P1-01

Theoretical calculations on proteins with fragment molecular orbital method

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Keywords: FMO, parallel processing, K-computer, geometry optimization, electron density

The fragment molecular orbital (FMO) method has been one of the most successful techniques in calculating proteins in a fully quantum mechanical way based on efficient parallel computations [1]. The ABINIT-MP system is our original FMO program by which a wide variety of applications have been performed, to date [2]. In particular, the four-body corrected calculation (FMO4) [3] is available only in ABINIT-MP, and the Cholesky decomposition (CD) for two-electron integrals [4] can be used for acceleration. This poster will summarize the activities with ABINIT-MP including recent topics.

Recently, ABINIT-MP has become usable on the K-computer as a representative massively parallel supercomputer. High execution efficiency with better than 10% was observed for real proteins with a few hundreds amino acid residues, through the DGEMM processing of CD-based MP2 and HF calculations. It was found that an one-center restriction in CD (1C-CD) is effective in further reducing the computational time. The use of cc-pVDZ basis has been made possible within acceptable cost.

Another new feature of ABINIT-MP has involved the MP2 geometry optimization [5] for crucial ligand-binding regions of proteins. There are sizable differences in interaction energies between PDB structures and MP2-optimized structures. Additionally, the electron densities can now be efficiently generated at the MP2 level for the direct comparison with X-ray data.

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P1-02

FMO-based analysis on DsRed chromophore

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Keywords: FMO, Fluorescence protein, Excitation energy, Protein modeling, Crystallization water

DsRed is a kind of red fluorescent protein (RFP), which was first isolated from *Discosoma* coral ; excitation energy and emission energy are 558 nm (2.22 eV) and 583 nm (2.13 eV), respectively. [1].The crucial pigment of chromophore is the deprotonated *p*-hydroxybenzilidene-imidazolinone moiety formed from three amino acid residues of Gln66-Tyr67-Gly68 [2]. There were various theoretical studies for DsRed, so far, including ours [3,4]. However, the effects from both water molecules and configurations of neighboring residues in the chromophore have not yet been examined enough. Therefore, we have performed a series of calculations based on the fragment molecular orbital (FMO) method in these regards, as will be shown in the poster presentation.

As in Refs. [3,4], the "1ZGO" PDB structure of DsRed [5] was adopted in the present study, and the ABINIT-MP program was used in all FMO calculations. It was found that the existence of two water molecules with hydrogen-bonds to the pigment as well as the the conformation of Ser69 have critical influences on the excitation energy in comparison with the experimental value. The energy decomposition analysis [6] was performed to illuminate the importance of electrostatic interactions in the choromophore.

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P1-03

Efficient Implementation of 3D-RISM Theory to the FMO Method, and Its Applications

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Keywords: FMO, 3D-RISM, Electronic structure calculation, Ligand docking

An efficient implementation of the three-dimensional reference interaction site model (3D-RISM) theory to the fragment molecular orbital (FMO) method was proposed. The method is referred to as FMO/3D-RISM method. The method allows us to treat an electronic structure of whole part of macromolecules, such as protein, as well as a solvent distribution around the solute macromolecules.

The electrostatic potential should be calculated on grid point in the three- dimensional real space witch is used in 3D-RISM theory. Therefore, the reducing the computational cost to calculate the electrostatic potential is most serious concern. In this article, we propose a procedure to save the computational cost for calculating the electrostatic potential in the framework of FMO method. The strategy of this procedure is to evaluate the electrostatic potential and the solvated Fock matrix in different ways, depending on the distance between solute and solvent. In the vicinity of solute molecule, the electrostatic potential is evaluated directly by integrating the molecular orbitals of monomer fragments of solute molecule, whereas that is described as the superposition of multipole interactions by using Taylor expansion of the electronic distribution of monomer fragment.

The results are compared with those from the other methods. We apply the FMO/3D-RISM method to investigate the selective binding of cellulose by cellulose binding module (CBM).

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P1-04

Fragment Based Drug Discovery (FBDD) vs Molecular Evolution: Shared Strategy for the Induction of Substrate/Target Selectivity

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Keywords: FBDD, FABP, Hydrophobic effect, Hydration Thermodynamics

Previously, we computationally investigate the effects of water molecules on the binding energetics of over 20 fragment hits and their corresponding optimized lead compounds.[1] The analysis revealed that fragment hits tend to displace water molecules with notably unfavorable excess entropies relative to those displaced by the newly added moieties of the lead compound during the course of FBDD. The degree of difficulty in displacing hydrations sites by "design" increases as the excess entropy of the hydration site increase. Thus, the water molecules in the biding site can be classified into "difficult-to-replace" and "easy-to-replace" waters according to their excess entropies. There is a clear trend that the "difficult" waters are displaced by the fragment hits and the leads eventually evolved from them by displacing the "easy" water molecules.

Mitochondrial β-oxidation of long chain fatty acids (LCFAs) is a key metabolic pathway for energy generation in the skeletal and heart muscle. Transporters and carrier proteins, including fatty acid-binding proteins (FABPs), are required for the efficient cytosolic delivery of highly hydrophobic LCFAs. However, it remains unclear how such proteins recognize LCFA's flexible alkyl chains of different length (c10 – c18) that do not exhibit defined structure or noticeable electrostatic interactions. Recently, we suggested that the two clusters of water molecules found in the FA binding pocket might play a crucial role in the recognition of substrate FAs by FABPs.[2] Our investigation of the thermodynamic properties of the two water clusters revealed their distinctively different thermodynamic profiles.[2] Cluster 1 contains a well-defined hydrogen bonding network of well-ordered, "difficult", water molecules with highly unfavorable excess entropies. On the other hand, Cluster 2 consists of relatively disordered, "easy", water molecules. FABP's selectivity towards the substrate FAs can be explained by considering the preferential displacement of "easy" Cluster 2 water molecules. The entropy of the binding site water molecules plays the key role in these two seemingly unrelated processes.

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P1-05

Prediction of Protein-ligand Binding Affinities Using Molecular Simulations (II)

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Keywords: molecular dynamics simulation, protein-ligand binding affinity

Accurately predicting the binding affinities of small-molecule ligands to target proteins is one of the ultimate goals of structure-based drug design. A variety of computational methods have been developed and tested in reproducing experimental binding data for different target systems. The computational methods range from molecular docking to free energy calculations using molecular dynamics (MD) simulations.

In this study, we have studied the calculation of protein–ligand binding affinities by using MD simulations with external perturbations. Especially we focused on the steered MD simulations[1-3]. Steered MD simulation induces unbinding of ligand and conformational changes in protein on time scales accessible to the simulations. Time-depepndent external forces are applied to a system, and the responses of the system are analyzed. We have evaluated protein-ligand binding affinities using steered MD simulations toward the practical use of the drug discovery.

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P1-06

Toward precise determination of binding free energy from molecular dynamics simulation

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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. Since 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], we can smoothly set to work on the various case studies. Unfortunately, however, it is not always easy to obtain successful results. Comparing the simulated results with experimental one, we often disappointed the poor correlation among them. Thus it seems that the blind use of the result from the molecular dynamics simulation is not yet guaranteed. What is a reason for such discrepancy between numerical and experimental results? Maybe there are many possibilities, e. g. incorrectness of the force fields, incorrectness of classical mechanical description, limited length of simulation time scale, and so on. Here we will focus on checking how precisely we can calculate the free energies for a given force field. For this purpose, we will compare different protocols for the calculation of the free energies. Such research could be a inevitable fundamental step before entering many other issues.

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P1-07

How does water in the binding site affect intermolecular interaction between a protein and a ligand?

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Keywords: Intermolecular interaction, Binding affinity, Water, Polarization, FMO

We investigated the effects of water in the binding site on interaction energy between a protein and a ligand from the viewpoint of enthalpic bridging.

We selected human coagulation factor Xa and some ligands (RRP, RTR, RDR, RRR) as the test system of this study[1]. Three of these ligands (RRP, RTR, RDR) contain chlorobenzothiophene, which is placed inside of the binding site, while RRR contains chlorothiophene at the corresponding position. Firstly, we calculated interaction energy between each ligand and factor Xa using Fragment Molecular Orbital (FMO) method[2]. Considering the polarization effect caused by protein-ligand binding, the calculated interaction energy and the experimental binding affinity show a correlation except for the ligand RRR. If the displacement of water in the binding site[3] explains the exception, entropically unfavorable water should exists in the vicinity of chlorobenzothiophene; however, it does not exist in the vicinity of chlorobenzothiophene but chlorothiophene[4], so the displacement of water does not explain the relatively high affinity of RRR. Therefore, we focused on the role of the water to build a weak bridge between RRR and factor Xa. We calculated interaction energy between RRR and the water in the vicinity of chlorothiophene in addition to the one between RRR and factor Xa. The total interaction energy and experimental binding affinity show an excellent correlation (R^2 = 0.95). This result indicates that the specific water molecules in the binding site have a role to enforce a protein–ligand binding affinity by bridging the space between a ligand and a protein.

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P1-08

Binding Mode Analysis of Protein Kinase CK2 Inhibitors with a Purine Scaffold

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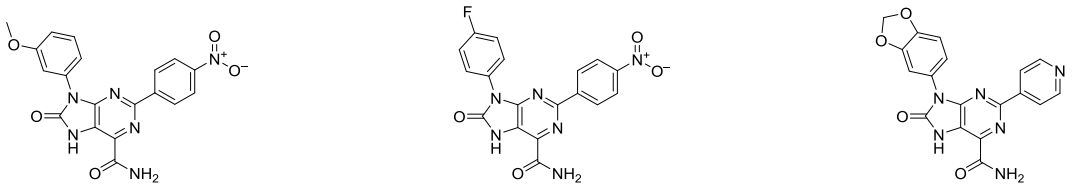
Keywords: Protein kinase CK2, docking study, MM-PBSA, AMBER11

Protein kinase CK2 (CK2) is a serine/threonine kinase that employs either ATP or GTP as a coenzyme. CK2 is a potential target protein for treating a wide variety of tumours and glomerulonephritis, since it is involved in regulation of growth, proliferation, and the survival of cell.[1] Several compounds with a purine scaffold were discovered through a virtual screening (Figure1). Binding modes of these compounds were elucidated by a docking study and binding energies were calculated using the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) method. The docking study was performed by using MOE2013 and binding energies were calculated using the MMPBSA.py script of AMBER11. The predicted binding modes showed that the polar groups such as nitro group, carboxamide group, and carbonyl group of purine scaffold might interact with Lys68 or Val116 of CK2. The calculated binding energies are well correlated with the experimental inhibitory activities (Table1).



Compound1 Compound2 Compound3

Figure1. Compounds discovered through virtual screening

Table1. Binding energies calculated by the MM-PBSA method (kcal/mol)

Compound	ΔG_{bind}	ΔE_{vdW}	ΔE_{ele}	ΔG_{PB}	$\Delta G_{non\text{-}ele}$	IC_{50} (μM)
1	-48.6	-49.4	-8.9	13.6	-3.8	6.1
2	-48.8	-48.4	-8.9	12.2	-3.4	3.3
3	-45.4	-45.8	-5.8	9.6	-3.6	19.6

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P1-09

Biomolecular Computational Simulations and Organic Synthesis: Prediction of the Enantioselectivity of Lipase-Catalyzed Biotransformations

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

Lipase-catalyzed biotransformatios have recently been utilized as a useful method in the synthesis of enantiomerically pure and biologically active organic compounds [1]. We have found in the recent literature that computational simulations have become gradually useful as a research method in the fields of organic chemistry and bioscience. The present effort involves a continuation of our interest in biomolecular computational simulations toward predicting the enantioselectivity of enzyme lipases in organic synthesis, such as *Burkholderia cepacia* lipase (BCL) and *Candida antarctica* lipase typeB (CALB) [2,3].

BCL- and CALB-substrate esters complexes in the presence of TIP3P water molecules were subjected to MD calculations over a period of 2000ps with ABMER11, and the C-O interatomic distance ($R_{C\text{-}O}$) between the carbonyl carbon of esters and the oxygen of the active site amino acid residue (BCL: SER87, CALB: SER105) side chain OH in each lipase complex was examined during the MD trajectory. After MD calculations, surrounding water molecules were removed and for the resulting lipase complexes FMO calculations with ABINIT-MP/BioStation program were carried out at FMO2-MP2/6-31G level.

MD calculations show that $R_{C\text{-}O}$ for the fast reacting enantiomer of substrate esters remains roughly unchanged during the computation, while $R_{C\text{-}O}$ for the slow reacting enantiomer of esters increases with the elapsed time. In addition, FMO computations indicate that for esters with high enantioselectivity, each fast reacting enantiomer shows strong interactions with some particular amino acid residues including a definite amino acid residue in each lipase, that is, HIS286 in BCL and THR40 in CALB, whereas for esters with low enantioselectivity, both (*R*)- and (*S*)-enantiomers interact with identical amino acid residues including HIS286 and THR40. We can safely say that HIS286 in BCL and THR40 in CALB play an important role in the chiral recognition of enantiomers using each lipase. Biomolecular computational simulations enable us to predict and to understand the reactivity and the enantioselectivity of lipases in organic synthesis.

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P1-10

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

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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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P1-11

Structural Recognition of Local Anesthetic Enantiomers in human Kv1.5 Channel Pore

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Keywords: Molecular Recognition, human Kv1.5, Local anesthetics, Enantiomer

Lethal arrhythmia and cardiac arrest are known as local anesthetic toxicity. Less toxic with enantio-selective ropivacaine and levobupivacaine are used in clinical practice. From animal experiment, cardiac toxicity increases ropivacaine (ropi) < *(S)*-bupivacaine (bupi) < bupi < *(R)*-bupi order [1]. Inhibition of potassium channel has some contribution to cardiac toxicity [2]. We choose here human Kv1.5 voltage-gated channel as a potassium channel model. *(R)*- and *(S)*-bupi bind to hKv1.5. (Kd=4.1, 27.3 uM, respectively), and *(R)*-bupi inhibits Kv1.5 current strongly [3]. I, V and A mutants of T507, L510S and V514S mutants do not have enantiomeric difference of the inhibition [3]. T507, L510, V514 residues at the bottom of filter region in pore contribute to enantiomeric difference of bupi inhibition. This study investigates enantiomeric local anesthetic binding to hKv1.5 channel using docking simulation, and surveys mechanisms of molecular recognition of R and S local anesthetic.

Method: The structure of the Kv1.2 channel from the Protein Data Bank (PDB:A79, 2.9 Å resolution) [4] was used for homology modelling. ASEDock2013.11.14 program [5] (Ryoka Systems Inc., Tokyo) of the MOE platform was used. Dissociated local anesthetic (LA's), lidocaine (lido+), mepivacaine (mepi+), enantiomer of ropi+ and bupi+ were docked Kv1.5 channel.

Results: (1) LA's bound around center of four T479 residues in pore region. (2) Binding energies (Udock kcal/mol) were *(S)*-bupi+(#59:-13.55) > *(S)*-ropi+(#35:-14.13) > mepi+(#1:-15.46) > *(R)*-ropi+(#3:-16.18) > *(R)*-bupi+(#1:-17.29) > lido+(#1:-17.97). (R>S) (3) Interactions of T479, hydrophobic residue, T507, L510 and V514 with LA's in pore were found. (4) Molecular recognition of LA's was determined by fitting of three groups around chiral nitrogen atom of amide LA's to biding site. R-body hydrophobic interactions of butyl- and propyl- to binding site augment to binding force and Kv1.5 inhibition.

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P1-12

Analysis of Barnase-Barstar Interactions with Interfacial Mutations, based on 3D-RISM Method

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Keywords: Protein-Protein interaction, 3D-RISM method, Barnase-barstar complex

Protein-protein interactions (PPIs) have a key role in numerous biological processes and are deeply concerned with the many diseases. For understanding the binding mechanism of PPIs and modulating these interactions by small molecules including water, quantitative information like binding energy is required. Toward the computational methods for quantitative analysis to predict the binding affinity of PPIs, we applied the 3D-RISM method [1], which is the statistical mechanics theory for liquid, to barnase-barstar complex. The 3D-RISM method is a powerful tool for studying solvation thermodynamics and properties of a solvation structure successfully. The barnase-barstar complex is one of the widely used PPI systems for investigating the protein-protein binding affinity, because they bind fast and with high affinity without drastic induced fit. Additionally, it was found that interfacial water molecules significantly contributed to the binding free energy.

In this study, we first comprehensively generated the 3D-structure models of 65 barnase-barstar complexes. We second calculated the binding free energy from these structures to compare with experimental values [2,3]. The desolvation energies on binding of each unit are obtained by 3D-RISM method and other energies (non-bonding and relaxation) are obtained by molecular mechanics (Amber99). As an instance, the distribution function of water at interface of wild-type complex was shown in Figure 1. As shown in this figure, the water molecules are concerned with the binding for complex. The distribution function reproduced the location of water molecule in X-ray structure including number of molecule.

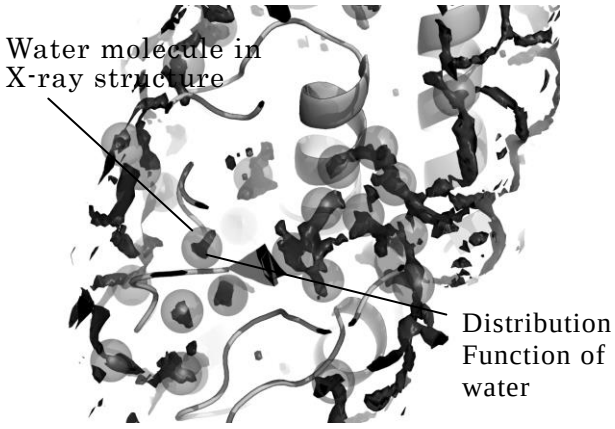

Figure 1. 3D distribution function of water between the in interfaces of wild-type complex.

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P1-13

Development and cellular application of novel streptavidin-binding small molecules

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Keywords: Protein-small molecule interaction, High-throughput screening, Fluorescence imaging

The interaction between streptavidin (protein from *Streptmyces avidinii*) and biotin (small molecule ligand) is known as the strongest non-covalent interaction that exists in nature (K_d = 10^{-13} M) [1]. Streptavidin-biotin system is used for many applications in biotechnology, such as affinity column chromatography (isolation), solid-phase sensing device (immobilization) or histochemical imaging (detection). However, for some applications, disadvantages of this system have been pointed out such as almost irreversible binding under physiological conditions. To disrupt a streptavidin-biotin complex, it is often necessary to denature the streptavidin under very harsh conditions, which destroys functions of many biomolecules. Therefore, in this research, we screened a large chemical library to search for novel chemical scaffolds that mildly bind to (and dissociate from) streptavidin, then aimed to apply the hit compounds to biological experiments.

First, we developed an assay system based on fluorescence polarization for the screening of ligands of egg avidin, which has identical tertiary structure with streptavidin. More than 160,000 compounds were screened, and we selected 773 compounds as primary hits. These compounds were subsequently screened for streptavidin, dose-dependently assayed, and analyzed by SPR (Surface Plasmon Resonance). As expected, several biotin analogs were detected among the hits, but we excluded these compounds. Finally, 6 compounds were selected as novel ligand scaffolds for streptavidin which exhibited fully reversible binding to the protein (K_d = 10^{-4}~10^{-6} M). We then proceeded to characterize the binding mode of the compound that showed the strongest affinity in detail. Based on information from X-ray crystal structure and derivative synthesis, the amide moiety in the 6-membered ring was most likely to be essential for the binding. Moreover, we applied the compound to provide reversibility to the recently-reported protein transport system inside cells [2]. When biotin was added to the system, protein transport occurred but it was irreversible because of its high affinity. In contrast, when our novel compound was used, the target protein moved back to the original positions after washout of the compound. We expect that this strategy may be a general method to control protein localization reversibly at a given time.

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P1-14

Molecular dynamics simulations of ATP or ADP bound form of SR Ca^{2+}-ATPase using CHARMM force field with updated polyphosphate parameters

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Keywords: sarcoplasmic reticulum Ca^{2+}-ATPase, force-field optimization, ATP-Mg^{2+} interactions

Sarcoplasmic reticulum (SR) Ca^{2+}-ATPase transports two Ca^{2+} from the cytoplasm into the SR lumen utilizing ATP hydrolysis. It is fundamental to know how ATP/ADP can bind stably in the cytoplasmic domains of the ATPase. We performed all-atom molecular dynamics (MD) simulations of the ATPase in the ATP/ADP bound forms [1, 2] with explicit solvent and a DOPC lipid bilayer. We observed the phosphates structure in the bound ATP changed rapidly into the extended form using the original CHARMM force field. After some modifications of the force field following the CHARMM protocol [3, 4], we could simulate the protein stably [5]. We examine the essential interactions between ATP bound with Mg^{2+} and the ATPase and discuss the relationship between the simulation results and experimental data.

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P1-15

Proposal for novel inhibitors to acetyl- and butyryl-cholinesterases by *ab initio* molecular simulations

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Keywords: Protein-ligand docking; molecular mechanics; molecular orbital calculation; inhibitor; acetylcholine

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) play important roles in the hydrolyzing mechanism of neurotransmitter acetylcholine (ACh). Recently, AChE and BChE have attracted much attention because of the possibility that they may contribute to ACh hydrolysis in the brain of Alzheimer's patients [1]. Since an adequate amount of ACh is contained in intact brains, the hydrolysis of some ACh causes no serious problem in living organisms. In contrast, the brain of a person diagnosed with Alzheimer's disease usually has a considerable shortage of ACh, so that the transmission mechanism of information between nerve cells is damaged. It is thus expected that if the hydrolysis reaction of ACh by AChE and BChE can be inhibited in the brain of Alzheimer's patients, the amount of ACh in the synapse will not be decreased so significantly and the transmission mechanism will become smoother. Many types of ligands have been produced for inhibiting the hydrolysis reaction by AChE.

In the previous study [2], we investigated the specific interactions between AChE and several carbamates and proposed some potent inhibitors for AChE, using molecular simulations based on classical molecular mechanics (MM) and *ab initio* fragment molecular orbital (FMO) methods.

In the present study, we investigate the specific interactions between BChE and some types of ligands, whose biochemical properties were investigated by our previous experiments [3], using the same molecular simulations. Based on the results simulated for AChE and BChE, we attempted to propose novel ligands, which have strong binding affinity to both AChE and BChE. The details of the results will be shown in the conference.

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P1-16

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

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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 further study on how accurately the binding energy of the protein-ligand systems by using FMO method. We tested the binding energies with some computational conditions for FMO method and have validated the computational conditions for molecular design toward the practical use of the drug development [4].

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P1-17

Molecular Similarity Based on Electronic Interaction Topology

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Keywords: Electronic structure calculation, molecular similarity, molecular shape

Prediction of biological activity of compounds is essential for drug discovery. One of the promising methods for this purpose is to investigate "structural similarity" among molecules because it is believed to be directly related to biological activity.[1] In this study, we would like to introduce another type of similarity which can reflect characteristics of intermolecular interactions exerted in biological systems. Herein, we suggest a method defining a shape of a molecule, which should reflect topological features of hydrogen-bond interactions.

In our approach, molecular shape is defined in two steps: one is geometry optimization of a target compound, and another is the calculation of the interaction energy. The geometry of the target molecule was optimized at the DFT(B3LYP)/6-31G** method. The three-dimensional gird for potential-energy-surface (PES) mapping, the target compound is placed at the origin of the Cartesian coordination. Then, grid points for proton are generated. At each grid point, the interaction energy between the target molecule and the probe (proton) is evaluated by using the PM6 method. These calculations were implemented with GAUSSIAN09.

For demonstration, we discuss about the application of the present method to investigate topological similarity between glucose and psicose. The latter compound attracts recent interest because of its biological activity for suppressing absorption of glucose in the small intestine.[2]

The molecular shape of psicose was calculated as follows. Here we focus on the ring-form compound of glucose and psicose called β -D-glucopyranose and β -D-psicopyranose, respectively. For these structural isomers, conformation search was carried out by using the CONFLEX software in order to find stable conformers within energy tolerance of 100 kcal/mol. Further selection of conformers was made by referring to the Boltzmann distribution in the compound space. Then the DFT method was applied to finally determine the most stable conformer. Its PES calculated using the PM6 method is shown in Fig. 1. It is seen that proton is energetically stabilized when it is close to the oxygen atoms (oxygen lone pairs) of the target compound. In the poster presentation, we will discuss about quantitative molecular similarity on the basis of the molecular shapes obtained with the present method.

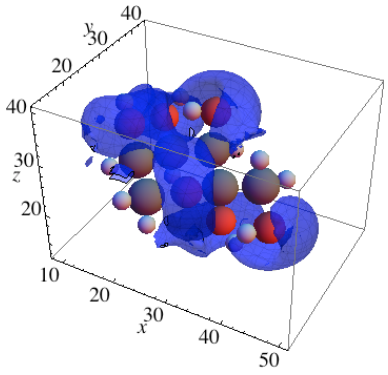

Fig.1 Molecular shape of β -D-psicopyranose

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P1-18

Electronic-Structure Data Mining For Molecular Property Analysis

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Keywords: Electronic-structure calculation, Data mining, Structure-property relationship

Computational chemistry is one of the promising methods for drug discovery. Among a variety of computational methods, the electronic-structure calculation is expected to be insightful for understanding structure-property relationships of a molecule, because both its structure and property are essentially determined by its electronic structure. In this study, we aim at developing an analytical method for data mining which would be useful for molecular design and discovery in Chemistry and Pharmaceutical Sciences. Herein we will also discuss its application to bio-related compounds.

We have developed a computer program that automatically discovers a mathematical correlation function between an electronic descriptor and a chemical property. Such a correlation is revealed through a variety of fittings of experimentally measured values for various electronic factors such as orbital energy levels, ionization energies, reorganization energies, and so on. A correlation between an experimental and theoretical parameters is automatically discovered by our computer program in which versatile fitting functions are applied: they consist of polynomial fittings, the Gaussian fitting, exponential functions, the Rayleigh distribution, sigmoid functions, the Box-Lucas model function, and power law functions as shown in Fig. 1. Throughout such fittings we can know a hidden mathematical relation which may be called “data mining”.

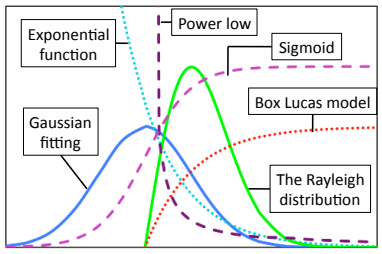

Fig. 1 Mathematical functions applied for data mining

In this presentation, an application of the above computational method will be discussed for an organic pigment for bio-imaging. Herein we focus on a derivative of fluorescein which is called TokyoGreen (TG) [1]. We have tried to discover an electronic factor that is well correlated with experimental fluorescent quantum yield of TokyoGreen derivatives. For this purpose, we have investigated electronic factors discussed above. After a large number of function fittings, it has been shown that the best fitting is obtained when the orbital energy of the molecular orbital localized on the benzene moiety is chosen as a descriptor (Fig. 2). This computational finding is consistent with the conclusion obtained by Urano et al.

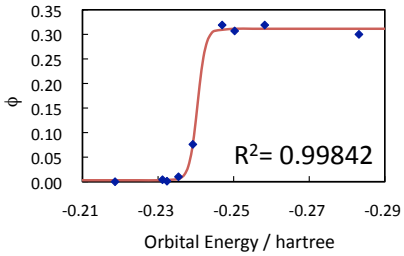

Fig. 2 Computationally discovered correlation function for the fluorescence quantum yield (ϕ) of TGs. The horizontal axis was computationally suggested as the benzene-localized orbital energy.

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P1-19

Classification of Bioisostere Based on Similarity Evaluated by Electronic-Structure Calculation

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Keywords: Quantum chemistry, Electronic structure calculation, Molecular similarity

Discovery of similar molecules is one of the key processes in Pharmaceutical Sciences. It has been believed that similar molecules should be similar in their functionality. One of the interesting ideas for drug discovery is "bioisosterism". This is a concept useful for chemical-substructure replacement. Its knowledge is indispensable in lead optimization. Compounds showing similar biological activity are called "bioisosteres". The classification seems essentially empirical, so that its *a priori* deviation seems challenging. This work has been motivated by a simple question: can quantum chemical similarity be a good measure for bioisosterism ? Herein we apply our method of quantum-chemical similarity[1] for classifying molecules, which is expected comparable to bioisosterism.

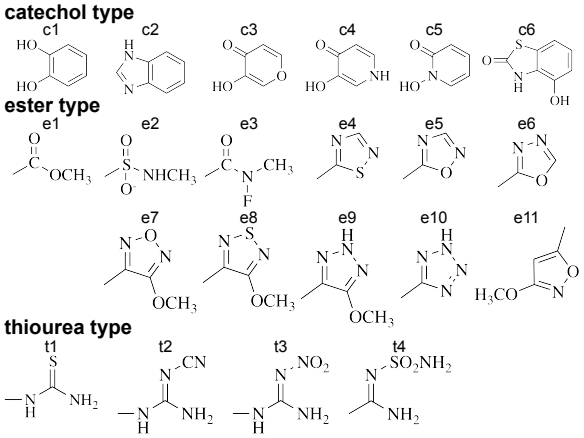

Fig. 1. Chemical structures of molecules investigated in this work.

We have studied 21 molecules in Fig. 1. It includes bioisosteres of catechol, ester, and thiourea. Their electronic ground states and vibrational states are evaluated using the DFT(CAM-B3LYP)/6-31G(d,p) method. In order to obtain overall electronic spectrum, electronic excited states are calculated using TDDFT(CAM-B3LYP)/6-31G(d,p) method. Throughout these calculations, we evaluate "electronic similarity", "vibrational similarity", "quantum chemical similarity", and structure-based similarities. Based on numerical values of these similarities, a statistical analysis method called cluster analysis is applied. The result is compared with the bioiosteres defined in Ref. [2] where correct answer rate (CAR) is evaluated.

Cluster analysis and CAR evaluation were performed in every possible combination among the above-mentioned descriptors. Comparison shows that CAR is large when either of HOMO, Raman spectrum, or molecular geometry is chosen as a descriptor: the CAR for the HOMO descriptor is 62.5 %. A cluster analysis for the catechol bioisostere successfully includes c1, c2, c4, c5 and c6 given in Fig. 1, while only c3 is excluded in the cluster. This reasonable agreement between our similarity-based classification and the empirical bioisostere is encouraging for further study. In the poster presentation, we will discuss about more extensive cluster analysis for other bioisosteres.

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P1-20

Dynamics of the Large Progenitor Toxin Complex of *Clostridium Botulinum*

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Keywords: Normal mode analysis, Molecular dynamics, Principal component analysis

Clostridium Botulinum produces one of the potent toxins called a botulinum neurotoxin and several proteins called a non-toxic component. The non-toxic components consist of a non-toxic non-hemagglutinin and a few hemagglutinins and can form various stoichiometric complexes with botulinum neurotoxins. As the complex becomes larger, the oral toxicity tends to be stronger and one of the complexes is referred to as a large progenitor toxin complex (L-PTC). Recently, the three-dimensional structure of the L-PTC has been revealed by X-ray crystallography and electron microscopy [1,2,3]. However, most of the dynamics of the L-PTC are unclear. Therefore, in this study, to estimate the dynamics of the L-PTC, three analyses are conducted by constructing three types of computational models, each of which has a different force between particles, and then solving an equation of motion. The first analysis called normal mode analysis with anisotropic network model considers a harmonic potential between particles and gives an analytical solution of the differential equation [4]. The second and third analyses are molecular dynamics with united-atom and coarse-grained models, respectively [5,6]. These analyses consider both harmonic and non-harmonic potentials between particles and give a numerical solution of the differential equation. As the result, when we compared the three analyses after a principal component analysis was performed to each of the trajectories of the molecular dynamics, a characteristic motion of the L-PTC was obtained. We are presently considering associating the characteristic motion with the oral toxicity.

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P1-21

Binding Model Construction of GPCRs Specifically Activated by Lysophosphatidylserine

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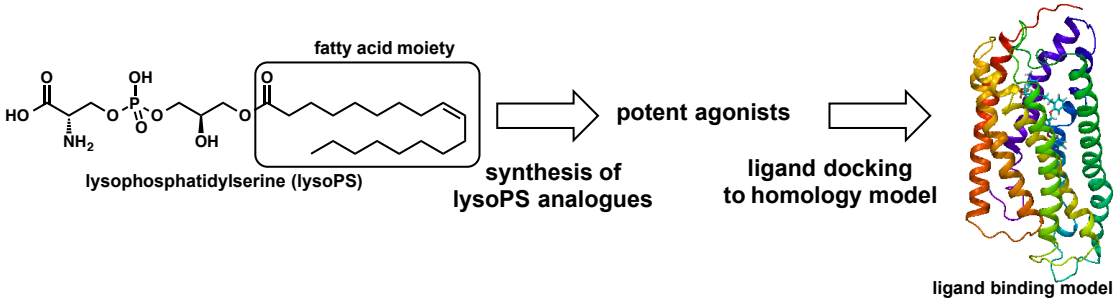
Keywords: G-protein-coupled receptor, Lysophosphatidylserine, Homology modeling, Ligand docking

Recently, three orphan G-protein-coupled receptors, GPR34, P2Y10 and GPR174 have been shown to recognize lysophosphatidylserine (lysoPS) as an endogenous ligand, a kind of lysophospholipids generated from plasma membrane lipid, phosphatidylserine (PS).[1, 2] These receptors are suggested to control immune system, but their detailed biological roles are still unknown. To design and synthesize active and/or subtype-selective agonists as useful tools to study the functions of these receptors, we aim to explore how lysoPS and its analogues bind to the receptors. In this study, we will propose plausible binding models, which are virtually consistent with the experimental structure-activity relationships.

LysoPS consists of three parts, a polar head group (phosphoserine), a hydrophobic tail (fatty acid) and a glycerol linker connecting them. In present modeling, we focused on the effect of fatty acid moiety such as structures of non-lipid surrogates. Homology models were constructed and active compounds were successfully docked into the model. Stability of these binding models was evaluated using molecular dynamics simulation. We found plausible hydrophobic pockets, which are thought to accommodate the non-lipid fatty acid surrogates effectively.



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P1-22

Random Matrix Theory and Principal Component Analysis of Protein-Ligand Interactions by Using Molecular Dynamics Simulation

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Keywords: Molecular dynamics, All atom MD with solvent, Random matrix theory, Principal component analysis, Protein-ligand interaction, Correlation matrix, Variance-covariance matrix

We apply the random matrix theory [1,2,3] and the principal component analysis (PCA) to study explicit and hidden interactions between protein and ligand. The time series data of motion of atoms for proteins-ligand complex which is produced by all atom molecular dynamics simulation [4] with solvent is used.

The PCA is a multivariate technique that analyzes a data table in which observations are described by several inter-correlated quantitative dependent variables. Its goal is to extract the important information from the table, to represent it as a set of new orthogonal variables called principal components, and to display the pattern of similarity of the observations and of the variables as points in maps.

In the PCA, the first several components are considered to be meaningful as the collective motion of protein and the higher order components are discarded in usual. It works well when the size of the collective domains is fairly big. When the size of the domains are small, for example, the protein-ligand interaction, we should take into account the higher order PCA components and reduciton of the noise is essential. The Inverse Participation Ratio analysis in the random matrix theory gives a criterion to distinguish the meaningful higher components from noise in PCA, which enables us to reduce the noise. We also calculate the fundamental statistical quantities which characterize the universality class in the random matrix theory. We find that the eigenvalue spacing agree well with the Gaussian orthogonal ensembles. Our method is an attempt to improve and refine the principal component analysis of protein domain [5]. As an example, we demonstrate our method to PDBID:2B18 to study the protein-ligand dynamical interactions.

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[3] The first attempt to the protein is reported by R. Potestio, F. Caccioli, and P. Vivo, Phy. Rev. Lett. 103, 268101, 2009 by the solvable elastic networks models. Here, we perform all-atom molecular dynamics simulation with water.

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P1-23

Benchmark for new fragmentation breaking peptide bonds

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Keywords: FMO method, fragmentation method, peptide bond, protein-ligand complex,

In the Fragment molecular orbital (FMO) method [1-3], the way of fragmentation directly influence the accuracy of calculation. Intra-molecular fragmentation has been usually done at the position of sp3 carbon as the bond detached atom (BDA). In such a manner, fragmentation of protein broke bonds between alpha carbon and carbonyl carbon of main chain, and therefore units of fragment and amino acid were different. Such inconsistency has caused misinterpretation of the inter-fragment interaction energy (IFIE) when a carbonyl group of main chain was important for residue-residue or residue-ligand interactions. Here, we examined benchmark calculations of new fragmentation which breaks peptide bond to conform units of fragmentation to amino acid one.The systems used for this benchmark were two peptides (polyalanine; (Ala)n, n=1-10, and chignolin) and two protein-ligand complexes (estrogen receptor and influenza virus neuraminidase with their ligand). Accuracy of fragmentation in total energy was acceptable by using many-body expansion of FMO method (FMO3/FMO4) [4,5].

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Prediction of activity cliff among Pim1 inhibitors using FMO and MM-PBSA calculations

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Keywords: FMO, MM-PBSA, Activity cliff, Serine/threonine kinase Pim1

We have predicted activities among serine/threonine kinase Pim1 inhibitors (compounds **1** to **6**, as seen in figure) [1] based on fragment molecular orbital (FMO) method in combination with molecular mechanics-Poisson Boltzmann surface area (MM-PBSA) scheme [2]. Nakano *et al.* [1] has reported that activities of five compounds including 7-azaindole derivative (compounds **2** to **6**) are shown various values. The compound **2** is a potent inhibitor as well as **1** including indole ring, however, compound **6** shows 200-fold decrease in potency by comparison with **1** and **2**. This result is a typical example of "activity cliff", because the slight difference in structure among the inhibitors with only replacement of a carbon atom with a nitrogen one and its position. Additionally, X-ray structures of complexes with compounds **1**, **3**, **5** and **6** are almost no change in conformation of pharmacophore, the origin of variation in inhibition remains enigmatic. Since dramatic change in activity are caused by the difference of electronic state derived from the replacement of atom, an approach by only MM calculations is not sufficient to predict the activities and explain the origin. Hence, we examine the prediction of activity by using FMO and MM-PBSA calculations.

In this study, we estimate a binding free energy ΔG^{bind} as a sum of interaction energy ΔE^{int} *in vacuo*, deformation energy of ligand in its complex form from free state ΔE^{def}, and solvation energy ΔG^{solv}. FMO calculations with many-body correction at FMO3-MP2/6-31G* level were achieved for the complexes with the protein divided into main/side chain of amino acids and each ligand into two functional parts by using ABINIT-MP/BioStation Viewer [3]. The ΔE^{int} was here estimated as a sum of inter-fragment interaction energy between Pim1 protein fragments and each inhibitor. On the other hand, the ΔE^{def} and ΔG^{solv} were obtained from MM-PBSA calculations with AMBER10. Moreover, we prepared three structures of the complex, which are X-ray crystal structure and MM and QM/MM optimized structures based on template using complex between Pim1 protein and compound **1**, and verified the appropriateness of construction protocol. The ΔG^{bind} obtained from the FMO-PBSA calculation are highly correlated with $log\mathrm{IC}_{50}$ more than that of MM-PBSA. In addition, the result of the QM/MM optimized structure is preferable to that of the X-ray and the MM optimized structures. This approach to prediction activity will be helpful tool to rational drug design.

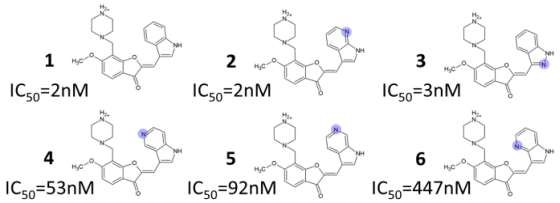

Figure Serine/threonine kinase Pim1

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P1-25

Study on QCLO method for canonical molecular orbital calculation of cofactor-containing protein

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Keywords: All-electron calculation, CMO, QCLO, DFT, Glucose oxidase

All-electron calculation of protein is becoming an indispensable tool to analyze protein electronic structure and accurate intermolecular interactions. However, convergence of SCF calculation is difficult for canonical molecular orbital (CMO) method, especially density functional method, and the effective initial guess creating method is required for the practical use.

We have developed QCLO method [1, 2] to make a precise initial guess of large systems. The QCLO, quasi-canonical localized orbital (LO), has the character of both CMOs and LOs. An initial guess of large system is created by gradually lengthening the QCLO of the subunit, which is called frame molecules. Since it assumed dividing at Cα, sp_3 carbon, it was difficult to apply to protein containing hetero molecules.

In this study, in order to remove the restriction, we have developed the new program which can divide by arbitrary subunits and create QCLO. The partial structure of the active center of Glucose oxidase (GOD, Fig. 1) [3] was applied for a verification calculation. Since the several ionic functional groups exist around cofactor FAD the whole calculation is very difficult.

By the new QCLO method, CMO calculation of not only protein but various nano-molecules is attained. It is also applicable for the analyses of intra- and/or inter- molecular interactions between the arbitrary subunits [2].

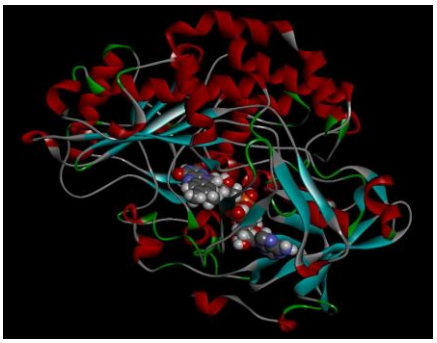

Fig.1 Structure of GOD (FAD is shown by CPK model)

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P1-26

Improvements of a method to predict interfaces for GPCR oligomerization

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Keywords: G protein-coupled receptor, Oligomerization, Interface

G protein-coupled receptors (GPCRs) interact with each other to form homo- and/or hetero-oligomers. Some GPCR oligomers are known to be associated with diseases. An accurate prediction of the residues that interact upon oligomerization interface would further our understanding of signal transduction and the diseases in which GPCRs are involved. We had previously developed a method to predict interfaces for GPCR oligomerization. The method requires both homologous sequences and a closely related structure of a target GPCR. Homologous sequences are used to calculate conservation scores of residues. A template structure is used to identify conserved residue clusters on the template structure as interfaces.

Recently, we modified the method as follows. Firstly, the source code of the method was rewritten in Java, which made the execution speed faster than that of the previous version. Secondly, a template can be selected from more than one hundred GPCR structures, which have been registered in Protein Data Bank in the past few years. Thirdly, a user can predict the interfaces of a GPCR structure model without inputting homologous sequences. These two modifications improved the usability of the method. The prediction server is available at http://grip.b.dendai.ac.jp.

P1-27

Complexity of Moving Robots on a Graph with Group Constraints

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Keywords: Computational Complexity, Reconfiguration

Given a graph G and a set of k robots, we consider the scenario of moving the robots on some vertices to some other vertices under a certain group constraint, such as independent set constraint, dominating set constraint, clique constraint, and so on. As an example, suppose that we are given two independent sets Fb and Fr of a graph such that $|Fb|=|Fr|=k$. and imagine that robots are located on Ib. We call an independent set of size k a formation. The problem that we consider here is to determine whether we can change the initial formation Fb to the target formation Fr by moving a robot along an edge one by one with keeping the independent set (or dominating set, clique). The problem for independent set is shown to be PSPACE-complete even for planar graphs, and also for bounded treewidth graphs, by using the results in [1], In the poster presentation, we review some known results and new results for several group constraints.

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P2-01

Identification of novel potential antibiotics against *Mycobacterium* using pharmacophore-based *in silico* screening targeting thymidine monophosphate kinase

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Keywords: Antibiotics, *in silico* screening, *Mycobacterium*, Pharmacophore model, Thymidine monophosphate kinase (TMPK),

The increasing prevalence of drug-resistant tuberculosis (TB, i.e. multidrug-resistant TB: MDR-TB, extensively drug-resistant TB: XDR-TB), which is resistant to several effective antibiotics, presents a major global health concern [1]. Advances in the identification of novel anti-TB drugs have been progressed against several biological pathways, such as mycobacterial cell wall synthesis, energy production, and DNA/RNA synthesis [2]. The *Mycobacterium tuberculosis* thymidine monophosphate kinase (mtTMPK) is a potential enzymatic target for the treatment of TB. In fact, several research groups have reported that the successful development of inhibitors of mtTMPK with potential antibacterial activity against *Mycobacterium* strains [3].

In this study, we attempted to identify novel chemical compounds specifically targeting the mtTMPK. We performed pharmacophore-based *in silico* screening [1,4,5] using mtTMPK crystal structure data (PDB ID: 1MRN) and the virtual chemical library including 461,383 chemicals. We then evaluated the antibacterial effects of the candidate chemicals, and we found one hit (KTP3), which was able to inhibit the growth of model mycobacteria *M. smegmatis*. We also performed similarity analysis to identify four additional chemicals (KTPS1-KTPS4) with similar structures to the active chemical from ChemBridge web-based database. The most potent inhibitor (KTPS1) does not have any toxic effects on model intestinal bacteria (*E. coli* BL21 and JM109 strains) and several mammalian cells (MDCK, SH-SY5Y, THP-1, HL-60, and K562 cells). Moreover, we also confirmed that these chemicals directly inhibit the enzymatic activity of mtTMPK.

In conclusion, the structural and biological assays data regarding these novel chemicals are likely to be useful for the development of novel antibiotics for the treatment of TB. Furthermore, our screening methodology presented in this study could contribute to the further identification of novel hit chemicals for other candidate medicinal drugs.

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P2-02

Ligand Based Pharmacophore Modeling and *In silico* Screening of Protein Tyrosine Phosphatase 1B Inhibitors for Identification of Novel Antidiabetic Lead Compounds

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Keywords: Diabetes mellitus; PTP1B inhibitors; Pharmacophore mapping; Hydrophobic domain.

Diabetes is a metabolic disorder of the pancreas, in which the pancreas loses its functionality to produce insulin hormone properly in the body [1, 2]. It has been effecting to the middle-aged and elderly, while there is still lack of efficient drug with improved efficacy and tolerability against this disease. The purpose of this study is to evaluate therapeutic applications of drug like compounds as antidiabetic activity through computational studies. Data set consist of reported antidiabetic compounds belongs to benzylidene-2, 4-thiazolidinedione derivatives; after substitutions on the phenyl ring at the ortho or para positions of the thiazolidinedione (TZD) group of compounds were synthesized as PTP1B inhibitors with biological activity (IC_{50}) [3]. PTP1B is a negative regulator of the insulin signaling pathway and is most promising potential therapeutic target for discovery of antidiabetic drugs [4-6]. A ligand based pharmacophore model has been mapped for 59 new antidiabetic compounds with significance for the development of new drugs by using Ligand Scout software and distance estimation using effectual software visual molecular dynamic (VMD). The best pharmacophore model in terms of predictive value consisted of two features like two hydrogen bond acceptors (HBA) and one hydrophobic domain (HYD). The derived pharmacophore models were then filtered using Lipinski's rule of five criteria. Therefore the results obtained in this study hope that the model generated will be helpful in identification of structurally diverse novel and potentially active lead compounds with improved activity against diabetes.

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P2-03

Predicting drug efficacy and bias for GPCRs by molecular dynamics simulation

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Keywords: molecular dynamics, drug discovery, agonistic activity, biased signaling, GPCR

The strength of signal transduction at a target protein induced by a drug is closely associated with the benefits and side effects of the drug. Especially, partial agonist activity and β-arrestin-biased agonist activity for G-protein coupled receptors (GPCRs) are key issues in drug discovery for central nervous system disorder[1]. However, it is challenging problem to design the GPCR drugs with appropriate efficacy and bias, because the structural mechanism underlying the differences in the signaling levels is still not clear. Here, we utilized molecular dynamics simulation to characterize the differences of the dynamic fluctuations that associate with agonistic activation in the various drug[2]–β2-adrenergic receptor (β2AR) complexes. Our computational analyses revealed that the drug efficacy and bias correlated with the rms fluctuation and the cross-correlated fluctuation at the sub-microsecond time scale in the transmembrane region, which compose the ligand-binding site and the G-protein-binding site. These findings show that the simulation of the dynamic fluctuations enables the prediction of the efficacy and the bias for drug candidates.

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P2-04

Identification of ‘dynamic hotspots’ for lead discovery using MD simulation and spatiotemporal cluster analysis

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Keywords: *in silico* drug discovery, dynamic hotspots, molecular dynamics simulation, spatiotemporal cluster analysis, induced-fit binding

Lead compounds with high potency and selectivity often take advantage of induced-fit binding, which implies that design of lead compounds in consideration of induced-fit binding enhances their potency and selectivity. On the other hand, driving forces of ligand-protein binding are characterized as hotspots, which contribute to the binding free energy. If we can identify hotspots for target protein, they are helpful for design of lead compounds[1]. We therefore establish a method for identification of hotspots involving induced-fit binding, ‘dynamic hotspots’, using molecular dynamics (MD) simulation and spatiotemporal cluster analysis of the MD trajectory.

As a test case, we attempted to find dynamic hotspots of the dimeric BTB domains of BCL-6, which interact with several transcriptional regulators such as SMRT and BCOR. At first, we performed 1 μsec MD simulation of BCL-6 in the presence of multiple organic solvents. From the entire MD trajectory, spatiotemporal clusters of organic solvents with long residential time in substrate-binding region were obtained by cluster analysis. Then, we classified the solvent cluster, according to their 3D localizations, their pharmacophores, and the contemporary structural states of BCL-6, and identified the several groups of solvent clusters as dynamic hotspots. Finally, we confirmed that the identified dynamic hotspots corresponded to free energy maps calculated based on the MD trajectory and the SMRT and BCOR peptides in the reported crystal structures.

Our method is similar with the X-ray crystallographic method in aqueous solution of organic solvents (MSCS)[2] and the MD simulation-based method with the probability distributions of organic solvents (SILCS)[3]. However, these methods reveal only static or time-averaged hotspots, and are unable to detect hotspots specific to the particular structural state of the protein. Our new method overcomes these problems and is expected to guide the design of lead compounds with high potency and selectivity.

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P2-05

In silico cardiac safety assessment of drug effects using heart simulator

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Keywords: Heart simulator, Multiple ion current assay, Cardiotoxicity testing

To save time and cost for drug discovery, a paradigm shift in cardiac safety assessment is required. Here, we present the results of a novel assay system using heart simulation technologies coupled with the multi-ion current assays. Our heart simulator, UT-Heart [1], is a finite-element-based model of human heart to each element of which molecular models of electrophysiology and excitation-contraction coupling process are implemented. For the detailed analysis of ECG, we coupled the heart model with the finite element model of human torso and solved the bidomain equations with the novel solving technique [2],[3]. The effect of the drugs under various plasma concentrations was reproduced in UT-Heart by changing the parameters of each channel model according to the experimentally obtained dose-inhibition relations. We show that our system combining in vitro and in silico technologies can predict drug-induced arrhythmogenic risk reliably and efficiently.

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P2-06

Construction of the Protein 3D Fragment Library System based on Glycine Neighboring Environment

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Keywords: glycine neighboring environment, fragment library, similarity search, motif pattern PROSITE, Folylpolyglutamate synthase

With the rapidly increasing number of proteins of which three-dimensional (3D) structures are known, the structure database is one of the key elements in many attempts being made to derive the knowledge of structure-function relationships of proteins. It is known that a glycine residue appears in various positions such as a turn domain and has important roles in protein structure [1]. In the present work, the authors have constructed the 3D fragment library which focused on the glycine neighboring environment. It has also applied to the structural feature analysis using known motif patterns.

We have focused on a peptide fragment which contained the target glycine residue and its first/second neighboring residues in N-, C-terminal sides. The fragment is characterized with structural information such as secondary structure assignment [2], dihedral angles (φ,ψ), and a distance matrix for alpha-carbon atoms. Two types of library have been constructed for the purpose of structural feature analysis: (1) full dataset consists of 3,283,178 glycine-targeted 3D fragments taken from 87,090 proteins in PDB 2013, and (2) the representative dataset consists of 34,009 fragments from 2,616 proteins.

The library system provides us a fragment condition search using PDBID, sequence pattern, secondary structure, and/or dihedral angle information. The sequence pattern search with regular expressions, e.g., T-[NK]-G-K-x which is a subsequence of *folylpolyglutamate synthase* motif (PS01011) taken from PROSITE database [3], is available in our system. In addition, we have also implemented structure similarity fragment search based on dihedral angle or distance matrix in the present work. *Folylpolyglutamate synthase* motif has rich glycine residues and could play a role in the catalytical activity [4]. For this subsequence pattern, 1,656 fragments were found in the full dataset library, and then structure similarity search was carried out with the query of the known motif 3D fragment (1JBVA 47-51). As the result, similar fragments close to the substrate binding sites were identified in several proteins. The result shows the potential applicability of our approach for 3D structural data mining of proteins.

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P2-07

Discovery of InhA inhibitors with anti-mycobacterial activity through matched molecular pair approach

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

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

In this study, by combination of the MMP and docking simulation approaches, we tried to discover novel InhA inhibitors alternative to a compound (KES4) we previously identified [4]. 461,384 compounds in the virtual chemical library were cut at all exocyclic single bonds, and single-, double-, triple-cut [5] unique MMPs were generated. After that, 10 candidate compounds associated with KES4 were identified through the docking simulation with *M. tuberculosis* InhA structure. We then evaluated the antibacterial effects of these compounds on growth of mycobacteria *M. smegmatis* as a model bacteria of *M. tuberculosis*, and all of 10 compounds showed inhibitory effects on growth of *M. smegmatis*. In addition to this, 6 of 10 compounds have lower experimental IC_{50} value than 50 μM and do not have any toxicity against enterobacteria (*E. coli* BL21 and JM 109) and mammalian cells (MDCK and SH-SY5Y cells).

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

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P2-08

Predicting Molecular Mechanisms of Action Associated with Binary Endpoints

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Keywords: Safety and efficacy profiling, in silico drug discovery, chemoinformatics, brain cancer

The in silico identification of mechanisms of action related to drug candidates can help elucidate therapeutic targets and discover off-target effects, providing information to better understand the underlying pathways involved in the efficacy and adverse effects of drugs. Additionally, predicting the mechanism of action of chemicals is of utmost importance to determine their environmental fate, and to predict the effects of long term exposure.

To address these needs, we have used Prous Institute's computational platform SYMMETRY to train a model that predicts around 650 mechanisms of action based on a training set extracted from literature and patent analysis. The training set contains approximately 1.5 million Structure-Activity Relationships (SAR) with a ratio of ~1.6 SAR per training set structure, and it is continuously updated. The prediction quality of the Mechanism of Action (MoA) model has been assessed in a 10% hold-out external validation yielding an average recall of 93%.

One interesting application of SYMMETRY's MoA model is to predict the mechanisms of action associated with a binary endpoint and using that information to find differentially expressed mechanisms, i.e. mechanisms associated with positives but unrelated to negatives for a given endpoint. A methodology is proposed to find the relevant molecular mechanisms of action associated with a binary training set, and avoid spurious MoA relationships.

As a case study, a binary data set of brain cancer cell line SNB-78 tumor growth inhibitors has been analyzed and MoAs related to this endpoint have been highlighted. The resulting MoAs associated with the anticancer activity in the SNB-78 cell line are compared with those described in the literature.

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P2-09

OEDocking 3.1: Pose Prediction in One Awesome Package.

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Keywords: Ligand docking, Pose Prediction

The history of molecular docking has been to generate a single algorithm that can do everything. This strategy ignores that the two problems where docking has been successfully applied to (posing prediction and virtual screening) are very different problems. The strategy at OpenEye is to develop applications that are optimized to produce the best performance for a single task. We present the first of these applications, POSIT for posing, and show that the new application is best class for generating pose predictions for lead optimization.

P2-10

Computational Studies for Drug Design

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Keywords: Molecular Modeling, Drug Design, Molecular Screening

In early stage of drug development process, “seed” compounds as drug candidate are detected from huge chemical compound library by experimental and computational screenings. Recently, computational screening using molecular docking plays an important role in this process. In general, molecular docking treats protein and ligand as flexible and rigid molecules and predicts binding pose and affinity of a ligand to a target protein. In living cell, proteins are obviously not static objects and conformational changes are key elements in ligand binding. Therefore, it is essential to introduce protein flexibility into molecular docking for detection of various seed compounds.

In this work, we performed molecular simulations of some proteins to understand the key protein-ligand interactions and the importance of protein flexibility to molecular docking. Based on these results, we will extend the information of protein flexibility to the drug design.

P2-11

Development of a novel anti trypanosoma drug

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Keywords: *Trypanosoma*, Spermidine synthase, Molecular dynamics, Protein-Ligand docking, Allosteric inhibitor

Chagas' disease, African trypanosomiasis, and leishmaniasis are some examples of tropical neglected diseases (NTDs) caused by the parasite *Trypanosoma* [1-2]. While nifurtimox and benznidazole are currently available for treatment of Chagas' disease, they have major drawbacks, such as side effects and their insufficient effectiveness in the chronic stage [3-4].

In order to discover a new anti-trypanosoma drug, this study focused on spermidine synthase (SRM) as target protein. SRM is one of the proteins involved in the spermine synthesis pathway, and is believed to be a promising target protein of trypanocidal agents.

While docking simulation is carried out exclusively for drug discovery, a protein can assume conformation states different from the one determined by X-ray crystallography due to thermal fluctuation. Therefore, we performed molecular dynamics (MD) simulation for SRM as target protein (PDBID: 3WBC) using Desmond [5]. Docking simulation was performed on the open structure obtained from MD simulation and approximately 4.8 million drug-like compounds using Glide [6]. We successfully obtained active compounds with IC_{50} values of the 10 μM order against SRM.

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Estimate of Pharmacophore for Anti-Trypanosome Drug Development Using Fragment Molecular Orbital Method

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Keywords: Chagas disease, Dihydroorotate dehydrogenase, Pharmacophore, Fragment molecular orbital

Chagas disease is caused by the parasite *Trypanosoma cruzi* (*T.cruzi*) and is one of the neglected tropical diseases. This disease affects people from approximately 20 countries, particularly those living in the southern United States and Latin America [1-3]. Currently, nifurtimox and benznidazole are available for treatment of Chagas disease. However, there are serious problems including adverse effects and limited effectiveness during the chronic phase of this disease [4-5].

In order to develop a novel anti-trypanosoma drug, we analyzed interaction energy for dihydroorotate dehydrogenase (DHODH) as target protein using fragment molecular orbital (FMO) method [6].

As a result, FMO calculation revealed that polar amino acids of DHODH and the cofactor flavin mononucleotide (FMN) interact with the orotate derivative by hydrogen bond and π-stacking. Thus, effective inhibitors of TcDHODH require hydrogen bond donor, acceptor and aromatic cycle. Based on these results, we estimated pharmacophore model that was effective for development of anti-trypanosoma drug.

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P2-13

Statistical analysis of hydrogen-bond preferences between chemical fragment and amino acid in Protein Data Bank

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Keywords: Electronic structure calculation, Ligand docking, Protein modeling

In a drug-discovery process, hit to lead optimization is a complicated task to achieve multiple optimization of sufficient potency, physical properties, ADME profile, and toxicity. To assist the process, many chemoinformatics approaches for bioisosteric transformation [1] in order to change physiochemical properties retaining potency against target protein are reported. Previously, such bioisosteric transformations were derived from examples of successful chemical transformations in public and/or in house screening data. Compared to such data mining from screening data, the number of data mining efforts for lead optimization from 3D structural information has been limited. In this study, we focused on the hydrogen-bonds between small molecules and receptor proteins, and tried to derive bioisosteric transformation from protein-ligand complex structures in Protein Data Bank (PDB),

At first, hydrogen bonding pairs of small molecules and amino acids were collected. Then, the frequencies of the interacting chemical fragment and each amino acid were analyzed. To clarify the differences between amino acids, we focused on the hydrogen bonds with side chain atoms in this study. Using this data, each chemical fragment was characterized the frequencies of observed hydrogen bonds with each of the amino acids. Using Kohonen Self-Organizing Map [3], the fragments were categorized into 5×5 map according to their preferences on amino-acids as their hydrogen-bond partners. The results proposed the possible bioisosteric transformations by which similar hydrogen bond preferences to the original compound can be expected.

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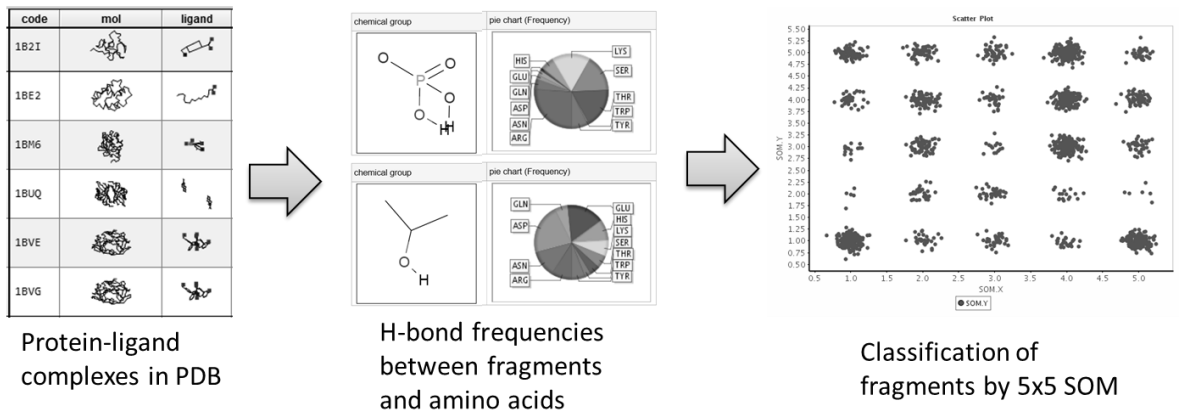

Figure 1. SOM analysis of chemical fragments by their H-bond preferences on amino acids

P2-14

A New Virtual Screening Method for Identifying Novel Active Compounds Based on Protein Promiscuity

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Keywords: virtual screening, protein promiscuity, ligand based virtual screening, scaffold hopping, in silico screening,

We propose a new virtual screening method for identifying novel compounds that acts against a target protein of interest. The method performs three-dimensional structural comparison to "the protein structure based multiple template", using a geometric hashing method, to rank database compounds. Here, the protein structure based multiple template is consists of a number of known active ligands that bind to the target protein or its close homologs and is obtained by protein structure superposition. This approach enables us to simultaneously exploit the diversity and universality of protein-ligand interactions for a target protein, and thus it may be suitable for finding novel active compounds.

We evaluated its performance on several protein targets from the directory of useful decoys (DUD) and compared it to other popular screening programs. The results indicate that the performance of our approach is comparable to that of one of the most famous screening program Glide. We also show that our method can identify active compounds that are not similar to any of the ligands used in the multiple template. These results suggest that our approach is useful for identifying novel compounds that acts against a target protein.

P2-15

Quantum-Chemistry-Based Chemoinformatics System for Molecular Discovery: Database, Search Engines, and More

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Keywords: Electronic structure, Database, Data mining, Text mining, Molecular discovery, Molecular design

We propose a chemoinformatics system for molecular discovery on the basis of electronic-structure calculations. In this presentation, we will provide the overview of our project consisting of developments of electronic-structure database, search engines for quantum chemical similarities reflecting electronic spectra and interaction topology, data-mining tools for "electronic structure-property" relationships, and knowledge search based on text-mining. Each of the research topics will be separately given in the other three presentations in this Meeting from our group. See the poster presentations by Iwane and Sugimoto, Doi and Sugimoto, and Kurogi and Sugimoto.

By combining these developments, we aim at constructing an informatics system by which users can learn, study, design, and discover functional molecules according to their individual needs. As a demonstration, we will discuss about our application of our system to predict tyrosinase inhibitors.

Some parts of the system will be open to public. Therefore we would like to discuss with the audience about what characteristics the system should have for enhancing applicability, reliability, and efficiency for molecular discovery with a particular focus on drug discovery.

This work is a result of collaborations with Naoki Yoshida, Toshihiro Ideo, Ryo Iwane, Takayuki Kurogi, Tatsuhiro Doi, Hideyuki Nagaya, Erika Makiyama, Mizuki Iida, and Kimitaka Kuroiwa of Kumamoto University. Their contributions are gratefully acknowledged. A part of the present work is financially supported by Grant-in-Aid for Scientific Research (KAKENHI) on Innovative Areas (#2601) by The Ministry of Education, Culture, Sports, Science and Technology(MEXT).

P2-16

Quantitative prediction of binding affinity for theophylline-RNA using alchemical transformation method

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Keywords: drug design, free energy, alchemical transformation

Recent advances in computing and communication power provide us the realization of the computer-aided rational drug discovery and design using high performance computing (HPC) system. Alchemical free energy calculation is nowadays well understood and widely accepted to compute the quantitative estimation of free energy change between two different equilibrium states, e.g., from the unbound state to the bound state.

In this work, the standard binding free energy of theophylline-RNA complex (PDB code 1O15 [1]) is calculated using alchemical transformation method with an application of a harmonic restraint. The amber14sb force field and general amber force field (GAFF) are used for describing potential function of RNA and for that of theophylline molecule, respectively. To restrict the conformation sampling of theophylline molecule to a finite volume within the binding site, we use the harmonic potentials restraining the orientation of the molecule relative to RNA [2]. The long range dispersion correction EXP-LR is also added [3]. The multistate Bennett's acceptance ratio (MBAR) is employed in evaluating free energies [4]. Our obtained value is -8.8 kcal/mol for theophylline-RNA complex, which agrees well with the experimental one (-8.9 kcal/mol).

We believe that this methodology leads to a standard approach of drug development in HPC system.

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P3-01

Approach to Meta-Analysis of Microarray Datasets Reveals Muscle Remodeling-related Drug Targets and Biomarkers in Duchenne Muscular Dystrophy

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Keywords: Pathway analysis, Drug target, Biomarker, Systems biology, Omics data analysis

We suggested a computational approach for drug target and biomarkers discovery that relies on the aggregation of individual gene expression profiling experiments and the use of a Sub-Network Enrichment Analysis (SNEA) algorithm and a literature-extracted network of biological relations to find key regulators of differentially expressed genes (1). We applied this approach to publicly available human muscle gene expression studies of Duchenne muscular dystrophy (DMD). Our analysis revealed possible regulators of transcriptional changes (e.g. AMPK, TORC2, PPARGC1A) within a skeletal muscle remodeling pathway. Some of the regulators had not been associated with Duchenne Muscular Dystrophy previously. Our principal findings were later confirmed by independent experiments.

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P3-02

Identifying Potential Indications for Drug Repositioning Using Text Mining Analyses

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Keywords: Humira, Adalimumab, Drug repositioning, Text mining, Bioinformatics

There is increasing pressure on pharmaceutical companies to continue producing new therapies while still returning shareholder investment. While research and development continue to look for discovery of new compounds and targets, pharmaceutical companies are also looking to supplement their drug discovery pipelines with drug repositioning of their existing drug portfolio. This is beneficial to maximize the value of their compound portfolio, particularly for compounds that may have limited patent protection left.

Text mining analysis can be used to help in drug repositioning efforts in several ways. Text mining is much better than keyword searching as text mining allows for searching with particular semantic relationships, e.g. finding only the items where adalimumab inhibited disease. Doing so reveals true positive results that contain semantically relevant information, whereas keyword searching may lead to very high noise and false positives as it relies on document level co-occurrence in which keywords could occur but are not semantically related. Furthermore, text mining analyses can be automated thereby allowing higher throughput analyses of content and overall project team efficiency. We validate the methodology of using text mining analyses to assist with drug repositioning by finding additional indications from the literature for adalimumab, a well-known TNF inhibiting drug.

Text mining analyses were performed on several content sources (biomedical literature, clinical trials, and patents) to identify all relevant documents in which adalimumab was used as an effective treatment condition, and also those in which other anti-TNF drugs were used. Different disease conditions, compound information, and supporting reference information were then extracted. Our data show 820 diseases, syndromes, physiological effects and toxicities reported with up-regulation of TNF-alpha activity. Data analyses confirm the top approved indications of adalimumab and also suggest other indications that may be of interest for potential drug repositioning. This information could be used to guide additional research and development efforts.

P3-03

What factors influenced the estimation of HIV-1 CRF07_BC evolutionary history in China

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Keywords: HIV-1, Virus origin, Evolutionary Analysis

It has been known that subtypes B' and C from Thailand and India, respectively, were introduced into China in the late 1980s and then the two subtypes became a recombined subtype CRF07_BC. There is a controversy on evolutionary history of CRF07_BC (HIV-1) in China by two studies, and both of the studies used Bayesian Evolutionary Analysis by Sampling Trees (Beast) program. The first paper published in 2011 suggested that CRF07_BC probably spread form Xinjiang, whereas another paper published in 2012 demonstrated that the virus originated in Yunnan province. Because the two studies used different viral samples and also analysed the data in different styles, there is a great concern that these differences might have affected the results. Therefore, we conducted a comprehensive analysis to clarify this issue.

We retrieved the viral samples from more than one public database and created five datasets: 1) *env* gene, 2) *gag* gene of subtype C origin, 3) *gag* gene of subtype B origin, 4) *pol* gene of subtype C origin and 5) *pol* gene of subtype B origin. These datasets included more locations (15 provinces), more sampling time points (1996-2011) and the longer genome regions of viral genes. In addition, the most recent version of BEAST was used in this study.

The results showed that: (1) outgroup can affect the estimation of viral origin, (2) no significant difference between recombinant and non-recombinant genome analyses and (3) sampling bias and early samples impact on the estimation of viral origin.

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P3-04

The regulatory mechanisms of adipocyte and osteoblast differentiation revealed by the integrated transcriptome analysis

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Keywords: Transcriptome, Osteoblast, Adipocyte, Differentiation

Mesenchymal stem cells can differentiate into various cell types. Particularly the regulatory mechanisms of the balance for differentiation to adipocyte and osteoblast are closely linked to the pathogenesis of metabolic syndrome and osteoporosis. We have displayed the gene regulatory networks for osteoblastogenesis and adipogenesis of mesenchymal cells, and several genes and microRNAs (miRNAs) were identified as regulators for those differentiation. Meanwhile, it has been reported that expression of several RNA variants in which such as their transcription starting site or exon usage are different can be occurred from a gene locus, and they are functionally discriminated among body tissues and cell types. In addition, it is revealed that various types of noncoding RNAs (ncRNAs) as well as miRNAs are involved in most of biological function. Therefore we aim to identify the RNA variants and ncRNAs that are involved in regulation of osteoblastogenesis and adipogenesis, and perform detailed RNA expression analysis using total RNAs which are obtained from the time-course samples during osteoblastogenesis and adipogenesis. Differentiation of the cells into adipocyte and osteoblast were confirmed by the increased expression of marker genes for each differentiation, then differential expression of each exon and ncRNAs are comprehensively analyzed using exon array, miRNA array and RNA-sequencing by next generation sequencer. As a result, we could detect specific increasing of PPARγ2 expression, which is a variant of PPARγ transcription factor gene and has crucial function for adipogenesis using exon array, suggesting that this tool is effective for identifying the functional RNA variants for adipogenesis and osteoblastogenesis. In this analysis, we could detect various RNA variants and ncRNAs that are differentially expressed during both types of differentiation. We estimate that specific RNA variants which regulate differentiation are involved in those differentially expressed RNA variants, and currently the experiment for their validation are underway.

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P3-05

A novel role of sphingosine-1-phosphate receptor in proliferation of breast cancer stem cells

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Keywords: sphingosine-1-phosphate, S1PR3, antagonist, cancer stem cells

Growing evidence suggests that breast cancer is originated from breast cancer stem cells (CSCs), which are defined by their ability for self-renewal and high tumorigenicity. Although targeting CSCs is believed to be a more effective approach for therapy, the regulation of CSCs at the molecular level has not been fully understood. We have previously reported that CSCs can be isolated from human breast cancer MCF-7 cells by aldehyde dehydrogenase (ALDH) activity-based flow cytometry analysis. In the present study, we investigated a receptor that regulates proliferation of CSCs by the ALDH assay system.

Using microarray screening in ALDH-positive cell population of MCF-7 cell, we found that S1PR3, a receptor for a lipid mediator sphingosine-1-phosphate (S1P), was highly expressed in the ALDH-positive cell population. Stimulation with S1P increased the proportion of ALDH-positive cell population in a dose-dependent manner. The effect of S1P was inhibited by s selective S1PR3 antagonist, suggesting that S1P increases breast CSCs via S1PR3.

S1P is produced by sphingosine kinase (SphK) from sphingosine. Overexpression of SphK1 increased the ALDH-positive cell population, whereas overexpression of SphK2 did not. S1PR3 antagonist inhibited SphK1-induced increase in the ALDH-positive cell population. These data suggest that S1P, which is produced by SphK1, stimulates S1PR3 and leads to increase breast CSCs.

We next performed nude mouse xenograft assay using breast CSCs. SphK1-overexpressing ALDH-positive cell population exhibited high tumorigenicity, compared with vector-overexpressing ALDH-positive cell population. Knockdown of S1PR3 inhibited the tumorigenicity of SphK1-overexpressing ALDH-positive cell population. In addition, chronic administration of S1PR3 antagonist inhibited the tumorigenicity of SphK1-overexpressing ALDH-positive cell population.

Furthermore, we examined expression of S1PR3 using CSCs from breast cancer patients. Similar to MCF-7 cells, S1PR3 was highly expressed in ALDH-positive cell population derived from breast cancer patients. In addition, ALDH-positive cell population co-expressed SphK1 and S1PR3. These data suggest that SphK1-S1P-S1PR3 signaling is present in CSCs from breast cancer patients.

Taken together, our data suggest that S1P induces breast CSCs via S1PR3. Thus, S1PR3 is a potential therapeutic target in breast CSCs.

P3-06

Analysis of alternative splicing in the cerebellum of spinocerebellar ataxia type 6 knockin mice

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Keywords: RNA-seq, spinocerebellar ataxia type 6, alternative splicing

Spinocerebellar ataxias (SCAs) are a group of neurodegenerative diseases characterized by a loss of motor coordination and balance capability. Among them, SCA type 6 (SCA6) is a late-onset autosomal dominant neurodegenerative disease caused by a polyglutamine (poly-Q) expansion in $Ca_v2.1$ voltage-gated calcium channel subunit. The development of effective therapy for SCA6 has been long-awaited as it is one of the most prevalent inherited ataxias in Japan. We previously reported that the poly-Q expansion causes Purkinje cells (PCs) degeneration without affecting the basic properties of the channel, but the detailed pathogenesis is still unclear[1,2]. We hypothesized that the altered gene splicing may be involved in the disease pathogenesis of Sca6 MPI-118Q mice, in which 118 CAG repeats were inserted into the corresponding *Cacna1a* locus.

Here, we aimed to detect genes differentially transcribed or spliced between cerebellar tissues of a wild-type mouse and an Sca6 MPI-118Q mouse by deep sequencing of transcriptome. The TopHat package (http://ccb.jhu.edu/software/tophat/) was used for the alignment of the short reads to the mm10 reference genome, followed by Cufflinks (http://cufflinks.cbcb.umd.edu/) and MATS (http://rnaseq-mats.sourceforge.net/) for transcript assembly, read counting, and alternative splicing analysis.

We found several genes differentially spliced between the wild-type and the SCA6 model mice by CummeRbund and Integrative Genomics Viewer. We found several genes had alternative splicing between the wild-type and the SCA6 model mice. For Example, a retained intron was found in the gene X, which is an enzyme necessary for glycolipid metabolism. Moreover, the gene X has been reported to be associated with lysosomal diseases and neurodegenerative disorders. Further validation study is required to identify the role of the gene X in the Sca6 and is being planned. These genes might be associated with pathology of SCA6 such as degeneration of PCs and will be possible novel targets for drug discovery.

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P3-07

Prediction of gene expression networks in hepatocellular carcinoma using genome-wide gene expression data

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Keywords: Hepatocellular carcinoma, gene expression network, genie3

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related mortality worldwide in 2008. The number of patients of the hepatocellular carcinoma is increasing, especially in Europe and the United States. Because molecular-targeted therapy for HCC is currently limited to sorafenib, novel drugs are strongly demanded to improve prognosis.

In this study, we used gene expression profiles from patients to identify pathway biomarkers, which are the molecular networks representing the malignancy of HCC. We also aimed to obtain the knowledge of candidate molecules for a novel therapeutic target. Identification of an abnormal branch in the molecular networks can contribute significantly to the promotion of new therapies development of HCC.

Gene expression profiles of 140 HCC specimens were obtained by DNA microarray (Affymetrix HG-U133 Plus 2.0), and the data set is available at the Integrated Clinical Omics Database (iCOD). We used 40 specimens for cancerous tissues including 20 specimens for each of good and poor prognosis group, respectively. We also evaluated the gene expression patterns of 23 non-cancerous specimens that contained eight specimens for good prognosis group and 13 for poor prognosis group. In addition, 15 normal liver specimens of adjacent non-cancerous tissue from metastatic liver cancer patients were also utilized for construction of a reference network. Co-expression molecular networks were predicted using genie3[1] under R statistical software (version 3.1.1).

In HCC, growth factors such as vascular endothelial cell growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and hepatocyte growth factor (HGF) were found to be strongly interacted in cancerous specimens[2]. Furthermore, interactions among a variety of signal transduction system such as ERK / MAPK pathway, PI3K / AKT / mTOR pathway, and Wnt pathway were observed.

Focusing on these signal transduction systems, gene expression networks in HCC were visualized using a Cytoscape (version 3.1.1). By interpreting the difference of gene expression networks in each group, molecular network abnormalities that possibly included the candidates for novel therapeutic target were identified.

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P4-01

Basic approach to optimize the small drug seeds with minimized CV risk on the early stage of drug discovery by using small molecule-based *in silico* models for prediction of hERG/Nav1.5 inhibitions

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Keywords: small molecule-based *in silico* models, prediction of hERG/Nav1.5 inhibitions

It is generally known that many drugs, which show proarrhythmic effects including prolongation of QT/QTc interval and a ventricular tachycardia of torsade de pointes (TdP) type in clinical use, exert inhibitory effects on cardiac ion channels. Although the risk assessment of those channel inhibitions has become more important in drug development, the elimination of the undesirable effects is challenging despite the use of *in vitro/vivo* screening technologies. Under these circumstances, *in silico* models for ion channel inhibitions are receiving increasing attention as an effective tool to select compounds with reduced risk of ion channel inhibition in the early stage of drug discovery.

At Eisai, we have an increasing need for a versatile prediction system that can screen a huge number of structurally variable virtual compounds and that can search the structure-activity relationship (SAR) for channel inhibition virtually. We are therefore applying two small molecule-based *in silico* models of hERG/Nav1.5 inhibition to the early stage of drug discovery, including providing predicted annotations of library compounds, comparing prediction results and actual wet data for the key family of compounds identified by high-throughput screenings both of which will help find well-balanced seed compounds with minimized cardiac safety risks attributed to the channel inhibitions. It is expected that this kind of *in silico* model can give concrete directions for drug design to avoid CV liability. In this presentation, the details of the approach to find/optimize the drug seeds by using *in silico* models developed in-house will be presented.

P4-02

Establishment of small molecule-based *in silico* models for prediction of hERG and Nav1.5 inhibitions for drug discovery

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Keywords: small molecule-based *in silico* models, prediction of hERG/Nav1.5 inhibitions

The struggle with cardiac ion channel inhibition in drug discovery and development process began more than 10 years ago when several non-cardiovascular drugs were reported to prolong the QT/QTc interval and induce a severe ventricular arrhythmia (torsade de pointes - TdP) due to human ether-à-go-go-related gene (hERG) channel inhibition. Drug induced arrhythmia and cardiac dysfunction caused by cardiac sodium (Nav1.5) channel inhibition are also problems. Therefore risk assessment of these channel inhibitions has become necessary in drug development. To minimize the inhibitory effects on these ion channels and identify good drug candidates, we established *in silico* models of hERG and Nav1.5 inhibition to support drug discovery. These models have been constructed based on the platform of PipelinePilot (Accerlys Co.ltd.) and the training wet-data set of hERG and Nav1.5 has been obtained from in-house data by using automated patch clamp systems (IonWorks and Q-patch) and/or a commercially available data set (BioPrint®). These established *in silico* models show high accuracy of around 80% for in-house library compounds. In the results, *in silico* models of hERG and Nav1.5 inhibitions, which have expected specifications for drug discovery, were established in-house, so we will present about details of the specifications in this presentation.

P4-03

Visualisation of drug delivery by using high resolution microscopic mass spectrometry

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Keywords: Drug delivery, Molecular imaging, MS imaging, Cancer

Pharmacokinetic (PK) and pharmacodynamic (PD) studies are important to evaluate the efficacy and toxicity of the drugs. In these analyses, tissue homogenates are generally used for the quantification by high-performance liquid chromatography (HPLC) or liquid chromatography mass spectrometry (LC-MS). However, they lack the information regarding the drug distribution in a specific anatomical area. The information of the drug distribution allows us to optimize the drug design enabling more efficient targeted delivery. We studied the tissue distribution of paclitaxel (PTX) and its micellar formulation (NK105) using a microscopic mass spectroscopy (MMS). A MMS in which a microscope is coupled with an atmospheric pressure matrix-assisted laser desorption/ionization (MALDI) and quadruple ion trap time-of-flight (TOF) analyser was used. The matrix-coated drug sample is ionised and then separated on the basis of its mass-to-charge ratio (*m/z*). Images were acquired from imaging mass spectrometry (IMS) or tandem mass spectrometry (MS/MS) data. (1) We established the drug imaging system with enhanced resolution and sensitivity. In the analysis, MS and MS/MS were used for quantification and validation, respectively. (2) NK105 showed much stronger antitumor effects on a human pancreatic cancer BxPC3 xenograft than PTX. In the drug imaging, we demonstrated that NK105 delivered more PTX to the whole tumor tissue (including the center lesion). In the mouse model, PTX caused the peripheral neurotoxicity but NK105 did not. Multiple high drug-signal areas surrounding and inside the caudal nerve were observed in the case of PTX, whereas the signals after NK105 injection were significantly low. We succeeded in corroborating the EPR effect using MMS. The data obtained by the drug imaging may be useful for facilitating DDS-drug design.

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P4-04

Evaluation of Human iPS cell-derived Hepatocytes for the Application to ADME/Tox Tests in Drug Development

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Keywords: iPS cell derived hepatocyte, drug safety test, ADME/Tox, functional evaluation

Tests for the prediction of drug pharmacokinetics (ADME: absorption, distribution, metabolism, excretion), drug adverse effects, and drug induced liver injury are conducted at earlier stage of drug development process. Such tests are common and important for the development of a safe new drug. Today, these depend on the human primary hepatocytes, however there are some difficulties in them, such as the inter-individual differences of donors. To solve such difficulties, much effort has been made to obtain hepatocytes from stem cells. The establishment of human iPS cells accelerated such studies and hepatocytes differentiated from human iPS cells are available commercially now.

Our laboratory has been evaluating human iPS cell derived hepatocytes about activities of drug metabolizing enzymes, induction abilities by chemical inducers, and responses to hepato-toxic chemicals. In this presentation, we will report the results of our evaluation of commercially available human iPS cell derived hepatocytes obtained from different venders together with those of HepaRG cells. In the evaluations, metabolic activities of CYP1A2 and CYP3A4 were measured by P450-Glo Assay kit (Promega), and expressions of CYP1A1, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A7, GSTA2, UGT1A1, ABCB1, ABCC2, PXR, CAR, RXRα, AhR, ARNT, CEPBα, and HNF4A were measured by TaqMan qPCR (Life Technologies). Induction of CYP1A2, CYP2B6, and CYP3A4 by typical inducers such as omeprazole, phenobarbital and rifampicin were also evaluated. The toxicity test was done by exposing cells to the different concentrations of acetaminophen.

Based on our observations, we are going to discuss the possibilities of applying these cells to the drug development process from the points of 1) stability of differentiation protocol, 2) reproducibility of the hepatocyte products, and 3) accessibility to users.

P4-05

Prediction of rat clearance using in vivo-in vitro extrapolation and Genetic Algorithm-based multiple linear regression

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Keywords: Clearance, Intrinsic clearance, Prediction, In vitro-in vivo extrapolation, Multiple linear regression

Drug clearance (CL) is a key determinant of animal and human pharmacokinetics (PK) for optimizing its property during lead optimization stage of drug development. Recently, several CL predictive models have been developed; however, the in vivo-in vitro extrapolation (IVIVE) which was shown the good accuracy required many in vitro experiences and statistical methods were difficult to identify outliers. The aim of this study was developed better predictive CL model that could calculate by minimum in vitro experiments and show the applicability.

We applied multiple linear regression (MLR) to predict CL of rat which is widely used in lead optimization stage. To minimize the experimental data used, we used rat hepatic intrinsic clearance (CLh) which was based on the basic well-stirred model as a predictor variable in the function. Therefore, CLh was only calculated by in vitro $t_{1/2}$ in rat hepatic microsomes and unbound fraction in rat serum (fu). Other predictor variables were selected from MOE 2D descriptors and kier-atom type descriptors by Genetic Algorithm (GA). Additionally, we applied Biopharmaceutical Drug Disposition Classification System (BDDCS) which was classified drugs according to their extent of metabolism and solubility for the selection of training set [1]. The number of training set, test set and validation set were 100, 26, 499 compounds, respectively. Validation set was used to define the applicable compound clusters.

GA led to nine descriptor variables contributing to the MLR model, and these variables were assessed by the relative importance (RI). The most important descriptor was the Q_VSA_PNEG (RI = 1.0) followed by total polar negative Van Der Waals surface area, and the next was the CLh (RI = 0.81). This CL model showed good predictive accuracy (r^2 = 0.693, q^2 = 0.609, average fold error = 1.5 to 2.2-folds). Additionally, we could define applicable clusters for our model by specific features of physical property (extent of metabolism, solubility and vsa_pol) and functional groups (carboxyl and sulfonyl groups).

In conclusion, we developed a novel model for prediction of rat CL to combine GA-MLR with IVIVE and BDDCS. Using our model could accelerate the optimization of CL in drug development to support successful drug discovery.

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P4-06

Detailed Analysis of the Binding Mode of Vanilloids to Transient Receptor Potential Vanilloid Type I (TRPV1) by Mutational and Computational Study

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Keywords: Structure property relationships, Pharmacophore,

Transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel and a multimodal sensor protein. Although TRPV1 has been extensively characterized, detailed structural information concerning the binding site is lacking. In this study, a human TRPV1 homology model was constructed to predict amino acid residues involved in the binding of vanilloids. The involvement of these residues in ligand binding was then evaluated by mutational and computational analyses. As a result, five amino acids (L515, I573, F587, F591 and L670) were newly identified as being involved in ligand binding and/or modulation of proton sensitivity. The locations of these residues range from TM3 to TM6, which indicated that the binding pocket of TRPV1 consists of TM3-5 in the same monomer and TM5 and 6 in the adjacent monomer. These results provide novel insights into the binding mode of TRPV1 and will be helpful in developing a mode specific TRPV1 modulator.

P4-07

Transforming Quantity into Quality: a Highly Optimized System to Rapidly Process High Throughput Ion-channel Screening Data

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Keywords: Ion channel, Auto patch clamp, High throughput, Screening, Quality control, Time trace, Electrophysiological, Parallel processing, Database

Electrophysiological Ion channel measurements generate a significant amount of signal data with time traces often consisting of hundreds of thousands data points. Furthermore, multiple events must be inspected, analyzed and combined for an optimal analysis result. This, together with high-data volumes, makes data processing very time consuming and laborious. Recent advances in automated electrophysiology enable the screening of thousands of compounds per week. However, data handling/processing infrastructures have not kept pace with these developments. In the absence of efficient computational platforms, valuable information can be overlooked while technology falls short of meeting its high-throughput discovery expectations.

Genedata has addressed these bottlenecks and challenges. Through extensive consultations with researchers at some of the world's largest pharmaceutical companies and with leading instrument vendors, Genedata has developed a specific solution for automated, high-throughput patch-clamping experiments. The Genedata Screener® software package integrates the trinity of raw data – analysis – storage/reporting into one highly optimized workflow with a flexible interface. This specialized and intuitive interface and the tight integration of components enables scientists to tailor each step and detail with all changes quickly propagated throughout the entire workflow.

This presentation will demonstrate how to simultaneously handle and analyze all plates and compounds from an automated electrophysiology campaign, enabling thorough quality control and accurate hit assessment. In total, over 3200 compounds were analyzed ranging over 10 384-well plates with 18,000 time points per well, and 2 (pre/post) channels – for approximately 140 million data points. The whole data processing procedure takes less than 30 minutes from raw data input to export of detailed reports inclusive of quality control and hit identification. The software system also features a flexible API, which enables the implementation of custom data processing algorithms, results export to company data warehouses, and much more. Customized algorithm development examples will be presented as well.

P4-08

In Vitro Integrated Assessment for Prediction of Drug-Induced Arrhythmia

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Keywords: Drug induced arrhythmia, Cardiac ion channels, Action potential, Trafficking

In vitro evaluation of the drug effects on a rapidly activating component of delayed rectifier potassium current (I_{Kr}) is recommended in the ICH S7B guideline to assess the potential risk of drug candidates for QT interval prolongation in pre-clinical studies. However, the arrhythmogenic mechanism is complicated and *in vitro* I_{Kr} assay alone is not sufficient to identify drugs with proarrhythmic risk. Recently, a new paradigm focusing on a comprehensive assessment of effects on multiple cardiac ion channels to predict the proarrhythmic risk of drugs has been proposed.

To predict proarrhythmic potential of drugs more properly, we investigated the effects of 10 drugs on action potential (AP) and several cardiac ion channels [I_{Kr}, L-type I_{Ca} ($I_{Ca,\,L}$) and I_{Na}] in male guinea pig cardiac muscles, and on human ether-a-go-go-related gene (hERG) currents, which are responsible for I_{Kr}, in hERG transfected CHO cells. In addition, we investigated the acute and delayed effects of pentamidine, known as the hERG trafficking inhibitor, on hERG current in hERG transfected CHO cells. [Methods] AP and each current measurement were performed using microelectrode and patch clamp technique, respectively. [Results and Conclusion] Each drug changed AP parameter depending on the inhibitory effects of I_{Kr}, $I_{Ca,\,L}$, and I_{Na}. There is little difference between the 50% inhibitory concentrations (IC_{50}) for I_{Kr} and hERG current for each drug. The IC_{50} for I_{Kr} [$IC_{50}(I_{Kr})$] of TdP-negative drugs (verapamil, diltiazem, and lidocaine) were comparable to IC_{50} ($I_{Ca,\,L}$) or IC_{50} (I_{Na}), and the IC_{50} ($I_{Ca,\,L}$)/ IC_{50} (I_{Kr}) or IC_{50} (I_{Na})/ IC_{50} (I_{Kr}) values were less than 7. The IC_{50} ($I_{Ca,\,L}$)/ IC_{50} (I_{Kr}) and IC_{50} (I_{Na})/ IC_{50} (I_{Kr}) values of TdP-positive drugs (bepridil, quinidine, and astemizole) were greater than 60. These results showed that drugs have IC_{50} for $I_{Ca,\,L}$ or I_{Na} similar to IC_{50} for I_{Kr}, did not induce TdP despite their potent inhibitory effect on I_{Kr}. IC_{50} ($I_{Ca,\,L}$)/ IC_{50} (I_{Kr}) and IC_{50} (I_{Na})/ IC_{50} (I_{Kr}) values were useful for the prediction of drug-induced arrhythmia. Pentamidine has no acute inhibition of hERG current up to 10 μmol/L; however, it induced delayed inhibition of hERG current with IC_{50} of 4.2 μmol/L, the effect assumed to be due to the hERG trafficking inhibition. These results suggest that *in vitro* assessment for drug candidates in multiple cardiac ion channel assays and hERG trafficking assay, in addition to *in vitro* I_{Kr} assay, is useful for proper prediction of drug-induced arrhythmia.

P4-09

Molecular Dynamics Simulation of Shiga Toxin, II

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

To develop a drug against infection by Shiga toxin (Stx)-producing *Escherichia coli* O157:H7 [1], this study investigates docking of Stx2 B subunit (Stx2 B) and a peptide neutralizer, using molecular dynamics simulations [2].
In the previous meeting (CBI2013: C-1-08), we reported the direction of this study based on the results of the short-time simulations (10-20 ns) about a single neutralizer, four unconnected neutralizers, and a dendritic neutralizer with four connected peptides. In this meeting, we will show the results of subsequent simulations of a dendritic neutralizer in which the simulation time was more than 100ns. The temporal analysis of the arrangement of the residues exhibited the following typical features.
(1) The number of residues which were bonded by electrostatic interactions increased as 7, 6, 6, 9, and 13 at time 10, 30, 50, 90, 130ns, respectively, providing the time scale of the binding of the Stx and neutralizer.
(2) Among the 13 bonds at 130ns, the 9 bonds were already formed and fixed at 90ns.
(3) Although the 3-5 bonds of these 13 bonds were formed at 10, 30, and 50ns, the arrangement of the residues still varied and fluctuated.
The bonding of residues occurred frequently between arginine of the neutralizer and asparagine or aspartic acid of the Stx, and in addition, the bonding occurred between methionine of the neutralizer and tryptophan of the Stx. This latter bonding results from hydrophobic interaction of residues. In the meeting, quantitative analysis of the above features will be presented in detail.

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P4-10

Comparison of *in silico* human ventricular model assay with *in vivo* canine PK/PD analysis for predicting the cardiac safety of antipsychotic drugs

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Keywords: ADMET, Computational toxicology, Pharmacokinetics, Pharmacodynamics

In new drug development, current approaches of cardiac safety assessment require *in vitro* I_{Kr} assay and *in vivo* QT assay in the preclinical stage and thorough QT/QTc prolongation test for supratherapeutic drug exposures in the clinical stage. Such approaches have been shown, however, not enough to predict the proarrhythmic potential of drugs. To improve the success rate of new drug development by efficient and low-cost operation, we compared a new technique, *in silico* prediction using human ventricular model, to an *in vivo* prediction based on the pharmacokinetics and pharmacodynamics (PK/PD) analysis in anesthetized dogs at preclinical stage.

Transmural ventricular wall model, of which membrane kinetics was represented by modified O'Hara-Rudy dynamic model of human ventricle, was constructed to simulate electrocardiogram. To predict clinical effects of antipsychotic drugs on QTc, we incorporated experimental data on their ion channel inhibition at unbound blood concentrations into the model. For PK/PD analysis, correlations between antipsychotic drug-induced QTc changes and plasma concentration of the drugs in anesthetized dogs were examined. Their effects on QTc in human at the therapeutic concentration were extrapolated based on the correlations. The QTc prolongation at the therapeutic concentration was classified into 4 categories; none, mild, moderate, or severe degrees.

In cases of Zipracidone and Quetiapine, the human *in silico* model predicted clinically-observed QTc prolongations more appropriately than the PK/PD analysis. In case of Thiolidazine, of which metabolites are suggested to contribute to the QTc prolongation, both human *in silico* and canine PK/PD analysis models underestimated the clinically-observed QTc prolongation at the therapeutic concentration.

Although the current version of the *in silico* human ventricular model needs to be improved, we can at least conclude that *in silico* assay would offer a powerful tool compared to canine *in vivo* conventional QT assay for predicting cardiac safety of new drugs at their early discovery stage.

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P4-11

QSAR analyses for thermodynamic driving force of antihistamines in their binding affinity for human H1 receptors

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Keywords: Affinity, Antihistamines, Enthalpy, Entropy, Histamine H_1 receptor, QSAR

[Introduction]
Differences between the thermodynamic driving forces of first and second generation antihistamines for human H1 receptors and their structural determinants were evaluated with quantitative structure-activity relationship (QSAR) analyses for the thermodynamic driving force.
[Methods]
The binding enthalpy and entropy of 20 antihistamines were estimated with the van't Hoff equation using their dissociation constants obtained from their displacement curves against the binding of tritium-labeled mepyramine, a typical antihistamine, to membrane preparations of Chinese hamster ovary cells expressing human H1 receptors at various temperatures. Structural determinants of antihistamines for their thermodynamic binding properties were assessed by QSAR analyses with genetic algorithm-partial least squares (GA-PLS) regression.
[Results]
Entropy-dependent binding was more evident in second than in first generation antihistamines, resulting in enthalpy-entropy compensation between the binding forces of first and second generation antihistamines. QSAR analyses indicated that enthalpy-entropy compensation was determined by the sum of degrees, maximal electrostatic potentials, water-accessible surface area and hydrogen binding acceptor count of antihistamines to regulate their affinity for receptors.
[Discussion]
It was revealed that entropy-dependent hydrophobic interaction was more important in the binding of second-generation antihistamines. Furthermore, the responsible structural determinants for enthalpy-entropy compensation were explored. These findings may contribute to understanding the fundamental mechanisms of how the affinity of ligands for their receptors is regulated.

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P5-01

In vivo quantification of pharmacologically blocked neuronal slow K^+ current and compensatory changes of HCN and persistent Na^+ currents

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Keywords: ion channel, threshold tracking, slow potassium current, HCN current, persistent sodium current

Background: Axonal excitability is tightly balanced by multiple ion channels such as slow K^+ channels, persistent Na^+ channels (INap), and HCN channels (Ih). For drug development, an ion-channel modulating drug is clinically promising. Quantitative *in vivo* assessment of ion channel functions is useful to confirm the intended effect. Threshold tracking (QTRAC) has elucidated dysfunction of ion channels in various neurological diseases.

Objective: To quantitate the *in vivo* effects of blocking slow K^+ channels on other ion channels

Methods: $BaCl_2$ (20 mg/kg) was given i.p. to seven normal, 6-week-old, male mice. Sensory nerve action potential was recorded at the tail. Axonal excitability was recorded by threshold tracking (QtracW, Digitimer, UK) before and 30 min post-infusion. Mathematical modeling assessed the interval changes of the ion channel functions.

Results: After the injection of $BaCl_2$, there were the following interval changes: (1) increased supernormality (P = 0.001) and decreased late subexcitability (P = 0.009) (recovery cycle), (2) increased threshold by long hyperpolarization (threshold electrotonus (P = 0.01)). Modeling showed the following changes of the channel parameters: 82% decrease of nodal slow K^+ current, 55% decrease of INap, and 56% decrease of Ih.

Conclusions: (1) Inhibition of neuronal slow K^+ current could be assessed *in vivo*, which was counterbalanced by reduction of INap and Ih. (2) Accommodation by other channels results in tight maintenance of axonal excitability. (3) *In vivo* axonal excitability testing is a non-invasive technique that can be easily applicable to human and animals. It can be a useful biomarker to monitor effects of ion channels by channel modulating drugs.

P5-02

Estimating fitness values and appearance time of evolvable self-replicating molecules from time series of frequencies in an evolution reactor

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Keywords: data mining; next generation sequencing; experimental evolution; fitness; quasispecies; evolutionary dynamics; synthetic biology

We have established a translation-coupled RNA replication system within a cell-like compartment, and conducted an experimental evolution of RNAs in the system [1]. As a result, we obtained a time series of the occurrence frequencies of various individual genotypes through next-generation sequencing technique. The time series showed a complex clonal interference [2] and a polymorphic population called the “quasispecies” [3]. Let Yi(t) be the observed mole fraction of a genotype i at generation t. By analyzing the time series of Yi(t), we estimated the fitness value and appearance time for each of the major genotypes. The analysis was conducted as follows. First, we assumed that the dynamics of a genotype i obeys the simple differential equation, dXi(t)/dt=(Fi-D(t))*Xi(t), where Xi(t) and Fi represent the mole fraction at generation t and propagation rate constant (=fitness) of the genotype i, respectively. D(t) is the dilution rate. Next, defining the sum of the relative entropy over all observed generations, $S = \Sigma_t \Sigma_i Yi(t) \log Yi(t)/Xi(t)$, and minimizing the value of S, we obtained the estimated fitness value Fi and appearance time for each genotype. As a result of the minimization, Xi(t) was well fitted to Yi(t) showing the correlation coefficient between Yi(t) and Xi(t) was 0.984. The estimated fitness values for 11 genotypes arbitrarily chosen were well consistent with the measured ones by the clonal measurement. These results support the validity of our estimation. We then analyzed the sequence-fitness relationship and concluded that the mutational effect is roughly additive.

This approach can clarify the evolutionary dynamics of quasispecies of viruses or other pathogens, and it may contribute to the drug design against the quasispecies.

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P5-03

Bifurcation Analysis in Reprogramming Process of Somatic Cells

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Keywords: Systems biology, Reprogramming, Bifurcation theory, Robust control theory

We study a mathematical model [1] that represents a mechanism for generating induced pluripotent stem cells (iPS cells). From dynamical systems theory, we find that saddle-node bifurcation occurs in the iPS model. We further address the robustness analysis problem to show that the bifurcation plays an essential role for inducing pluripotency even in actual iPS cells.

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P5-04

Oriented reconstitution of an ion channel in a cell-sized liposome

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Keywords: Model cells, Membrane proteins, Vesicles, Molecular robots.

We introduce a method for the reconstitution of the KcsA potassium channel with an appropriate orientation in cell-sized liposomes, using droplet-transfer technique. First, we prepared cell-sized water-in-oil (W/O) droplets covered with a lipid monolayer. When solubilized KcsA was encapsulated in the droplet, it accumulated at monolayers of phosphatidylglycerol (PG) and phosphoethanolamine (PE). The droplet was then transferred through an oil/water interface having a preformed monolayer. The interface monolayer covered the droplet so as to generate a bilayer liposome. By creating chemically different lipid-layers at the droplet and oil/water interface, we obtained liposomes with asymmetric lipid compositions in the outer and inner leaflets. KcsA was spontaneously inserted into liposomes from the inside or outside. Integrated insertion into the liposome membrane and the KcsA orientation was examined by functional assay, exploiting the pH sensitivity of the opening of the KcsA when the pH-sensitive cytoplasmic domain (CPD) faces toward acidic media. KcsA loaded from the inside of the PG-containing liposomes becomes permeable only when the intra-vesicular pH is acidic, and the KcsA loaded from the outside becomes permeable when the extravesicular pH is acidic. Therefore, the internal or external insertion of KcsA leads to an outside-out or inside-out configuration so as to retain its hydrophilic CPD in the added aqueous side [1].

Recently, self-moving liposomes are trying to construct as a molecular robot. It is known that liposomes tabulate their membranes in presence of membrane proteins or under hypertonic conditions [2-3]. Oriented reconstitution of ion channels to the tabulated liposomes using this method may allow them to achieve ameboid movements and illustrate the self-moving liposomes.

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P5-05

Positive Quadratic Systems Modeling and Stability Analysis of Chemical Reactions in Cells

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Keywords: Chemical reactions, Nonlinear Systems, Positive Systems

Chemical properties in a cell are analyzed by statistic, information theory, mechanics system, and etc. In particular, the intensive study on the relationships between signals outside a cell (extracellular signals such as antigen, growth factor, cytokine) and properties inside the cell (dynamics of intracellular signal transduction pathway, responsible for cell growth, differentiation or cell death) has been performed in the field of systems biology, where several dynamical models of chemical reactions in the cell including the signals outside the cell [1]: S-systems [2-4] or ordinary differential equations (ODEs) consisting of Hill functions [5].

It is one of important problems to "control" chemical properties in a ce ll through stimulus outside the cell as a control input. However, few models of chemical reactions in a cell that is suitable to design an optimal control input, more specifically, that expresses specified properties on chemical reactions in a simple and explicit way. We propose a new model of chemical reactions in a cell to express two biological properties: concentrations of chemical materials are non-negative and the dynamics of the chemical reactions depend on some multiplications of chemical material concentrations. Next we analyze stability of the new model by using a positive system analysis [6] and the LaSall invariant theorem [7].

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P5-06

Establishment of neuron-specific toxicity evaluation system using human induced pluripotent stem cell-derived neurons

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

Human induced pluripotent stem cells (hiPSCs) hold potent possibility in the drug development, because they possibly overcome difference between species in drug efficacy and toxicity. Neurons have specific damage process called ‘exictotoxicity', which is caused by excessive stimulation of excitatory neurotransmitter receptors. Because Ca^{2+} influx via NMDA type L-glutamate (L-Glu) receptors play a main role in this process, we have been searching for the hiPSC-derived neurons which stably express functional NMDA receptors. We first confirmed the correlation of NMDA receptors with the excitotoxicity in hiPSC-derived neurons by fura-2 Ca^{2+} imaging of neurons differentiated from 253G1, and commercially-available hiPSC-derived neurons (iCell® neurons [iNeurons]). 253G1-derived neurons showed stable but weak Ca^{2+} responses to L-Glu (100 μM) from 10 days *in vitro* (DIV). They did not exhibit the responsiveness to NMDA (100 μM) until 40 DIV. On the other hand, iNeurons showed sharp Ca^{2+} responses to L-Glu from 1 DIV. The L-Glu responses were sometimes inhibited by AP5, an NMDA-specific antagonist, however, the efficacy was not stable. We then tried to evaluate the excitotoxicity using these two lines. The toxicity was quantified by three parameters, i.e., propidium iodide (PI) / calcein staining, lactate dehydrogenase (LDH) release, and reduction of MTT. Although L-Glu (100 μM, 1 hr) did not cause cell damage in 253G1-derived neurons (three independent experiments), it caused significant cell damage in iNeuron-culture batches in which AP5 responsiveness had been detected. Staurosporine, an apoptosis inducer, caused significant toxicity to both of 253G1-derived neurons and iNeurons. These results indicate that the hiPSC-derived neurons which stably express NMDA receptors are necessary for the excitotoxicity evaluation system. We have examined 6 lines of hiPSC-derived neurons so far, and found two new lines most of which cells have functional NMDA receptors.

This work was partly supported by a Grant-in-Aid for Young Scientists from MEXT, Japan (KAKENHI 21700422), the Program for Promotion of Fundamental Studies in Health Sciences of NIBIO, Japan, a Health and Labor Science Research Grant for Research on Risks of Chemicals, a Labor Science Research Grant for Research on New Drug Development from the MHLW, Japan, awarded to K.S., and a Health and Labor Science Research Grant for Research on Publicly Essential Drugs and Medical Devices, Japan, awarded to Y.S.

P5-07

Biomarker-responsive supramolecular hydrogels

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Keywords: Hydrogel, Stimuli-response

Soft matters such as supramolecular hydrogels composed of self-assembled nanofibers exhibiting stimuli-response under aqueous conditions are attractive owing to their numerous potential bio-applications. However, installing macroscopic response toward structurally complex biochemical stimuli into them still remains a challenge. Here we show that redox-responsive peptide-based hydrogels[1] have the ability to encapsulate enzymes whilst retaining their activities and co-operative coupling of enzymatic reactions with the gel response allows us to construct unique stimuli responsive soft materials capable of sensing a variety of disease-related biomarkers. The programmable gel-sol response even to biological samples is visible to the naked eye. Furthermore, we built the Boolean logic gates, OR and AND gates into the hydrogel-enzyme hybrid materials, which were able to simultaneously sense plural specific biochemicals and execute a controlled drug release through the logical calculation.[2] The intelligent soft materials that we developed may prove valuable in future medical diagnostics or treatments.

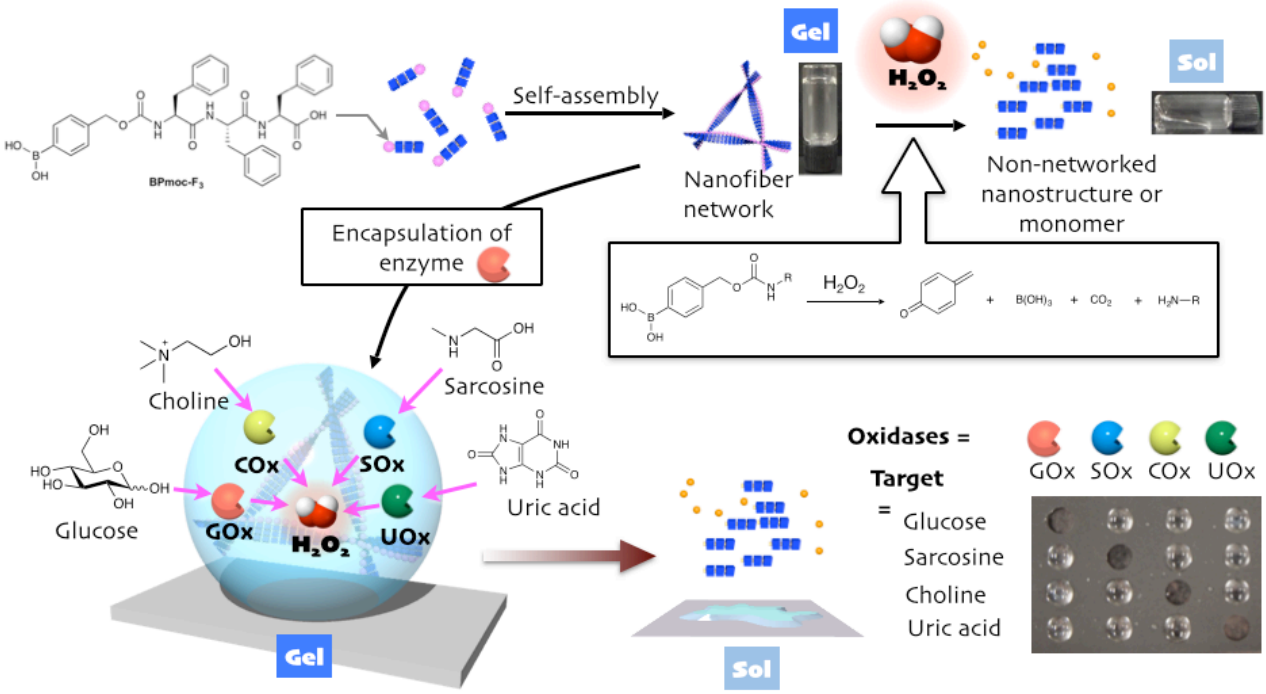

***Figure* 1** Schematic representation of Ox⊂**BPmoc-F$_3$** hybrid gels. Information in the form of molecular input is received by enzyme (oxidases) and converted into H_2O_2, which eventually gives rise to gel-sol change as output through the degradation of matrix consisting of H_2O_2-responsive nanofiber.

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P5-08

Live-Cell Super-Resolution Imaging with Spontaneously Blinking Fluorophores

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Keywords: Super-resolution imaging, Fluorescent probe, Rhodamine

Single-molecule localization microscopy (SLM) is used to construct super-resolution images by repeated detection and high-precision localization of individual fluorophores. However, to achieve an initial dark state and subsequent blinking of conventional fluorophores for SLM, additives such as thiols, an oxygen scavenging system, and high-intensity laser irradiation prior to measurement are needed, and these requirements are unfavorable for live-cell imaging. From this point of view, fluorophores that spontaneously blink in the absence of any additive and without the need for special conditions are highly desirable. Herein, we report a new class of spontaneously blinking fluorophore, utilizing intramolecular spirocyclization, which is suitable for live-cell SLM without additives or special conditions [1]. We focused on the phenomenon that rhodamine derivatives bearing an intramolecular nucleophile exist in thermal equilibrium between a fluorescent open form and a non-fluorescent spirocyclic form in the ground state. In order to utilize this thermal equilibrium to achieve spontaneous fluorescence blinking suitable for SLM, we optimized two parameters, the ratio of bright state and the duration until the open form reverts to the closed form. We prepared a series of rhodamine derivatives bearing various intramolecular nucleophiles and/or fluorophores, evaluated the properties, and selected **HMSiR** as an appropriate fluorophore. Then, we performed live-cell SLM of microtubules in HeLa cells with **HMSiR**. β-Tubulin-SNAP was expressed and labeled with benzylguanine derivative of **HMSiR**, and SLM was carried out in culture medium after washing. Spontaneous blinking of **HMSiR** was observed in the absence of any additive in the intracellular environment and microtubule structures were successfully constructed at far higher resolution than the projection image.

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P5-09

Introduction of DNA nanostructures and multiple μm-sized objects into live cells by a novel cell-GUV electrofusion method

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Keywords: GUV, electrofusion, artificial cell

Here we report a novel method for introducing large objects, up to a micrometer level, into live HeLa cells by electrofusion with an artificial giant unilamellar vesicle (GUV) [1]. We prepared the GUVs using the water-in-oil (w/o) emulsion centrifugation method [2], by which artificial objects were encapsulated in them. HeLa cells and GUVs were placed into an electrofusion chamber, then exposed to an AC field to align cells and GUVs linearly. After this positioning, DC pulses were applied to induce transient electrofusion and rapid transfer of objects from GUVs into cells. We successfully demonstrated that plasmids (GFP, mCherry), designed DNA nanostructure (DNA origami) and magnetic beads can be introduced into live cells by this approach. We also confirmed that multiple artificial objects (an expression plasmid for mCherry protein and 1 μm-sized fluorescent beads) can be transferred simultaneously into live cell. The treated cells divided normally and reached confluency in culture without significant damages. In addition, Hela cells, in which magnetic beads were introduced by this method, can be moved externally by a neodymium magnet.

Based on these results, we believe that our method can be used for simultaneous transfer of multiple genes and artificial objects, which paves a new way for elucidation of cell mechanisms, drag delivery system, and even creation of artificial cells.

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P5-10

A bright future: optogenetics and the FLIPR for voltage gated ion channels

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Keywords: optical, Ion channel, screening

Channelrhodopsin-2 (ChR2) is a light-activated microbial nonspecific cation channel, which can be used to depolarize neurons through the incidence of blue light.[1] In this regard it is possible to optically control the plasma membrane potential. This process opens new and interesting perspectives for the characterization of voltage-gated ion channels and the search for modulators.
A proof of principle study was performed to verify the applicability of this tool to the development of cell based assays suitable for High Throughput Screening (HTS).
We generated a stable cell line, which expresses both ChR2 and the human voltage-gated calcium channel Cav1.3 (hCav1.3). In addition, the inward rectifier hKir2.3 channel was stably transfected to control the plasma membrane potential by adjusting the extracellular K^+ concentration.
We used a ChR2 variant which carries a single amino acid mutation. This results in a prolonged lifetime of the ChR2 conducting state and reduces the light energy required for its activation. Two FLIPR^{TETRA} protocols were created to perform light stimulation of ChR2, with subsequent Cav1.3 activation, and also to induce a light dependent Cav1.3 partial inactivation. Known Cav1.3 state dependent blockers were tested in the resting versus the partial inactivated conditions and the obtained results were in very good agreement with the ones obtained with both the classical "external K^+ protocol" and patch clamp experiments (Q-Patch).
Our study shows for the first time the application of an optogenetic tool on the FLIPR instrument, for the purpose of running screening on voltage-gated ion channels in physiological extracellular potassium concentration.

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P5-11

Theoretical analysis of the formation of membrane tubes in giant liposomes induced by electrostatic effect

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Keywords: Lipid bilayer membranes, shape transformation, electrolyte, numerical calculations

Liposomes are closed vesicles formed by lipid bilayer membranes. They have been well studied as simplified models of biological membranes, and are considered as a possible component for molecular robots. It is well known that some cytoskeletal proteins and cytosolic proteins induce shape transformation of liposomes, such as the formation of long and thin membrane tubes from globular vesicles. However, membranes can undergo spontaneous tubulation without proteins. We observed giant liposomes composed of negatively charged lipids (PC/PI) and containing a sugar solution, and found that when they were exposed to a hypertonic KCl solution, very thin membrane tubes protruded into the interior of the spherical liposomes. To investigate the mechanism of this phenomenon, we numerically calculated the free energy of curved membranes, which is determined from the surface charge density, the distribution of electrolytes in the aqueous solution, and the membrane elasticity. The result showed that unless the lipid molecules have bulky heads, a cylindrical charged membrane may have lower energy than a flat one. This fact suggests that the thin tubes observed in the experiment may be induced by the electrostatic effect of the membrane charge.

P5-12

Loosely-stabilizing Algorithms for Leader Election in Population Protocols

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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 (we call this event an *interaction*). The PP model is suitable to molecular robotics because typical molecular devices are not fixed and they can sense only nearby devices.

In this poster, we introduce loosely-stabilizing leader election algorithms in the PP model. A loosely-stabilizing algorithm [3] ensures that, starting from any initial configuration, a system satisfies the problem specification within a relatively short time; after that, the system holds the specification for a sufficiently long time. Such an algorithm is highly reliable because, even if states of devices are changed due to some fault, the system recovers within a relatively short time. Since molecular devices can fail, high reliability of algorithms is important. We introduce two loosely-stabilizing leader election algorithms: Both algorithms elect a device as a leader within a polynomial time and keep the unique leader for an exponential time. The first algorithm [3] assumes that interaction between any pair of devices happens uniformly at random, and the second algorithm weakens this assumption.

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P5-13

pH-Responsive High-Density Lipoprotein for Drug Delivery System

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Keywords: Nanobiotechnology, Nanoparticle, Internalization, Endosomal escape

Endosomal escape of drug-loaded nanoparticles is a key step to achieve the efficacy and specificity of the drugs. High-density lipoprotein (HDL) is a natural drug carrier, and we succeeded in development of a cell-penetrating high-density lipoprotein (cpHDL) *via* genetic fusion with a cationic peptide derived from human immunodeficiency virus [1]. cpHDL was able to deliver an anticancer drug [1], an photothermal dye [2], and metal nanoparticles [3] into human cancer cells, and to potentiate their therapeutic actions. However, the majority of the cargoes delivered by cpHDL was entrapped in the endosomes. Polyanions (PA) bearing both pendant carboxylate groups and alkyl chains are able to destabilize the endosomal membrane in response to mildly acidic endosomal pH. Here we sought to develop cpHDL/PA complexes for cytosolic drug delivery by cpHDL.

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P5-14

Mathematical models of RNP-mediated synthetic translational systems for precise control of the differentiation of iPS cells

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Keywords: RNP-mediated synthetic translational systems, mathematical modeling, synthetic biology

Recently, it is possible to induce cellular differentiation and to develop various tissues and organs with iPS cells which are generated from human somatic cells, such as epithelial cells. The application to regenerative medicine of this biotechnology is expected. However, because of dispersion of the states of iPS cells or error of the handling, efficient induction of differentiation is difficult to achieve.
Here we exhibit the strategy to induce aimed differentiation of iPS cells by applying RNP-mediated synthetic translational system. Our synthetic translational systems detect the cellular state autonomously, and will provide appropriate stimuli for differentiation at the each stage of the cell, respectively.
To design a desired synthetic translational system, we developed mathematical models which can simulate the behavior of its dynamics in a cell. There exists an RNA/protein interaction-based synthetic translational switch to create a feedback system that tightly controls the expression of proteins [1]. It is the simplest and the most efficient system among possible synthetic switch, however there is no RNA/protein interaction-based synthetic translational switch which may behave as a positive feedback system. Based on this fact, first we designed a desired synthetic systems by applying artificial genetic circuits which consist of cis- and trans-regulatory modules [2] in addition to RNP-mediated synthetic translational systems. We simulated the behavior of sequential and oscillation circuit.
Simulation results suggested that time delay or nonlinear systems are required to produce sequential and oscillation circuit. Our goal is to establish the new strategy which can provide safe and precise autonomous system to control the fate of cells by RNP-mediated synthetic translational systems.

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P5-15

Novel voltage protocol for the characterization of hNav1.5 channel inhibition of drug candidates

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Keywords: Drug screening, Ion channel, Automated-patch clamp system

The human cardiac sodium channel (hNav1.5) plays important roles such as generation of action potential and propagation of excitation-conduction in the heart. Blockade of sodium channel can be the causes of arrhythmia or cardiac dysfunction in clinical use. Therefore, hNav1.5 inhibition may be considered as a risk factor in drug development. Detection and mitigation of Nav1.5 inhibition for drug candidates at early stage of drug development is very important. The patch clamp assay was conducted using hNav1.5-CHL cells and the automated patch-clamp system, IonWorks-Quattro. The voltage protocol used in this assay was a double pulse protocol which including 1 sec duration of recovery period at -100mV to evaluate the state-dependency and binding kinetics. The inhibitory activities of 6 typical sodium channel inhibitors such as disopyramide (Dis), flecainide (Fle), lidocaine (Lid), mexiletine (Mex), propafenone (Pro) and quinidine (Qui) were evaluated and the peak amplitude of sodium current at 1st and 2nd depolarization pulses was analyzed. The IC50 values at 1st pulse and 2nd pulse of Dis, Fle, Lid, Mex, Pro and Qui were 183.4 and 217.0 uM, 2.0 and 3.0 uM, 107.1 and 466.0 uM, 5.4 and 69.1 uM, 0.3 and 0.7 uM, 7.4 and 9.8 uM, respectively. The concentration-inhibition curves for the 1st pulse of Lid and Mex were dramatically shifted to the left, but those of Dis, Fle and Qui were not shifted so much. These variations of curve shift are thought to be due to the differences of dissociation kinetics of each drug at -100 mV. It is concluded that the double pulse protocol is useful for elucidation of Na channel inhibition kinetics and will contribute to the integrated risk assessment for the cardiac function.

P5-16

In Vitro Selection of Functional Peptides with Pore-Forming Ability to Liposomes

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Keywords: Antimicrobial peptide, cDNA display, liposome

Antimicrobial peptides that bind to the cell membrane of the bacteria, indicating bactericidal activity are found naturally in a wide variety of organisms, including amphibians, insects, humans, and plants[1].This mechanisms of action is due to the fact that these peptides form pores in the cell membrane, and to flow out the cytoplasm. Recently these peptides are also attracting attention in the application to the Drug Delivery System and new therapeutic drug development[2]. Structural features of antimicrobial peptides are amphipathic with hydrophobic and hydrophilic clusters, are to take the secondary structures.

By using cDNA display method which is an in vitro display technology, we have already succeeded in vitro selection of functional peptides having liposome membrane anchoring ability from a random peptide library of 28 amino acids in length. Then we discuss about the next experimental system to obtain the functional peptide with pore-forming ability to the liposome membrane by considering the application to Drug Delivery, by cDNA display method using a Fluorescence Activated Cell Sorting (FACS).

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P5-17

Image-based drug profiling reveals a dual inhibitor of EGF receptor tyrosine kinase and microtubules

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Keywords: High contents analysis, Drug profiling, Membrane traffic, Signal transduction

Small-molecule inhibitors are widely used as tools for biology and therapeutic drugs, and uncovering the target specificity provides a valuable insight into both basic and clinical studies. Here, we present image-based inhibitor profiling of EGF receptor pathway by quantitating their effect on both receptor trafficking and signal transduction. Unbiased multivariate analysis classified fourteen inhibitors into four clusters. Interestingly, EGFR inhibitor, a highly uni-specific tyrosine kinase inhibitor, was classified with a microtubule depolymerizer. Actually, EGFR inhibitor had microtubule depolymerizing activity both in vivo and in vitro, and also had an antimitotic activity. Our work indicates that image-based multivariate analysis is a powerful tool for discovering an unexpected drug properties, and EGFR inhibitor become as a novel seeds for multi-targeting cancer drug.

P5-18

Novel Cell- or Tissue-Markers Based on Giant vesicles

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Keywords: Giant vesicle, Magnetic resonance imaging, Infrared fluorescence imaging

The development of new technologies for imaging of living bodies or organs using cell- and tissue-markers to ensure good spatial resolution, sensitivity, and detectability is one of the most important subjects in pharmaceutical and medical treatment research. Here we introduce cell- or tissue-markers based on giant vesicles (GVs) developed by our group.

High-field magnetic resonance imaging (MRI) with contrast medium is already used clinically, and the design of new MRI probes for pharmaceutical and medical applications has attracted significant attention. Super-paramagnetic iron oxide (SPIO) is a promising candidates for the development of smart and functional probes. Here GVs that encapsulate SPIO particles at high number density have become a research focus.[1] Phospholipid membranes are natural membranes that are degradable *in vivo*. The encapsulation of SPIO particles at a high number density in GVs yields SPIO-containing GVs (SPIO-GVs) that can be used as highly sensitive MRI probes. The SPIO-GVs were composed of a single lamellar membrane with a diameter of 4.7 ± 2.2 μm. After injection of the SPIO-GV into one cell of a 4-cell stage medaka embryo with a glass capillary and fixing with paraformaldehyde, dark spots (typical diameter, 200 μm) were observed at the blastomeres of the injected cells in micro MRI. This indicated that the GV-based SPIO MRI contrast agent could be effective for enhancement of the MRI signal at the cellular level.

Rapid *in situ* imaging of human tissues is required for surgical applications. Specifically, various techniques using preoperative or intraoperative gastrointestinal endoscopies to assist with the intraoperative localization of tumors have been proposed. Near-infrared (NIR) fluorescent molecules, such as indocyanine green (ICG)[2], have drawn much attention because the NIR light can permeate organ tissues and can be excited and detected with a fluorescence CCD camera. GV-based tissue markers, including ICG, are prepared using three steps[3] : (i) mixing of ICG with lecithin GVs;, (ii) suspension of the ICG-containing giant vesicles (ICG-GV) in an oil phase dissolving polyglycerol-polyricinoleate (PGPR), and (iii) centrifugation of the suspension layered on a buffered solution to obtain a giant polymer vesicle (polymerasome) containing ICG-GV. When the tissue marker was injected into the inner gastric surface of an anesthetized pig using an endoscopic syringe and the injection site was observed using a laparoscopic fluorescence camera, the diameter of the spot blur was approximately 2 cm over a 5-h period, which can be used to determine the location of early-stage tumors of several centimeters in diameter.

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P5-19

Hybrid Systems as Controllers of Molecular Robots --- A Case Study

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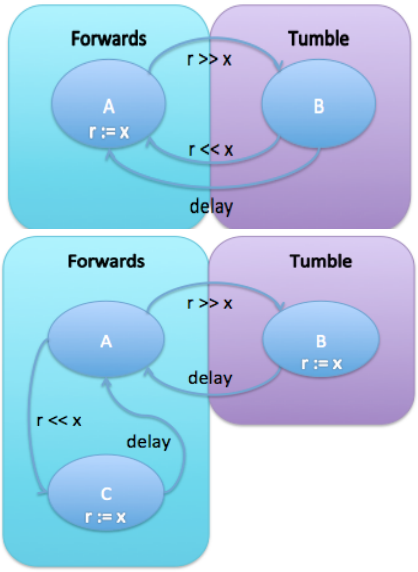
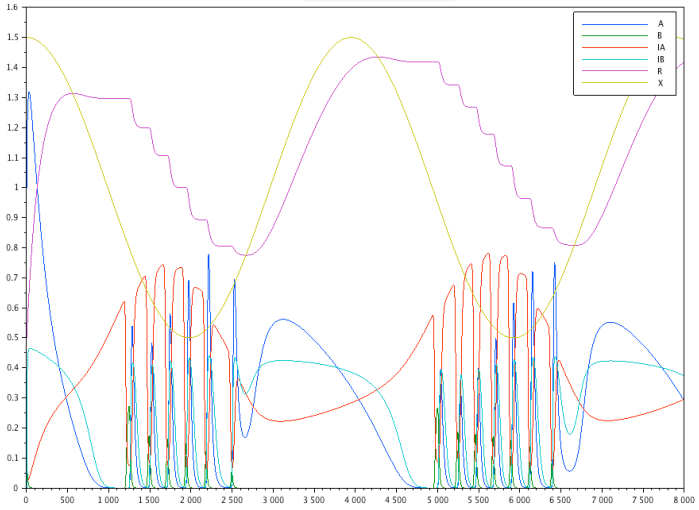
Keywords: Hybrid systems, molecular robotics, DNA computing, DNA PEN toolbox, Seesaw gate

Hybrid systems such as hybrid automata are often used to model controllers that process continuous values from sensors measuring the environment. Controllers for molecular robots are no exceptions. In this case study, we design a hybrid system that controls a "Paramecium Robot", which senses the concentration of "food", and moves on until the position with the highest concentration is found.



The robot senses the concentration of "food" at its current position. When the food concentration "x" gets higher, the robot stays in the state A (see Figure) and goes straight forwards. In this state, the concentration of "r" is used to store the value of "x". When the food concentration gets lower or the robot stays in A for a long time, it transfers to B and tumbles to direction. In this state, "r" remains constant. Furthermore, after the state B becomes unstable and the robot returns to A.

We use the PEN Toolbox[1] to realize the states and state transitions. A typical bi-stable system[2] consisting of the states A and B is used, where the transitions between the two states are controlled by "r" and "x". We use the Seesaw Gate[3] to approximate the assignment of "r:=x". The delay was realized by making the bi-stable system asymmetric so that B is weaker than A. Time trace for specific distribution of "x" is shown to the right.



However, while this system can be implemented by chemical reactions, it cannot be formulated as a hybrid automaton, prompting us to design another model with three states. Both the states A and C are for "going straight forwards" and B is for "tumbling to change direction". The value of "r" is updated (by r:=x) in B and C. When "x" falls down, the robot transfers to B, and when "x" rises, it transfers to C. After some delay, it returns to A.

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P5-20

Creation and manipulation of an artificial cellular membrane

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Keywords: lipid bilayer, photo-control, membrane interaction, vesicle transformation

In living cells, bio-molecules self-assemble in a predetermined fashion under specific conditions into highly organized supramolecular structures with specific functions. The constructive and synthetic approach to biological cells is an important and interesting challenge that spans across several disciplines, including physics, chemistry and biology. A closed two-dimensional surface consisting of a lipid bilayer is an essential compartment of living organisms. We have developed an artificial cellular membrane that exhibits biological functions [1]. We utilized synthetic photosensitive molecules to achieve the photo-based manipulation of membrane transformations [2]. The successful photo-manipulation of mesoscopic membrane structures in a noncontact and reversible manner may see wider application, such as in micro-reactors and smart drug-delivery systems (SDDS). We then investigated the association of nano-materials, such as nano-particles (NPs), on a model membrane surface [3]. We found that lateral heterogeneity in the membrane mediates the partitioning of NPs in a size-dependent manner. These studies may lead to a better understanding of the basic mechanisms that underlie the association of nano-materials within a cell surface, and to a design of artificial cell systems that recognize contacting objects.

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P5-21

Oscillatory Population Protocols

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Keywords: Population protocols, Oscillatory behavior, Distributed algorithms, Molecular robots

Understanding how autonomy emerges in biological systems and applying it in giving artificial distributed systems autonomous properties motivate our study. Precisely, we focus on self-oscillations that play crucial roles in autonomous biological reactions, and investigate them as a phenomenon in distributed computing. Self-oscillations are often understood as a chemical oscillator provided, for example, by the Belousov–Zhabotinsky reaction. In biological systems, the oscillatory behavior is used as a natural clock to transmit signals and hence transfer information. In artificial distributed systems, self-oscillations could be used to distributely and autonomously implement a clock. This problem emerges in the project of designing molecular robots [2].

In our investigation, we use the population protocol (PP) model introduced by Angluin et al. [1]. PP is used as a theoretical model of a collection of finite-state mobile agents that interact with each other in order to solve a given problem in a cooperative fashion. Computations are done through pairwise interactions and the interaction pattern is unpredictable. PPs can represent not only artificial distributed systems as sensor networks and mobile agent systems, but also natural distributed systems such as chemical reactions and biological systems. We aim in our work at designing algorithms that make a given population exhibits an oscillatory behavior by itself whatever its initial state.

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P5-22

Development of a hybrid potassium ion probe for detection of local potassium ion transition on cellular membrane

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Keywords: fluorescent probe, potassium channel, protein labeling, imaging

Potassium ion (K^+) is the most abundant metal cation inside living cells, which determines the level of resting membrane potential. Hence, real-time analysis of potassium ion (K^+) dynamics near cellular membrane brings us important biological insights. Lately, several fluorescent K^+ probes have been reported, but they are not capable of telling the difference between average extracellular [K^+] change and local [K^+] transition around the cells of interest, due to the suboptimum localization of the probes. To solve this problem, we conceived a strategy to selectively tether a fluorescent K^+ probe to the outer surface of plasma membrane by HaloTag technology (Figure 1a).

We synthesized TLShalo, a K^+-selective, photoinduced electron transfer (PeT) -regulated fluorescent Halo ligand. As fluorophore we adopted boron dipyrromethene (BODIPY) derivative, which has excellent optical properties for cell imaging. As a chelator moiety we selected triazacryptand (TAC) structure, which was reported to have favorable affinity for K^+ and selectivity against Na^+. To give sufficient membrane-impermeability to the probe, we introduced multiple anionic functional groups to the structure. As a result, fluorescence intensity of TLShalo elevated up to 10-fold as K^+ concentration increased from 0 to 150 mM, even in the presence of Na^+ (Figure 1b). TLShalo selectively labelled cellular surface of HeLa cells expressing HaloTag-fused protein and detected K^+ concentration change in extracellular medium. With the system, we could detect K^+ efflux induced by K^+ ionophore on the cellular membrane of HeLa cells under a cover glass that restricted K^+ diffusion. Further, we demonstrated that TLShalo could monitor local [K^+] transition near the cellular surface at subcellular resolution by using microinjection apparatus (Figure 1c, d).

We are now trying to detect real-time K^+ channel activity by labeling potassium channel directly with TLShalo.

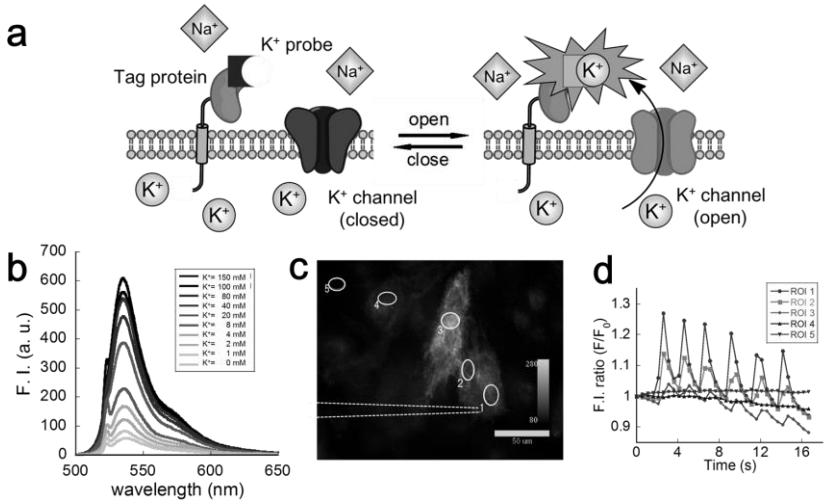

Figure 1 (a) Schematic image of fluorescent K^+ probe-based K^+ detection system. (b) Emission spectra of 1 μM TLShalo in HEPES buffer containing indicated concentration of K^+. (c)Epi fluorescent microscopic image of HeLa cells labelled with 2 μM TLShalo. (d) Fluorescence intensity ratio (F/F0) transition in each ROIs as a function of time. During experiment, 150 mM KCl aq. was effused every 2.5 seconds from a micro-injector needle.

P5-23

Reaction Graphs Controlled by External Signals

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Keywords: Molecular robotics, DNA nanotechnology,

In the field of molecular computing, researchers aim to implement information processing by chemical reaction of biopolymers. Especially computation using DNA is prosperous. There has been proposed many logic circuits using DNA([1], [2], [3], etc.). In particular, seesaw gate advanced by Qian and Winfree is a very promising molecular device with which we can construct large scale logic circuits. The progress of DNA computing technology may contribute to that of DNA nanotechnology. Yin, et al. developed an elegant DNA computing system which controls, or programs, bio-molecular self-assembly pathways of DNA nanostructures. They formulated the self-assembly reaction by the use of graphs, where each node represents DNA motif and each edge represents assembly or disassembly reaction. The system is called a "reaction graph".

Photochemical devices are also regarded as important nano-tools for DNA computing. There has been some studies on the application of photo-reactive devices to the implementation of molecular computing models. For example, Fujimoto's photo-reactive devices were used to construct a DNA logic circuit, full adder([5]). Tanida, et al., utilized azobenzene to the implementation of photonic automaton([6]). Thus, it is interesting to apply photochemical devices to the control of self-assembly pathways of DNA nanostructures. In this poster presentation, we will study on the computational model for the control of self-assembly pathways of DNA nanostructures by external signal. As external signals, we consider photo-irradiation of specific wavelength or temperature change, etc. In this study, we start from the computational model, "reaction graph", proposed by Yin, et al., and extend it so that it can deal with signals from outside the system.

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P5-24

Automated Microtubule Path Tracking on Gliding Assay Using Dynamic Programming

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Keywords: Bio-image informatics, object tracking, microtubule gliding assay, microtubule movement mechanism

Microtubule gliding assay is a biological experiment observing the dynamics of microtubules driven by motor proteins fixed on a glass surface when ATPs are dosed, video data of microtubules' movement can be obtain from gliding assay. In order to analyze the microtubule gliding mechanism from video data, computational methodologies that can automatically track the paths in microtubule gliding assay video data are urgently needed.

In microtubule gliding assays, object tracking becomes non-trivial due to the occurrences of compound objects such as crossing and snuggling of microtubules as well as sudden appearance and disappearance of microtubules. In order to solve these issues, we discuss the object tracking methodology using a dynamic programming.

These microtubule tracking paths can enhance our understanding of the dynamics of microtubule movement. Our future work will focus on the improvement of the recognition accuracy and mechanism of microtubule turning.

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P5-25

Comparison of field potential measurement and clinical information with *in vitro* system to evaluate arrhythmogenic potentials of compounds using human iPS-cell derived cardiomyocytes

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Keywords: drug development, safety pharmacology, human iPS-cell derived cardiomyocytes, field potential measurement, arrhythmogenic

To provide a better *in vitro* assay system to predict arrhythmogenic during the early stages of drug development including preclinical study, we propose here a drug screening system using the field potential (FP) measurement with human induced pluripotent (iPS) stem cell-derived cardiomyocytes. To accurately predict arrhythmogenic property of test compounds, several indices of FP have been proposed. In this study, we examined whether FPD prolongation with STV increase and expression of early afterdepolarization (EAD) were effective for the prediction of arrhythmogenicity *in vitro*.

We collected FP recording data from 36 compounds. Exposure of 31 compounds to the iPS-derived cardiomyocytes was conducted according to plasma concentrations in clinical studies. EAD or triggered activity was detected at concentrations within 1000 times more than human plasma concentration in 15 of 18 drugs, which are characterized as arrhythmogenic. Combined with the observation of FPD prolongation and STV increase, all 18 drugs were evaluated as arrhythmogenic. On the other hand, EAD or triggered activity was detected in only 1 of 13 drugs, in the interview form of which, no description on the risk of torsades de pointes was seen. In summary, concordance rate was found at 30/31 (97%), the false positive was 1/31 (3%) and the false negative was 0/31 (0%).

The screening system presented here showed an ability to predict the arrhythmogenic property of known compounds under the indices taken here. In this study, antiarrhythmic drugs were mainly tested in order to obtain a fundamental data in initial trials. Other compounds including anti-cancer or anti-psychiatric drugs will be the next challenge to improve sensitivity in the prediction of arrhythmogenicity with the system.

P5-26

Chronic probucol treatment decreases the slow component of the delayed-rectifier potassium current in CHO cells transfected with KCNQ1 and KCNE1

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Keywords: Ion channel, cardiac safety and pharmacology

Voltage-gated potassium (KV) channels can form heteromultimeric complexes with a variety of accessory proteins, including KCNE subunit. KCNQ1 (KV7.1) together with KCNE1 generates the slow component of delayed rectifier potassium current (IKs) that important for cardiac repolarization. We had reported that acute treatment of an anti-hyperlipidemic drug probucol did not inhibit IKs current in CHO cells coexpressing KCNQ1 and KCNE1 but chronic treatment of the probucol inhibited IKs current of the cells dose-dependent manner. Western blotting analysis suggested the disruption of the heteromeric complex formation could be the inhibition mechanism. Then, we had tried to reveal in detailed mechanism of chronic probucol's effect on the IKs current. Heterologous expression studies have demonstrated different functional modulation effects of probucol between IKs complex and KCNQ1 molecule. Probucol completely inhibited the IKs current in CHO cells expressing both KCNQ1 and KCNE1 genes (KCNQ1/KCNE1-CHO) at a concentration of 1 μM. However, KCNQ1 current in CHO cells expressing KCNQ1 gene alone (KCNQ1-CHO) was not reduced by probucol at concentrations up to 10 μM. These results suggest that chronic probucol treatment may contribute to KCNE1 molecule mediated IKs function. Then, because of probucol has cholesterol-lowering effect in the cells; we examined the effects of another type of cholesterol-lowering drugs, simvastatin and triparanol, on the IKs and KCNQ1 current using KCNQ1/KCNE1-CHO and KCNQ1-CHO cells, respectively to determine detailed inhibition mechanisms of the probucol on the IKs current.

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P5-27

Monitoring Cellular Response to Ion Channel Blocker with Motion Vector Prediction Method

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Keywords: Cellular motion, Ion channel, Induced pluripotent stem cell

We present a noninvasive method for the characterization of cellular motion behavior and its applicability for cardiac and neural cell. Motion Vector Prediction (MVP) method based on a block matching algorithm calculates motion vectors, which have a velocity dimension, with various spatiotemporal resolutions of the series of time-lapse phase contrast microscopic images. Especially, the MVP method with high spatiotemporal resolution images allows us to analyze the motion vector length with sub-micrometer resolution. We have established an evaluation for contractile behaviors of human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs). Simultaneous measurements of contractile motion and field potential revealed that the electrophysiological behaviors of hiPS-CMs are quantitatively reflected in the contractile motion. We also demonstrated that contractile motion prolife of hiPS-CMs was affected by various ion channel blockers in a dose-dependent manner. Furthermore, by using this method, we could detect sub-micrometer motion of human iPS cell-derived neural cells (hiPS-Neurons) and rat primary cultured cortical neural cells. Surprisingly, voltage-gated and ligand-gated Na^+ channel blockers inhibited the neural cell motion. Extracellular Na^+ deprivation also inhibited neural cell motion, suggesting that cellular ion flux affected neural cell motion. We will discuss the biological basis of neural cell motion and the applications of our high spatiotemporal resolution motion analysis to monitor cellular ion flux in various cells.

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P5-28

Contribution of quantitative change in funny current (I_f) to electrophysiological properties of developing embryonic ventricular cells: a simulation study

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Keywords: myocardium, pacemaker channel, embryonic development, mathematical model

In early embryonic development, the heart develops and gains new functions while continuously pumping blood, and heart abnormalities progress to congenital heart malformations; therefore, the developmental program of the heart, including the expression of the genes responsible for various ionic channels, is likely to be tightly regulated. The quantitative changes in ionic channels, pumps, exchangers, and sarcoplasmic reticulum Ca^{2+} kinetics are responsible for the changes in electrophysiological properties of the developing cardiomyocytes. Previously, we demonstrated that the developmental changes in action potentials (AP) of ventricular myocytes were well represented, as Na^+ current (I_{Na}) increased before the disappearance of and funny current (I_f), followed by a 10-fold increase in inward rectifier K^+ current [1] via simulation using a comprehensive cardiomyocyte model, the Kyoto model [2]; briefly, the relative conductances of the 9 components were switched between early embryonic (EE) and late embryonic (LE) values and simulated the 512 (2^9) combinations of the model. Here, we constructed a model to represent "middle" embryonic (ME) stage of guinea pig ventricular cell on the basis of experimental data, shifted relative current densities of the 9 components among EE, ME, and LE stages, in order to further overview the functional landscape of developmental changes in embryonic ventricular cells. As a result, we show that the relative conductance of I_f should be decreased by 80% by ME stage in order to avoid unphysiological behavior of ventricular cell in latter half of embryonic development.

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P5-29

Revised Modeling of Self-oscillating Gel including Experimental Results

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Keywords: dissipative particle dynamics method, OCTA, self-oscillating gel, meso-scale simulation

The self-oscillating gel is the gel which pulsates spontaneously itself using the dynamics of BZ reaction inside the gel. Recently, it is applied to several kinds of devices as an actuator or micro pump. To clarify its mechanism, we have made the model based on dissipative particle dynamics method, and perform the simulations of self oscillating gel.

In our previous model, the oscillation occurs along the change of the interaction parameter between polymer and solvent, and its value is changed along the sin-type oscillation according to our computational convenience. In the real self oscillating gel, BZ reaction occurs in the reaction-diffusion system, and the real oscillation of BZ reaction is not like the sin-type oscillation. Therefore we need the revision of our simulation model.

To represent self oscillating gel more realistically, we must include the real oscillation of BZ reaction from the experimental results. In the BZ reaction, change of reaction step can be observed by its color. Color is changed periodically from yellow to green. In the self oscillating gel, the changes of color also occur. In the shrinking state, color is from pale orange to yellow. In the swelling state, the colors change in an opposite manner. These changes of color are analyzed using our OCTA system. The color of gel is observed as a RGB value using the snapshots in each steps from the movie of self oscillating gel. From our analysis, in the swelling steps, the color changes rapidly, and in the shrinking steps, the color changes much slower.

These changes are included in our revised model. The change of interaction parameter is set along the change of RGB value. Using this parameter changes, we perform the DPD simulation. From our simulations, the result of change of thickness in swelling-shrinking dynamics is modified from our results in our previous model. Detail of our simulation results and difference from our previous model will be shown in the session.

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P5-30

On Time Responsive DNA Analog Computing Devices

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Keywords: DNA computing, Molecular robotics, DNA analog circuit

Since Adleman's seminal work on a DNA implementation of an algorithm for solving a directed Hamiltonian path problem, it is well recognized that the property that nucleic acid hybridization based on Watson-Crick complementarity enables us to construct chemical reaction systems with computational function ([1]). There has been many research results on the design and implementation of logic circuits using nucleic acid strands ([2] [3], etc.). In particular, Qian's work on seesaw gates made it possible to largely scale up the size of circuits ([3]). In most of the previous works, however, the proposed circuits are not time-responsive, 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]). Although time-responsive circuits were proposed in some previous works, they deal with only discrete systems ([4] [5], etc.).

In this poster presentation, we will propose a new approach to the implementation of time responsive DNA analog computing elements. Distinct features of the proposed devices is that they utilize kinetic relationship of concentrations of input and output molecules at steady state and that the change of the concentration of the input molecule will cause the change of the concentration of the output molecule according to the kinetic relationship. Therefore, the proposed analog device is time-responsive.

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P5-31

Optimization and pharmacological characterization of iCell cardiomyocytes for electrophysiological assessment in a multielectrode extracellular recording

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Keywords: iPS, cardiomyocyte, safety pharmacology

Human iPS cell derived cardiomyocytes (hiPS-CM) have become a valuable tool for safety assessment in drug discovery. In order to translate the results obtained in these cells into in vivo effects in humans, basic physiological or pharmacological properties of these cells should be well characterized. Present studies examined the effects of temperature, cell density and representative ion channel modulators with MED64 in iCell cardiomyocytes.

When the experimental temperature was changed from 35 to 38.5°C, the corrected field potential duration (FPDc) decreased from 370 to 285 msec (24 msec/ºC) almost linearly, but beating rate increased from 42 to 66 bpm at 37ºC and did not increase further even at 38.5ºC.

The cell density of plating affected the response to Iks blockers. The concentration-response curves for 293B and HMR1556 shifted to the right when cell densities were decreased from 30000, 15000, 7500, and 3750 cells/well. In lower density conditions, cell sizes enlarged and expression of the KCNQ1 gene decreased. The hERG channel blocker, E4031, consistently prolonged the FPDc at concentrations higher than 3 nM regardless of the cell density.

Various ion channel modulators indicated pharmacological characteristics of hiPS-CM.

These results indicate that the basic information on experimental conditions and pharmacological effects are very important for translation of the data.

P5-32

Fracture of Biomaterials

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Keywords: Surface energy, Elastics, Young's modulus, Fracture

Living cell is consisted of various biomaterials such as lipids, microtubule, DNA, RNA, and etc., and spontaneously forms various morphology after cellular differentiation. To understand the reason why the various morphology are formed, it is necessary to understand the mechanical properties of biomaterials as elastics. Here, we present the fracture phenomena of microtubule [1], which is an example to evaluate the mechanical strength of biomaterials.

The experimental strategy is as follows. First, we fixed a microtubule on the surface of a PDMS substrate. Next, we stretch the substrate by suitable stretching rate and observe the microtubule to determine the fracture conditions of microtubule. Based on the fracture conditions, we evaluate the Young's modulus of a microtubule. We found that the fracture happens when deformation energy of a microtubule is equal to surface energy of two fracture surfaces as

$$E = (1/2)E_{Young}\varepsilon^2 V = 2\gamma S$$

where E_{Young} is the Young's modulus of a microtubule, V is the volume of the microtubule before fracture, S is the area of a fracture surface, and ε is strain. As a result, we obtain that the mechanical strength of a bare microtubule is 10 MPa, which is a good agreement with previous studies.

Relation to this, we also present blooming phenomena of chocolate, the fracture phenomena of fatty acids [2].

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P5-33

Characterization of synaptic transmission induced synchronized population bursts of the iPSC-derived neurons

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Keywords: Human iPSC-derived Neurons, seizure, MEA

Many drugs have been reported to cause seizures. It has been reported that the causes of drug-induced seizures are $GABA_A$ antagonism, $GABA_B$ agonism, adenosine antagonism, and enhanced excitation through NMDA in the neurons. So far, there is no good *in vitro* assay system for predicting drug-induced unexpected seizure-risks. Spontaneous neuron activity recordings by multi-electrode array (MEA) system from networks of cultured neurons could be a good risk evaluation system for such drug-induced seizure events [1]. It was reported that long-term electrophysiological activity and pharmacological response of human induced pluripotent stem cell (hiPSC)-derived neurons were accelerated by co-culture with rat astrocytes. [2].

In this study, we observed time course generation of population burst spikes from iCell neurons with conditioned medium of mouse primary astrocytes by MEA system. Humoral factor(s) from mouse primary astrocytes was sufficient to generate synchronized population burst signals. GABA antagonism enhanced and calcium channel blockers eliminated the astrocyte-induced synchronized population bursts. We concluded that the observed astrocyte-induced population bursts by MEA system are mediated by synaptic transmission and the periodic synchronized population burst signals could be a good prediction marker of $GABA_A$ antagonism.

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P5-34

Single-molecular Activity Measurement of Enzymes Attached to DNA origami

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Keywords: Molecular Robotics, DNA nanotechnology, DNA origami, enzyme

DNA is a suitable material to make various functional devices [1,2]. One of such application is a measurement of chemical reactions at the single-molecule level. Nanostructures made by DNA origami can be used as a platform for this purpose [3]. Here, we propose a system to restrict the reaction space of enzymes on DNA origami substrates. We have attached ssDNA to polymerase and nicking endonuclease [4], and the reaction space of the enzymes is controlled by the length of the ssDNA. The experimental results of the measurement will be reported in the poster.

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P5-35

Making Logic Circuits with a Single Component in a Virtual Kinematic Environment

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Keywords: Virtual kinematic environment, logic circuit, molecular robotics

The final target of molecular robotics is to achieve a small robot composed of molecules. One need to make a variety of functional parts, such as motor, sensor, computer, and structure, using as small number of molecules as possible since too many molecules are difficult to control in atomic scale. It is ideal that a single component can work as computer as well as motor, sensor, and structure. The final goal of this research is to find such a component in a virtual kinematic environment and to construct a preliminary robot in the environment.

In the current work, we first made a logic circuit in a virtual kinematic environment using Unity, which is a computer-game development environment widely used recently. We used a single component like a cube, which two faces are input, and two are outputs. As it is a mechanical logic circuit, the input and output are expressed by the moving of the face into or out of the stable position. In a sense, the component already include the minimum function of sensor and motor as well as computer. By combining a lot of components, it can also form a structure of the robot. Therefore, it is a candidate for a component of a molecular robot, though the environment is virtual.

Second, we showed that the cubic component works to make a simple logic circuit like half adder, ring oscillator, and so on. The logical function of the cube is the combination of AND and NAND logic elements. The first output is the AND output of two inputs, and the other face is that of NAND. Therefore, we can theoretically make arbitrary logic circuit by connecting cubes. As it has a certain delay inside the logic, we can make an oscillator, which works like an engine of the robot. We plan to make a vehicle using this mechanism.

Stevens[1] showed self-replicating machine can be made by combining several cubic components in a virtual environment. His target was self-replication and he used many different functional cubes, such as sliding, rotating, wiring, and fusing. In our case we use a single component and self-replication is still a future target.

The problem of the current implementation is that it is too slow to achieve complicated logic circuit which needs to construct a robot. Tens of cubes would be the limit of this Unity system. The collision detection seems the bottleneck of the calculation. We plan to switch to a simple software layer, such as CUDA, which is a GPU computing environment. Then we could get more speed. We already compared the calculation speed between Unity and CUDA in a simple particle collision simulation, and found that CUDA is much faster for large number of particles. We plan to make a molecule like structure for a component, since the collision detection becomes simpler with spheres.

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P5-36

Construction of Computational Elements by a Swarm Network with Brownian Motion

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Keywords: Swarm network, Universal Turing machine, Brownian circuit

Designing computational devices will be of importance for molecular-sized robot systems that are composed of chemical or biomolecular elements, in order to be able to control these systems. Micrometer-sized or Nanometer-sized robots can have only limited complexities, thus building the conventional types of information processing structures may be quite difficult due to their complexity. The alternative approach to establish molecular robotics–constructing complex machines from a cloud of simple and homogeneous machines–represents a type of architecture that have been well studied in the context of Cellular Automata (CAs). In this model identical finite automata–called cells–arranged in a regular structure interact locally according to a set of rules.

To conduct information processing in molecular-sized robots, we have proposed the so-called *Swarm Network* model and have shown it to be Turing universal through the construction of computational elements [1]. Swarm Networks are a generalization of CA in which the neighborhoods and functionalities of cells are determined by the presence or absence of connections between cells. An agent, which corresponds to a cell in a CA, can have a wide range of functionalities. One functionality is the exertion of a force on the agent in a particular direction, but this requirement is unnatural when implementing swarm agents by biological molecules, because it remains unclear where this force originates from.

This presentation proposes a Swarm Network that is more realistic by assuming that agents are subject to Brownian motion. Moreover, connections between agents can be changed dynamically, allowing for the formation of structures and the dynamic transfer of information between agents. These characteristics make the model mimic behavior of biological organisms more closely than its previous incarnation. We show that this model is capable of universal computation by constructing a universal Brownian circuit [2] based on it.

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P5-37

Metabolomic approaches for compound annotation of volatile organic compounds in headspace of *Allium fistulosum* using in-tube extraction devices

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Keywords: Metabolomics, annotation, volatile organic compounds

Plants produce a multitude of volatile organic compounds (VOCs), which are important components of the metabolome. VOCs play a dominant role in biotic and abiotic stress responses as well as plant-plant and plant-insect interactions. To determine what kinds of VOCs can be emitted from plants and how much their levels change, a comprehensive and a reproducible analytical method is required. In the study, we employed one of the state-of-the-art techniques, in-tube extraction (ITEX), to conduct headspace (HS) sampling of Allium fistulosum (Japanese bunching onions). The collected HS samples were analyzed by gas chromatography–time-of-flight–mass spectrometry (GC-TOF-MS) because it enables us to rapidly identify peaks by combining our metabolomics pipeline. In parallel, we estimated how many volatile peak s in the mass spectra overlapped among the mass spectral libraries for the tentative identification of VOCs. Using the VOC profiling pipeline based on HS-ITEX-GC-TOF-MS, we conducted VOC profiling of the samples that were emitted from 12 Allium cultivars. We will present how to annotate VOCs by comparing each MS spectrum obtained by HS-ITEX-GC-TOF-MS analysis and MS spectral database.

P5-38

A Computational Framework to Analyze Microtubule Gliding Assay

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Keywords: Molecular Robotics, Microtubule Gliding Assay, Bio-imaging, Real-time Simulation

Microtubules and motor proteins are expected to play important roles in molecular robotics as molecular actuators and skeletons, to name a few [1,2]. Microtubule gliding assay is a biological experiment observing the dynamics of microtubules driven by motor proteins fixed on a glass surface when ATPs are dosed [3]. In order to understand control mechanism of microtubules, we propose a computational framework to analyze microtubule gliding assay video data and to reproduce the behavior of microtubules on 3D real-time simulation [4]. Novelty of this framework is in cooperative feedback between bio-imaging and 3D real-time simulation to estimate control parameters of microtubule gliding assay. Bio-imaging play an essential role to estimate control parameters of stochastic microtubule head movement by means of microtubule tracking analysis. 3D real-time simulation enables us to confirm the validity of the control parameters by reproducing overall dynamics of thousands of microtubules.

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P5-39

Hemodialysis treatment weakens the contraction force of central sinoatrial nodal cell

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Keywords: Na^+/Ca^{2+} exchanger, Contraction force, Cardiac myocyte, Hemodialysis

In the hemodialysis treatment which is one of the renal replacement therapies, it is difficult to provide adequate serum calcium (Ca) level to patients [1]. Pun *et al*. reported that patients who were exposed to larger serum dialysate Ca gradient had a greater fall in blood pressure during treatment and increased a risk of sudden cardiac arrest [2]. It is well-known that the blood pressure depends on the cardiomyocyte contraction force which correlates significantly with cytosol Ca^{2+} concentration (excitation-contraction coupling) [3]. However, the question of whether any effect of hemodialysis treatment is observed on the Ca dynamics in cardiac myocyte remains unclear. In this paper, we constructed a massive integrative mathematical model for which a change of Ca^{2+} concentration in interstitial fluid during treatment affects on the excitation-contraction coupling of the central sinoatrial nodal cell. Using this model, we explored a mechanism of the fall in blood pressure during treatment from a viewpoint of single cell biology.

Prescription of the low Ca concentration dialysate (2.5mEq/L) for hypercalcemic patient gradually decreased Ca^{2+} concentration in interstitial fluid during hemodialysis treatment. Although the decrease in Ca^{2+} concentration in interstitial fluid had little effect on amplitude in membrane potential, the peak level of inward current on cellular membrane increased during treatment. A componential analysis of the inward current demonstrated that the increase in peak level of inward current was induced by an activation of Na^+/Ca^{2+} exchanger. This finding implied that the intracellular Ca^{2+} and extracellular Na^+ were excessively transported to extracellular and intracellular space, respectively. As a result, the cell showed both a 2% decline in the beating rhythm and an 8% decrease in the contraction force during treatment.

In summary, the hemodialysis treatment caused an activation of Na^+/Ca^{2+} exchanger and weakened the contraction force of central sinoatrial nodal cell in hypercalcemic patient. The minimization of serum dialysate Ca gradient was shown to be useful in the prevention of a fall in blood pressure during treatment.

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P5-40

Development of a Molecular Sensor that Works on a Liposomal Membrane

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Keywords: DNA, lipid, conjugate, vesicle

In the molecular robotics project, molecular robots that is based on a vesicle or a gel as a body consist of molecular sensors, molecular computers, and molecular actuators.[1] These components are communicated with single-stranded DNAs each other through the hybridization. We cover a development of molecular sensor that is conjugated molecules between DNA strands and lipid.[2] A structure of the conjugated molecules has a long hydrophobic part at the center of molecule and two DNA strands at the both ends. The long hydrophobic parts of sensor molecules sticked in a liposomal membrane. A complementary DNA/RNA strand that represented an exterior environmental information was hybridized with the two DNA strands of conjugate molecules. The hybridization caused an approach of the two molecular sensors, consequently, two short DNA strands of conjugate molecules on the inner surface of liposomal membrane behaved as a long DNA molecule. The approached DNA strands that showed high melting temperature caused a strand displacement between a DNA duplex that was labeled with a fluorescent and a quenching dye. Then, a single-stranded DNA that was labeled with the fluorescent dye was released into an inner water pool of liposome. The released DNA can be a trigger molecule for molecular computers and molecular actuators encapsulated in the liposome.

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P5-41

Designing an Artificial Ion Channel From DNA origami

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Ion channel, which electric signal transduction molecule across the cell membrane, is one of the most indispensable devices of living organisms. In natural case, the channel molecule of membrane proteins attach on cell membrane, pass ions, and regulate the ion flux. In the point of view for molecular robotics [1,2], it is quite important to construct such a channel device passing small information molecule. Controllable synthetic channel molecules would expand possibility of molecular nanobiotechnology[3].

Here we report an artificial ion channel using DNA origami–a DNA self-assembly methodology to create predesigned nano-scale structure[4]. Our DNA origami channel is constructed of two vertically positioned parts, a flat plate part and a cylindrical tubular part. The flat planner part will play a role to be pressed onto the membrane by inputted voltage on account of DNA negative-charge, and will lead the cylindrical part insert into the lipid bilayer. Electrophoresis and AFM imaging indicated that the DNA origami channel structure was constructed as designed.

Their function identification of current measuring was also accomplished by using home-made droplet interface bilayers system [5]. The current value showed about 70pA per contact area when the potential difference inputted 100mV across a lipid bilayer. The current-voltage correlation was proportional in the range of -100mV to +100mV. The result supports that the designed origami worked as voltage-independent channel. The pore was designed as in the size of 4x4 nm^2. By modify the gate of the tubule structure, we are now investigating the new functions of the origami channel. The possibility of some high-selectivity function such as diodicity, specific ssDNA transportation, and size controlled molecular transportation. These researches would show the clue to help constructing compartmentalized molecular robot or artificial cell.

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Effects of substrate elasticity on gene expression profiles of human iPS-derived cardiomyocytes

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Keywords: human iPS cells, cardiac toxicology, hydrogel culture, motion vector analysis.

Human induced pluripotent stem cell (hiPSC)-derived cardiomyocyte (CM) provides promising possibilities to generate a new cell resource for drug safety screening as well as patient-specific lineages for a variety of translational research. However, the suitable cardiac differentiation method has not been attained yet both for toxicological and clinical applications. Because previous studies showed that mechanical stimulation regulates differentiation and maturation of stem cells, we tested the effects of substrate elasticity on contractile behaviors of hiPSC-CMs cultured on hydrogel ranging from 12 to 50 kPa and rigid plates using a high-speed video imaging system with motion vector prediction algorithm [1]. The motion vector prediction analysis revealed that substrate elasticity affects velocity of contraction-relaxation without altering beating rates. The cells cultured on 12kPa hydrogel showed the largest velocity of contraction-relaxation and pharmacological responses. In order to pursue the underlying mechanisms of the effects of substrate elasticity, gene expressions in hiPSC-CMs (iCell-CM, CDI) provided from cultured with rigid plates and 12kPa hydrogel were compared by microarray analysis (TORAY) or real-time PCR. Differentially expressed genes were identified using a fold-change cut-off > 2.0 (188 up-regulated genes and 63 down-regulated genes). Gene Ontology analysis and pathway analysis (DAVID) identified enriched functional-related gene groups such as ion transport, response to stress and calcium regulation for up-regulation, and cell adhesion for down-regulation. The responses of drugs will be tested. These results suggest that appropriate mechanical force may be an important factor for maturation of hiPSC-CMs.

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Building An Artificial Protein Capsid

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The long-term goal of bionanoscience is to be able to programmably construct designed nanoscale objects using biological molecules. Such objects could have a myriad of uses as templates for inorganic materials, as interfaces between biological and silicon systems, as highly sensitive disease detectors and as smart, robot-like drugs. Proteins and DNA are widely used molecules in this area but offer different possibilities and challenges. We have used proteins to construct a variety of nano-shapes such as self-assembled nanotubes and in particular, an artificial virus capsid1. This capsid is an example of an engineered protein cage, which we hope will have numerous applications, including drug delivery devices and nano reaction vessels. Our cage is based on a modified ring-shaped TRAP protein as the starting material. Addition of a metal catalyst to the protein leads to the efficient formation of a hollow ~21-nm diameter spherical protein cage. The cage displays high stability under various conditions, yet readily disassembles under reducing conditions. Structural analyses have revealed that it is composed of 24 identical TRAP rings arranged in a unique symmetry.

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