of computational elements and their circuits at nanometer-scale feature sizes has gained much attention as fabrication technology has progressed. One promising candidate for a computing system based on molecules uses 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, shortly called DDQ molecules [1]. These molecules are uniformly arranged on an Au (111) surface with a honey-comb structure. A DDQ molecule has four distinct states that are distinguished by the numbers and positions of excess electrons, whereby the state of a DDQ molecule changes as a result of interactions with its neighboring DDQ molecules. Seven types of rules for transitions between molecular states have been discovered. Though it has been shown in [1] that Boolean gates capable of information processing can be constructed based on DDQ molecules on an Au(111) surface, the construction relies on the averaged behavior of a large number of molecules, and it is yet unclear how circuits can be built that connect these gates to each other.

This presentation proposes novel constructions of logic gates based on DDQ molecules on an Au(111) surface. The logic gates use tokens as signals, which are represented as molecules in certain states, and the gates can be connected to each other to form more complex circuits. The circuits are simulated on a computer by software implementing a Cellular Automaton that mimics the behavior of DDQ molecular systems on Au(111) surfaces. We also evaluate the potential for universal computation by the constructed computational elements through simulations.

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P5-16

Small-molecule screening of fluorinated chemical library using SPR, ITC and ^{19}F-NMR in FBDD

Satoru Nagatoishi[1,2]
nagatoishi@bioeng.t.u-tokyo.ac.jp

Sou Yamaguchi[1]
6711877632@mail.ecc.u-tokyo.ac.jp

Keita Kajita[3]
kajita@nard.co.jp

Etsuko Katoh[4]
ekatoh@nias.affrc.go.jp

Hiroyuki Akiyama[5]
akiyama@kishida.co.jp

Satoru Kanai[6]
s-kanai@peptidream.com

Toshio Furuya[6]
furuya@pharmadesign.co.jp

Kouhei Tsumoto [1,2,7]
tsumoto@bioeng.t.u-tokyo.ac.jp

[1] Department of Bioengineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
[2] Drug Discovery Initiative DDI, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
[3] Nard Institute, Ltd., 5-4-1 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan
[4] National Institute of Agrobiological Sciences NIAS, 2-1-2 Tsukuba, Ibaraki 305-8602, Japan
[5] Kishida Chemical Co.,Ltd., 2-11-8, Shin-ohashi ,Koutou-ku ,Tokyo 135-0007, Japan
[6] PharmaDesign, Inc., 2-19-8, Hatchobori, Chuo-ku, Tokyo 104-0032 Japan, Japan
[7] Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Keywords: Fluorine, Fragment-based drug discovery, ^{19}F-nuclear magnetic resonance, Surface plasmon resonance, Isothermal titration calorimetry

Fluorine compounds have had a profound impact on the development of drugs for the modern pharmaceuticals market [1]. Recently, fluorine NMR-based spectroscopy (^{19}F-NMR) has emerged as an efficient tool for performing binding assays. The chemical shift or intensity change of fluorine NMR is especially a powerful marker of the fluorine local environment. This method can become a useful tool for hit validation in primary screening of drug discovery.

In the primary screening, fragment-based drug discovery (FBDD) has emerged as one of the successful approach to design high-affinity ligands for biomacromolecules of therapeutic interest. The interactions between the fragments and a target macromolecule are relatively weak with submilimolar affinity. It is expected that these weak binders provide a starting point for the development of inhibitors with submicromolar affinity. The fragment bindings can be detected using sensitive biophysical techniques such as NMR, surface plasmon resonance (SPR), or isothermal titration calorimetry (ITC) [2].

Here, the FBDD was performed using a chemical library containing a fluorine atom. We have used the extracellular signal-regulated kinase 2 (ERK2) as a model target protein. ERK2 binds to an inhibitor (FR180204) and shows binding responses in SPR and ITC. We performed ^{19}F-NMR, SPR and ITC analysis to obtain the hit compounds binding specifically to ERK2. ^{19}F-NMR and SPR are suitable to use in the primary screening due to the high sensitivity in the NMR signal and the exclusion of false positive signals in the SPR response, respectively. ITC was used to validate thermodynamically the ERK2 binding in hit candidates of NMR and SPR.

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P5-17

Photoactivation of Thermosensitive Ion Channel by Plasmonic Nanoparticles

Tatsuya Murakami[1] murakami@icems.kyoto-u.ac.jp
Hirotaka Nakatsuji[2] nakatsuji.hirotaka.36z@st.kyoto-u.ac.jp
Tomohiro Numata[3] numata@fukuoka-u.ac.jp
Nobuhiro Morone[1] morone@icems.kyoto-u.ac.jp
Shuji Kaneko[4] skaneko@pharm.kyoto-u.ac.jp
Yasuo Mori[3] mori@sbchem.kyoto-u.ac.jp
Hiroshi Imahori[1,2] imahori@scl.kyoto-u.ac.jp

[1] Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan
[2] Department of Molecular Engineering, Graduate School of Engineering, Kyoto University, Kyotodaigaku-Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
[3] Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyotodaigaku-Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
[4] Department of Molecular Biology, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan

Keywords: TRPV1, nociceptive neuron, pain, gold nanorods, high-density lipoprotein

Recently, much attention has been focused on regulation of cellular function by light. Meanwhile, such methods require genetic modification of cells of interest to introduce light-sensitive targets, which limits their diverse applications. One of the strategies to overcome this limitation is targeted cellular delivery of light-sensitive materials. Gold nanorods (AuNRs) are a photothermal conversion nanomaterial capable of absorbing minimally invasive near-infrared (NIR) light. In this study, we focused on photoactivation of a thermosensitive cation channel, TRPV1, in the plasma membrane of mammalian cells by using highly cell adhesive AuNRs.

Electrostatic interaction was utilized for plasma membrane targeting of AuNRs because cell surface is negatively charged. Plasma membrane-targeted AuNRs (pm-AuNRs) were prepared according to the surface chemistry we developed with a cationized form of high-density lipoprotein [1]. Control AuNRs were also prepared by a layer-by-layer assembly method with conventional cationic polymers.

Among the cationized AuNRs prepared, pm-AuNRs were found to bind most adequately to the plasma membrane with the weakest cytotoxicity. Upon NIR illumination of TRPV1-expressing cells bearing pm-AuNRs on the plasma membrane, Ca^{2+} influx was observed. This influx was dependent all on TRPV1 expression, NIR illumination, and pm-AuNRs treatment, clearly demonstrating photothermal activation of TRPV1 by AuNRs. Importantly, similar illumination of the control AuNRs resulted in the plasma membrane disruption with no signs of TRPV1 activation. Our method using pm-AuNRs is an unprecedented cell engineering means for thermosensitive ion channel activation by light [2].

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P5-18

Construction of a DNA Generation Circuit for a Molecular Robot

Ken Komiya[1] **Chizuru Noda**[1]
komiya@dis.titech.ac.jp

Masayuki Yamamura[1]
my@dis.titech.ac.jp

[1] Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology, 4259, Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa, 226-8503, Japan

Keywords: DNA-based state machine, Physiological temperature, Molecular robot

We had developed a DNA-based state machine that allows single-molecule programming [1], and later expanded it into a DNA-responsive DNA-based state machine that implements signal-dependent computation at a physiological temperature [2]. Our state machine requires sequential addition of distinct DNA species for triggering each computational step. We then constructed a single-stranded (ss) DNA generation reaction system that generates distinct ssDNA species [3], for achieving an intelligent molecular robotic system by combining it with the state machine. In contrast to the enzyme-free DNA logic circuits that require elaborate preparation of DNA complexes containing DNA strands as signals [4], DNA generation circuits utilizing DNA polymerase might allow easy preparation and fast operation. Although our ssDNA generation reaction system enables some functions via cascading, including signal amplification at a physiological temperature, it seems infeasible to solely apply it as a control circuit to a molecular robot. For directing our DNA-responsive state machine or other DNA-responsive DNA nano-machines [5], there remain various problems to be solved. In the present study, we report an experimental investigation of the characteristics of DNA generation behavior of our reaction system. And then, we discuss the requirements for integrating our reaction system with DNA-responsive nanomachines. The proposed intelligent molecular system would contribute to a novel means for controlling biochemical process and automation of the common procedure in medical treatment, as a controller embedded in a solution.

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P5-19

Efficient Universal Computation by Molecular Co-transcriptional Folding

Cody Geary[1]
cody@dna.caltech.edu

Pierre-Etienne Meunier[2]
pierre-etienne.meunier@aalto.fi

Nicolas Scabanel[3]
nicolas.schabanel@cnrs.fr

Shinnosuke Seki[4]
s.seki@uec.ac.jp

[1] California Institute of Technology, Pasadena, CA 91125, USA
[2] Department of Computer Science, Aalto University, P.O.Box 15400, FI-00076, Aalto, Finland
[3] CNRS – Universite Paris Diderot (LIAFA), France and IXXI – Universite de Lyon, France
[4] Department of Communication Engineering and Informatics, University of Electro-Communications, 1-5-1, Chofugaoka, Chofu, Tokyo, 1828585, Japan

Keywords: Nano-engineering, Self-Assembly, Co-transcriptional folding, Turing completeness

Self-assembly is an omnipresent phenomenon in which relatively-simple objects (atoms, molecules, cells, etc.) interact with each other locally so as to build up a complex structure without being controlled externally. It has been intensively applied for manufacturing structures at nano scale including logic circuits and molecular robots. It already became possible to design molecules (e.g. square-like DNA tiles) so that they are guaranteed to autonomously combine and assemble a single target nanoscale structure at atomic precision, even in a well-mixed chaotic chemical soup. At this scale, programming matters becomes a significant cost-effective alternative to industrial tools (easier, cheaper, and more powerful).

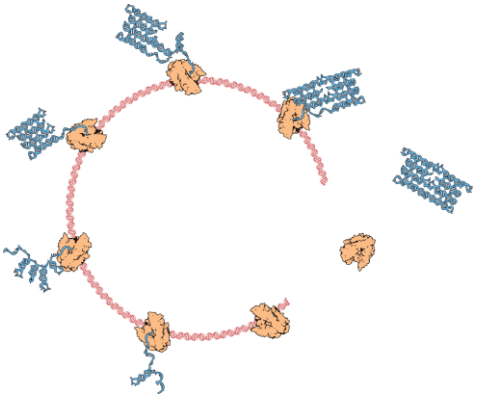

Figure 1. RNA origami [1]. RNA polymerase binds to a DNA template (circular arc), scans it, and transcribes it. The product RNA single-stranded sequence co-transcriptionally folds into a rectangular RNA tile.

Theory of self-assembly has been intensively studied mainly in the abstract tile self-assembly model (aTAM) proposed by Winfree [2] in 1998. It is the self-cohesion of DNA tiles that is studied in aTAM. We have just launched the collaborative research of self-assembly based on an essentially different phenomenon: cotranscriptional folding. Transcription is a process in which RNA polymerase binds to a DNA sequence and produces its RNA copy sequence nucleotide by nucleotide while scanning the DNA template from one end towards the other. Being unstable if being single-stranded, the product RNA sequence starts folding into itself as soon as a few of its nucleotides are transcribed. Using this phenomenon called the cotranscriptional folding, Geary, Rothemund, and Andersen [1] developed an experimental technique called *RNA origami* (see Figure 1) to manufacture nanoscale structures autonomously. In this work, we propose a mathematical model of computation by cotranscriptional folding called *oritatami system*, and prove that it can compute all computable functions efficiently.

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P5-20

Toward Implementation of Cellular Automaton by Encapsulated Chemical Reaction Network

Ibuki Kawamata[1] **Takuto Hosoya**[1] **Satoru Yoshizawa**[1]
kawamata@molbot.mech.tohoku.ac.jp hosoya@molbot.mech.tohoku.ac.jp yoshizawa@molbot.mech.tohoku.ac.jp

Fumi Takabatake[1] **Ken Sugawara**[2] **Satoshi Murata**[1]
takabatake@molbot.mech.tohoku.ac.jp sugawara@cs.tohoku-gakuin.ac.jp murata@molbot.mech.tohoku.ac.jp

[1] Tohoku University, Aramakiazaaoba 6-6-01, Aobaku, Sendai, Miyagi, 980-8579, Japan
[2] Tohoku Gakuin University, Tenjinzawa 2-1-1, Izumiku, Sendai, Miyagi 981-3105, Japan

Keywords: DNA-enzyme chemical reaction network, cellular automaton, molecular robotics

We propose a theoretical reaction-diffusion model that can emulate simple maze-solving cellular automaton [1]. The model is based on experimentally well-characterized bistable switch that is driven by DNA and enzymatic reactions [2]. Assuming a space discretized by hydrogel is our novel idea to realize state transitions of cellular automaton by spatio-temporal chemical reaction system. Since our model employs realistic parameters such as rate constants and diffusion coefficients, it is reasonable to assume that the model can be implemented as real chemical experiment. In the poster presentation, we show *in silico* simulation results and further discuss about preliminary experiments using basic DNA logic gates and alginate hydrogel. Our results indicate that it is possible to program a dynamic chemical reaction system capable of evolving both in space and time. Our programmable spatio-temporal chemical reaction system using DNA has a potential to be a reaction field for molecular robots that may contain sensors and actuators [3].

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P5-21

The development of photochemical DNA toolbox toward construction of intelligence on DNA robotics

Shigetaka Nakamura
S7kmr4gtk6a@jaist.ac.jp

Kenzo Fujimoto
kenzo@jaist.ac.jp

School of Materials science, Japan Advanced Institute of Science and Technology, 1-1 Asahi-dai, Nomi, Ishikawa, 923-1292, Japan

Keywords: DNA robot, photochemical DNA manipulation, DNA strand displacement

The specificity of the A-T and G-C hydrogen bonded Watson-Crick interaction is an emerging scientific discipline that seeks to engineer nanoscale system created out of DNA strand. Based on this DNA specificity, various nanoscale DNA device and DNA structure have been reported. Hence, the research toward construction of DNA robot is done actively all over the world.

The aim of construction of DNA robot is the design and fabrication of dynamic DNA nanostructure that perform specific tasks via a series of state changes. In previous report, dynamic DNA nantechnology often uses toehold-mediated strand displacement and enzymatic method for controlling reaction kinetics.

Attempts to design and implement chemical reaction circuits to serve as computational components in DNA robots. However, this approach is not always appropriate, given the physical properties of the actual chemical reactions. In order to introduce intelligence in molecular robots, it is important to explore various chemical reactions that might be more suitable for DNA robotics.

In our laboratory, various type DNA photochemical manipulations were reported. In particular, We previously reported an artificial nucleoside in which a nucleobase was reported with 3-cyanovinylcarbazole moiety (^{CNV}K)[1]. An oligodeoxynucleotide containing ^{CNV}K can photo-cross-link to a pyrimidine base in complementary DNA or RNA molecules within a few seconds of photoirradiation[2]. This reversible photo-cross-linking is applicable for antisense strategies[3], photo-induced chemical shift change[4], and the construction and stabilization of nanostructed DNA[5]. Now, we study construction of intelligence toward DNA robotics based on photochemical DNA manipulation.

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P5-22

A Loosely-stabilizing Algorithm for Leader Election on Arbitrary Graphs in Population Protocols

Yuichi Sudo[1]
y-sudou@ist.osaka-u.ac.jp

Fukuhito Ooshita[2]
f-oosita@is.naist.jp

Hirotsugu Kakugawa[1]
kakugawa@ist.osaka-u.ac.jp

Toshimitsu Masuzawa[1]
masuzawa@ist.osaka-u.ac.jp

[1] Graduate School of Information Science and Technology, Osaka University
Yamadaoka 1-5, Suita, Osaka 565-0871, Japan
[2] Graduate School of Information Science, Nara Institute of Science and Technology
Takayama 8916-5, Ikoma, Nara 630-0192, Japan

Keywords: Distributed algorithm, Loose-stabilization, Population protocols, Leader election

Recently molecular robotics, which uses molecules as robot components, has received a lot of attention as a new paradigm for artifacts [1]. To realize molecular robots, it is necessary to assemble a large number of molecular devices in a self-organizing manner. That is, each molecular device operates autonomously based on local information (obtained with their sensors), but the molecular devices as a whole should work cooperatively as a robot. This behavior is similar to that of distributed systems, where computing devices operate based on local information but they work cooperatively as a system. For this reason, it is natural to apply distributed algorithms (i.e., algorithms for distributed systems) to molecular robotics.

As a model to design distributed algorithms for molecular robotics, we focus on the *population protocol* (PP) model [2]. In the PP model, devices are passively moved, and two devices communicate with each other only when they come sufficiently close to each other. The PP model is suitable to molecular robotics because typical molecular devices are not fixed and they can sense only nearby devices.

In our previous work, we proposed a loosely-stabilizing leader election algorithm on arbitrary graphs in the PP model [3]. The algorithm ensures that, starting from any initial configuration, exactly one device is elected as a leader within a relatively short time; after that, it is kept as the leader for a sufficiently long time. Such an algorithm is highly reliable because, even if states of devices are changed due to some fault, one device is elected as a leader within a relatively short time. Since molecular devices can fail, high reliability of algorithms is important. However, this algorithm assumes that each device has a unique identifier or can use random numbers.

In this poster, we remove both of the assumptions, that is, we present a loosely-stabilizing leader election algorithm on arbitrary graphs in the PP model without identifiers nor random numbers. That is, our new algorithm can be applied to devices with more restricted capability.

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P5-23

Formation Problems by Autonomous Mobile Robots in the Three Dimensional Euclidean Space

Yukiko Yamauchi yamauchi@inf.kyushu-u.ac.jp
Taichi Uehara taichi.uehara@inf.kyushu-u.ac.jp
Shuji Kijima kijima@inf.kyushu-u.ac.jp
Masafumi Yamashita mak@inf.kyushu-u.ac.jp

Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

Keywords: Swarm of mobile robots, rotation groups, symmetry, formation problem

We consider a swarm of autonomous mobile robots each of which is anonymous and oblivious (memory-less), synchronously executes the same algorithm, and moves in the three-dimensional Euclidean space (3D-space). *The plane formation problem* requires the robots to land on a common plane without forming any multiplicity from a given initial configuration and *the pattern formation problem* requires the robots to from a given target pattern from a given initial configuration.

The pattern formation problem in the two dimensional Euclidean space (2D-space) has been investigated by Yamashita et al. [2] and Fujinaga et al. [1]. They characterized the pattern formation problem by using the notion of *symmetricity* of an initial configuration that shows the symmetry that the robots can never break. The symmetricity of an initial configuration is intuitively the order of the cyclic group of the positions of robots. It has been shown that oblivious fully-synchronous (FSYNC) robots can form a target pattern from an initial configuration if and only if the symmetricity of the initial configuration is a subgroup of the symmetricity of the target pattern.

We extend the notion of symmetricity to 3D-space by using the *rotation groups* that is defined by a set of rotation axes and their arrangement. We define the symmetricity of positions of robots in 3D-space as the set of rotation groups formed by rotation axes that the robots can never eliminate. We show the following necessary and sufficient condition for the pattern formation problem which is a natural extension of the results in 2D-space: Oblivious FSYNC robots in 3D-space can form a target pattern from an initial configuration if and only if the symmetricity of the initial configuration is a subset of the symmetricity of the target pattern. As a corollary, we can rephrase a necessary and sufficient condition for the robots to form a plane shown in [3] as follows: Oblivious FSYNC robots can from a plane if and only if the symmetricity of an initial configuration consists of cyclic groups and dihedral groups. We then show a pattern formation algorithm and a plane formation algorithm for oblivious FSYNC robots.

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P5-24

Stimulus-Responsive Control of Peptide Structure and its Functions Directed toward Molecular Robotics

Shin Mizukami[1,2] smizukami@mls.eng.osaka-u.ac.jp
Masayoshi Kashibe[1] kashibe@molpro.mls.eng.osaka-u.ac.jp
Kazuya Kikuchi[1,2] kkikuchi@mls.eng.osaka-u.ac.jp

[1] Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
[2] Immunology Frontier Research Center, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Keywords: peptide, photoswitch, molecular robot

Recently, lots of attempts to develop a molecular robot based on self-assembly and self-organization have attracted increasing attentions. In order to create a high-performance molecular robot, it is important to develop the individual elements such as sensor, actuator, etc. Therefore, we focused on the development of a molecular robot element, an actuator that shows one of the most basic operation, reversible catch and release. We are developing artificial peptides that reversibly catch and release their specific target molecules in response to external stimulation.

As the external input, light was chosen because light enables the precise control of irradiation area and time, wavelength, repetitive frequency, intensity, and so on. In addition, light is non-invasive if correctly applied. To achieve the synchronized movement of the actuator with the light irradiation, a photochromic compound was utilized. In this study, we are aiming at development of peptide aptamers that reversibly control their affinity to the target molecule.

At the first step, we hypothesized that the affinity of peptide aptamers toward target molecules can be controlled by the structural changes in the scaffold structure induced by light irradiation. As the scaffold, we chose the artificial twisted β-turn structure stabilized by tryptophan (Trp) zipper. It is reported that Trp zipper structure is very stable (T_m = 72 °C),[1] and peptide aptamers based on Trp zipper bound various target proteins with high affinity (K_d: ~50 nM).[2] Their high affinity is considered to be guaranteed by the scaffold structure. Thus, affinity of the peptide aptamer is supposed to be dramatically changed by the structural changes of Trp zipper. In this conference, we will report characteristics of newly synthesized azobenzene derivatives in terms of the kinetic parameters of photoisomerization, and the structural changes of the artificial peptides when the azobenzene moiety was incorporated into the amino acid sequences of Trp zip.

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P5-25

Stability evaluation of DNA origami in living cell

Masahiro Endo[1]
endo@molbot.mech.tohoku.ac.jp

Ibuki Kawamata[1]
kawamata@molbot.mech.tohoku.ac.jp

Shin-ichiro M. Nomura[1]
nomura@molbot.mech.tohoku.ac.jp

Satoshi Murata[1]
murata@molbot.mech.tohoku.ac.jp

[1] Tohoku University, 6-6-01, Aoba Aramaki-Aza, Aoba-ku, Sendai, Miyagi, Japan

Keywords: Molecular Robotics, DNA nanotechnology, DNA origami, HeLa cell, FRET

Recently, the research on using DNA to construct nano divices capable of sensing, computation and actuation has been actively conducted [1, 2]. These devices are expected to apply to nanomedicine, such as DNA nanorobot that transports molecular payloads to cell, taking advantages of high accuracy in nano scale and biocompatibility [3]. Considering the applications as nanomedicine, assessing the stability of DNA device *in vivo* environment is essentially important. The stability of DNA origami, which is widely used substrates for DNA devices, in cell lysate has been investigated [4]. However, the evaluation inside living cell has not been reported yet. Here, we propose a way to evaluate the stability of DNA origami in various environments, especially in a living cell. Fluorescent and quencher molecules are attached at the center of origami and are designed to be apart each other when a part of origami is damaged, by which deformation can be easily detected as a change of fluorescent intensity.

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P5-26

Toward Efficient Computation of Chemical Equilibria of Interacting Nucleic Acid Strands Including Pseudoknots

Mizuki Yokoshima[1] **Satoshi Kobayashi**[1]
kobayashi.satoshi@uec.ac.jp

1 Department of Communication Engineering and Informatics,
Graduate School of Informatics and Engineering,
University of Electro-Communications,
1 – 5 – 1, Chofugaoka, Chofu, Tokyo 182-8585, Japan

Keywords: Computing chemical equilibria, Interacting nucleic acid strands,

The analysis of chemical equilibria of interacting nucleic acid strands is important for the design of chemical reaction circuits using DNAs. Dirks, et al., proposed a method for computing chemical equilibria of interacting nucleic acid strands with pseudoknot-free secondary structures ([1]). However, their method uses a dynamic programming algorithm in couple with a concave programming, which runs in exponential with respect to the maximum length and the maximum number of nucleic acid strands. Kobayashi proposed a new approach, called symmetric enumeration method (SEM), to computing chemical equilibria of complex chemical reaction system ([2] [3]). SEM has been applied to the equilibrium analysis of one-dimensional tile assembly systems ([4]), interacting nucleic acid strands ([5]), and also to kinetic simulation of RNA folding process ([6]). In [5], Kobayashi and Kawakami considered the problem of computing chemical equilibrium of interacting nucleic acid strands under the restriction that the strands will form only linear secondary structures. They showed that the problem can be reduced to a concave programming problem with a set of variables whose cardinality is polynomial with respect to the maximum length and the maximum number of strands. In this poster presentation, we will challenge to the problem of computing chemical equilibria of interacting nucleic acid strands under the restriction that the strands will form only secondary structures which can be generated by simple linear tree adjoining grammars ([7]).

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P5-27

Toward Design and Analysis of Analog Computing Chemical Reaction Circuits

Daiki Matsuwaki[1] **Satoshi Kobayashi**[1]
kobayashi.satoshi@uec.ac.jp

1 Department of Communication Engineering and Informatics,
Graduate School of Informatics and Engineering,
University of Electro-Communications,
1 – 5 – 1, Chofugaoka, Chofu, Tokyo 182-8585, Japan

Keywords: DNA computing, Molecular robotics, Analog circuit

Since Adleman's seminal work on the DNA solution to directed Hamiltonian path problem ([1]), there has been proposed many DNA computing devices and circuits ([2] [3] [4] [5], etc.). Among them, Qian's seesaw gate is one of the promising DNA computing devices toward the scalable DNA computing ([3]).

In order to build up molecular robots, one of the most important properties required for the computing devices is *time-responsiveness* , where we say that a circuit system is time-responsive if, when inputs to the circuit change after the initial computation, the output is re-computed to reflect the new inputs ([4]). Kobayashi, et al., proposed a time-responsive analog computing device using DNA strand displacement reaction ([6]), where they proposed time-responsive thresholding devices and comparators, etc., and showed their behavior by numerical simulation. However, it is not so easy to find the parameters (i.e., concentrations of dominating strand complexes, and toehold length of DNA strand exchange reactions) which make the circuits work in the intended manner. In this poster presentation, we will consider a problem of designing time-responsive analog computing circuits based on the idea proposed in [6], but do not restrict our attention only to DNA strand displacement reactions, but also to any type of general chemical reactions. We then try to design a more complex chemical reaction circuit.

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P5-28

Reconfiguration of molecular device on lipid membrane

Koichiro Katayama[1] katayama@molbot.mech.tohoku.ac.jp
Shinichiro M. Nomura[1] nomura@molbot.mech.tohoku.ac.jp
Ibuki Kawamata[1] kawamata@molbot.mech.tohoku.ac.jp
Satoshi Murata[1] murata@molbot.mech.tohoku.ac.jp

1 Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Sendai, Miyagi 980-8579, Japan

Keywords: DNA nano-engineering, Molecular robotics, Liposome, DNA origami

Controlling cell membrane is fundamentally important for living organisms because the membrane is related to various kinds of rolls like signal transduction and cell reduction. In nature, membrane proteins have various functions from molecular transportation through membrane to mechanical deformation of membrane. There is the field called the artificial cell engineering which realize such functions by synthesized molecules. Various artificial channels made of DNA origami nano-structures have been reported.

Here we propose a novel DNA origami device capable of deforming membrane by external signal. The device attaches on lipid membrane via cholesterol moieties. By adding single stranded DNA as an input, the device transforms from open to close state, which can pull and deform lipid bilayer. In the poster presentation, we will discuss preliminary experimental data observed by atomic force microscopy.

Artificial membrane deformation is essential function to reproduce natural behaviors of the cell. We would be able to control cell membrane as desired in the future. Another application is DNA sequence-responsive liposome. If the molecular devices can break the membrane by its transformation, the liposome release its payload. This expands the possibilities of DDS using liposome and DNA nanostructure.

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P5-29

Construction and functional analysis of DNA origami base DNA-RNAP hybrid nanomachine

Takeya Masubuchi[1] kk147326@mgs.u-tokyo.ac.jp
Hisashi Tadakuma[1] tadakuma.hisashi.8s@kyoto-u.ac.jp
Masayuki Endo[2] endo@kuchem.kyoto-u.ac.jp
Hiroshi Sugiyama[2] hs@kuchem.kyoto-u.ac.jp
Yoshie Harada[2] harada.yoshie.4r@kyoto-u.ac.jp
Takuya Ueda[1] ueda@k.u-tokyo.ac.jp

[1] Graduate School of Frontier Science, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa-shi, Chiba 277-8562, Japan
[2] Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Yoshida-Ushinomiyacho, Sakyo-Ku, Kyoto 606-8302, Japan

Keywords: Molecular robotics, DNA nanostructure, RNA polymerase, gene expression

In the cell, gene expression is highly controlled. To create biologically inspired nanoscale device enabling the control of gene expression, we made hybrid nanomachine (T7-tile) using DNA origami tile as the skeletal structure and T7 RNA polymerase (T7-RNAP) as the functional module (Miyazono et al., *EMBO J*, 2010). T7-tile hybrid allowed us to evaluate the effects of intermolecular distance of enzyme (T7-RNAP) and substrate (target gene containing T7 promoter). We will show our recent achievements.

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P5-30

Synthesis of Cell-Sized Liposomes and Droplets by Centrifugal Capillary Based Microfluidic Device

Masamune Morita[1]
morita.m.ah@m.titech.ac.jp

Masahiro Takinoue[1]
takinoue.m.aa@m.titech.ac.jp

[1] Tokyo Institute of Technology, G3-53, 4259, Nagatsuta, Midori-ku, Yokohama, Kanagawa 226-8503, Japan

Keywords: Liposome, Water-in-oil Droplets, Microfluidic device

We developed a simple generation method of cell-sized liposomes and water-in-oil (W/O) droplets using a centrifugal capillary-based microfluidic device in a tabletop mini-centrifuge [1-3]. Cell-sized liposomes and W/O droplets have been used in a wide range of fields in biochemistry and biophysics. In recent years, microfluidic techniques have been developed to generate cell-sized liposomes and W/O droplets. However, some difficulties hamper the use of a micro channels, such as complicated microfabrication processes to make the micro channels, precise control of the liquid flow in the micro channels using a syringe pumps, and requirement of a large amount of samples into the syringe pumps. Our device was constructed from a microtube, a glass capillary, and a polyacetal holder for the glass capillary. This device does not need complicated microfabrication processes. We demonstrated the production of monodisperse liposomes and W/O droplets from a small sample volume (0.5-5 μL). To test the applicability of our method, we encapsulated a cell-free gene expression system into liposomes and W/O droplets. We believe that this method will assist biochemical and biophysical experiments.

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P5-31

Building molecular robot made of biological molecular motor and DNA-origami

Hiroyuki Fujino[1]
fujino@molbot.mech.tohoku.ac.jp

Ibuki Kawamata[1]
kawamata@molbot.mech.tohoku.ac.jp

Shin-ichiro Nomura[1]
nomura@molbot.mech.tohoku.ac.jp

Shoichi Toyabe[2]
toyabe@m.tohoku.ac.jp

Satoshi Murata[1]
murata@molbot.mech.tohoku.ac.jp

[1] Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Aramaki-aza-aoba 6-6-01, Aoba-ku, Sendai, Miyagi 980-8579, Japan
[2] Department of Applied Physics, Graduate School of Engineering, Tohoku University Aramaki-aza-aoba 6-6-05, Aoba-ku, Sendai, Miyagi 980-8579, Japan

Keywords: Molecular robotics, DNA-origami, F_1-ATPase, Self-Assembly

Realizing autonomous molecular robots is one of the goal of nanotechnology. We will build the micro-scale vehicle composed of the body, the motor, and the helical shaped propeller. The body is the colloidal particle coated Ni^{2+} ion. The motor is the F_1-ATPase which is a rotational molecular motor working in biological cells. The propeller is made of the DNA-origami which is the nanostructures build by programmed self-assembly of a long DNA strand and many short DNA strands [1].

To create the helical shaped propeller, we designed the linked DNA-origami structures. The structures are the bend [2] and twisted [3] cylindrical DNA-origami and connected each other by complement bases. The cylindrical DNA-origami is composed by bundling some helixes of DNA. When you change the number of bases of the helixes partly and adequately, the pressure occurs in the structure so that the cylindrical DNA-origami are bent and twisted. The curvature and the torsion of the propeller are controlled by changing the number of bases added or removed with respect to the cylindrical DNA-origami.

The micro-scale vehicle is assembled by using the selective binding between biotin and streptavidin II, and also Ni^{2+} ion and Histidine-tag. Biotin is modified to the top of the rotator shaft of the F_1-ATPase and the DNA-origami. Histidine-tag is modified to the bottom of the stator of the F_1-ATPase. To demonstrate the assembly, we observed the complex of the colloidal particle and the F_1-ATPase and the streptavidin coated beads, and also the rotation of the proved DNA-origami connected to the F_1-ATPase.

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P5-32

Random Matrix Theory of Rigidity in Protein

Masanori Yamanaka[1]
yamanaka@phys.cst.nihon-u.ac.jp

[1] Department of Physics, College of Science and Technology, Nihon University, 1-8 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8308, Japan

Keywords: Protein, Molecular Dynamics, Random Matrix, Variance-Covariance Matrix, Rigidity, Softness, Time Series

The softness or rigidity is defined by the Young modulus or the Poisson ratio to a macroscopic object. The definition is also valid in microscopic levels such as a macromolecule. Although the measurement is extremely difficult, there have been several approaches to measure the Young modulus of proteins [1,2,3,4]. When we consider the dynamics and fluctuation of a molecule, such definition of the softness of a single molecule or a fraction may be ambiguous in the true sense, because we have to consider dynamics in atomic level. In protein, the dynamics and the softness in this sense are believed to play an important role in folding, molecular recognition, and structural change. One of the definitions of the softness in microscopic level is the root mean square deviation (RMSD) of the normal mode and this has been extensively investigated.

We study the rigidity of protein [5] in microscopic level using the theory of characteristic scale of coupling formation developed in random matrix theory [6]. The eigenvalues and the eigenvectors of the time-dependent variance-covariance matrices, which are calculated from the time series data of the atomic coordinates of a protein produced by the molecular dynamics, are analyzed. We demonstraight the method to a lysozyme, PDBID:1AKI as an example. We find that there are at least three different time scales involved in the coupling formation of correlated sectors of atoms and at least two different time scales for the size of the correlated sectors. In short time scale, there were many atom sectors in which the atoms had a strong correlation inside the sector but almost no correlation among sectors. When making an analogy with classical elastic theory, the protein exhibits a type of plasticity. In long time scales, there are a lot of large sectors of atoms in which the correlation of atoms extends over the whole protein. These features occur simultaneously and their nature is very complicated.

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P5-33

Reaction Diffusion Model to Understand Slime-mold Morphological Reconfiguration

Masahiro Shimizu[1]
shimizu@sys.es.osaka-u.ac.jp

Koh Hosoda[1]
hosoda@sys.es.osaka-u.ac.jp

[1] Department of Systems Innovation, Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama-machi, Toyonaka, Osaka, 560-8531, Japan

Keywords: Reaction diffusion model, Slime-mold, Morphological reconfiguration, Molecular Robotics

The paper deals with reaction diffusion model to understand slime-mold morphological reconfiguration. We specifically modeled by modular robots and discusses a control method by which fluidity of a modular robot is enhanced. A living true slime-mold utilizes “sol-gel transition” so that fluidity is encouraging enough to make “protoplasmic streaming” inside the body. Based on this fact, we so far advanced the modular robot which has decentralized control system consisting of coupled oscillators [1]. It is shown from simulation results that as fluidity is made higher, the modular robot is enhanced in its abilities. Therefore when attention is anew paid to the fact that the control system and mechanical system as direct objectives for design are in existence on the origin of the interaction dynamics, it might be intrinsically proper enough for them to be dealt with as having the equalized weight. We believe that above consideration is indispensable for development methodology for molecular robotics [2] [3].

Acknowledgements. The authors would like to thank Prof. Akio Ishiguro, Tohoku University, for stimulating, and helpful suggestions. This work was supported partially by Grant-in-Aid for Scientific Research on 15H02763 and Innovative Areas “Molecular Robotics”(No. 15H00820) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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