P1-01

An elucidation of STING phosphorylation mechanism by a combination of molecular dynamics simulations and molecular biological analyses

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Keywords: STING phosphorylation, molecular dynamics simulations, vaccine adjuvants

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

In this study, we adopted an interdisciplinary approach and predicted the dynamics of STING by in silico simulations, followed by the characterization of candidate residues by molecular biological techniques. Based on the results, we propose a putative mechanism of STING phosphorylation upon binding of a potent ligand.

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P1-02

Molecular Dynamics Simulation of Shiga Toxin, IV

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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 [1,2], this study investigates docking of Stx B subunit (Stx B) and a peptide neutralizer, using molecular dynamics simulations [3].
In the previous CBI meetings, we reported the binding of Stx1 B and MMA-tet: Stx1 in the former is a family of Stx, and the latter is a neutralizer developed by Nishikawa and collaborators with the peptide library technique [2]. The MMA-tet is a branched peptide oligomer with four arms; the amino-acid sequence of each arm is MMARRRR. We have shown that (1) the MMA-tet recognized and docked on the three classes of binding sites in Stx1 B, in agreement with the deduction based on the experiments; (2) the binding of Stx1 B and MMA-tet occurs inhomogeneously on the Stx1 B surface, implying that in fact more than one neutralizer bind to one Stx1 B in actual situations; (3) it remained unclear why the neutralizer binds strongly one of the three binding-site classes which consists of arginine or tryptophan residues.
In this 2016 meeting, in addition to further analysis of the binding of Stx1 B and MMA-tet, we will report the results of PPP-tet, in which the amino-acid sequence of its arm is PPPRRRR. The biological experiments have shown that the PPP-tet binds to Stx2 B (B subunit of another family of Stx) but not to Stx1 B [1]. This study demonstrates that our MD simulations can reproduce this feature and therefore it can be an effective method for developing drugs against toxic proteins. .

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P1-03

3D reconstruction program for coherent diffraction patterns obtained by XFEL

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Keywords: X-ray free electron laser, Single particle analysis

3D structural analysis for single particles using X-ray free electron laser (XFEL) is a new structural biology technique, which enables us to observe the molecules that are hard crystallized such as biological molecules and living tissue in the state close to nature[1,2]. In order to restore the 3D real structure of the molecule from the diffraction patterns obtained by XFEL experiments, computational algorithms are necessary as one needs to estimate the beam incidence angles to the molecule and retrieve the phase information in Fourier space[3,4].

We are developing a program package for XFEL analysis based on XMIPP[5], which is commonly used for image processing of single-particle 3D cryo electron microscopy. Our program successfully restored 3D structure of the molecule.

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P1-04

Interaction analysis of MDM2 inhibitor by fragment molecular orbital method

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Keywords: MDM2, p53, Protein-protein interaction inhibitor, FMO, IFIE

The p53 protein is a transcription factor that controls the cellular response to DNA damage or other stress stimuli through the induction of cell-cycle arrest, DNA repair, apoptosis, and senescence. Since it is well known that lack of p53, which is inhibited by MDM2, can cause cancer, the MDM2-p53 interaction was proposed to be a promising therapeutic strategy for cancers. A number of inhibitors have been reported by several groups [1-5]. In order to obtain more information for designing novel inhibitors using the fragment molecular orbital (FMO) method [6], we computed inter-fragment interaction energies (IFIE) of co-crystal structures of MDM2 with its inhibitors, and evaluated the relationship between interaction energies and the reported experimental IC50 values. 13 structures were adopted in current study. These structures were prepared by the following steps before the FMO calculations using software package MOE2014: modifying PDB structures with structure preparation module, adding hydrogen atoms using protonate 3D module, and optimizing these hydrogen positions. Subsequently, the FMO calculations were carried out by ABINIT-MP6.0+ and the calculation level was set to MP2/6-31G*. Finally, the correlation coefficients between the interaction energies and the reported pIC_{50} were calculated. As a result, we obtained a good correlation between IFIE-SUM and experimental values (R = 0.777). Importantly, an amino residue, Phe55, played vital roles in interacting with inhibitors.

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

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P1-05

Hydration Thermodynamics Study of Activation of Hydrolytic Water in Acyl-Trypsin Intermediates

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Keywords: WaterMap, Catalytic rate constant, Hydrolytic water, Gibbs free energy

[Backgrounds]

The force driving the acyl intermediate to the tetrahedral intermediate in the deacylation step of serine proteases is not well understood. In the deacylation step, a hydrolytic water attacks the carboxyl carbon of the acyl group, which results in a tetrahedral intermediate. We investigated the hydration thermodynamics of activation of the hydrolytic water in acyl-trypsin intermediates.

[Materials and Methods]

First, representative solution structures of five different acyl-trypsin intermediates (see [1] and other related reports) were obtained from the trajectories of molecular dynamics simulations using Desmond 4.3 (Schrödinger). Then, hydration thermodynamics analysis was performed using WaterMap^{TM} 2.4 (Schrödinger) [2]. Among the predicted water sites, the hydrolytic water site was identified in each structure, and thermodynamic properties of the hydrolytic water were analyzed.

[Results and Discussions]

All calculated Gibbs free energy (ΔG) values of hydrolytic water exhibited positive values, and logarithms of experimental catalytic rate constant values and calculated ΔG values were positively correlated with a remarkably high correlation coefficient ($R^2 = 0.925$). The result suggested that unfavorable ΔG of the hydrolytic water may contribute to the deaylation in the catalysis.

Calculated enthalpy values and number of hydrogen bonds between acyl-trypsin and the hydrolytic water per frame showed a negative correlation. The hydrolytic waters seemed to be stabilized through hydrogen bonding to surrounding residues. Also, calculated entropy values and occupancy values were negatively correlated. The occupancy seemed to increase when surrounding residue prevented a water molecule escaping from the hydrolytic water site.

Consequently, we suggest that the environment of serine proteases leads to unfavorable ΔG of hydrolytic water in cooperation with substrates, and when this hydrolytic water turns to tetrahedral intermediate hydroxyl group, deacylation may be accelerated in proportion to the ΔG value. In the future, the methodology developed in our study will accelerate researches on hydrolytic enzymes.

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P1-06

Approach to the quantitative prediction of the binding affinity in biomolecular system

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Keywords: binding affinity, free energy, metadynamics, alchemical transformation, bootstrap

Current “state-of-the-art” free energy calculations based on alchemical transformation for ligand binding have been widely accepted in computational ligand design and optimization. Here, the prediction of the preferred orientation of the ligand bound to the target receptor is necessary. In this work, to construct the free energy surface for ligand binding pose, metadynamics is applied. Consequently, we propose the following workflow based on the free energy calculations coupled with exploring metastable structures.

1. Search several metastable ligand-receptor complexes using the well-tempered metadynamics in a suitable collective variables space.
2. Extract the equilibrium geometries using conventional molecular dynamics procedure.
3. Run equilibrium simulation at each intermediate state along the alchemical transformation pathway.
4. Calculate free energies from the collected uncorrelated samples using the multiple trajectories, multistate Bennett acceptance ratio.
5. Estimate statistical uncertainty with the bootstrap method.

We believe that our workflow described above will become a promising approach in innovative drug design.

P1-07

Theoretical Comparison of the Conformational Affinity between hOGG1 DNA Repair Protein and DNA Holding Different Numbers of 8-OxoG

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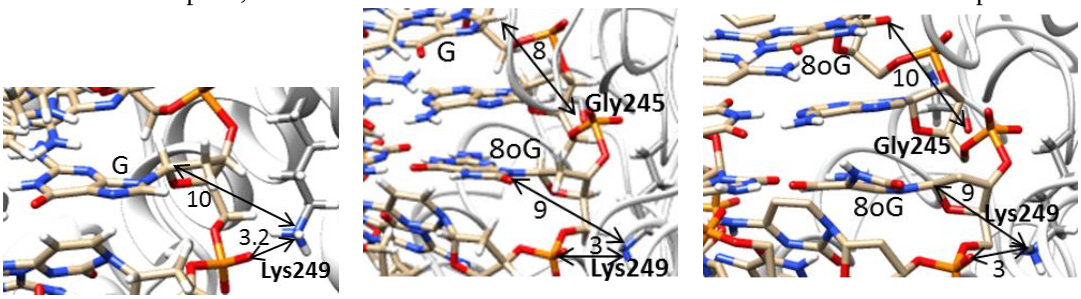
Keywords: 8-oxoG, hOGG1, Accelerated Quantum Chemical Molecular Dynamics

Introduction: The human repair enzyme 8-oxoguanine (8-oxoG) DNA glycosylase (hOGG1) is known to particularly remove the 8-oxoG and ignores undamaged bases, and the numbers or the position of 8-oxoG in DNA changed the frequency of mutation induction[1,2]. To elucidate the mechanism of the phenomenon, the conformational affinity of various DNAs to hOGG1 was investigated.

Theoretical Methods and Models: The Protein Data Bank ID 2I5w[3] was applied to model the initial interrogation stage of hOGG1. Energy minimization for the DNA/hOGG1 complex was conducted for 20000 steps with 0.1 fs by the accelerated quantum chemical molecular dynamics method, which reduces computational load with maintaining accuracy to solve Shrödinger equation, HC = SCε, with the first-principle parametrization for ionization potential, H and overlap integral, S.

Results: Conformational affinity of the DNA/hOGG1 complex (Fig. 1a) composed from double stranded 10-base pair-DNA, 5'-CCACGAGGCT. (ca. 640 atoms) and hOGG1 (ca. 4800 atoms) was compared with 8oxoG-DNA/hOGG1 including 5'-CCAC[8-oxoG]AGGCT carrying single 8-oxoG (Fig. 1b), and 2 ×8oxoG-DNA/hOGG1 including 5'-CCAC[8-oxoG]A[8-oxoG]GCT carrying tandem 8-oxoG (Fig. 1c) in the transcribed strand. Catalytic Lys249 for the rotation or cleavage of 8-oxoG-ribose interatomic bond came closer to the 8-oxoG in single and tandem 8-oxoG containing DNA than no adduct DNA. Gly245 acted repulsively at carbonyl oxygen of and kept away about 10 Å in case of 2×8oxoG/hOGG1 complex, which is farther than 8 Å in case of 8oxoG/hOGG1 complex.



(a) DNA/hOGG1 (b) 8-oxoG-DNA/hOGG1 (c) 2×8oxoG-DNA/hOGG1

Fig. 1 (a) DNA/hOGG1, (b) 8oxoG-DNA/hOGG1, (c)2×8oxoG-DNA/hOGG1 complexes. Numericals in these structures are inter-atomic distances (Å).

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P1-08

Fragment Molecular Orbital Calculations on the Interaction and Binding Energies of Human Protease Renin and its Inhibitors

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Keywords: Fragment Molecular Orbital (FMO) Calculation, Renin, Inhibitor, Interaction energy, Binding energy, Pair interaction energy decomposition analysis (PIEDA)

We have focused our attention on the Fragment Molecular Orbital (FMO) simulations of the complexes of proteins with ligands to elucidate the function of proteins by analyzing of the interaction between protein and ligand [1,2]. A human protease, renin, is the first component of the renin-angiotensin system in hypertension. This presentation describes the FMO calculations on the interactions of renin and its inhibitors. The PDB structures of 22 different inhibitor-bound renin complexes were optimized respectively by an energy minimization with explicit water molecules such as TIP3P model using AMBER9. After energy minimization, the surrounding water molecules were removed and for the resulting renin complexes FMO calculations with ABINIT-MP/BioStation program were carried out at FMO2-MP2/6-31G* level on the K computer. We computed the inter-fragment interaction energy (IFIE) between inhibitor and the amino acid residues in renin, and estimated the sum of IFIEs as binding energy between inhibitor and renin. FMO computations indicated that the calculated binding energy can be correlated to the activity value on 50% inhibitory concentration (IC_{50}) of the inhibitors. In addition, we performed pair interaction energy decomposition analysis (PIEDA) calculation to examine the energy components.

This research was done in activities of the FMO drug design consortium (FMODD). The results were obtained using the K computer (project ID: hp150160 and hp160103). PIEDA calculation was done by using MIZUHO/BioStation software package.

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P1-09

Construction of a platform for protein-ligand binding affinity prediction: Application to colony-stimulating factor-1 receptor (CSF-1R)

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Keywords: free energy, binding affinity prediction, alchemical transformation, IT-Soyaku

Aiming to make a breakthrough in drug discovery, we have developed technology of IT-based drug design (IT-Soyaku)[1]. One of the key technologies is the protein-ligand binding affinity prediction using the alchemical free energy calculation[2]. The protocol has been implemented in the latest version of MAPLE CAFEE™ (Massively Parallel Computation of Absolute Binding Free Energy)[1]. For the execution of this scheme, we have to perform hundreds of molecular dynamics (MD) simulations on the large-scale computer systems (hundreds or thousands of cores). If system faults occur in some machines, we cannot execute calculations on schedule. Then, we have constructed a computing platform where all of the calculations are monitored under a program specialized for control, failure of the machine is automatically detected and simulation is continually executed on other machines. In order to build the robust system, we adapted the double surveillance function; each MD simulation is controlled by individual monitor processes, and all of monitor processes are supervised by other process.

On the constructed platform, we performed absolute free energy calculations for three co-crystal structures of CSF-1R[3,4,5], and obtained the results within 1 day using 2240 cores per a compound even if some troubles took place. Here, the protocols we used in these calculations are briefly described. The harmonic restraint between a protein and a ligand has been used to define the volume of sampling for the standard state correction[6,7]. We also applied the multistate Bennett's acceptance ratio to evaluate free energies[8]. Our obtained values agree well with the experimental ones. We expect that our constructed platform drives technology of IT-Soyaku.

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P1-10

Estimating the qualitative and quantitative capability of the MM/3D-RISM method

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Keywords: 3D-RISM, molecular dynamics, binding free energy

In-silico screening of drugs requires the prediction of a free energy difference between bound and unbound states of host and guest molecules in aqueous solutions. The binding free energy consists of three contributions: the interaction energy of the solute molecules, structural entropy change of the solute molecules, and the desolvation free energy. However, the estimation of the structural entropy change and the desolvation free energy is a difficult task, since these quantities can be estimated by exploring the vast extent of the configuration space of the solvent molecules in addition with the solute molecules.

Considering such a situation, we have started to apply the MM/3D-RISM method[1], which is the combined approach based on the molecular dynamics simulation and the 3D-RISM theory[2], to the binding free energy prediction. Since MM/3D-RISM method needs only the structural ensemble of the solute molecules for estimating the desolvation free energy, this method enables to reduce the computational cost for sampling of the solvent molecules. However, the quantitative capability of the MM/3D-RISM method have been ambiguous so far since this method is somehow novel and have been applied to a few systems.

In this study, we applied the MM/3D-RISM method to a relatively simple system, inclusion process of a cyclodextrin, and typical protein-ligand system, Pim-1 kinase and its inhibitors and tried to assess the quantitative capability of the MM/3D-RISM method. As a result, our procedure reproduces reasonable correlation of the experimental and calculated binding free energy both of inclusion process of the cyclodextrin[3] and Pim-1 kinase – inhibitor system. We conclude that the MM/3D-RISM method have an ability to distinguish the tightly bind compounds from the candidate molecules.

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P1-11

Computational analysis of interaction between HLA-B*13:01 with Dapson responsible for drug-inducing hypersensitivity syndrome

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Keywords: Homology modeling, Docking, Molecular dynamics simulation, HLA, Dapson

Drug-induced hypersensitivity syndrome (DIHS) is a severe drug eruption marked by fever, redness, liver dysfunction, and high mortality rate (10-20 %) [1]. Dapson is one of medicines causing DIHS. Recent study reports that human leukocyte antigen (HLA) allele, HLA-B*13:01 was identified as a marker of susceptible individual for DIHS related to Dapson [2]. Interestingly, HLA-B*13:02 having high sequence identity with HLA-B*13:01 over 99 %, is not reported to have related with DIHS. The different amino acid residues between HLA-B*13:01 and HLA-B*13:02 were only three. Ile118, Ile119, and Arg121 of HLA-B*13:01 are mutated to Thr, Trp, and Thr, respectively, in HLA-B*13:02. In order to investigate the reason why only HLA-B*13:01 is involved in the onset of DIHS from a structural point of view, we performed computational analysis of Dapson-HLA-B*13:01 and Dapson-HLA-B*13:02 interactions using homology modelling, molecular docking, and binding free energy calculation combined with molecular dynamics (MD) simulation and Molecular mechanics Poisson-Boltzman surface area (MM-PBSA) methods.

As a results of homology modelling, we identified a crucial structural difference in the antigen binding sites between HLA-B*13:01 and HLA-B*13:02. We observed an additional small hydrophobic pocket in HLA-B*13:01, as Trp119 of HLA-B*13:02 was substituted by less bulky Ile in HLA-B*13:01. All of three representative docking poses of Dapson for HLA-B*13:01 used this unique small pocket, indicating that it seems to be suitable for Dapson-binding. On the other hand, Dapson seems to bind to HLA-B*13:02 at several different locations. Finally, the bind free energy calculations suggested that the binding affinity of Dapson with HLA-B*13:01 is much greater than that with HLA-B*13:02. This result indicated that Dapson would give more influence to the antigen-recognition site of HLA-B*13:01 than that of HLA-B*13:02, which might be associated with the incidence of DIHS.

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P1-12

An approach for analyzing protein-ligand binding pockets using probe molecules

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Keywords: Binding pocket, Probe molecule, Water, Fluctuation of pocket

We present a novel and efficient approach for analyzing protein-ligand binding pockets. Firstly, a large number of probe molecules were randomly distributed in the unit cell of protein, and constrained molecular dynamics (MD) simulation was carried out using NVT ensemble. Then water molecules were distributed to fill the vacuum space of the unit cell, and partly-constrained MD simulation was carried out using NPT ensemble. We applied this approach to human coagulation factor Xa (fXa)[1]. As a result, we found that the fluctuation of the pocket has an important role for identifying the appropriate orientation of a probe molecule in the pocket. To summarize, partly-constrained MD with a large number of probe molecules provides useful information for analyzing protein-ligand binding pockets.

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Fragment ER : Efficient and Accurate Binding Free Energy Calculation Method for Protein-ligand Complex

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Keywords: Molecular dynamics simulation, Binding free energy, Energy representation

The accurate prediction of binding affinities (K_d) of small molecules to the target protein is a crucial interest issue in the drug discovery process from early hit finding phase through final optimization of drug candidates, as the logarithm of an experimental binding affinity is exactly proportional to the Gibbs binding free energy.

Although the various methodologies for computing binding free energy with atomistic molecular models have been developed, most of them have trade-off relation between accuracy and computational efficiency, i.e., rough though quick or accurate though time-consuming.

On the other hand, one of us (Matubayasi) has developed a theory of solution in the energy representation (ER) [1] in which the solvation free energy can be obtained from relatively short molecular simulations performed at two end-points, the pure solvent and solution system, and that does not involve any adjustable parameters. Since the theory does not take into account the intermediate states in the solute insertion process, the computation time to determine the solvation free energy is considerably reduced compared to the exact methods such as FEP that requires considerable samplings of a large number of intermediate states.

We have developed the “Fragment ER” method by applying the ER theory to “binding” free energy calculations of small molecules bound to the target protein [2]. Fragment ER calculates the relative binding free energy efficiently and accurately between two similar molecules having the common scaffold and differing in the substructure (“fragment”) such as substituents. The results and comparison with FEP and experimental data will be reported in this meeting.

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P1-14

Toward the rapid *in silico* screening of mutations to improve the thermal stability of proteins

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Keywords: Mutation, In silico screening, Protein modeling, thermal stability

The accurate prediction of the effect of mutations on the thermal stability of proteins is important in protein engineering, and is very useful for the rational design of modified proteins, keeping stable and active in unusual conditions such as at high temperature. The mutation effect on the thermal stability of proteins is associated with the difference of a melting temperature Tm, i.e. the temperature at which the proteins unfold. Since it is very complicated to predict the effect of mutations through Tm, a standard folding free energy dG is alternatively used to evaluate the mutation effect on the thermal stability. To estimate the precise value of dG, molecular dynamics simulation such as free energy perturbation method is generally used, which is very time-consuming. Here, we propose the rapid in silico screening method of mutations to improve the thermal stability of proteins using a short time molecular dynamics simulation with implicit solvent model. In this method, we use the difference in the potential energy (dE) between folded structure and unfolded structure as a measure of the thermal stability of proteins. The initial structure of the modified protein with mutations is modeled by using the crystallographic structure as a template with a modeling tool for protein side chain prediction. The unfolded structure is made by extending the conformation of the protein so that the shape is an almost straight bar. We apply this method to evaluate the thermal stability of protein G beta1 domain [1-2] and compared the calculated values of dE with experimental values of Tm. We succeeded in getting the correlation coefficient of the calculated and the experimental data of ~0.7.

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P1-15

Estimation of binding free energy for the Pim1 kinase-ligand system based on the MM/3D-RISM method

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Keywords: 3D-RISM, binding free energy

To seek drug candidate molecules, it is necessary to predict the binding free energies between a protein and its ligands. Although many computational methods for predicting the binding free energy have been developed, some of those methods have disadvantages for evaluating the contribution from the solvent molecules. On the other hand, 3D-RISM theory[1], a statistical mechanical theory for the molecular liquid, analytically calculates configuration integral about solvation, and can assess the solvation structure and solvation free energy along the protein structure in a reasonable computational cost. In this regards, the combination of the 3D-RISM theory and MD simulations, called MM/3D-RISM method[2], can reduce the computational cost for the sampling of the solvent molecules since this combination of the methods reduces the sampling space to only of the conformational space of solute molecules. However, the quantitative capability of the MM/3D-RISM method has been ambiguous since this method has been applied to a few system.

In this study, we apply the method to estimate binding free energies between Pim1 kinase and its inhibitors. Pim1 kinase is expected as a target protein of therapeutic agents for cancer, such as leukemia and lymphoma[3]. As a result, our procedure reproduces reasonable correlation between the experimental and calculated binding free energies. Furthermore, we also analyse the differences in the characteristics of the tightly bound ligands and unstable ligands.

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P1-16

Inhibition Mechanisms of Carbapenem Antibiotics on Acylpeptide Hydrolase: Docking Simulation Study

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Keywords: Protein modeling, Docking simulation, Drug-drug interaction

Valproic acid (VPA) has a broad spectrum of anticonvulsant activity and is the most commonly used antiepileptic drug. VPA is often applied in combination with other anticonvulsant agents. Numerous types of drug-drug interaction (DDI) between concomitantly administrated drugs were known. Among interactions, it was first reported in 1997 that serum level of VPA was drastically decreased by carbapenems (CBPMs) [1], such as panipenem and meropenem, which have a broad spectrum of antibacterial activity with moderate activity against Gram-positive bacteria and excellent activity against Gram-negative aerobics and anaerobics. This DDI results in insufficient concentration of VPA to prevent epileptic fit and increase of the risk of recurrence of epileptic seizures. After the first report by Nagai et al, several reports outlined a significant interaction between VPA and CBPMs with a marked decrease in VPA concentration and neurological adverse events [2].

Although several mechanisms are proposed to explain it, the mechanism of the DDI has yet to be solved. Among the proposed mechanisms, inhibition of deconjugation is thought to be the most reasonably explain the DDI. Suzuki *et al.* systematically investigated the DDI based on inhibition of deconjugation of VPA-glucuronide (VPA-G) by CBPMs and identified the key enzyme as acylpeptide hydrolase (APEH), in which hydrolysis of VPA-G to VPA is inhibited by CBPMs [3]. However, molecular mechanism of APEH inhibition has not been elucidated. To address this problem, we employed computational method to obtain structural information and inhibition mechanism of APEH by CBPMs. The structure model of APEH were constructed by homology modeling and interactions between APEH and several compounds, including VPA-G, meropenem and panipenem, were investigated by docking simulation. The results of the calculations and molecular mechanism of the DDI mediated by APEH will be discussed in this presentation.

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P1-17

Prediction of peptide binding to a major histocompatibility complex class I molecule based on docking simulation

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Keywords: MHC class I molecule; docking simulation; structure-based method.

Binding between major histocompatibility complex (MHC) class I molecules and immunogenic epitopes is one of the most important processes for cell-mediated immunity. Computational prediction of amino acid sequences of MHC class I binding peptides from a given sequence may lead to important biomedical advances. In this study, an efficient structure-based method for predicting peptide binding to MHC class I molecules was developed [1], in which the binding free energy of the peptide was evaluated by two individual docking simulations. An original penalty function and restriction of degrees of freedom were determined by analysis of 361 published X-ray structures of the complex and were then introduced into the docking simulations. To validate the method, calculations using a 50-amino acid sequence as a prediction target were performed. In 27 calculations, the binding free energy of the known peptide was within the top five of 166 peptides generated from the 50-amino acid sequence. Finally, demonstrative calculations using a whole sequence of a protein as a prediction target were performed. These data clearly demonstrate high potential of this method for predicting peptide binding to MHC class I molecules.

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P1-18

Molecular Simulation Analysis of RNA Aptamer to Human Immunoglobulin G

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Keywords: RNA aptamer, Fragment molecular orbital, Molecular dynamics

RNA aptamers are short single-stranded nucleic acids with high affinity and specificity for their target molecules, which can be nucleic acids, proteins, or small organic compounds. Aptamers, therefore, have many potential applications in medicine and technology.

In order to be used in biological applications, the sugar moiety of the RNA aptamer has to be suitably modified to make it resistant to nuclease digestion. Recently, an optimized 23-nucleotide aptamer was designed, which was shown to bind with high affinity to the Fc domain of human IgG (hFc1) [1]. The crystal structure of the hFc1 complexed with the RNA aptamer has been determined [2]. However, the structural basis of the interaction of the RNA aptamer with hFc1 is poorly understood.

In this study aimed at elucidating the specific binding mechanism, we carried out *ab initio* fragment molecular orbital (FMO) calculations for the aptamer/hFc1 complex (Figure). We analyzed the energy of interaction between each nucleotide and each residue by using the inter-fragment interaction energy (IFIE) analysis based on FMO calculations. Furthermore, we performed molecular dynamics (MD) calculations for some aptamers in order to elucidate the conformational behaviors and dynamical features of aptamers.

The final goal of this study was to evaluate the affinity of chemically modified aptamers for the target protein using this method.

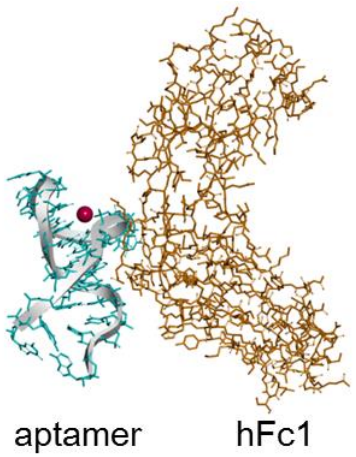

Figure. The crystal structure of the aptamer/hFc1 complex

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P1-19

Analysis of Intermolecular Interactions between Measles Virus Hemagglutinin and Receptors by the Fragment Molecular Orbital Method

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Keywords: Measles virus, Receptor, Interaction energy analysis, Fragment Molecular Orbital (FMO) method, IFIE (Inter-Fragment Interaction Energy)

Measles virus has been one of the major causes of childhood morbidity and mortality worldwide. Three measles virus receptors (SLAM, CD46, nectin-4) have been identified so far, and in this study, we use the Fragment Molecular Orbital (FMO) method [1] to investigate the interaction energy between the hemagglutinin of measles virus (MV-H) and the three respective receptors based on the PDB conformation of the complexes [2-4]. The calculation was performed at FMO-MP2/6-31G* level. On the basis of the calculated IFIE [5,6] (Inter-Fragment Interaction Energy) results between the MV-H and the three receptors, we found the strongly interacting amino acid residues in hemagglutinin which play an essential role in binding to receptors. Then we carried out FMO calculations also for different structures which were obtained by mutating some important amino acid residues in the conformation of hemagglutinin of measles virus. For example, by comparing the IFIEs, we observed that Arg533 in MV-H is important in binding to SLAM (the predominant receptor for wild-type), which was consistent with the earlier virological studies [7-9]. The present study has shown that the FMO method is a powerful tool to comprehensively search for those amino acid residues contributing to interactions with receptor molecules. It is also useful for the drug design of inhibitor and for a construction of engineered measles virus for cancer therapy.

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P1-20

Re-docking by Analyzing the Profile of Protein-protein Interaction

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Keywords: rigid-body docking, post-docking analysis

Various proteins exert molecular functions through forming a protein complex. Elucidation of a complex structure is essential to understand its molecular functions. At present, the number of complex structures in PDB is not enough for us to understand the information coded on the protein-protein interaction network. *In silico* analyses, docking simulation has been applied to augment the lack of complex structure information. In general, software for docking simulation rotates a protein (ligand) around its interaction partner protein (receptor) to generate many complex structure candidates. Hereafter, these candidates are referred to as decoys. Similar complex structures to the native structure (near-native structures) are expected to exist among the decoys. However, in some protein pair cases, there is no near-native structure in a set of decoys because of the shortage of docking space, that is one of the important problems to be solved.

This problem was addressed by expanding the docking space based on re-docking strategy [1]. In general, re-docking is a second round docking step after the initial docking. Besides, analyses of Interaction FingerPrints (IFPs) [2] have been proved to obtain near-native structures efficiently by focusing on the docking space corresponding to a decoy. To our knowledge, however, there is no tool for re-docking by analyzing IFPs.

Hence, we developed a software named Pftkool that is a tool for re-docking by calculating and classifying IFPs. Firstly, Pftkool performs the initial docking by MEGADOCK [3] to generate a set of 2,000 decoys. Secondly, IFP of a receptor (r-IFP) is calculated as an N-dimensional vector for each decoy. N represents the number of residues of the receptor. Each element of a vector is the number of residues of a ligand interacting with a receptor residue. Thirdly, decoys are classified into groups based on the distance calculated from r-IFPs. Then, decoys in each group have similar interacting residues of the receptor. Fourth, r-IFPs of the decoys in a group are assembled into an assembled r-IFP. Fifth, Pftkool performs re-docking by MEGADOCK to generate another set of 2,000 decoys by using the assembled r-IFP in a group. In this step, interacting residues shared in a group and their neighboring residues are exclusively used for re-docking. Thus, Pftkool enables us to perform initial docking, re-docking, and several other data processing. We intend to provide Pftkool free of charge.

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P1-21

Application of FMO-LCMO method to Protein-Ligand system

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Keywords: Electronic structure calculation, fragment molecular orbital method, one-electron orbital

One of the most popular strategy to obtain the protein-ligand binding energy by electronic structure calculations is to use the fragment molecular orbital (FMO) method. The FMO method was developed as a way of calculating the QM electronic states of whole systems through the fragment based type of strategy[1]. Recently, we proposed an efficient scheme for protein-ligand binding energy by using the FMO method and have shown the validity of our scheme[2].

On the other hand, we also have focused on the molecular orbitals of protein-ligand system. Recently, we have developed the FMO-linear combination of molecular orbitals (LCMO), which is an efficient post-process calculation of one-electron orbitals of the whole system after the FMO total energy calculation[3,4]. In this method, the Hamiltonian of the whole system is constructed from the Hamiltonian submatrices with respect to the MOs of each fragment as the basis set. The Hamiltonian submatrix elements are calculated by using the fragment orbital energies, fragment MO coefficients, and fragment overlap matrices during the FMO step. The one-electron orbitals and orbital energies of the whole system can be obtained by (one-shot) diagonalizing the whole Hamiltonian matrix.

In this study we report further study on the application of FMO-LCMO method to the protein-ligand systems and demonstrate the one-electron orbitals of protein-ligand system.

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P1-22

The molecular simulation for predicting RNA duplex dimerization free-energy changes upon mutations and three dimensional protein-RNA complex structures

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Keywords: RNA, protein, docking simulation, molecular dynamics simulation, thermodynamics

We are studying RNA with the method of molecular simulations. In this poster, we show following two results.

First, we show a prediction of RNA duplex dimerization free-energy changes upon mutations using molecular dynamics simulations, and its comparison to the experimental data.[1] A linear regression for nine pairs of double-stranded RNA sequences, six base pairs each, yielded a mean absolute deviation of 0.55 kcal/mol and an R^2 value of 0.97, indicating a quantitative agreement between simulations and experimental data. The observed accuracy indicates that the molecular dynamics simulation with the current molecular force field is capable of estimating the thermodynamic properties of RNA molecules.

Second, we show a prediction of RNA–protein three dimensional structures.[2] It is often difficult to determine the tertiary structure of these complexes experimentally, and thus an accurate rigid body docking for RNA–protein complexes is needed. In general, the rigid body docking process is divided into two steps: generating candidate structures from the individual RNA and protein structures and then narrowing down the candidates. In this study, we focus on the former problem to improve the prediction accuracy in RNA–protein docking. Our method is based on the integration of physicochemical information about RNA into ZDOCK, which is known as one of the most successful computer programs for protein–protein docking. Because recent studies showed the current force field for molecular dynamics simulation of protein and nucleic acids is quite accurate,[1] we modeled the physicochemical information about RNA by force fields such as AMBER and CHARMM. A comprehensive benchmark of RNA–protein docking, using three recently developed data sets, reveals a remarkable prediction accuracy of the proposed method compared to existing programs for docking. The highest success rate is 34.7% for the predicted structure of the RNA–protein complex with the best score and 79.2% for 3,600 predicted ones, which may be the world record.

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P1-23

Molecular dynamics simulation of binding free energy between protein and ligand

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Keywords: molecular dynamics simulation, protein-ligand binding affinity

For rational drug design, the computational estimation of the precise protein-ligand binding free energies is essential. Aiming at the selection of compounds in hit-to-lead and lead optimization process, rather high precision (around the order of thermal energy kBT=2.5kJ/mol) for prediction of the binding free energies should be established. Atomistic (classical mechanical) force fields optimized for organic molecules have been developed[1-2], and methods to calculate binding free energies by molecular dynamics simulations are proposed[3-7]. Nevertheless, it seems that prediction of binding free energy by the molecular dynamics simulation is not yet guaranteed for use in practical drug development. There is a room for further investigations to clarify the difficulties and for developing more efficient schemes.

Here we applied a variant of replica exchange methods [8] to predict the binding free energy between protein and ligands. Although we do not yet obtain conclusive results on two issues, (1) how precise estimation can be obtained not depending on the initial condition and (2) how long simulations must be done to obtain the reliable results, we will discuss the present preliminary results on the day.

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P1-24

Flexible Fitting to Cryo-EM Density Map using Ensemble Molecular Dynamics Simulations

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Keywords: Cryo-EM single particle analysis, Flexible fitting, Replica exchange molecular dynamics simulation

Cryo-EM single particle analysis provides structural information on biologically important molecular complexes. However, resolution is still not sufficiently high, and thus, high-resolution structures from other experiments are often used to create an atomic model from such low-resolution structural information.

Flexible fitting is a computational algorithm to derive a new conformational model, from a known structure, that conforms to low resolution experimental data to obtain conformational models in new functional states. However, the conventional flexible fitting algorithms cannot derive correct structures in some cases. In this study, we show the importance of examining conformational ensemble in the refinement process by performing multiple fitting trials using different force constants. Application to simulated maps of Ca^{2+} ATPase and diphtheria toxin as well as experimental data of release factor 2 revealed that for these systems, multiple conformations with similar agreement with the density map exist and a large number of fitting trials are necessary to generate good models. In addition, we show that an automatic adjustment of the biasing force constants during the fitting process, implemented as replica exchange scheme, can improve the success rate.

P2-01

Discovery of allosteric non-peptide ERK2 inhibitors through in silico screening and competitive binding assay

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Keywords: ERK2, allosteric inhibitor, in silico screening, crystal structure, binding assay

Extracellular signal-regulated kinase 2 (ERK2) is a drug target for type 2 diabetes mellitus. A peptide-type ERK2 inhibitor (PEP) was discovered in the previous study through the knowledge-based method and showed physiological effects on the db/db mice model of type II diabetes [1]. The crystal structure at 2.98 Å resolution revealed that PEP bound to the allosteric site without the interruption of the ATP competitive inhibitor binding to ERK2. An in silico biased-screening in regard to the allosteric site, using the focused library eliminating the ATP binders such as FR180204 previously discovered [2], rendered three compounds with inhibitory activity of IC_{50} < 100μM by ELISA-based enzymatic assay. Among them, two compounds displayed the concentration-dependent efficacy by a competitive binding assay against PEP and could be lead compounds for anti-diabetic medicine through the optimization process by structure-based approaches.

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P2-02

In Silico Prediction of Drug Inhibitory Activities to Monoamine Transporters using ADMEWORKS (R)

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Keywords: in silico prediction model, monoamine transporters, off-target

The monoamine transporters for norepinephrine (NET), dopamine (DAT) and serotonin (SERT) are localized to presynaptic neuron and responsible for the reuptake of their associated monoamines. In drug discovery, the reduction of the inhibitory activities to monoamine transporters is very important to avoid serious side effects related to the cardiovascular and central nervous systems. The *in silico* model with high accuracy for inhibitory activity of monoamine transporters is considered to be a powerful tool for finding good drug candidates in the lead optimization process.

We tried to build *in silico* models to predict the inhibitory activities to NET, DAT and SERT using in-house data of about 3,000 compounds and a commercially available platform, ADMEWORKS/ModelBuilder v7.0/Predictor v7.0 developed by Fujitsu Kyushu Systems Limited. ADMEWORKS allows the building of predictive models based on empirical data using advanced machine learning algorithms.

In this study, the usefulness of binary classification models (qualitative model) was examined. Data for NET, DAT and SERT were randomly selected to create corresponding training sets and validation sets. About 20 to 78 descriptors out of 1010 descriptors generated by the software were selected using a genetic algorithm and particle swarm optimization. Algorithms used for building the *in silico* models were AdaBoost (ADA), k-nearest neighbor (KNN), neural network (NN), support vector machine (SVM) and discriminant function (TILSQ). Each algorithm was applied to the selected descriptors to build several *in silico* models for comparison. Validation test results showed that the best models with high accuracy and a good balance of sensitivity, specificity and number of descriptors for prediction of NET, DAT and SERT inhibition were as follows: sensitivity: 76.6, 84.6 and 80.0%, specificity: 77.7, 87.6 and 87.0%, accuracy: 77.1, 86.1 and 83.5%, number of descriptors: 35, 63 and 60, respectively. The algorithms used in the best models were ADA and SVM.

Based on the above results, we established good *in silico* models in order to predict the inhibitory activities of monoamine transporters using ADMEWORKS.

P2-03

Interaction Analysis of FABP4 Inhibitors by X-ray Crystallography and Fragment Molecular Orbital Analysis

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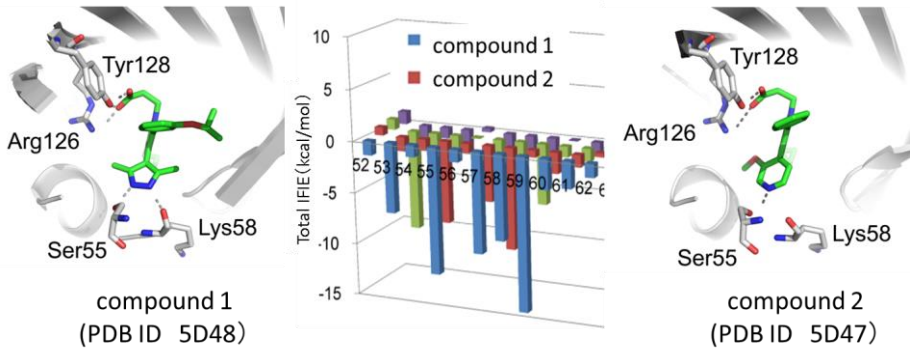
Keywords: FABP, aP2, inhibitor, X-ray crystal structure, FMO

X-ray crystal structural determination of FABP4 in complex with four inhibitors revealed the complex binding modes, and the resulting observations led to improvement of the inhibitory potency of FABP4 inhibitors. However, the detailed structure-activity relationship (SAR) could not be explained from these structural observations. For a more detailed understanding of the interactions between FABP4 and inhibitors, fragment molecular orbital analyses were performed.

These analyses revealed that the total interfragment interaction energies of FABP4 and each inhibitor correlated with the ranking of the Ki value for the four inhibitors. Furthermore, interactions between each inhibitor and amino acid residues in FABP4 were identified. The oxygen atom of Lys58 in FABP4 was found to be very important for strong interactions with FABP4. These results might provide useful information for the development of novel potent FABP4 inhibitors.



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P2-04

Exploring Structural Diversity and Ligand Binding Property Consensus from Protein Structure Database

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Keywords: Protein Structures, Pharmacophore elucidation, Ligand binding mode

There are more than 120,000 protein structures deposited in PDB as of August, 2016. A number of similar sequence data are deposited due to the fact that the structures will form different conformations according to their states (*e.g.*, active, inactive, ligand-bound, *etc.*).

Extracting 'information' such as structural diversity and ligand binding property accordingly to these states can be somewhat time-consuming. As a starting point, one will need to search the relevant data from the PDB database and verify if it is the optimal dataset needed. One may also need to perform a necessary preparation, such as sequence alignment and structure superposition before even looking at the dataset to extract the information. Then finally comes to look at each data and extract the necessary information. In any steps, human errors or biases can cause unreproducible results. It may also be desirable to go through the in-house data as well as the PDB database in extracting the information.

We therefore made two programs in order to reduce the necessity of going through the steps and going directly to the information from the query protein sequence or structure. Programs were coded by Scientific Vector Language of MOE[1] and also made use of PSILO[2], which can manage both the in-house data and the public PDB data. These programs will search, pre-calculate, and 1) show the structural diversity or 2) output the consensus ligand binding property that can be elucidated (namely, PSILO diverse search and PSILO pharmacophore consensus respectively) from the whole protein database.

We will demonstrate these two programs using a kinase sequence as an example. Given just the amino acid sequence as a query, PSILO diverse search clusters the structures automatically with exploring the structural diversity of the similar kinases (such as active and inactive state conformations, Fig. 1a). Then, PSILO pharmacophore consensus can be used for, but not limited to, each representative kinase and quickly elucidate the conserved ligand binding mode (Fig. 1b).

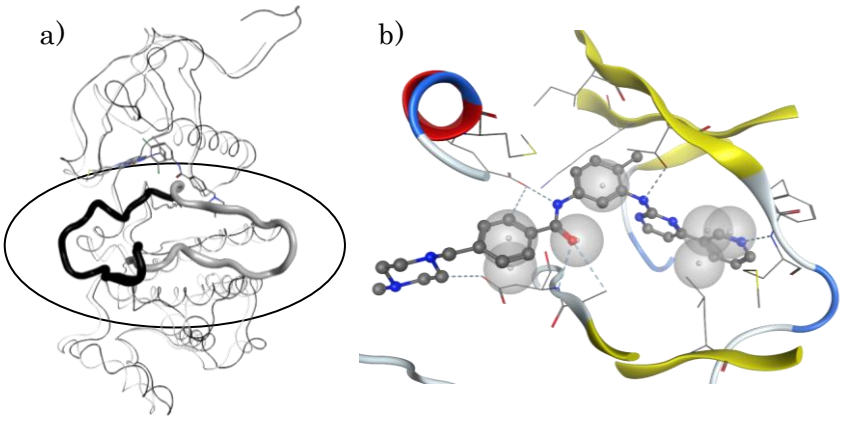

Figure 1. Structural information of c-Abl tyrosine kinase derived by PSILO diverse search and PSILO phamacophore consensus
a) Structural diversity of c-Abl kinase (diverse region circled in black)
PDB ID: 1IEP, 1M52
b) Consensus ligand binding property of active states c-abl kinase. The spheres represent the conserved binding properties
PDB ID: 1IEP

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P2-05

Reactivity evaluation of donepezil in the oxidation by CYP3A4 based on QM calculation

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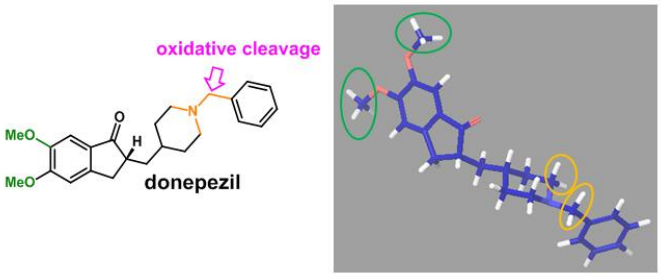
Keywords: Cytochrome P450 (CYP), donepezil, reactivity, radical intermediate, sites of metabolism (SOM), density functional theory (DFT), potential energy

CYP3A4 contributes to more than 50% of clinical drug metabolism. Therefore, optimization of drug candidates considering the CYP3A4 metabolism is critically important. Our purpose of this research is to establish methods for metabolically stable drug design. Generally, accessibility of each carbon atom to heme iron and oxidative reactivity of the atom in the substrate are important to predict the site of metabolism. In our previous study, we have investigated the mechanism of oxidation on carbamazepine (CBZ) using MM and QM calculation [1].

In this presentation, we focused the reactivity prediction on donepezil. We have evaluated the reactivity of the compound by the potential energy concerned with the radical intermediate using quantum mechanics by DFT calculations. We have compared a fast simple method such as SMARTCyp [2] with our QM calculations using the compound conformation in the CYP3A4 complex. In this evaluation process, we will discuss the protonated state of donepezil in the complex of CYP3A4.



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P2-06

Molecular dynamic approach to identify significant PTP1B inhibitors to combat diabetes

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Keywords: Diabetes mellitus; virtual screening, PTP1B inhibitors; ZINC database; Pharmacophore modelling

Abstract: Diabetes is a metabolic disorder of the pancreas, in which the pancreas loses its functionality to produce insulin hormone properly in the body. Protein tyrosine phosphatase 1B (PTP1B) interrupts insulin signaling by dephosphorylating both the insulin receptor and insulin receptor substrate. PTP1B is considered to be a negative regulator of insulin receptor signaling. PTP1B is a promising drug target for the treatment of type 2 diabetes, obesity, as well as cancer. In this research virtual screening was carried out on drug like compounds of Zinc database using several criteria. The 3-D coordinates in the X-ray crystal structure of PTP1B enzyme complex (PDB code: 3EAX) was selected as the initial model for structure based virtual screening to find active drug like PTP-1B compounds. The PTP-1B focused library (1625 compounds) was downloaded and filtered by Lipinski rule of five and OSIRIS server for ADME properties. 70 selected compounds were docked against the PTP-1B by CLC and MOE to identify low-energy binding modes within the active site of a macromolecule. Interactions like hydrogen bonds, ionic interaction, hydrophobic interactions and metallic bonds were analyzed. Two different docking algorithms were used to validate the docking results. For the characterization of novel and potent inhibitors for PTP1B, we explored selective features of our docked complex shown in Figure 1 by structure based pharmacophore modeling and finally select the features of two top scored models and subjected as pharmacophore query for screening of Zinc database of 4 million compounds. Significant hits were identified by pharmacophore based virtual screening of Zinc database. Hits with similar binding poses and top scores were selected for subsequent refinement and optimization by molecular dynamic approach. Structural features relevant to the interactions of the newly identified inhibitors with the active-site residues of PTP1B are discussed in detail. This study might be helpful in designing active lead for clinical studies that may serve as antidiabetic drugs in future.

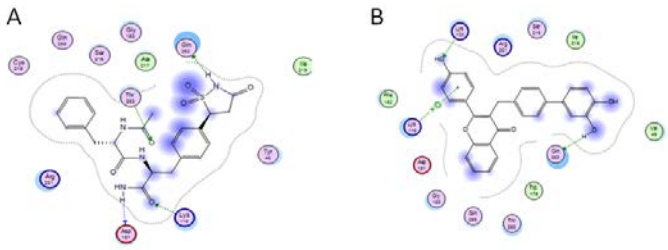

Figure 1: Schematic representation of the top scored PTP1B hits (A) ZINC13674403 and (B) ZINC40431189 in the binding Pocket (PDB ID: 3EAX) exhibits ideal binding mode and interactions to key residues.

P2-07

Bridging the dimensions: Seamless integration of 3D structure-based design and 2D structure-activity relationships to guide medicinal chemistry

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Keywords: quantitative structure activity relationships, structure-based design, visualization

The effective use of software can have a major impact on timelines and innovation in drug discovery. However, the traditional split between computational modellers and synthetic chemists has been blurred and software must be accessible across disciplines to quickly understand and predict structure-activity relationships (SAR). There has been a similar divide between tools for three-dimensional (3D) structure-based design and those for analysis of SAR based on a two-dimensional (2D) compound structure. Seamless integration of these approaches enables all of the structural knowledge to be used to guide the efficient design of high quality, active compounds.

In this poster, we will illustrate how information from 2D models of key physicochemical and absorption, distribution, metabolism, elimination and toxicity (ADMET) properties can be linked with 3D views of protein-ligand complexes. The influence of each atom or functional group on these properties can be highlighted [1] and combined with visualization of the key interactions contributing to binding affinity [2], enabling development of optimization strategies that balance potency with the ADMET properties required in a safe and efficacious drug [3].

Furthermore, 2D analyses, such as activity cliff detection and matched molecular pair analyses, are commonly used to explore compound data sets and quickly identify important SAR within a chemical series or library [4]. We will demonstrate how a seamless, highly visual link between the results of these analyses and related 3D structural information helps to understand and rationalize this SAR. This enables the efficient design of compounds with improved target affinity in a truly multi-parameter optimization environment.

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P2-08

Predicting inhibitory and activatory effects of drug candidate compounds from chemically-induced transcriptome data by a multitask learning with gene perturbation similarity

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Keywords: multitask learning, drug target, transcriptome, gene knock-down, gene over-expression, chemical treatment

Genome-wide identification of inhibitory and activatory effects of drug candidate compounds is an important issue in drug discovery. It is also necessary to appropriately evaluate the compound activities not only on a single target protein but also on other proteins (off-targets). Deciphering inhibitor and activator actions derived from the interactions with the primary target and off-targets can help to elucidate potential therapeutic effects and side effects of the compounds. In recent years, a variety of computational methods have been proposed for drug target prediction in the framework of chemogenomic approach [1] and phenotypic approach [2], but they cannot distinguish the difference between inhibitors and activators, and the performance is poor when the number of known ligands for a target protein of interest decreases. Genome-wide expression profiling of transcriptional responses to chemical and genetic perturbations on human cell lines is a promising unbiased approach for exploring the mode-of-action of bioactive compounds.

In this study, we propose a novel method to simultaneously predict inhibitory effects and activatory effects of drug candidate compounds on multiple target proteins from chemically-induced transcriptome data by a multitask learning with gene knock-down similarity and gene over-expression similarity. Transcriptome data were obtained from the Library of Integrated Cellular Signatures (LINCS: http://www.lincsproject.org/). For chemically-induced data, we analyzed transcriptomic changes in global patterns of gene expression profiles in response to the chemical treatments of 20,122 compounds (including approved drugs) on 71 human cell lines. For genetically-perturbed data, we analyzed transcriptomic changes in global patterns of gene expression profiles in response to the knock-down of 4,331 human genes and the over-expression of 2,946 human genes. The proposed method can predict inhibitory effects and activatory effects separately for drug candidate compounds from the integration of chemically-induced and genetically-perturbed transcriptome data. We demonstrated the usefulness of the method in terms of high accuracy and high coverage, and show advantages over the previous methods. The proposed method is expected to be useful for identification of potential drug actions that the previous methods failed to detect in various pharmaceutical applications.

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P2-09

Apo- and antagonist-binding structures of vitamin D receptor ligand-binding domain in solution studied by MD and SAXS hybrid approach

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Keywords: Vitamin D receptor, Molecular dynamics simulation, Small-angle X-ray scattering

Vitamin D receptor (VDR) is a ligand-dependent transcriptional factor regulating the expression of genes related to calcium homeostasis, immunomodulation, and cell differentiation and proliferation. Since functions of VDR and other nuclear hormone receptors (NRs) are involved in human diseases, their regulation by ligand binding is a typical strategy for pharmaceutical drug design. According to X-ray crystal structures of NRs and other experimental results, the ligand-binding domain (LBD) undergoes conformational change upon ligand binding, and a local conformational change around helix 12 is key to regulating agonism/antagonism.

The mechanism of agonism/antagonism of VDR is not understood clearly on the basis of X-ray crystallography. All the crystal structures of VDR-LBD are almost identical, regardless of agonist/antagonist binding. Since the conformation of helix 12 is key, the crystal structures of the VDR-LDB/antagonist complex do not provide structural insight into the mechanism of antagonism. In addition, no crystal structures of apo VDR-LBD has been reported, and the exact conformation of the apo form at atomic resolution remains unknown.

In order to reveal the apo and antagonist binding forms of VDR-LBD, we analyze them by a hybrid approach of small-angle X-ray scattering (SAXS) and molecular dynamics simulation (MD). SAXS analysis can reveal an overall shape of molecular structures in solution, and can capture both flexible structure in the apo form and the conformational change in response to antagonist binding. We obtained SAXS profiles of apo and antagonist binding rat VDR-LBDs, however, the profiles were different from the theoretical profiles calculated from crystal structures. To clarify the solution structures at atomic resolution, we conducted MD and collected each structural ensemble. Comparing the SAXS profiles and the MD results, we report a reliable structure for apo and antagonist binding forms of VDR-LBD. In apo form, helix 12 is partially unraveled and fluctuates around the canonical position. In addition, helix 11 bends outward by a kink-centered hinge-bending motion, resulting in opening of the entrance into the ligand-binding pocket. In antagonist-binding form, the position of helix 12 is unsuitable for creating canonical active position, thus preventing co-activator binding for transactivation. On the basis of these results, we propose a mechanism for the agonism/antagonism of VDR by ligand binding that is an alternative to the mouse-trap model.

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P2-10

Efficient Similarity Search Using Multiple Reference Molecules on PG-Strom architecture

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Keywords: k-Nearest Neighbors (k-NN), GPU acceleration, Query acceleration engine

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

Similarity searches using multiple reference molecules are one of data fusion methods, which are widely used in virtual screening. Various methods have been introduced to utilize multiple reference molecules, such as centroid fingerprints [3], k-nearest neighbors (k-NN) [3], and SVM-based ranking [4]. Data fusion methods generally improve virtual screening performance.

In this study, we attempted to implement the k-NN based similarity search system on PG-Strom architecture. It allows running the heaviest portion of the similarity search onto GPGPU which has more than thousands cores, then we could observe significantly accelerated similarity search results.
On the other hands, the entire workload is processed inside of the PostgreSQL database, thus, we can utilize flexibility of SQL for both of pre-process and post-process of the similarity search.
We will discuss about relationship between the SQL queries, adoption of GPGPU computing power inside of database management system, and its applications to chemoinformatics.

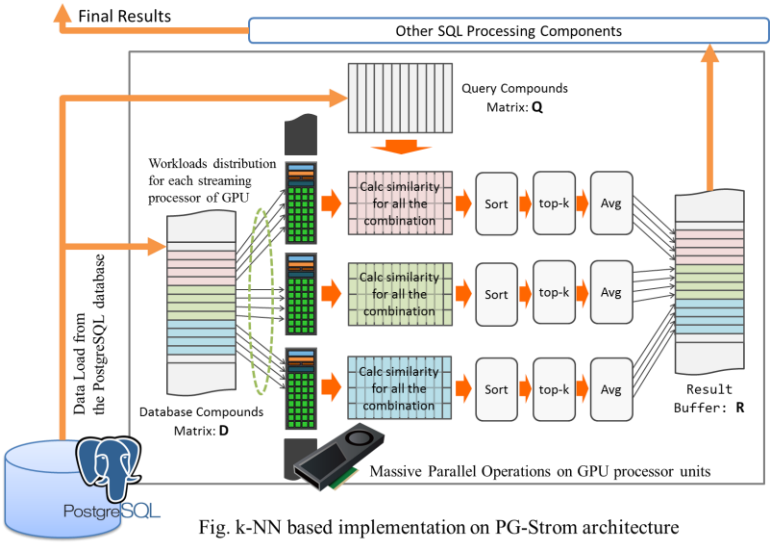

Fig. k-NN based implementation on PG-Strom architecture

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P2-11

Fragment-based Drug Design based on SZMAP Water Mapping in Protein Active Site

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Keywords: Fragment-based Ligand Design, Water Mapping, Protein Structure

Fragment-based drug design (FBDD) is widely used in modern drug discovery for designing novel ligands. Although it requires fragment mapping in an active site of a target protein structure, it is sometimes difficult to find appropriate fragments and their stable locations in the proteins from X-rays analyses. Therefore, alternative approaches to find appropriate fragments are required in order to apply this useful method against a lot of drug discovery targets.

SZMAP predicts the role of water molecules in molecular interactions around an active site of a protein using semi-continuum approach[1,2]. This approach combines one explicit water molecule with robust continuum solvent methods[3]. SZMAP uses classical statistical mechanics and thorough sampling of water molecules to analyze specific water orientations. GAMEPLAN, which is an application of SZMAP, analyzes environment on each atom of a bound ligand in a protein and suggests ways to modify the bound ligand per one or two atom(s) using water structure in the immediate environment of the ligand. This method helps to find reasonable directions by adding polar or non-polar fragments in dependence upon a protein active site environment. One complication with the fragments generated by GAMEPLAN is chemical synthesizability. This problem is solved by using our fragment replacement tool BROOD which searches a large fragment database generated from ChEMBL20 using shape and color similarity. Since BROOD does not require a readily synthesizable chemical structure for the query when searching the database, fragments suggested by GAMEPLAN can be easily applied as a BROOD query. The result is novel synthesizable fragment-sized R-groups that are added to the template of interest with chemistry suggested from solvent mapping.

In this poster, we will discuss how we can design novel potent ligands from a smaller compound by combining SZMAP/GAMEPLAN water mapping and BROOD fragment replacement approaches.

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P2-12

Application of deep learning for large scale data with standard performance workstation(s)

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Keywords: Deep Learning, Big data, cheminformatics, in-silico drug discovery

Deep Learning (DL) [1], a widely known method of machine learning, is used in various fields, such as drug development, image recognition and autonomous driving technology. DL is suitable for dealing with large scale data; therefore we have been studying application of DL for in-silico drug discovery and large scale biochemical assay screening.
There are powerful libraries/frameworks for DL, for example, Caffe [2], Chainer [3] and TensorFlow [4]. When applying DL to large scale (i.e. larger than the memory size) data, it is possible to use LMDB key-value storage with Caffe. However, Chainer and TensorFlow have no such database storage support, making them unsuitable for DL with large scale data.
In this presentation we will introduce our new framework that allows us to carry out DL with large scale data using Chainer. Our framework requires a workstation with GPU for training and applying the DL model. If the storage provided by a single workstation is not enough, multiple workstations interconnected by wide-bandwidth network such as 1Gbit Ethernet can be used. We have used our framework for applying DL to PCBA bioassay data which has 3072 columns and 1.1M rows (prepared by Dr. Okuno and his team). It took 7.3x10^2 seconds to train 1 epoch using a single workstation equipped GeForce GTX 980 Ti GPU and 6.7x10^2 seconds with the same GPU workstation plus 3 storage workstations.
PaaS providers (Amazon, Microsoft, Google…) are likely to support powerful GPU instances in the near future. Our framework has good compatibility for PaaS infrastructure and will be able to make use of them allowing us to carry out high performance DL.
This study is supported by Fujitsu Limited.

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P2-13

Hydrogen bond basicity prediction for common fragments in high-activity compounds

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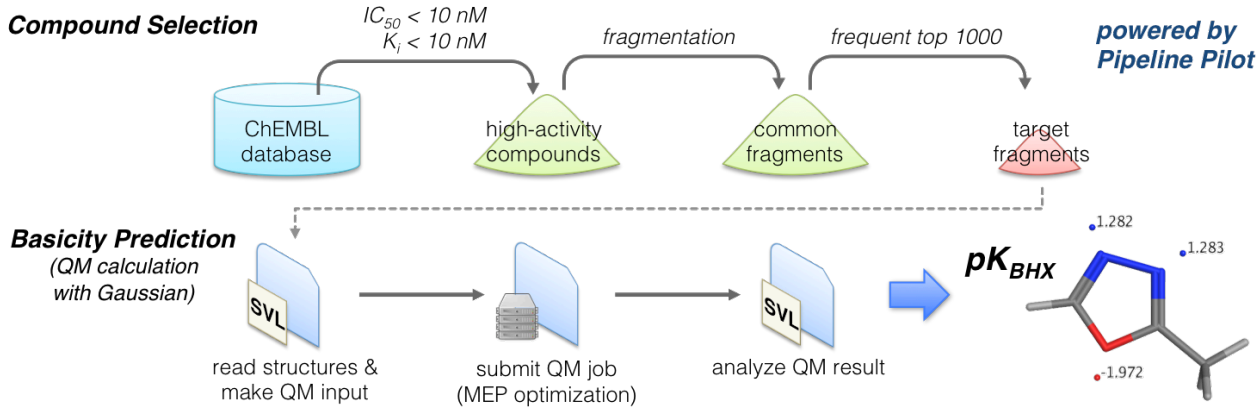
Keywords: Hydrogen bond acceptor, Lone pair, Molecular electrostatic potential, QM, ADMET

Hydrogen bond (HB) is strongly contributing to binding affinity of a drug molecule with target proteins or solubility in water and prediction of the strength in advance is very useful for drug design process. HB acidity and basicity, which are defined as the capabilities to make an HB for its donor and acceptor, respectively, play key roles in determination of hydrogen bonding strength between them.

Recently, Kenny et al. proposed a nonlinear regression model to predict HB basicity by using molecular electrostatic potential (MEP) minima near each lone pair as a descriptor [1]; for each HB acceptor type, MEPs of small molecules only with equivalent HB acceptors were quantum mechanically (QM) calculated and fitted to measured HB basicity values to determine the parameters of a regression equation, which are used to predict HB basicity for molecules with multiple, nonequivalent HB acceptors, whose basicity values cannot be measured individually.

In accordance with this methodology, we newly developed an automated protocol to evaluate HB basicity for multiple compounds and applied to a dataset where consists of frequently appearing fragments common to highly potent compounds registered in the ChEMBL database (see the figure below). Because the frequently used fragments are likely to be pharmacologically meaningful in rational drug design, predicted HB basicity data would provide useful information to enhance activity or improve physicochemical and ADMET properties from the bioisosterism viewpoint for medicinal chemists.



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P2-14

Human insight in drug discovery: docking pose selection in protein-ligand docking and hit-compounds selection in structure-based virtual screening

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Keywords: Drug discovery, Structure-based virtual screening, Visual inspection, Human computation, Crowd sourcing

Implicit knowledge still remains important in drug discovery process, while physicochemical simulation and machine learning methods are rigorously utilized in computational drug design. Such drug discovery-related knowledge includes chemical alert structure, synthetic accessibility, feasibility of ligand binding pose, etc. To take advantage of human knowledge, visual inspection of chemical structure and docking pose is commonly performed by researchers. However, the criteria of visual inspection-based filtering have not been systematized and the effectiveness of such heuristic filtering is unclear. Here, we examined the validity of visual inspection-based filtering, such as docking pose selection in protein-ligand docking and hit-compounds selection in structure-based virtual screening. Visual judgment by examinees was collected via vote system. Our human-assisted screening workflow was tested by participating in the IPAB 3rd Computer-Aided Drug Discovery Contest[1]. Based on the analysis of the vote result, we will discuss the feasibility of human-computer synergy in drug discovery.

(The author also presents this subject in the IPAB Focused Session FS6-1.)

[1] IPAB 3rd Computer-Aided Drug Discovery Contest (2016) www.ipab.org/eventschedule/contest/contest3

P2-15

Structure-Based Rational Design of Novel Oral Bioactive LXR Modulators

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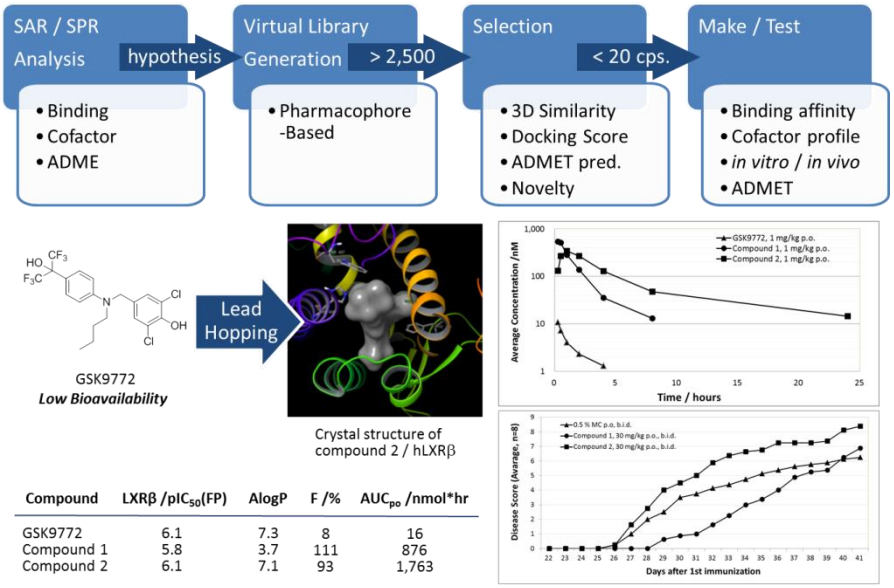
Keywords: LXR, nuclear receptor, corepressor selectivity, rational design, SBDD, LBDD

To achieve good balance between activities and drug-like properties is difficult in medicinal chemistry. Especially, like nuclear receptors, the targets which consist of hydrophobic binding site tend to prefer poor developable high lipophilic compounds.

Liver X receptors (LXRs), which have been of interest as targets for the treatment of atherosclerosis, inflammation, Alzheimer's disease, dermatological conditions, hepatic steatosis and cancer, are no exception to this rule. Although over a decade of efforts, no LXR modulator has successfully progressed beyond clinical trials[1][2].

Compared with LXR agonists, such as T0901317 and GW3965, GSK9772 shows potent anti-inflammatory activity without inducing lipogenic side-effects in vitro through stabilizing repressed state of LXR. But no additional work on this series including in vivo efficacy has been reported. It could be due to its low bioavailability derived from high lipophilicity (AlogP = 7.3)[3].

In this study, we have discovered novel oral bioactive LXR modulators which stabilize repressed state. Structure-based rational drug design made it possible not only to find novel oral bioavailable structure while maintaining desired activities and cofactor profile, but to reduce number of compounds being synthesized and project cycle time. Our SBDD approaches and compound profiles including in vivo activities will be discussed.



Compound	LXRβ /pIC$_{50}$(FP)	AlogP	F /%	AUC$_{po}$ /nmol*hr
GSK9772	6.1	7.3	8	16
Compound 1	5.8	3.7	111	876
Compound 2	6.1	7.1	93	1,763

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P2-16

Prediction of sites of metabolism of compounds for CYP1A2 by combined molecular simulations

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Keywords: Molecular dynamics, Molecular docking, Site of metabolism, Cytochrome P450 (CYP)

Cytochrome P450s (CYPs), a superfamily of heme-containing enzymes, are the major enzymes involved in drug metabolism. In humans, it has been estimated that CYPs metabolize approximately 75% of all marketed drugs, 95% of which are metabolized by CYP3A4, 2D6, 2C9 and 1A2 [1]. In the drug metabolism by the CYPs, identification of sites of metabolism (SOMs) on molecules and the structure of their metabolites can be decisive for the design of molecules with favorable metabolic properties. However, experimental techniques to determine SOMs and structures of metabolites are still highly resource-demanding and challenging [2]. Thus, developing fast and accurate computational methods to predict the SOMs/products of compounds metabolized by the CYPs is one of the important tasks for the optimization of ADME and toxicity properties.

In this study, we present a new computational method to predict the SOMs of compounds metabolized by CYP1A2. First, a long time molecular dynamics (MD) simulation of the solvated CYP1A2 system was carried out to observe the inherent dynamical multiple receptor (pocket) structures in physiological conditions, and then those sampled receptor structures were used for the molecular docking using multiple receptor structures (ensemble docking). Next, from the information on the docked compound poses obtained from ensemble docking, we evaluated the possibility on access of the compound to the active site of CYP1A2 at atomic level (accessibility). In the molecular docking, docking program GOLD with ChemScore parameters, which are optimized for CYP system [3], was used.

We prepared 19 test set compounds metabolized by the CYP1A2 and 10,000 receptor snapshots (which correspond to the one micro second MD simulation) for the ensemble docking simulation. The top three ranked atoms by the accessibility analysis are defined as possible SOMs for each compound in this study, and we found the success rate of the predicted SOMs was 78.9 %. For more accurate SOMs prediction, we then evaluated a new score function utilizing the accessibility score by the ensemble docking and the reactivity score obtained from the SMARTCyp software [4]. The success rate of the predicted SOMs using the new score function was 89.5%, indicating that the use of the new score function improves about 10 % as compared with the SOMs prediction from the ensemble docking alone.

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P2-17

Prediction of Tankyrase2-ligand Binding Affinities Using Molecular Mechanics and Quantum Mechanics Calculations

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

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

Protein-ligand systems exhibit various non-bonded interactions, such as π-stacking, charge transfer, polarization effects, and dispersion, as well as the usual and weak hydrogen bonds, as evidenced by structural studies[1,2]. These non-bonded interactions cannot be understood with sufficient accuracy by using classical MM force fields. On the other hand, application of quantum mechanical (QM) calculations considering such non-bonded interactions can significantly improve the accuracy of the MM force fields.

In this study, we evaluated prediction of binding affinities for tankyrase2-ligand systems by using combined approaches of MM and QM calculations. As our approach for reducing computational cost of QM calculation, ONIOM method and multilayer fragment molecular orbital method[3,4] were used. We will report the detailed results.

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P2-18

Towards Desktop laboratory from MD, docking, and virtual screening to bio assay: Computation performance

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Keywords: myPresto, Cloud computing, Virtual screening, Molecular dynamics, Performance

"*myPresto*"[1] is a free computer software suite for drug design, including more than 20 programs and a compound database: molecular dynamics (MD)[2] simulation, protein pocket search, protein-ligand docking[3], structure-/ligand-based virtual screening (VS)[4], molecular editor, solubility prediction, and synthetic accessibility. Several commercial programs with the graphical user interfaces enable us to easily operate these computational methods, to dramatically reduce cost of cloud configuration (installation of software environment of cloud computer) for these programs, and to help ordering chemical compounds and using an assay service.

We evaluated the VS and MD simulations on a workstation and cloud computing resources, where VS and MD simulations were examples of parallel and sequential computation applications. As a result, the required cost of the VS and MD simulations showed unique trends depending on the resources (processors, storages and network). The VS of 2-million compounds on an ordinary workstation costed 10 hours. On the contrary, it costed from 1 hour/100 nodes to 10 hour/10 nodes on a cloud computer. On average, the VS costed about 3,000 JPY on the cloud.

We will discuss the cost-efficient modeling of computations for cloud computing within short CPU time.

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P2-19

ESPRESSO: An ultrafast compound pre-screening method with segmented compounds

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Keywords: Virtual screening, Protein-ligand docking, Compound pre-screening, High-speed computation

Recently, the number of available protein tertiary structures and compounds has increased. However, structure-based virtual screening is computationally expensive due to docking simulations. Thus, methods that filter out obviously unnecessary compounds prior to computationally expensive docking simulations have been proposed. However, the calculation speed of these methods is not fast enough to evaluate more than 10 million compounds. In this presentation, we propose a novel, docking-based pre-screening protocol named ESPRESSO (Extremely Speedy PRE-Screening method with Segmented cOmpounds)[1]. Partial structures (fragments) are often common among several compounds; therefore, the number of fragment variations needed for evaluation is smaller than that of compounds. Our method increased calculation speeds ~200-fold compared to conventional methods. ESPRESSO is written in C++ and Python, and it is available as an open-source code (http://www.bi.cs.titech.ac.jp/espresso/) under the GPLv3 license.

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P2-20

Singular Value Decomposition in the FMO-IFIE Analysis for Ligand Screening

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Keywords: p38 MAP kinase, Interaction energy analysis, Ligand binding, Fragment Molecular Orbital (FMO) method, IFIE (Inter-Fragment Interaction Energy), Singular Value Decomposition (SVD)

In recent years, ‘‘In silico drug discovery’’ using high performance computing (HPC) technology such as large-scale molecular simulations attracts much attention. However, computer simulations have a number of difficulties in accuracy as compared with experiments. In this study, we evaluated the binding affinity between p38 MAP kinase[1] and various inhibitors by the fragment molecular orbital (FMO)[2] method at MP2/6-31G* level in comparison with experimental values of IC_{50}. Kinase p38 is a protein that plays an important role in the functional expression of various cellular processes that cause cell aging and autoimmune disease, and is activated by phosphorylation with external stress such as heat, osmotic pressure and ultraviolet rays.

It has been found that the calculated results of the FMO-IFIE[3, 4] (inter-fragment interaction energy) for 95 complex structures registered on Protein Data Bank were not well correlated with the activity data of IC_{50}. Therefore, we performed the singular value decomposition (SVD)[5] for the calculated results of the IFIE matrix (amino acid residues × ligands) to find the reason why there was not the good correlation and the direction for improving the correlation.

As a result, we succeeded to improve the correlation by rejecting some singular vectors that impair the correlation. Furthermore, we analyzed the rejected singular vectors and found an overestimation of hydrogen bond between GLU71 and the ligand.

This study was conducted as one of the activities of ‘‘FMO Drug Design Consortium’’, under the task of K computer ‘‘hp150160’’ and ‘‘hp160103’’. We thank the members of the FMO Drug Design Consortium for their providing the IFIE data on p38.

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P2-21

GPCR/Membrane Model Builder for Molecular Dynamics Simulation

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Keywords: GPCR, MD simulation, protein/membrane modeling

G protein-coupled receptors (GPCRs) are the most important drug targets in human bodies . For the purpose of elucidating the functions of GPCRs and their conformational changes in atomic level, molecular dynamics (MD) simulations are extensively employed starting from experimentally solved structures or homology based theoretical models. However, without taking account of explicit lipid bilayer environments, realistic MD simulations for GPCRs could not be carried out.

To prepare the initial model for the MD simulations easily, we developed a tool building coordinates of protein(s) embedded in membrane and generates a corresponding force-field file. The scheme is as followings; (i) locate ligand and cholesterols to GPCR. (ii) palmitoylate Cys in helix 8 if necessary. (iii) rotate and translate GPCR in suitable direction based on its charged residue positions. (iv) place appropriate phospholipids around the GPCR. (v) solvate GPCR/membrane complex in saline. (vi) carry out energy minimizations and MD simulation with NPT condition for equilibrium. (vii) perform product runs with NVT ensemble. The tool is implemented in myPresto program suit[1][2], which will be provided in our download site.

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P2-22

Postprocessing protein-ligand docking based on the distance among interaction energy vectors

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Keywords: Protein-ligand docking, Interaction analysis, Structure-based drug discovery

Protein-ligand docking is an important method in Structure-based Drug Discovery [1, 2]. Although many programs have been developed for docking [3], the accuracy is still insufficient due to the difficulty in the scoring function [4]. Interaction fingerprint is one of the solutions, which generate fingerprints of ligands using the interactions between the ligand and the protein. Interaction fingerprints use the information of known compounds so that new compounds that have similar interaction to the known active ligand are expected to find through the virtual screening. However, existing interaction fingerprints such as SIFt [5] and SPLIF [6] only assess the existence or the distance of the interactions and do not consider the strength correctly. In this study, we made a new scoring function of protein-ligand docking called SIEVE-Score (Similarity of Interaction Energy VEctor-Score), which can consider the strength of each interaction explicitly. SIEVE-Score is calculated based on the similarity of the interaction energy vector, which is the list of interaction energy between the ligand and each residue of the protein. We also evaluate the accuracy of virtual screening using SIEVE-Score after the docking by Glide [7].

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P2-23

Drug repositioning for Chagas disease by docking simulation and *in vitro* assay

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Keywords: Drug repositioning, Protein-ligand docking, Chagas disease, Spermidine synthase

Chagas disease is a kind of neglected tropical diseases (NTDs) caused by the parasite *Trypanosoma cruzi* [1-2]. Although nifurtimox and benznidazole are currently available for treatment of Chagas disease, these drugs are associated with serious side effects and exhibit limited effectiveness during the chronic phase of Chagas disease. Drug repositioning uses the drugs which were already approved for different targets, and has been recognized as an effective strategy because repositioned drugs have been through several R&D preclinical and clinical phases [5].

In order to discover a new anti-Chagas drug, we focused on spermidine synthase (SpdSyn) which synthesize polyamine as target protein, and conducted docking simulation and *in vitro* assay.

Docking simulation [6,7] was performed for SpdSyn to select drug candidate from among 39,218 compounds which were already approved or tried clinical phase. Moreover, we conducted *in vitro* assay to determine the half maximal inhibitory concentration value (IC_{50}). As a result, we successfully obtained active compounds with IC_{50} values of the 10 μM order against SpdSyn.

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P2-24

Development of the selective inhibitor of WNK1 using fragment compounds

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Keywords: Fragment library, WNK1, docking simulation

Pseudohypoaldosteronism type II has been known as a rare autosomal dominant disorder allegedly triggered by overexpression of WNK1 [with no lysine(K)] or mutations in WNK4. Thus far, there have been only a few reports regarding the specific inhibitor for WNK1 or WNK4.

The WNK1 has the back pocket just behind the ATP binding site (Figure 1). Therefore, we targeted the back pocket rarely existed in the common kinase rather than the ATP binding site in order to get more selective inhibitor.

In order to harness the peculiar structure and get more selective inhibitor, we targeted the back pocket less existed in the common kinase, rather than the abundant ATP binding site. A docking simulation was conducted using MOE with Amber 10: EHT, and ca. 9,000 kinds of fragment library compounds in Drug Discovery Initiative were assessed here.

Firstly, we classified the higher-frequency scaffolds that were in the top 20% of GBVI/WSA dG scores into three types (Figure 2) and then examined the interaction of the selected compounds with WNK1 by MOE. Using mobility shift assay and surface plasmon resonance techniques, we assessed the inhibitory activity for phosphorylation of WNK1 and the binding abilities to WNK1 as well.

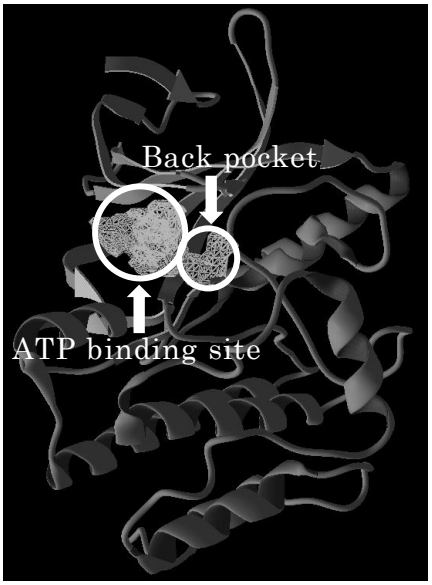

Figure 1. Structure of WNK1

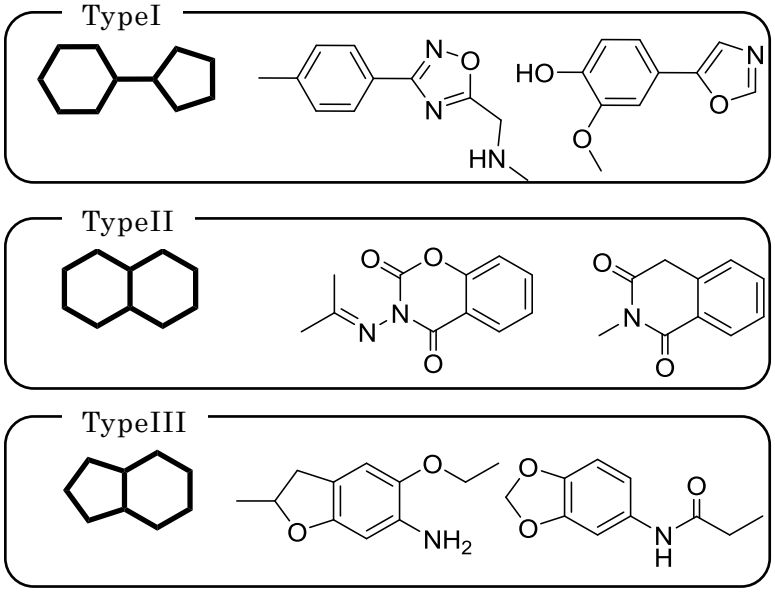

Figure 2. Three types of compounds and some examples.

P2-25

Discovery of Novel LPA-1 Antagonists - Computational Analysis for Molecular Design -

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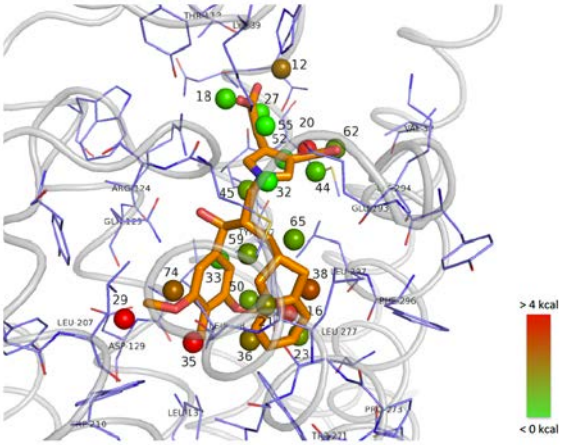
Keywords: LPA1, GPCR, Molecular Design, WaterMap, MD simulation

Lysophosphatidic acid (LPA) evokes various physiological responses through a series of G protein-coupled receptors known as LPA1-6. We conducted a high throughput screen against LPA1 to give a hit compound. Subsequent optimization of the hit compound led to the discovery of novel and potent LPA1 antagonists, which showed good efficacy *in vivo*.[1),2)] We also successfully determined the first crystal structures of LPA1 in complex with antagonists.[3)]

In recent years, various kinds of X-ray crystal structure analysis of GPCR have been reported. Some of such studies include molecular design based on GPCR X-ray structures, but it is still challenging to develop potent and selective ligands for GPCRs by SBDD approach.

In our presentation, we will show some examples of SBDD approach based on the LPA1 X-ray crystal structures. Molecular design based on the ligand-receptor interaction map and WaterMap (Schrödinger Inc.)[4)] analysis of the ligand binding pocket will be discussed.



LPA1 Complex WaterMap Result

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P2-26

Semi-automated FMO calculation protocol for construction of IFIE-database

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Keywords: Modeling, Optimization, MM-PBSA, FMO, Semi-automation, IFIE-database

Since 2014, we have performed fragment molecular orbital (FMO) calculations for various drug targets (e.g. Kinase, Nuclear receptor, Protease, PPI) with activity values (e.g. IC_{50}) as the activities of FMO drug design (FMODD) consortium. To efficiently calculate huge amount of different structures, we must refine modeling conditions. For example, appropriate structure preparation, which includes complementation of missing atoms and missing residues, addition of hydrogen atoms, and structure minimization, is necessary as pretreatment before FMO calculations. However, the appropriate method(s) for preprocess are not yet established. Thus we have discussed the modeling condition (e.g. complementation of heavy atoms, with/without water molecules, restraint of heavy atoms on minimization) in FMODD consortium based on the results so far. Know-how of the modeling has accumulated. Another issue is treating massive structure data (e.g. PDB having ca. 120,000 entries) to construct IFIE-database (IFIE-DB) in the future. There are limits to prepare huge amount of structures by manual labor. Thus, we are currently developing "semi-automated FMO calculation protocol" using pipeline pilot as described below.

Semi-automated FMO calculation protocol with Pipeline Pilot

1 Structure preparation of protein and ligand complex.
- 1.1 Correction of missing atoms and missing residues based on PDB files.
- 1.2 Structure optimization by MM method with MOE software.
- 1.3 Structure optimization by QM/MM method with Gaussian (option)

2 MM-PBSA/GBSA calculations
- 2.1 Input files generation of MM-PBSA/GBSA calculation.
- 2.2 Perform MM-PBSA/GBSA calculation on PC cluster machine with AMBER.
- 2.3 Analysis of solvation and binding energies between ligand and protein.

3 FMO calculations
- 3.1 Input files generation FMO calculation.
- 3.2 Perform FMO calculation on PC cluster machine or K-computer with ABINIT-MP.
- 3.3 Analysis of IFIE between ligand and protein and its energy decomposition with PIEDA.

This research was done in activities of the FMO drug design consortium (FMODD). The results of FMO calculations were obtained using the K computer (project ID: hp150160 and hp160103). Science Technology Systems supported to develop the FMO calculation protocol. The FMO calculation protocol is automated by BIOVIA Pipeline Pilot from Dassault Systè mes BIOVIA. Structure modeling and MM optimization were carried out by MOE software package. QM/MM optimization was using Gaussian09 and were used computational resources of the supercomputer, HOKUSAI (RIKEN Advanced Center for Computing and Communications).. MM-PBSA was performed by AMBER 12. PIEDA calculation was done by using MIZUHO/BioStation software package.

P2-27

Flexible ligand docking to multiple receptor: application to tankyrase2-ligand system

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Keywords: Molecular Screening, Multiple Protein Structures

In early stage of drug discovery, computational compound screening is widely used to find hit compounds. Molecular docking is one of the principal methods for the compound screening. This method generally docks a flexible ligand to a rigid (static) protein structure and predicts the binding pose and affinity for ligand in a practical time. As the different structural characteristics (chemotypes) of compounds induce different receptor's binding pocket conformations, molecular docking tends to predict that a specific ligand whose chemotype binds to a specific pocket conformation binds to other binding pocket conformation improperly and weakly. To resolve the problem, incorporation of protein flexibility into molecular docking is required. Molecular docking using multiple receptor conformations ("ensemble docking" [1-3]) is one of approaches to consider protein flexibility. These multiple receptor conformations can be obtained by X-ray crystallographic structures and/or structures sampled by molecular dynamics (MD) simulations. However, there still remains difficulty on proper generation and selection of multiple receptors used in ensemble docking.

In this work, we study the effective method of ensemble docking for tankyrase2-ligand system. From analysis of X-ray crystallographic structures on tankyrase2-ligand system, we found three tankyrase2-ligand complex structures where the chemotypes of ligands and the conformation of binding pocket are so different, respectively. In three receptor conformations, we found that positions of side chains of His and two or three Tyr residues at binding pocket are closely related to conformations of binding pocket and focused on these residues to classify receptor conformations. To generate various receptor conformations, several MD simulations of tankyrase2 with and without ligand were performed. We are researching various methods on classification and selection of receptor's binding pocket conformations obtained from MD simulations for effective ensemble docking and gives information on the relationship between chemotypes of ligands and receptor's pocket conformations.

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P2-28

An Application of Deep Learning for Classifying Chemical Structures

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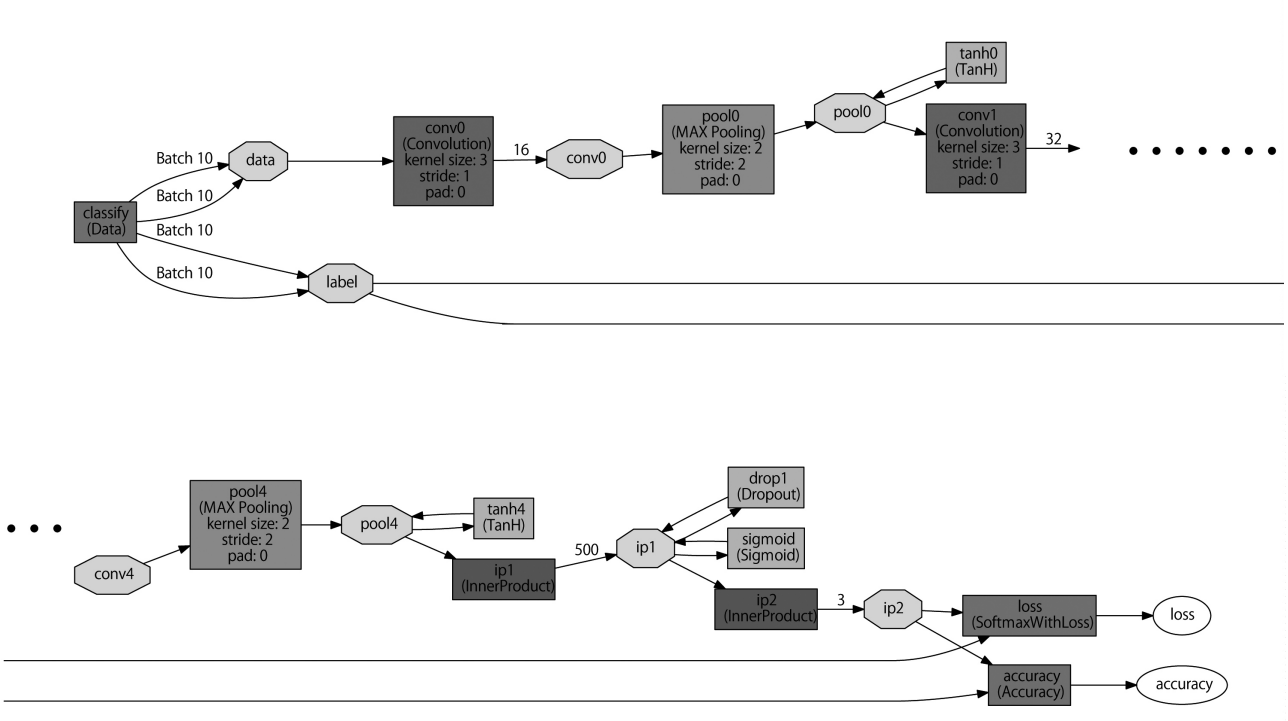
Keywords: Deep Leaning, Caffe, Chemical Structure

Deep learning, a branch of machine learning utilizing multiple processing layers, is rapidly advancing many areas including image, text and voice recognition, and now being applied to drug discovery and cheminfomatics. In this work we demonstrate how deep neural networks (DNN) trained on images of chemical structure can classify various drugs to therapeutic chemical categories based on the INN (international nonproprietary names) stems. We prepared an automated scheme to retrieve chemical table files from the KEGG database[1] and to transform them to chemical structure images. The Caffe framework[2] was used to construct DNN models. GPU-accelerated parallel computing platforms were developed for data training on Amazon Web Services(AWS), a suite of cloud-computing service. Cross-validation experiments showed that the DNN-based models achieved extremely high classification accuracy in predicting therapeutic category for each chemical structure image. This preliminary work indicates that DNN extract features from chemical structure images successfully. Our next goals are applying DNN to problems related to prediction of biological activities and drug repositioning.



[1] http://www.genome.jp/kegg/
[2] http://caffe.berkeleyvision.org/

P3-01

Generation of Amino Acid Indices to Distinguish Two Peptide Groups

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Keywords: Genetic algorithm, Amino acid index, Principal component analysis

Classification of data is a basic step for data mining, where multivariate analyses, such as principal component analysis (PCA), are widely used. The approach is also used for the classification of peptides in the field of proteochemometrics. In the analysis, a peptide is often expressed as a set of several numerical values with different amino acid indices. However, there are several problems in the approach with the amino acid indices. One of the problems is the number of amino acid indices. There are many amino acid indices, which are available from the KEGG database, but there is no clear criterion to select the indices for the analysis. The other problem is the result of the classification. Even if we applied PCA, for example, to the classification of peptides, there is no proof that the peptides are classified into groups, for which proper interpretation is possible. Both problems are intertwined each other. In this presentation, we propose a method to solve the problems simultaneously. Consider that we have two sets of peptides. The object is to find amino acid indices to distinguish the two groups in the space of reduced dimensions by PCA. Instead of using the currently available amino acid indices, we generate the amino acid indices to fit the object described above by the genetic algorithm (GA). The fitness for an amino acid index is defined as a product of a modified Fisher's ratio, which is used for the discriminant analysis, in the space of the reduced dimensions by PCA and the contribution ratio for GA. The chromosome consists of a set of amino acid indices and a set of two parameters. The size of the former set is equal to that of the latter. Therefore, a pair of parameters corresponds to an amino acid index. Each set of the two parameters, which is used to express a beta function, is introduced to treat the peptides of different length. As a result, the sets of amino acid indices and the parameters are optimized and the two groups are distinguished in the space of the reduced dimensions by PCA. The program was constructed by the C language. The performance of our method is discussed.

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P3-02

Network-based-Analysis of Glycosyltransferase and Protein Interaction

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Keywords: Network, Glycan chain

Most of the secreted and cell membrane proteins in mammals are glycosylated, and the glycan chains constitute a representative group of biopolymers [1]. Unlike nucleic acid and amino acid sequences, the glycan chain has a branched structure with a wide variety [2]. It is known that the glycosylation of proteins is deeply involved in the regulation of the function and the structural stability of proteins. However, the relationship between diseases and glycoproteins still remains unclear. Barabasi and his colleagues reported a method to derive the relationship between genes and that between diseases by marginalizing genes or diseases in a bipartite graph consisting of diseases and associated genes to constitute a network of only diseases or genes [3]. In this study, we tried to construct an interaction network of glycosyltransferases and target proteins based on the similar approach. At first, the information about the diseases related to proteins was obtained from OMIM. Then, the secreted proteins and membrane proteins including glycosyltransferases were collected based on the GO annotation. A bipartite graph between disease and collected proteins was generated. Finally, a network of the proteins was generated by the marginalization of diseases. The features of the network associated with the glycosylation are discussed.

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P3-03

Neuronal differentiation model constructed from combination of network motifs in the comprehensive molecular interaction map

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Keywords: Neuronal differentiation, Signaling pathway, network motif, CellDesigner

The regenerative medicine mediated by neurogenesis is expected to remedy refractory neurological disorders like spinal cord injury. Although various studies have been conducted to establish the therapy mediated by neurogenesis, it is a big problem because the underlying mechanism of neuronal differentiation remains to be elucidated. To understand a neuronal differentiation which has very complex biological processes, an approach of systems biology is expected to be effective. However, existing bottom-up or top-down approaches of systems biology is not appropriate for comprehensive and complex biological process like neuronal differentiation. To analyze such comprehensive and complex problem, we used hybrid method of bottom-up and top-down. In the method, network motifs which are important to cell dynamics are extracted from the comprehensive network of target biological process. And then, mathematical model is constructed by piecing together the motifs. The model is expected to maintain original cell dynamics of comprehensive network. We applied the method to comprehensive network of neuronal differentiation that was constructed by relations collected from literatures and pathway databases, and got simple mathematical model of neuronal differentiation. The model could simulate the characteristic dynamics of HES1 and ASCL1 changes before and after differentiation. This mathematical model will be useful to understand the mechanism of neuronal differentiation.

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P3-04

Prediction of interacting proteins from homologous protein-protein interactions

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Keywords: Prediction of protein-protein interactions, Homology, Machine Learning, AODE

The identification of protein-protein interactions (PPIs) is essential for a better understanding of biological processes, pathways and functions. However, experimental identification of the complete set of PPIs in a cell/organism remains a difficult task. To circumvent limitations of current high-throughput experimental techniques, it is necessary to develop high-performance computational methods for predicting PPIs. In this study, we propose a new computational method to predict proteins that interact with a given protein using three sequence-based features derived from known PPIs using Averaged One-Dependence Estimators (AODE; [1]). The three features are: (I) sequence similarities to a known interacting protein pair (F_{Seq}), (II) statistical propensities of domain pairs observed in the protein pair (F_{Dom}) and (III) a sum of edge weights along the shortest path between their homologous proteins in a PPI network (F_{Net}) [2].

The AODE models were trained on the highly imbalanced, realistic dataset, which contained only 0.13% of high confidence, direct physical human PPIs (43,060 positive PPIs / 33,098,951 negative PPIs) obtained from TargetMine [3], and evaluated in 3-fold cross validation. As a result, the AODE model trained with all the three features, F_{Net}, F_{Dom} and F_{Seq}, achieved the highest AUC of 0.887 ($pAUC_{FPR \leq 0.5\%} = 0.243$). These models were applied to the identification of all the proteins that would interact with a query protein. This new computational system can assess the potential interactions between a query protein and the proteins registered in the UniProt database at high speed, and then it prioritizes the predicted interactions according to their prediction scores. The proposed method and the new database scanning system will contribute to the identification of novel PPIs and the estimation of complete PPI networks.

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P3-05

Toxygates for toxicogenomics analysis: an update

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Keywords: Toxicogenomics, web application, microarray

In order to assess the safety of drugs based on their gene expression profiles, the Japanese Toxicogenomics Project consortium (TGP) [1] collected microarray data and pathology information for about 200 compounds and developed a toxicogenomics database, Open TG-GATEs [2], which is open to public and now widely used all over the world. Toxygates [3] was originally developed as a user-friendly interface to enable users to access the data deposited in Open TG-GATEs. Here we report recent updates in Toxygates, which include the “user data upload function”, “interactive heatmap”, “integration with TargetMine [4] for enrichment analysis” and “multi-species mode”.

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P3-07

Network inference from promoter activity in lung cancer

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Keywords: Lung cancer, cap analysis of gene expression (CAGE), gene regulatory network (GRN)

Various molecular mechanisms in carcinogenesis have been revealed by recent studies, and molecular targeted drugs have also been developed one after another. For example, it has been reported that specific mutations in the epidermal growth factor receptor (EGFR) gene are associated with sensitivity to EGFR inhibitors such as gefitinib and erlotinib in lung cancer patients [1]. However, many challenges in drug discovery (e.g., acquisition of resistance to therapy, different mechanisms in carcinogenesis and progression, and prediction of drug efficacy in individual patients) have been left for the future.

In this study, we aimed to investigate the gene regulatory networks (GRNs) in lung cancer in order to reveal the molecular characteristics of the disease subtypes. We used the surgical specimens of lung squamous cell carcinoma (SCC, n=22) and adenocarcinoma (AD, n=73) at the Department of Thoracic Surgery, Juntendo University. The expression profiles of gene promoters were obtained by the cap analysis of gene expression (CAGE) method [2] using the RNA samples extracted from each specimen. Then, we constructed GRNs for each subtype of lung cancer using GINIE3 software [3], which is one of the best algorithms to estimate GRNs from gene expression profiles. Then, we compared and interpreted the functional differences in the promoter activity among each subtype of lung cancer.

In the AD patients, we carried out comparison between of EGFR mutation-positive group and the wild-type group. By inferring the network among the expression regulatory genes, co-expression was found on the transcription factors that involved in cancer (HMGA1/2), homeobox genes(ONECUT2/3), and nuclear receptor (HNF4A). We will also investigate the network differences from patients with the gene mutations in KRAS.

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P3-08

An integrative systems analysis of gene expression profiles of the fibrotic lung from CD151 knockout mice reveals insights into the mechanisms of lung fibrosis

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, **Keywords**: Disease omics analysis, Pathway analysis, Translational systems biology

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, irreversible, and age-related lung disease associated with high morbidity and poor survival. Because the exact mechanisms responsible for the development of IPF remain largely unknown, there are currently no proven therapies targeting the fibrotic process itself. Previously, we found that tetraspanin CD151 knockout mice spontaneously developed fibrosis[1)]. In this study, we have employed integrative systems biology-based computational approaches[2)] to investigate the global gene expression profiles of the fibrotic lung from CD151 knockout mice. We analyzed genes that were differentially expressed in the mouse disease model by constructing protein-protein interaction (PPI) networks and performing pathway enrichment and topological analysis with TargetMine data analysis platform[3)]. Our analysis illuminated specific genes and pathways that explain the onset and progression of pulmonary fibrosis. We observed an overall upregulation of factors associated with extracellular matrix deposition and a specific involvement of the circadian clock pathway in the fibrotic process. Our analysis provided novel insights into the role of CD151 function pulmonary fibrosis and identified potential targets for better anti-fibrotic therapy and potentially novel biomarkers of IPF.

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P3-09

Pathway-based drug discovery and repositioning by large-scale chemically-induced transcriptomics

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Keywords: bioactive compound, drug repositioning, drug target, modes of action, transcriptome

The identification of the modes of action of bioactive compounds is a major challenge in chemical systems biology of diseases. Diseases are caused by dysfunction of the human biological system, which consists of genes, proteins, and pathways. Most drugs are bioactive compounds that modulate the activity of biological systems for the treatment of diseases. However, drugs may interact not only with their primary target proteins but also with other proteins (off-targets) and thereby cause unexpected side effects. Side effects derived from off-target interactions, although typically undesired, may occasionally enable new therapeutic indications [1] because different diseases often share same or similar pathogenic mechanisms. Therefore, understanding the complex responses of the human body to the treatments of bioactive compounds is a vital important issue in medical and pharmaceutical research. A promising unbiased approach is genome-wide gene expression profiling of the transcriptional response to chemical perturbations of human cell lines.

Here we developed a novel computational approach to elucidate the modes of action of bioactive compounds using large-scale chemically-induced transcriptome data acquired from the Library of Integrated Network-based Cellular Signatures, and analyzed 16,268 compounds (including most approved drugs) and 68 human cell lines (e.g., MCF7, PC3 and HL60). First, we performed pathway enrichment analyses of regulated genes to reveal active pathways among 163 pathways. Next, we explored potential target proteins (including primary targets and off-targets) with cell-specific transcriptional similarity using chemical–protein interactome. Finally, we predicted new therapeutic indications for 461 diseases based on the target proteins. We demonstrated the usefulness of the proposed approach and confirmed its advantages over the previous related approach [2] in terms of prediction coverage, interpretation, and large-scale applicability. We also validated the new prediction results experimentally by an in vitro cellular assay. The approach has a high potential for advancing drug discovery and repositioning.

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P3-10

Evaluating immune related function and characteristic analysis of the Okinawan lactic acid bacteria

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Keywords: Lactic Acid Bacteria, Immune function, IL-12, IgE

Lactic acid bacteria (LAB) are bacteria that produce lactic acid. It is reported there are LAB that have various functions. In Okinawa, there is variety of bioresources that have adapted to the subtropical climate. Our laboratory made the LAB library from bioresources of Okinawa. In this library, both Enterococcus faecalis had an anti-allergic function and one that didn't have it was found. In this study, we evaluated and compared immune stimulating activity and anti-allergic function of the two Enterococcus faecalis strains, named No.5 and No.6. In addition, the causes in the difference function are investigated by genome sequencing and genome analysis. As a result, No.5 strain increased the amount of Il-12b mRNA expression as index of immune stimulating activity and decreased IgE which is the cause material of allergy. No.6 strain didn't have these functions. We conducted genome sequencing and compared CDS of these strains, No.5 strain only had CRISPR related genes that work as immune system against phage. Enterococcus faecalis is reported that its derived extracts are effective for hay fever sufferers. Therefore, we concluded that No.5 strain had this function because the defensive system should work against phage that negatively affected the function of LAB.

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P3-11

Using gene expression profiles to identify the underlying mechanism of adverse drug events

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Keywords: Osteonecrosis, Gene expression profiling, Machine learning, adverse drug reactions

Understanding of the effects of adverse drug events (ADE) on gene expression can help in understanding the underlying mechanism of ADE; it can also be utilized for prediction of these events in newly discovered drugs. In this study, we aimed to explore the underlying mechanism of adverse drug events using bioinformatics' tools. We used four publicly available databases, The Japanese Toxicogenomics Project (TGP) database (Open TG-GATEs[1]), curated and standardized FAERS (FDA's Adverse Event Reporting System (FAERS[2]), SIDER[3], and Medical dictionary for regulatory activities (MedDRA®[4]).

We included in our analysis only those compounds for which Open TG-GATEs provided gene expression profiles. We identified drugs that had a statistically significant association with adverse events reports in FAERS using Fisher's exact test ($p < 0.001$). The other compounds in open TG-GATEs were considered controls. We obtained gene expression profiles for these compounds from Open TG-GATEs. TGP measured gene expression across different time intervals and dose levels.

We applied machine-learning methods, and enrichment analyses to find out the pathways affected by these drugs, and estimate the underlying mechanism of these ADE.

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P3-12

Garuda Platform – Connecting Analytics for Open Innovation

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Keywords: Computational analytics platform, open innovation, integrated data analytics pipeline, privacy preserving queries

To understand the disease states and pathogenesis at the molecular level, or to achieve a meticulous patient stratification leading to drug discovery, there is a need to understand the dynamic behavior of each layer of the living systems by examining a large set of high dimension data. With recent advancements in data collection techniques for various domains, data analysis methods have been steadily diversified and rapidly expanding including the introduction of AI and machine learning. This becomes an urgent challenge for researchers worldwide, requiring immediate need for a platform that enables easy access to up-to-date and diverse analysis workflow and can apply to systematic analysis both in life science and clinical research. Further, the complexity and multi-dimensionality of data and analytics make it extremely challenging to develop a single, stand-alone system that can integrate a broad range of dataset and analysis techniques. Instead, a platform that connects the diverse components through open interfaces, and allows the community to discover and navigate through them, can provide a unique collaborative eco-system.

One of the efforts to build such platform is Garuda, which is a community-driven open platform that provides a framework to connect, discover and navigate through different analytics applications, databases and information services in biology and medicine. Garuda provides programming language independent APIs to connect software/tools as gadgets, explore them through a gateway and operate them through the dashboard, supported by a global alliance of leaders in computational biology and informatics. Now, Garuda is extending its wings to data security. In the poster, we would illustrate some potential user scenarios for privacy preserving queries over clinical data or in-development compound structure information, which would serve as a gateway to the life science/drug discovery workflow.

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P3-13

Genetic analysis of mitochondrial disorder

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Keywords: Mendelian genetic disorders, Metablic disorders, Mitochondria

Mitochondrial disorder has the highest incidence among congenital metabolic disorders characterized by biochemical respiratory chain complex deficiencies. It occurs at a rate of 1 in 5,000 births, and has phenotypic and genetic heterogeneity. More than 250 genes that cause mitochondrial disorder have been reported to date. However exact genetic diagnosis for patients still remained largely unknown. Recently, we published genomic analyses for 142 juvenile patients with mitochondrial respiratory chain complex deficiencies. We identified 37 novel mutations in known mitochondrial disease genes and 3 mitochondria-related genes (*MRPS23*, *QRSL1*, and *PNPLA4*) as novel causative genes, and also achieved genetic diagnoses in 49 of 142 patients (34.5%). The high proportion of novel mutations indicates the different ethnicity in Japanese population compared with those so far reported mainly by studies on Caucasian patients. Genetic causes for a large number of cases are still unknown. Therefore, to further clarify these unsolved cases, we are currently focusing on the analysis of exonic deletions as well as SNVs/INDELs/CNVs. By increasing the number of cases analyzed by exome sequencing, we will present our latest results and discuss about the contribution of these variants for the pathogenesis of mitochondrial disorder.

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P3-14

A novel ChIP-Seq spike-in strategy enables detection of global histone modification changes across the genome

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Abstract:

Chromatin immunoprecipitation and DNA sequencing (ChIP-seq) has been instrumental in understanding genome-wide distribution of chromatin proteins and their modifications (e.g., histone modifications). However, current standard ChIP-seq methodologies do not allow quantitative analyses and thus make it difficult to directly compare ChIP-Seq data among different samples (e.g., disease vs normal or treated vs untreated). To overcome this issue, we have developed a universal chromatin spike-in strategy that introduces Drosophila melanogaster chromatin and a D. melanogaster-specific antibody into standard ChIP reactions with human chromatin samples. To prove the principle of this concept, we performed ChIP-Seq of human cell samples treated with DMSO or small molecule inhibitors of the methyltransferase EZH2. The Drosophila ChIP-Seq reads were used to normalize human ChIP-Seq data. The spike-in strategy allowed detection of dramatic and global reduction of histone H3 lysine 27 trimethylation (H3K27me3) in inhibitor-treated samples, which was invisible with standard analysis methods. Thus, our ChIP-Seq spike-in strategy proves useful to compare ChIP-Seq data among different samples and to understand the effect of drug treatments.

P3-15

Development of the Protein Motif Extraction System based on an Artificial Bee Colony Algorithm

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Keywords: protein motif, motif extraction, artificial bee colony, swarm intelligence, motif length, zinc-finger

It is well known that the amino acid sequence of a protein is closely related to its structure and function. This is especially true for particular structural features called motifs, and they are considered to be well-reserved sites in the genomic sequences. Some sequence motifs contains several gaps which corresponded to insertions or deletions in the biological evolution. Since it is also known that two or more motif sites in a particular protein sequence, the motif extraction is regarded as a multimodal problem. To extract novel motif candidates from unaligned protein sequences, the authors have employed an artificial bee colony (ABC) algorithm which has high search ability of multivariable functions.

ABC is an optimization algorithm based on the intelligence behavior of honey bee swarm, which contains three types of bees: employed bees, onlookers and scouts [1]. The position of a food source represents a possible solution of the problem, i.e., a set of the peptide fragment that corresponded to the potential motif site for given protein sequences. A peptide fragment is shown using the starting and terminating residues in the sequence, so that a solution is described a 2N-dimensional vector. Here, N denotes the number of given protein sequence. The nectar amount of a food source corresponds to the quality of the associated solution. In this case, an approximated multiple alignment score (SP score) for a set of peptide fragments is used for the fitness function that is a measurement for the motif probability. The range of motif length is specified by the user for flexible motif extraction. At first, the ABC generates a randomly distributed initial population of solutions. An employee bee modified specific fragment on a solution for finding a new solution. An onlooker bee chooses a solution depending on the probability value, and tries to improve the solution using selected index. If a position cannot be improved further through a predefined limit number of cycles, a scout replaces new food source by random selection. Our system iterated these process to extract novel motif candidates.

The motif extract trials were carried out using the DNA binding proteins which contained five entries taken from the UniProt database. The range of fragment length was set to 21-24. BLOSUM-62 substitution matrix was employed for alignment, and gap penalty was set to -1. As the result, our system successfully identified the known zinc-finger C2H2 related motif sites [2]. In addition, several novel motif candidates were also extracted for each solutions. These results show the potential applicability of the present approach for sequence feature analysis of proteins.

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P3-16

Clustering of Flaviviridae based on RNA sequences using Deep Learning

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Keywords: Deep Learning, Classification, Flaviviridae

Infections caused by Flaviviridae are virulently poisonous and the diseases are often threats to people. Many of Flaviviridae can often spread widely because they are spread by mosquitoes [1]. If we construct a method of treatment after the confirmation of infection to people, the treatments are not completed in time and virus spreads over many people and areas. It is necessary to grasp the characteristic of prevailing virus in order to understand what types of virus are spread. Accurate classification is needed to analyze the characteristic of virus scientifically. However, the shape of virus is similar to the homologous ones by observing through electron microscopes or considering the genes. In this research, we used one of machine learning, Deep Learning. Because Deep Learning is good at feature extraction [2], we applied it to analyzing virus. Stacked Autoencoder, one of Deep Learning, can compress the dimension of inputted data with little information loss [3]. We compressed the dimension of the information of the base sequences of the viruses until it becomes two by applying this technology and evaluated it by plotting the results on a two dimensional plane. When we inputted the sequences of six kinds of viruses (West Nile virus, dengue virus, Zika virus, Japanese encephalitis virus, yellow fever virus, Hepatitis C virus), clusters for each virus were generated. Also, the result suggests that the differences of species are expressed as distances between the clusters. In addition, when we inputted only the data of dengue virus, it was classified into four clusters depending on four kinds of models of the virus. This result shows that Deep Learning can classify things in detailed way. We would like to apply this technique to examine how viruses evolve.

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P3-17

Deep Learning and Human Recognition of Biomolecules for Drug Design and Screening

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Keywords: Deep Learning, Autoencorder, Mitochondrial DNA, Virus, Antibody

In drug discovery research, methods are required to link biological properties and the compounds. Deep Learning (DL) potentially has an overall understanding ability of such relations.

1) Structural analysis of the compound having the sequence as protein

We have been analyzing influenza virus, flavivirus, human mitochondrial DNA and human antibodies. The major concern is the meaning of the entire sequence. In the case of mtDNA, haplogroups are commonly identified by SNPs, very small parts of DNA sequences. The Deep Lerning (Autoencoder) analysis of mtDNA gave a specific distribution corresponding to the haplogroup. The result indicates the positive relation of entire sequence and the haplogroup. However, they are not identically same [1].

Flavivirus group virus gene have been analyzed to identify the viruses. A clear separation forming the clusters of the virus species [2].

Influenza virus has been examined to elucidate the year-by-year and pandemic change of the gene. Entire sequence analysis was effective to classify the virus.

Variable regions (100 amino acid residues , 5932 lines) of the H chain of a human antibody were analyzed in a similar manner as formed for each particular antigen (virus). The positions of the clusters were sensitive to the change of the amino acids.

2) Cognition made by Autoencoder

Cluster formation of the haplogroup, species and the antibodies are commonly found by the sequence analysis. As far the virus classification, human naming and the classification by Autoencorder are almost identical. Human mtDNA analysis by Autoencorder gave specific characteristics as the haplogroup.

The comparison gives a conclusion that the human cognition and Deep Learning cognition are substantially same. Application of Deep Learning to the biological molecules could be possible for medical and pharmaceutical uses.

[1] Masato Shimabayashi et al in this symposium.
[2] Shuya Yamamoto et al in this symposium.

P3-18

Analysis of mitochondrial DNA based on whole sequence using Deep Learning

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Keywords: Deep Learning, mitochondrial DNA, classification

Human haplogroups has been studied by using indexes as blood type, virus infection, Y-chromosome. Mitochondrial DNA (mtDNA) is one of the most popular substance for the analysis. It is regarded as a key factor to study human evolution for three reasons. First, mtDNA gives a magnified view of the diversity present in the human gene pool, because mutations accumulate in this DNA several times faster than in the nucleus. Second, because mtDNA is inherited maternally and does not recombine, it is a tool for relating individuals to one another. Third, mtDNA molecules are abundance within a typical human [1].

In general, an approach for differentiation of identical mtDNA haplogroups is the analysis of single nucleotide polymorphisms (SNPs). In this research, we focused on whole sequence mtDNA-based classification with Deep Learning.

A mechanical learning method “Deep Learning” opened a promising way for artificial intelligence. Deep Learning has dramatically improved the state-of-the-art in speech recognition, visual object recognition, object detection and many other fields such as drug discovery and genomics [2].

We used Deep Learning to classify whole sequence mtDNA. We plotted the results compressed by deep auto encoder in two dimensions (The number of bases: about 16570, The number of data:396). The dimensional compression results suggest that mtDNA sequences should be classified in some groups. The meanings of the spatial dispersion and the correlation distances are discussed with reference to the divergence of human haplogroups. We think that the relative relationship of haplogroups from mtDNA data can be expressed using deep auto encoder.

We classified human haplogroups by deep convolutional neural network. We evaluated this approach with six haplogroups dataset (The number of bases:16581, The number of training data:360, The number of test data:90) and achieved over 90% accuracy. We also found that classification may not be based only on existing haplogroup definition. The main conclusion of our study is that whole sequence mtDNA-based classification with Deep Learning seems to be a new index to study human evolution.

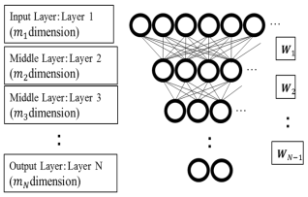

Fig1: An illustration of deep auto encoder.

[Acknowledgements] I would like to thank Yuhei Kaneshita for his supporting this work.

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P3-19

VaProS: A Data Cloud Approach for Integration of Protein and Genome Information

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Keywords: Big data analysis, Lysosomal storage disease, Protein 3D structure

Current life science research depends heavily on all sorts of omics data, such as genome sequences, transcriptome, protein-protein interactome, protein three-dimensional (3D) structurome, phenome and others. The knowledge compiled by all the omics research is so huge that a computer-aided search of databases is now a starting point for a new study. Especially, a combinatorial search of multiple databases has a big chance to retrieve new idea and new hypotheses that can be tested by wet-lab experiments. We therefore tried to launch an application that virtually integrates the related databases on the Internet. Specifically, we have built a new web application that facilitates life science researchers for retrieving experts' knowledge stored in the databases and for building new hypothesis for their research targets. The web application is named VaProS and puts stress on the interconnection between the functional information of genome sequence and protein 3D structurome, such as structural effect of gene mutation. In this poster presentation, we will demonstrate the overview of VaProS, and the databases and tools that can be accessed without any knowledge of database locations and data formats. We will further demonstrate the power of search on VaProS using lysosomal storage disease as an example for the application. VaProS is freely available at http://p4d-info.nig.ac.jp/vapros/.

P4-01

Evaluation of drug-likeness on bioavailability by *in vitro* screening

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Keywords: Drug-likeness, Bioavailability, Hybrid parameter

Analysis of launched drug by *in vitro* ADME/physicochemical properties screening (*in vitro* screening) is important to improve drug-likeness in early drug discovery stage. Although bioavailability (BA) is a useful parameter which represents the degree of oral absorption in pharmacokinetic profile, details of the relationship between BA and *in vitro* screening data are not sufficiently clear. In order to obtain a new parameter reflecting drug-likeness focused on BA, analysis of launched drugs was investigated by the use of *in vitro* screening dataset which measured four parameters of solubility, permeability, metabolic stability and protein binding.

We could obtained BA potential score (BAP score) as a new parameter. BAP score were calculated by absorption and hepatic availability potentials that expressed as hybrid parameters of *in vitro* screening. Drugs with high BAP score exhibit higher BA potential. Forty-eight percent of launched drugs existed in area above 3 of BAP score. As shown in Figure 1, BA potential map (BAP map) is valuable to better understand the relative positioning between launched drugs and in-house compounds, and the balance of absorption and hepatic availability potential.

Finally, it was considered that BAP score and BAP map could visually show the direction of drug design, and that this analysis was a practical and useful tool in effective improvement of drug-likeness.

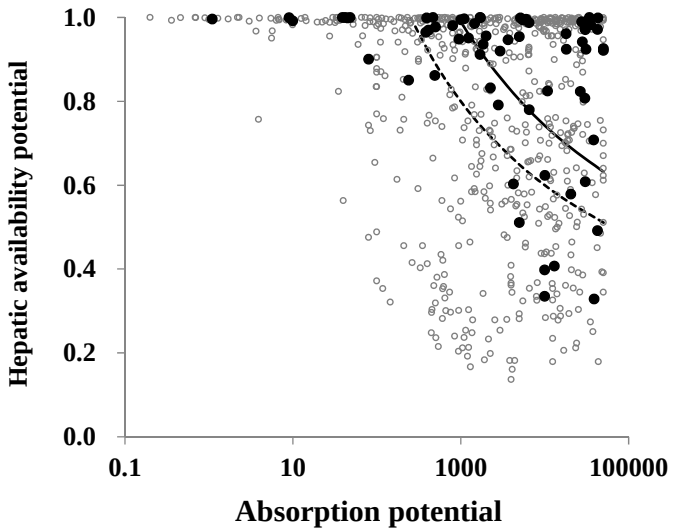

Figure 1 BA potential map based on absorption and hepatic availability potentials
●: launched drug, ○: in-house compound
Solid line: BAP score = 3.0, dashed line: BAP score = 2.4.

P4-02

Proposal of *in silico* mechanism-based methodology for prediction of toxicity

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Keywords: Structure-Property-Activity-Relationship, toxicity prediction, mechanism-based

In silico toxicity prediction becomes to be of importance in drug discovery and development. Especially, quantitative-structure-activity-relationship (QSAR) currently attracts attention because the ICH M7 guideline was issued. QSAR predicts toxicity with some structural alerts which are related to the toxicity outcome in experience or in statistics. In many cases, the relationship between the mechanism of toxicity and the structural alerts is uncertain. On the other hands, new statistical methods, *the random forest* and *the deep learning*, have been used to predict some toxicities. These methods have a relationship with the mechanism of toxicity by using physico-chemical-properties and experimental data. However, these methods are complicated system to discuss their results for common toxicologists. Thereby, toxicologists desire new *in silico* methodology for toxicity prediction based on the mechanism of toxicity with simple procedure.

We have been developing a new methodology for toxicity prediction to call positive or negative, which is based on the mechanism of toxicity. The methodology was consistent with 5 steps as follows: 1) translate the mechanism of toxicity to some physic-chemical-properties (descriptors); 2) put the criteria of prediction power (sensitivity, specificity, concordance); 3) set the cut-off lines; 4) repeat the 3rd step for each descriptor; 5) combine descriptors to call one result for one compound. Among these steps, the 1st and 3rd steps are important to achieve good prediction power. Especially, the 3rd step is the key-point of our method. In many case, there is a “gray zone” which is co-existed of positive and negative compound at similar rate on 2D plot of descriptor and toxicity. The discriminatory analysis is unsuitable at the above situation because of existing many false predictions. Therefore, we used the decision tree-like method to set cut-off lines and to define “gray zone”. By using our methodology, we achieved to establish new toxicity prediction methods for phototoxicity and phospholipidosis [1], [2]. Each new prediction method shows high concordance (>80%) with 2 or 3 descriptors. In conclusion, our methodology proposal here is considered to be useful in establishing new toxicity prediction methods for the mechanism-well-known toxicity.

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P4-03

Prediction of chemical-induced developmental and reproductive toxicity in human using the machine learning

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Keywords: *In silico* prediction, Developmental and reproductive toxicity, Decision tree, SVM

Introduction: The developmental and reproductive toxicity (DART) in humans is one of the crucial adverse reactions. However, because pregnant women are largely excluded from clinical research the data available for research is limited. The toxicity has too complex and unknown mechanisms to be empirically predicted, and the results of the time consuming and animal-intensive studies are not always extrapolated to humans. In this study, we try to predict the humans' DART from the physical and chemical properties of drug molecules using two machine learning methods, decision trees and support vector machine (SVM).
Methods : The DART in humans is classified according to FDA pregnancy risk categories (A, B, C, D or X) which indicate the potential of a drug to cause birth defects if used during pregnancy. In our work compounds were defined as "non-toxic" if they are categorized as A or B, "unknown" (n=227) if they are categorized as C, and "toxic" (n=162) if they are categorized as D or X. The *in silico* model for human DART was built on the data set of compounds with 39 atom-type descriptors and 4 molecular properties (molecular weight (MW), p*Ka*, topological polar surface area and log *P*). RapidMiner Studio 7.0 was used for the generation of decision tree and SVM, with under-sampling methods: Nearmiss-1 (NM1), Nearmiss-2 (NM2), Most distant [1], and Random sampling. Forward features selection and backward elimination were used with the SVM model. The performance of generated model was evaluated using 10-fold cross validation method.
Results and discussion: In order to avoid models with high false-negative, model sensitivity was considered in addition to its accuracy. The best performance model of decision tree was obtained with NM2, yielding a mean (±SD) accuracy, sensitivity and specificity of 69±6%, 84±10% and 58±7%, respectively. The resulted decision tree was simple enough to assess the DART of "unknown" compounds in a short time. The tree showed that DART is associated with S3 (sp3 sulfur in thioether), MW and N3 (sp3 nitrogen). SVM with NM2 and backward elimination has also produced a good prediction yielding a mean (±SD) accuracy, sensitivity and specificity of 76±7%, 83±11% and 70±7%, respectively. The DART of "unknown" compounds was also evaluated by the SVM and compared with the results of decision tree.
Conclusion: Present results showed that data mining algorithms such as decision tree or SVM can be useful in predicting the human DART *in silico* not only before screening of new chemical entities but also at the selection of safer therapeutics for pregnant women.

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P4-04

Informatic assessments of global gene expression data obtained from the analysis of hepatic stellate cell deactivation process

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Keywords: hepatic stellate cell, hepatic fibrosis, GneChip, informatic assessment

【Introduction】Hepatic stellate cells (HSCs) are pericytes presenting in a small area between the sinusoids and hepatocytes, and storing vitamin A as quiescent state in normal liver. When liver is damaged, HSCs are activated and playing a key role in hepatic fibrogenesis by secreting collagen. HSCs cultured on a normal culture plate usually changes into the activated state. We found that when the HSCs were cultured on VECELL membrane, activated HSCs turned into deactivated state. In this research, gene expression pattern of HSCs cultured on VECELL membrane was compared to that cultured on normal culture plate or on matrigel coated plate, and the effect on HSCs deactivation was evaluated.

【Methods】The data used in this study were obtained from the following experiments. Hepatic stellate cell line LI90 was cultured on VECELL, on matrigel coated plate or on normal culture plate. Total mRNA was isolated from cells cultured for four days or for seven days. Gene expression profiles were determined by the human genome U133A Gene Chip DNA Microarray. Gene expression data were filtered according to the following criteria: (i) genes with a signal above 500, (ii) genes with more than 2.0 fold change, (iii) genes with p-values less than 0.05. Statistical analysis of the expression data was performed using ANOVA. The genes remaining after filtering were analyzed using MetaboAnalyst3.0 [1], DAVID [2] and DiRE [3].

【Results】There were 203 genes selected in common with four days and seven days of culture. Gene expressions in VECELL culture and in matrigel culture were compared to those in normal plate culture. The partial least squares-discriminant analysis (PLS-DA) was performed on these genes by MetaboAnalyst3.0. VECELL culture and matrigel culture formed a cluster separated from normal plate culture. When the gene list was analyzed by DAVID on the base of gene ontology function annotation, the gene groups related to HSCs activation (TGFβ pathway, p53 pathway, immune response and extracellular matrix) were found to be enriched. DiRE analysis showed that SRF, NFκB and HMEF2 were associated with down-regulated genes in VECELL culture or in matrigel culture. These transcription factors are reported to be related to HSCs activation.

【Conclusion】 In this research, gene expressions among three culture conditions were compared and those changed both in VECELL culture and in matrigel culture in common were identified. Furthermore, informatic analysis suggested pathways, functions and transcription factors relating to HSCs deactivation were regulated in VECELL culture and matrigel culture.

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P4-05

Prediction for chemical-induced hepatomegaly and computational analysis of feature motifs

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Keywords: Hepatomegaly, Machine learning, Molecular features, QSAR

Hepatomegaly is often observed in the repeated-dose toxicity studies of chemicals [1], and frequently affects the derivation of the No Observed Adverse Effect Level (NOAEL). So, we are expected to understand and predict hepatomegaly in toxicological research.

In this study, we have developed the prediction Quantitative Structure Active Relationship (QSAR) model for the hepatomegaly from chemical substance's descriptors by machine learning methods known as deep learning, support vector machine and random forest. Our original data sets were composed of hepatomegaly-positive or hepatomegaly-negative compounds based on the risk assessment reports of pesticides, food additives, and veterinary medicine products that were published by Food Safety Commission of Japan [2]. These our prediction models had the nearly 80% prediction accuracy.

However, it is difficult to understand their contents in machine learning methods [3]. In order to understand what molecular features are important for hepatomegaly, we analyzed about molecular descriptors by Dragon, the application for the calculation of molecular descriptors [4]. Then, we calculated 154 functional groups to examine characteristic partial structure of hepatomegaly. Results showed that Pyrazoles, halogen on aromatic ring and secondly amides are characteristic of hepatomegaly-positive compounds. It might be possible to distinguish hepatomegaly by these structural features combination. For the screening of chemicals, these results are expected to be useful.

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P4-06

The importance of the applicability domain in prediction QSAR model for chemical-induced hepatomegaly

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Keywords: Applicability domain, Hepatomegaly, Machine learning methods, QSAR

It is important to predict hepatomegaly, which is often observed in repeated-dose toxicological studies, in the toxicity assessment [1]. The *in silico* predicting methods are expected to be used as the alternative to animal testing from the point of not only 3Rs in animal study but also the efficient toxicity study. So, we have developed hepatomegaly prediction Quantitative Structure Active Relationship (QSAR) model based on the structure of the chemicals.

In this study, we used two databases. One is our original toxicological database from the risk assessment reports of pesticides, food additives, and veterinary medical products that were published by Food Safety Commission of Japan [2]. The other is Hazard Evaluation Support System Integrated Platform (HESS-DB) [3] by National Institute of Technology and Evaluation. First, we extracted rat repeated-dose toxicological data from these databases. Then, chemical substance's descriptors were calculated and selected using Dragon 6.0 software [4]. Finally, we constructed QSAR model for hepatomegaly prediction by machine learning methods.

The applicability domain (AD) is important to construct a reliable QSAR model, which generally limits the application for chemicals structurally similar to the training compounds [5]. Therefore, the definition of the AD is necessary to improve the performance of the prediction model. Then, we defined the AD based on data density [6]. As a result, we could confirm the validity of the AD because the accuracy of predictions and the area under the curve (AUC) were increased.

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P4-07

Estimation of Mechanism-of-Action of Pharmaceutical Compounds Based on Systems Biology Approach

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Keywords: Modeling and Simulation, Metabolic profiling, Endocrine disruptors

Systems biology provides a framework for constructing mathematical models of biological and physiological systems from systematic measurements of multiple molecular levels. Recently systems biology is in an exponential development stage and has been widely used in drug discovery and development to deeply understand molecular basis of disease and pharmacological action. However, most of these conventional researches have been based on the exploratory approach, and comprehensive technologies for quantitative estimation of mechanism of drug action based on differentially omics analysis, such as metabolic profiling, are not widely reported. We have proposed useful computational approach for elucidation of mechanism of adrenal action of endocrine-disrupting compounds based on differentially metabolic profiling as an application of systems biology for risk assessment of novel pharmaceutical compounds [1].

Adrenal toxicity is one of the major concerns in drug development. To quantitatively understand the effect of endocrine-active compounds on adrenal steroidogenesis, and to apply to assess the human adrenal toxicity of novel pharmaceutical drugs, we developed a mathematical model of adrenal steroidogenesis in human adrenocortical carcinoma NCI-H295R cells. The computational model includes cellular proliferation, intracellular cholesterol translocation, diffusional transport of steroids, and metabolic pathways of adrenal steroidogenesis. It reconstructed in an experimental dynamic patterns of cholesterol and 14 steroids from an in vitro steroidogenesis assay using NCI-H295R cells. Results of dynamic sensitivity analysis suggested that 3β-HSD enzyme plays the most important role in the metabolic balance of adrenal steroidogenesis. Based on differentially metabolic profiling of 12 steroid hormones and 11 adrenal toxic compounds, we could estimate which steroidogenic enzymes were affected in this mathematical model by using a hybrid optimization method of genetic algorithm (AREX/JGG) and local search (non-linear least squares). In terms of adrenal steroidogenic inhibitors, the predicted action sites were approximately matched to reported target enzymes. On the other hands, vasculotoxic agents were estimated to have no effect according to the results obtained by our system. Thus, our computer-aided system based on a systems biological approach may be useful to understand the mechanism of action of endocrine-active compounds and to assess the human adrenal toxicity of novel pharmaceutical drugs.

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P4-08

Intestinal absorption of citrus fruits-derived heptamethoxflavone in rats

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Keywords: Intestinal absorption, Absorption clearance, Intestinal metabolism

3, 5, 6, 7, 8, 3', 4'-heptamethoxyflavone (HMF), which is present in citrus fruits, has been reported to induce brain-derived neurotropic factor (BDNF) production, and have an anti-inflammatory effect [1-3]. However, HMF pharmacokinetics has been unclear. Therefore, in the present report, intestinal absorption of HMF was studied using the upper (jejunum) and lower (ileum) parts of the small intestine isolated from rats [4].

HMF appeared on the serosal side, and the absorbed amount increased with time. Absorption clearance (CLabs) of HMF increased from 0.81 to 1.6 μL/min/cm as HMF concentration increased from 10 to 50 μM in the jejunum, whereas in the ileum the CLabs of HMF was nearly constant to be around 2.4 μL/min/cm. The CLabs of 10 μM HMF in the jejunum was significantly higher in the presence of verapamil than that in its absence, whereas in the ileum the CLabs of 10 μM HMF in the presence of verapamil was almost equal to that in its absence.

These results suggest that HMF is metabolized in the jejunum, and its metabolism impede HMF absorption, whereas in the ileum, verapamil does not have any significant effect on the HMF absorption under this condition.

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P4-09

Prediction of hERG binding using a nondominated sorting genetic algorithm and Support Vector Machine

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Keywords: hERG, machine learning, genetic algorithm

Inhibition of the human ether a-go-go related gene product (hERG) has been identified as a major contributor to QT interval prolongation. It can cause critical cardiovascular side effects and has accounted for the withdrawal of several drugs from the market. Therefore, Blockade of hERG has been extensively investigated since the early 2000s, and in silico models for hERG prediction related studies have been continuously reported [1,2]. Most of the previous studies have undertaken the small and/or biased datasets, the predictability and/or scope of models were not sufficient for drug discovery process. To overcome this limitation, we have collected a large dataset containing 317,232 diverse compounds (positives (<10 uM): 6,619, negatives: 310,613) with IC50 values or % inhibition determined by the biological assay. To reduce the computation cost, we selected the 13,238 structurally diverse compounds (ratio (positives/negatives) = 1/1) covering the original chemical space for machine learning use. Then, support vector machine (SVM) was employed to build classification models with ECFP4 and 15 molecular descriptors selected by nondominated sorting genetic algorithm-II (NSGA-II [3]) were used. NSGA-II is an algorithm given to solve the multi-objective optimization problems. It was used to find the Pareto optimal solutions in which both high predictability (Kappa statistics) and the small number of descriptors in the SVM models are compatible. The best model showed Kappa = 0.772 and 0.791 for the 5-fold cross-validation and external test, respectively.

In this poster session, we will compare predictability of the model with those of previous studies (e.g. Pred-hERG[1]) and commercial software.

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Characterization of cardiac phenotype and drug responses in MiraCell™ Cardiomyocytes from ChiPSC12, a new product of iPS-derived Cardiomyocytes

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Keywords: iPS cells, cardiomyocytes, drug toxicity testing, Multi-electrode array

Cardiomyocytes derived from human iPS cells (iPS-CMs) have potential application for regenerative medicine as well as cardiac toxicity testing. Recently, it has been reported that iPS-CMs can predict drug induced QT-prolongation, which is a major safety issues around drug development.

Production methods of the iPS-CMs have been developed by many labs including us. It's important to clarify the basic characters and drug responsiveness of iPS-CMs produced by each method because the differences in production method may affect their characters.

We have developed novel production method for highly purified (>95%) iPS-CMs without drug selection using cardiomyocyte specific promoter and drug resistant gene (in preparation). Currently, the iPS-CMs produced by the method are commercially available as MiraCell™ Cardiomyocytes (from ChiPSC12).

The aim of this study is to evaluate phenotypic characters and the response to 4 reference drugs for cardiac toxicity testing using MiraCell™ Cardiomyocytes (from ChiPSC12). Furthermore, we compared MiraCell™ Cardiomyocytes with two types of commercially available iPS-CMs provided by other vendors (vendor X and Y) in drug response.

Immunostaining of MLC2V and MLC2A, a maker for ventricular and atrial cardiomyocytes respectively, revealed that majority of the MiraCell™ Cardiomyocytes showed MLC2V positive cells, suggesting ventricular-like cardiomyocytes.

In order to assess the response to the 4 reference drugs, we estimated field potential duration (FPDs), which is correlate to QT intervals *in vivo* ECG, and beats per minute (BPM) of the cells by multi-electrode array system (MED64). We observed prolonged FPDs against E-4031 (Ikr blocker), chromanol 293B (Iks blocker) and mexiletine (Na and Ikr blocker), and shortened FPDs against verapamil (Ca blocker). In addition, early after depolarization could be detected by E4031 and mexiletine. While the degree of FPD prolongation by chromanol 293B was similar among three iPS-CMs we compared, those by E-4031 was most moderate in MiraCell™ Cardiomyocytes. The treatment of Verapamil caused slightly increase of BPM in MiraCell™ Cardiomyocytes in dose dependent manner, but the degree of increased BPM was significantly lower than that of the iPS-CMs provided from vendor Y. The iPS-CMs provided from vendor X showed decrease of BPM in dose dependent manner of Verapamil.

These results demonstrated that MiraCell™ Cardiomyocytes showed expected response of 4 reference drugs. And we have confirmed some differences in the responsiveness among three iPS-CMs we compared in this study. Further studies are needed for elucidating the reason for these differences.

P4-11

Study of Effects of NF-κB for Reporting Frequency of Adverse Drug Reactions based on Tox21-AOP Database

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Keywords: Tox21, JADER, NF-κB, Adverse Drug Reaction, Adverse Outcome Pathway

Introduction

NF-κB is one of the adverse outcome pathways (AOPs). In this study, comprehensive analyses of a dataset consisting of the Tox21-AOP database merged with the Japanese Adverse Drug Event Report (JADER) database were performed to investigate adverse drug reactions involving NF-κB.

Methods

Molecular information was added to JADER. NF-κB activity scores described in the Tox21-AOP database were then merged with JADER via the molecular information. Odds ratios and P-values were calculated by Fisher's exact test based on the relation between NF-κB-activating properties of drugs and the reported frequency of whole cases in the dataset. Adverse drug reactions related to NF-κB were extracted by using these statistical values.

Results and Discussion

Dermatological inflammatory diseases, such as generalized rash, erythema multiforme, and drug rash, as well as many other adverse reactions, were significantly related to drugs with NF-κB activity. These findings are consistent with reports in which NF-κB is one of the responsible pathways for generation of proinflammatory cytokines. Our results may be useful for both drug discovery and clinical management of disease.

P4-12

HGS is a new molecular target for antitumor medicine.

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Keywords: ESCRT, Signal Transduction, Tumor, Peptide

The endosomal sorting complexes required for transport (ESCRT) machinery plays multivesicular body (MVB) biogenesis, cellular abscission, and viral budding [1]. The ESCRT machinery is made of ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III [1]. The ESCRT-0 complex plays a vital role in the generation of MVB and in the signal transduction mediated by cytokines and growth factors [2]. The ESCRT-0 complex is a 1:1 heterodimer of Hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) and Signal transducing adapter molecule (STAM) through antiparallel coiled-coil domains [1]. This dimerization is necessary to play the role of the ESCRT machinery. We demonstrated that HGS was involved in HGF and TGF-β-SMAD signal transduction and promoted cancer properties. The HGS overexpression caused carcinogenesis in normal cell lines. The HGS overexpression also aggravated cancer properties such as TGF-β-SMAD signal transduction, cancer cell metastasis, angiogenesis ability, and tumor growth ability in a mouse melanoma B16 cancer cell line. On the other hand, the overexpression of C domain (HGS/C) protein of HGS obstructed the TGF-β-SMAD signal transduction and suppressed the cancer properties. Furthermore, HGS/C oligopeptides (10 amino acid residues in length) which constituted HGS/C protein suppressed in vitro cancer properties in the B16 cell line and in a human colorectal COLO205 cell line. HGS/C oligopeptides also suppressed the tumor growth in vivo by a subcutaneous administration and an intravenous administration. These findings suggested that HGS was a new molecular target for antitumor medicine. The result of this study is expected to contribute to medicine design and development for antitumor medicine that targets HGS molecule.

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P4-13

Construction of the integrated database for hERG blocking small molecules

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Keywords: hERG, human ether-a-go-go, QT prolongation, database

The inhibition of hERG potassium channel is closely related to the prolonged QT interval[1], and to assess the risk could greatly contribute to avoid delay of the development of therapeutic compounds or withdrawal of marketed drugs. Utilizing the recent increase of information about hERG inhibitors in public databases, many successful applications of machine learning techniques to predict hERG inhibition were reported[2]. However, most of such researches constructed their dataset from only one database because the difference of the data format and ontology made the integration of the databases difficult. This incompleteness of the dataset could limit the prediction performance and applicability domain of the statistical models. In this study, we curated existing dataset in ChEMBL, PubChem, GOSTAR, and hERG Central[3], and integrated them into a largest database for hERG inhibition by small molecules (Table 1). Assessment of structural diversity of the hERG inhibitors in each database using structural clustering revealed that the integrated database contains more than 1.5 times as many clusters as any of the existing databases.

Table 1

Database	Value type	hERG inhibitors (IC50≦10uM)	Inactive compounds (IC50>10uM)	Compounds (total)
ChEMBL(21)	IC50	3,986	3,937	7,923
GOSTAR	IC50	2,772	2,729	5,501
PubChem (AID588834)	IC50	572	1,136	1,708
ChEMBL(21)	% inh.	587	1,212	1,799
GOSTAR	% inh.	494	921	1,415
hERG Central	% inh.	231	303,108	303,339
integrated hERG database		5,650	309,560	315,210

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P4-14

Analyses of Chemical Properties Associated with Reporting Frequency of Erythema Exudativum Multiforme Major in Adverse Drug Reaction Database

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Keywords: Erythema exudativum multiforme major (EEMM), JADER, Adverse Drug Reaction, Chemical Structure

Introduction

Erythema exudativum multiforme major (EEMM) is a group of diseases, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), characterized by fever, general malaise, systemic erythema, and erosion. EEMM occurs as an adverse reaction to a variety of medications. The mechanism of development of EEMM is unclear. The purpose of the present study is to clarify properties of drugs related to EEMM.

Methods

Japanese Adverse Drug Event Report (JADER) database added chemical structures of drugs were used to characterize molecular features, such as structural and physicochemical properties, related to the reported frequency of EEMM.

Results and Discussion

Many kinds of molecular descriptors related to molecular refractive index, partial equalization of orbital electronegativity, and logP were involved in the reported frequency of EEMM in the JADER database. Discrimination models for compounds could be constructed by using these descriptors. These findings might be useful to avoid usage of risky drugs in patients with EEMM constitution.

P4-15

Analyses of Chemical Properties for Reporting Frequency of Thrombocytopenia in Adverse Drug Reaction Database

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Keywords: JADER, Thrombocytopenia, Adverse Drug Reaction, Chemical Structure

Introduction

Thrombocytopenia as an adverse drug reaction may involve thrombocyte production-suppressing effects resulting from myelosuppression by the drug. This side effect may be difficult to predict from pharmacological aspect. The Japanese Adverse Drug Event Report (JADER) database published from Pharmaceutical and Medical Devices Agency (PMDA) includes many cases of thrombocytopenia and is useful for the identification of drugs associated with this adverse reaction.

Methods

JADER added chemical structures of drugs were used to characterize molecular features, such as structural and physicochemical properties, related to the reported frequency of thrombocytopenia.

Results and Discussion

Many molecular descriptors of drugs, especially those related to van der Waals surface area, van der Waals surface area, and logP were statistically significantly associated with thrombocytopenia. Understanding of the common chemical properties of drugs associated with thrombocytopenia may be helpful to prevent this adverse drug reaction.

P4-16

Analyses of Chemical Properties Associated with Reporting Frequency of Interstitial Lung Disease in Adverse Drug Reaction Database

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Keywords: Interstitial Lung Disease, JADER, Adverse Drug Reaction, Chemical Structure

Introduction

Interstitial lung disease (ILD) has a variety of symptoms, such as difficulty breathing, cough, and shortness of breath due to thickening of the lung stroma with overproduction and accumulation of collagen. ILD has the highest reporting frequency among all adverse drug reactions listed in the Japanese Adverse Drug Event Report (JADER) database. Therefore, characterization of the chemical structures of drugs associated with ILD might be helpful to improve the safety of drug usage and to avoid the use of risky seed compounds in early stages of drug development.

Methods

JADER added chemical structures of drugs were used to characterize molecular features, such as structural and physicochemical properties, related to the reporting frequency of ILD.

Results and Discussion

Numerous significant descriptors were found by analysis of variance (ANOVA). Descriptors related to van der Waals surface area and partial equalization of orbital electronegativity were especially useful to identify drugs associated with ILD. Construction of discrimination models for ILD-causing drugs is planned with the use of these descriptors.

P5-01

Lipoproteins for DDS

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Keywords: High-density lipoprotein, gold nanorods, carbon nanotubes, phototherapy

High-density lipoprotein (HDL) is a ca. 10 nm nanoparticle that consists of lipids and lipid-binding proteins. HDL has long been known as a cholesterol transporter from the periphery to the liver, but recent studies have clarified its broader functions, including anti-inflammation, anti-oxidation, anti-thrombosis, etc. HDL is easily reconstituted with phospholipids and recombinant apoA-I proteins and incorporates or binds a wide variety of therapeutic agents. HDL as a drug carrier has at least distinct two advantages over well-known drug carriers, liposomes and polymeric micelles, which are now used in clinics and tested in clinical trials, respectively. They are (1) no need of polyethylene glycol for its formulation and (2) its much smaller size. Polyethylene glycol has been most widely used for DDS development but recently it is reported to be immunogenic under some conditions. Smaller drug carriers (but >8 nm) are able to penetrate deeper into tumor tissues. We have chemically and genetically engineered HDL to explore its DDS potential [1-4]. In this meeting, we will present our recent HDL researches for development of local and systemic DDSs.

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P5-02

Spontaneous pseudorotaxane formation methods targeting on nucleic acids

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

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

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

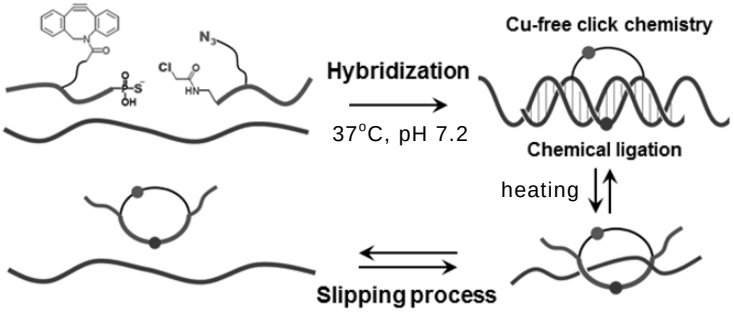

Fig. Schematic representation of pseudorotaxane formation

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P5-03

Construction of Organic-Inorganic Nanostructures by Multiple Site-Specific Precipitations on DNAs Using Artificial Peptides.

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Keywords: Biomineralization, Artificial peptide, DNA, Organic-inorganic nanostructure

Biomineralization, mineralization (precipitation of inorganic compounds) in a biological system, is controlled by biomolecules including proteins. These biomolecules can control spatiotemporally both the size and the shape of the inorganic deposits with high reproducibility and accuracy during the biomineralization processes. Few studies have demonstrated controlled site-specific mineralization on organic compounds for creating novel nano-structures. Development of such a process would enable the easy construction of inorganic-organic nanocomposites. Consequently, focusing on SiO_2 and $CaCO_3$ mineralization, we attempted to construct organic-inorganic nanocomposites by site-specific precipitation using DNA and silica precipitating peptide [1] with peptide nucleic acids (PNA) [2].

The results of micro-scale observation such as TEM and TEM-EDX showed that precipitating structures composed of submicrometer-sized chains and submicrometer-sized spheres. We also observed oxygen, silicon and calcium (SiO_2 and $CaCO_3$) in the spheres and calcium, oxygen and phosphorus in the chains. In addition, we conducted macro-scale observation such as blue silicomolybdic assay (quantitative analysis of silicic acid) and chelate titration with ethylendiaminetetraacetate (quantitative analysis of calcium hydrogen carbonate). These macroscopic results also agreed with microscopic results. Additionally, we attempted to control the spheres and chain diameters by changing the incubation time and calcium hydrogen carbonate concentration.

In conclusion, we succeeded in shape control by site-specific precipitation of two different inorganic compounds [3]. This method could contribute to manufacturing novel functional organic-inorganic nanomaterials.

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P5-04

Construction of self-assembled DNA microstructures for synthesis of molecular robots

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Keywords: DNA microstructures, water-in-oil droplets

One of the ultimate goals of material science is to create and control self-organized systems inspired by the living systems. Recently, molecular robotics has been attracting much attention as a novel bottom-up approach to accomplish the artificial cell-like systems by using DNA, RNA and lipids [1]. Bottom-up DNA nanotechnology has achieved the construction of various-sized DNA structures such as nanometer-sized DNA origami and macroscopic DNA hydrogels [2, 3]. Self-assembled DNA microstructures have potential application to create a body of molecular robots. However, it is difficult to control the form of self-assembled DNA microstructures under conventional methods.

Here, we present a self-assembled DNA microstructure with fractal-like patterns (Fig), and the investigation of its formation mechanism. The self-assembled DNA microstructure is formed at a water-oil interface of a water-in-oil (W/O) microdroplet coated with a cationic lipid monolayer. The electrostatic interaction between the lipid and DNA induced gelation of DNA on the interface of the W/O microdroplets. Because of the kinetic process of the DNA self-assembly, DNA gel exhibited the fractal-like pattern at the interface of the W/O microdroplets. We hope that this generation method can lead to novel way for construction of DNA microstructures.

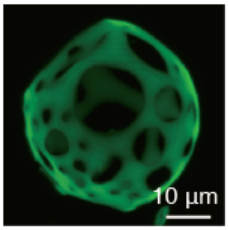

Fig. A DNA microstructure with fractal-like pattern

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P5-05

Allosteric control of nanomechanical DNA origami devices

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Keywords: Molecular Robotics, DNA nano structures

By introducing two groups of ligands to a pliers-shaped DNA origami nanostructure (DNA pliers and DNA chopsticks)[1, 2], allosterically controllable nanomechanical DNA origami devices have been constructed. Four pairs of complementary chain attached to one side of the lever to provide allosteric site, which have photo-cleavable linker at the end of complementary chain, and tightly fix DNA pliers into parallel closed form. This structural change of DNA pliers arranges two specific ligands for the chemical target of detection, which are attached to the opposite side of the levers as anchors, in close proximity to promote ligand–target–ligand bidentate binding. Successive irradiation of UV cleave the PC zipper, and leaves the second bridge, formed by the anchors and the target, to keep the parallel closed form, visually distinguishable under AFM. Significantly enhanced binding to miRNA target in a pre-formed binding site as well as bidentate conjugation of thiolated DNA to single AuNP were also clearly demonstrated with the present system.

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P5-06

Creation of Phototactic Liposome by Photo-Responsive Peptide

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Keywords: Molecular Robotics, Phototaxis, Liposome, DNA-peptide conjugate, Photocleavage, Nanofiber

Spaciotemporal control of peptide nanofiber growth was achieved by photocleavage of a DNA-conjugated β-sheet forming peptide which is linked through a photoresponsible amino acid residue. Peptide nanofibers were selectively formed by photocleaving the conjugate on complementary DNA-immobilized glass substrate [1] and giant liposome. Photo-cleavage of the conjugate on phase-separated giant liposome caused phototaxis of giant liposome due to Marangoni convection.

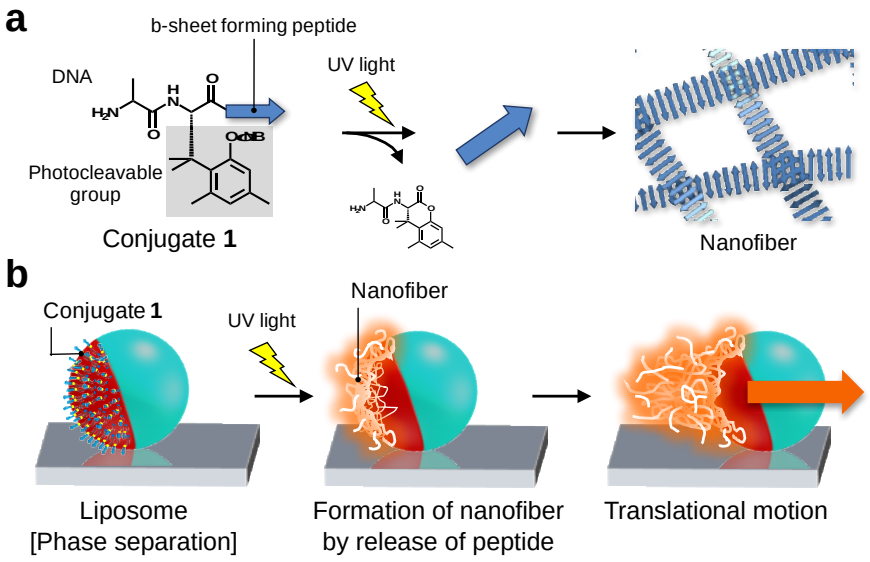

Fig. 1. (a) Photo-induced peptide nanofiber growth (b) Spatiotemporal modulation of translational motion of liposome by modification of conjugate **1**

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P5-07

Density Functional Theory and Random Matrix Theory in Proteogenesis

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Keywords: Protein, Molecular evolution, Random matrix, Density functional theory

Proteins are synthesized from amino acids based on the genetic information encoded into DNA. This is the central dogma, to which almost all of life on Earth adheres. It is obvious that the first protein was not synthesized according to this dogma, because another protein must have participated in the role of as an enzyme. By what kind of mechanism was the first protein synthesized? Because this question addresses the very origin of life itself, answering it is a difficult and important problem.

Meanwhile, a fine-tuning of the localization of the existing amino acid sequence in its entire configuration space and the existence of the upper bound of the total number of protein tertiary structures are a mystery in the study of biological evolution. [1] For example, assuming 20 types of amino acids and 100 amino acid residues, the possible number of proteins is 20^{100}. However, the fine-tuning results that the upper bound of the total number of proteins in an existing protein fold is only 400-1000 and that in an existing protein superfamily is only 1000-30000. Despite the fact that the above estimates of the upper bounds were made over 10 years ago, they are found to be fairly accurate compared with recent estimates from recent, vigorous analyses of a proteins tertiary structure. Fine-tuning of the sequence localization was accomplished via the process of molecular evolution itself, and it is essential to existence. However, it is not known whether the fine-tuning of the localization is a coincidence or an inevitability.

Recently, physics-based approach was proposed by [2], where the electronic state of the valence electrons for macromolecules are calculated using the extended Huckel method and multi-fractal and random matrix analysis are performed. Their analysis showed that the system is critical and the wave functions are multifractal.

We reexamine their results in detail and study the energy-level statistics of amino acids by random matrix theory and calculate the orbital energy of 20 kinds of amino acids using the density-functional formalism. [3] To generate statistical data, we performed a multipoint calculation on 10000 molecular structures for each amino acid produced via the molecular dynamics simulation. For the valence orbitals, the energy-level statistics exhibit level repulsion, but the universality in the random matrix cannot be determined. For the unoccupied orbitals, the energy-level statistics agrees with an intermediate distribution between the Gaussian orthogonal ensemble and the semi-Poisson statistics for all 20 kinds of amino acids. These amino acids are considered to be in a type of critical state.

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P5-08

Morphological control of tubulin-encapsulating giant-liposomes induced by change of hydrostatic pressure, temperature and osmotic pressure

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Keywords: Molecular robotics, Giant liposome, Microtubule, Morphological control

We examined the effect of hydrostatic pressure, temperature, and osmotic pressure on the tubulin-encapsulating giant-liposomes (TEGLs). Many of TEGLs kept a bipolar shape with a central sphere and two tubular protrusions that aligned in a straight line at room temperature and ambient pressure as reported previously [1]. Immediately after the application of high pressure (80 MPa) or low temperature (4ºC), the protrusions shrunk within several tens of seconds. This process was reversible; after the pressure was released or the temperature increased to 25ºC, the protrusions regenerated within several minutes [2]. Surprisingly, TEGLs showed poly-spine shape, which had a hundred of membrane spines, when microtubules elongated after hypertonic treatment. Our results demonstrate that the shape of giant liposomes is controllable by changing the polymerization and depolymerization dynamics of microtubules. The findings should help to develop molecular robotics to construct an artificial motile cell model driven by physical stimulation cycle.

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P5-09

Challenge to Develop the Platform of Personalized Medicine and Point-of-Care for Elderly People in Local City of Japan

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Keywords: Personalized medicine, pharmacogenomics, genetic testing, point-of-care

The rapid growth of personalized medicine is being supported by emerging new technologies together with accumulating knowledge of pharmacogenomics. Basic technologies of molecular diagnostics play a role in expanding pharmacogenomic information, particularly with respect to SNP genotyping. Diagnosis is thus integrated with therapy for selecting treatments as well for monitoring results. Cost-effective methods should be developed for genotyping, however, and it would be desirable to include this information in the patient's record as guidance for physicians to individualize the treatment. The accurate measurement of allele frequency variations among population groups with different sensitivities to diseases and/or different responses to drugs is fundamental to genetic epidemiology.

Development of personalized medicine, including point-of-care technology, requires the integration of various segments of biotechnology, clinical medicine, and pharmacology. Multiple players should be involved in the development of personalized therapy. In particular, pharmaceutical and biotechnology companies would take leading roles in this venture in keeping with their future roles as healthcare enterprises.

Nevertheless, the reality is different from such scientific thoughts. It is not easy to develop the platform for personalized medicine and point-of-care in local cities in Japan, where most people are very conservative. Since 2014, I have been concentrating my efforts and time on that challenge in Saijo city (ca. 100,000 population) and have recently realized that education of medical doctors, pharmacists, as well as citizens of the local city is utmost critical issue.

[1] Ishikawa, T., https://www.facebook.com/personalized.medicine.healthcare/?

P5-10

Causality Analysis for Finding Potential Cancer Driver Genes Using a Differential Gene Expression profile in Gene Regulatory Network

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Keywords: gene regulatory network, cancer driver gene, RNA sequencing

Finding driver genes which affect cancer growth has been a major challenge for personalized medicine. One of the latest technological advances for elucidating potential driver genes is next-generation RNA sequencing (RNA-seq). RNA sequencing technology can be used to derive gene expression profiles for normal and cancer cells. In this research, we develop a computational method derived from mathematical model of gene expression process. The method integrates gene expression data from RNA sequencing and transcription factor gene data to discover potential driver gene candidates.

Potential cancer driver genes may have different gene expression levels from normal cells. The genes affected by the driver genes may also have different gene expression levels. Such genes are called passenger genes. In order to find the driver genes, we need to develop a method that can distinguish the driver genes from the passenger genes in differential RNA expression profile in cancer cells.

We hypothesize that analysing the expression difference within a regulatory network is the key to discovering the driver gene candidates. We assume that if transcription factor genes are differentially expressed, then the target genes will have a great chance to be differentially expressed. Based on this assumption, we can derive a relationship between transcription factor genes and target genes. When this potentially causal relationship has been inferred, we can distinguish which transcription factor genes influence the changes in cancer and normal cells and which genes are affected by the transcription factor genes. This method is applied to a gene regulatory network so that we can see a broader view of causality relationship among the genes. We use the Wnt signalling network as an example.

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P5-11

Microtubules Flow Dynamics Analysis on Gliding Assay

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Keywords: Microtubule dynamics, fluid flow, Structure tensor

Various complex microtubules patterns including rings and streams can be observed in microtubule gliding assay experiment. Understanding microtubule motion patterns has important implications for establishing nanometer level robots. We investigate a “fluid flow” of microtubules assuming microtubules as indicators of the fluid flow. A structure tensor provides knowledge about a local orientation of microtubules at each pixel in microtubule images. This knowledge can used to investigate specific microtubule flows. We adopt this method for measuring the collective behavior of microtubules driven by kinesin fixed on a gliding assay. This method enables us to characterize the degree of transition of vortex like motion with regards to an order parameter. A gradient tensor is obtained from the Cartesian product of the gradient vector of an image. The order parameter helps us to estimate how microtubule ring-like swarm behavior is undertaken through the first frame to the last frame. In this work, we describe the image processing steps and algorithms in detail. Numerical analysis demonstrates the existence of large ring like motion and stream like motion on our images.

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P5-12

PROLOQL: Integration of SPARQL and Prolog for Sematic Web Systems

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Keywords: Semantic Web, Linked Open Data, SPARQL, Text mining

In recent years, the World Wide Web mainly written in HTML for data description has been also standardized to the Semantic Web for data linking description. In keeping with this trend, enormous LOD (Linked Open Data) have been published by academia, industry and government, so far. However, effective uses of LOD are still limited mainly due to the difficulties in programming and data access performance. In order to solve these issues, we propose an inference system PROLOQL to use LOD with SPARQL and Prolog.

Firstly, we combined a logic programming language Prolog and SPARQL, a query language for searching RDF[1], the data format for LOD[2]. RDF format and Prolog clauses are very similar in language structures and would be compatible with each other. In RDF, the relationship of two data is described as Subject, Predicate and Object: predicate(subject, object) in Prolog clause.

In PROLOQL, data getting from LOD are treated as atomic clauses. This means that an enormous amount of various data on the webs can be accessed from Prolog programs just describing SPARQL queries for LOD.

Secondly, we developed a LOD based text mining system. In this system, we can collect the meaning and related concepts of given words through dictionary web site such as Wikipedia. We can also solve the problem of a spelling inconsistency and synonyms in similar way. The most remarkable point is that these functions are very similar to what human does in web surfing.

Lastly, we apply above two systems to understand “Tenpu-Bunsho”, a Japanese medical document for drug usages, as an example for our natural language understanding system. There is a lot of spelling fluctuations and synonyms in Tenpu-Bunsho as well as technical words. So, it is difficult for a computer to understand the Tenpu-Bunsho in general. We believe our system will be an effective solution to analyze domain specific texts using LOD as live dictionaries.

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[2] W3C:W3C Recommendation 10 February 2004 (http://www.w3.org/TR/2004/REC-rdf-primer-20040210/)

P5-13

Liposome-based Liquid Handling System

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Keywords: Liposome, Fusion, Division, Liquid Handling

This paper describes the utilization of giant unilamellar vesicles (GUVs) as a platform for handling miniature amount of chemical and biochemical reagents. GUVs with diameters of 5 to 10 μm and containing chemical/biochemical reagents together with inert polymers were fused with electric pulses (electrofusion). After reagent mixing, the fused GUVs spontaneously deformed to a budding shape, separating the mixed solution into sub-volumes [1]. We utilized a microfluidic channel and optical tweezers to select GUVs of interest, bring them into contact, and fuse them together to mix and aliquot the reaction product. We also show that, by lowering the ambient temperature close to the phase transition temperature T_m of the lipid used, daughter GUVs completely detached (fission). This process performs all the liquid-handing features used in bench-top biochemistry using the GUV, which could be advantageous for the membrane-related biochemical assays [2].

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P5-14

Photochemical RNA editing using 3-vinylcarbazole analog containing oligodeoxynucleotide

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Keywords: RNA editing, Photo-cross-linking, Nucleic acid medicine

DNA/RNA editing is powerful tool for the treatment of genetic disease, and enzymatic or chemical editing have been reported. In previous literature, the photochemical C→U transition occurred via [2+2] photocycloaddition between C and 3-cyanovinylcarbazole(^{CNV}K)[1,2]. This reaction is distinctive and modifies the C to U in a sequence specific manner. However, this reaction required heating at 90°C for 1h, which prevents the application of this method to living cell. In this study, we designed and demonstrated the C→U transition via [2+2]photocycloaddition of 3-vinylcarbazole derivatives to overcome this advantage of the C→U transition.

We synthesized 3-carboxyvinylcarbazole(^{OHV}K), 3-methoxycarbonylcarbazole(^{OMeV}K), and 3-carbonylamidevinylcarbazole(^{NH2}K) to demonstrate C→U transition. The oligodeoxynucleotides (ODN) containing 3-vinylcarbazole derivatives and complementary ODN was photo-cross-linked and then its photo-cross-linked dsDNA was incubated to perform C→U transition. After C→U transition, it was analyzed by denaturing PAGE. The C→U transition in the DNA duplex using ^{CNV}K was processed to yield 46% by heating at 70°C for 4h. In contrast, the yield of C→U transition using ^{OHV}K increased to 61%. The C→U transition activity was in the order of ^{OHV}K > ^{CNV}K > ^{NH2V}K > ^{OMeV}K, suggesting that the C→U transition yield was influenced by the substitution of 3-vinylcarbazole.

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P5-15

The effect of 5-substitution in pyrimidine base in DNA photo-cross-linking using 3-cyanovinylcarbazole

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Keywords: DNA photo-cross-linking, 3-cyanovinylcaarbazole, LUMO

DNA photo-cross-linking and photo-ligation via [2+2]photocycloaddition is a useful for the manipulation, regulation, and detection of nucleic acids. The famous photo-cross-linker such as psoralen, cumarin, and stilbene and their application have been widely reported. We have already reported that the oligodeoxynucleotide (ODN) containing 3-cyanovinylcarbazole (^{CNV}K)[1,2] can cross-link to pyrimidine base in the complementary DNA or RNA with a few seconds of photoirradiation. The biological application using ^{CNV}K such as detection of miRNA based on large shift of 19F MR signal[3], photoregulation of the antisense effect in living cell[4],acceleration of DNA strand displacement. Thus, the ^{CNV}K has potential for biological applications; however, the detail of the photo-cross-linking, such as the effect of 5-substituent group of pyrimidine base in DNA photo-cross-linking, are still unclear.

In this study, we investigated the effect of 5-substitution of the pyrimidine base in DNA photo-cross-linking using ^{CNV}K. The duplex of ODN 1 containing ^{CNV}K and ODN 2 containing pyrimidine derivatives such as thymidine(T), Uridine(U), Fluorouridine(^{F}U), Hydroxyuridine(^{HM}U), and Trifluorothymidine(^{TF}T) was photoirradiated at 366 nm and then those was analyzed by UPLC. Each purimidine base having another substituent group in the C5 position had deiverse photoreactivity with ^{CNV}K. There was a good correlation with the photoreactivity of each pyrimidine base and the LUMO level of pyrimidine base.

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P5-16

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

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Keywords: Molecular robotics, DNA nanostructure, RNA polymerase

In the cell, gene expression is highly controlled. To create biologically inspired nanoscale device enabling the control of gene expression, we create a molecular device consisted of transcription factors (T7-RNAP and target genes) and DNA origami tile scaffold as the functional module (Miyazono et al., *EMBO J*, 2010). The molecular device had unique features such as orthogonality, where the device transcribes only its own gene, and rational designability, where the transcription activity can be designed by molecular layout.

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P5-17

A Universal Asynchronous Cellular Automaton with Cyclic-States Cells

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Keywords: Molecular Robotics, Cellular Automaton, Turing Complete, Cyclic State

Cellular Automata (CA) have attracted increasing attention as architectures for computers with nanometer-scale devices, because their regularity has the potential for bottom-up manufacturing, like molecular self-assembly. Fewer efforts, though, have gone into biological implementations of CAs, even though their features, such as their modular structure, make them suitable for such a framework. Research in this direction has aimed to implement the enclosures surrounding each cell compartment by DNA-based gels that are dissolved or reestablished depending on control asserted from a supervising mechanism [1]. Another mechanism for microscale control of computation by DNA has been touched upon recently [2,3], but one of the problems it faces is the lack of a well-defined chemical environment in which operations are enclosed. Such control is important, because it is a precursor to the successful realization of molecular robots [4], which to date remains a futuristic, though exciting, goal.

This paper presents a CA model designed for possible implementation by the reaction and diffusion of DNA strands. The proposed CA works asynchronously, whereby each cell undergoes its transitions independently from other cells and at random times. The state of a cell changes in a cyclic manner, rather than according to an any-to-any mapping. The transition rules are designed as totalistic, i.e., the next state of a cell is determined only by the number of states in the neighborhood of the cell, not by their relative positions.

Universal circuit elements are designed for the CA as well as wires and crossings to connect them, which implies that the CA is Turing-complete. The designed CA consists of 14 types of cyclic state cells and 57 rules for cells' transitions.

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P5-18

Development of signal amplification circuit composed of acyclic D-threoninol nucleic acid (D-*a*TNA) for detection of RNA.

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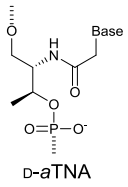
Keywords: Artificial nucleic acid, Signal amplification

DNA circuit[1],[2] can detect small amount of target nucleic acid by signal amplification system. However, it is difficult to detect the target RNA precisely, because DNA is not only affected by impurities, but degraded by nucleases in cell. Therefore, we attempted to develop a signal amplification circuits composed of D-*a*TNA[3] (*acyclic* D-threoninol nucleic acid). D-*a*TNA cannot hybridize with either DNA or RNA, while it forms duplex with D-*a*TNA. Moreover, D-*a*TNA has high nuclease resistivity due to its unnatural scaffold. We synthesized D-*a*TNA circuits (Fig. 1) and measured fluorescence spectra. The results showed that the D-*a*TNA circuits amplified fluorescence signal by the addition of input strand. Additionally, the D-*a*TNA circuit was affected neither by non-targeting DNA nor nuclease, whereas DNA circuit didn't operate normally in the presence of irrelevant DNA or RNA due to undesired hybridization. Furthermore, we revealed that D-*a*TNA circuit could be applied to the detection of natural nucleic acids via SNA[4] as an interface.



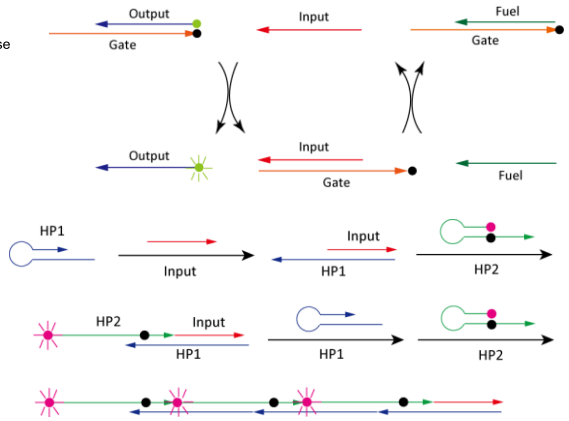


Fig. 1 D-*a*TNA signal amplification circuit

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P5-19

Protein Structure Analysis Platform for Academia-based Drug Discovery

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Keywords: Crystal structure, High-throughput screening, Inhibitor, Ligand, Robotics

High-throughput (HTP) structural analysis of drug-targeted proteins in complexes with their ligands is now critical for rational drug development. The Division of Structural and Synthetic Biology (DSSB) at the RIKEN Center for Life Science Technologies (CLST) has been developing one-stop protein structure analysis platform for academia-based drug discovery in collaboration with the RIKEN Program for Drug Discovery and Medical Technology Platforms (DMP). We have developed and improved several methodologies aimed for HTP protein structure determination, including large-scale cell-free protein synthesis system, bacterial co-expression system, and protein/nucleoprotein complex preparation systems [1-5]. By taking advantages of these proprietary technologies, we are now able to perform high-quantity protein production, HTP crystallization of ligand-bound proteins, and HTP crystal structure determination through intimate collaborations with several RIKEN platforms such as the Drug Discovery Computational Chemistry Platform Unit in CLST and the SR Life Science Instrumentation Unit in the SPring-8 Center. Examples of our structure-based drug development along with our protein synthesis technologies will be presented.

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P5-20

Toward the Molecular Artificial Intelligence by using DNA reactions

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Keywords: Molecular Robots, Strand Displacement Reactions, Molecular Artificial Intelligence

We have developed molecular Artificial Intelligence system by using DNA molecules, where "intelligence" means that the reaction system can "select" DNA molecules to sustain their reactions. We have bio-chemically implemented the reaction system by using Chemical Reaction Systems, CRS; CRS have been bio-chemically implemented by using the DNA strand-displacement reactions [1], in which two strands with partial or full complementarity hybridize, displacing in the process one or more pre-hybridized strands[2]; we have bio-chemically implemented self maintaining reactions by using the CRS of DNA reactions and it can sustain the reactions under *noisy* environment, where *noises* mean mutations of DNA sequences that interact with an input sequence; in our system the input DNA sequence A interacts with a DNA sequence B and transform into a sequence C then it interacts with a sequence D and the sequence A is re-generated; hence the CRS is able to maintain A. We have obtained several mutated DNA sequences that can sustain the reactions and we have investigated behaviors of reactions when there are several mutated DNA sequences.

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P5-21

Microfluidic Formation of Lipid Bilayer-stacking

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Keywords: Lipid bilayer, Microfluidics, Five-layered flow

This paper describes planar lipid bilayer stacking by using the microfluidic technique. The microchannel consisted of five channels. An organic solvent containing lipid molecules flowed along the walls and lipid bilayers were formed at the parallel aligned walls. Although extensive studies for preparing the planar lipid bilayers by microfluidics have been reported, the most of them have focused on single-stacking bilayers. In this study, we propose the method of a double-stacking lipid bilayer formation by using the microchannel which has five-layered flow channels and four micro walls.

Artificial lipid bilayers with membrane proteins are widely used as artificial models of cell membranes [1]. Planar typed lipid bilayers have been attracted attentions as an evaluation system of channel membrane proteins because the transport activity of the transmembrane proteins can be evaluated by electrical measurement. We have previously proposed the stable and reproducible planar lipid bilayer formation by using a droplet contact method and measured ion channels or pore-forming proteins with single membrane system [2]. If we can form double-stacking planar lipid bilayers and reconstituting cell adhesion proteins in the stacking lipid bilayers, it should not only be useful for the understanding of the cell-to-cell communication mechanisms but also be utilized as an interface for a living and artificial objects. However, it is difficult to stack planar lipid bilayers in micro-space by using the conventional droplet contact method because of the millimeter scale chambers and droplets. Recent years, some groups have been reported miniaturized artificial lipid bilayer systems by using microfluidic technologies [3]. In this paper, as a first step in the reconstruction of cell-to-cell communications, we develop the microchannel for lipid bilayer stacking and demonstrate to stack two planar lipid bilayers.

The formation process of the double-stacking lipid bilayer is as below. First, an aqueous solution and the organic solvent containing lipid alternately flowed in each channel and the organic solvent passes along the walls. Lipid monolayers are formed on the interface between the aqueous solution and the organic solvent. Then, the organic solvent layers between the walls get thin and lipid bilayers are formed by contacting the monolayers. The organic solvent and the center and both ends of aqueous solution flowed at 4.0, 7.0 and 3.5 μL/h, respectively. The organic solvent flowed along the walls and lipid monolayers were formed on the interface between the aqueous solution and the organic solvent. Then, the organic solvent layers were become thin by clamping tubes to increase the pressure and lipid bilayers were formed at the parallel aligned walls. We succeeded in preparing and stacking planar lipid bilayers by using the microchannel. The double-staking lipid bilayer prepared microchannel has an enough potential to be applied for the cell-to-cell communication model reconstruction.

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P5-22

Novel Compensation method for Gene Expression Fluctuation by Real-time Single-cell Selection

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Keywords: Single-cell analysis, secretion imaging, real-time selection

It has been known that single-cells show gene expression heterogeneity [1], and therefore the averaged data from the bulk cells collected by conventional method would miss some unique information of each cells. For instance we previously demonstrated that the onset of secretion of simultaneously stimulated immune cells were widely varied from cell to cell[2,3]. The fluctuation of cell response ranged over hours, indicating that time-varying gene expression of cells are no longer the same state. To overcome this, we developed a novel compensation method of gene expression fluctuation by real-time single-cell selection technique. We monitored onset of type2 immune response of IL-33 stimulated mouse type2 innate lymphoid cells (ILC2) by monitoring IL-13 secretion on Real-time Single-cell secretion imaging platform. The cell starting secretion of IL-13 was collected in a certain time by the glass capillary single-cell picker. These two process, Single-cell secretion imaging" and "Single-cell Selection", proceeded in parallel for several hundreds of cells. We found that the amount of IL-13 mRNA of the cells collected at 30 min after onset of IL-13 secretion were less varied from that of the cells collected regardless of elapsed time from onset of IL-13 secretion. This result clearly shows that our method could eliminate gene-expression fluctuation and may contribute to explore the dynamic gene-expression profiles.

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P5-23

Live Controlled Real-time Microtubule Gliding Assay Simulation for Understanding Dynamic Behavior

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Keywords: Live Control, Real-time, Microtubule Gliding Assay, GPU

Microtubule dynamics is now an area of research interest, with the goal of being able to control the dynamics for use in molecular motors. However gaining a clear understanding from experiments alone can prove difficult. As a solution to this we have developed a 3D live controlled simulation environment for microtubule swarm dynamics, which is able to run in real-time[1]. The simulation is able to deal with very large test cases while maintaining the desired performance, speed, needed for easy 3D viewing[2]. This was accomplished by using GPU cards, for both general purpose computing and graphical rendering; optimized parallel CUDA algorithms for calculations and DirectX for rendering[3]. With the use of these technologies the simulation is capable of simulating tens of thousands microtubules, while allowing for live control by the user[4]. The interactions being computed allow for the recreation of microtubule interactions, such as snuggling and overriding, naturally due to forces such as the Lennard-Jones potential.

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P5-24

Automatic AFM-Image Classification with Deep Learning

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Keywords: Deep Learning, Atomic Force Microscope (AFM), Image Processing

Recently DNA-Origami has attracted a lot of attention and it is studied and developed actively all over the world for making DNA nano structures[1] and molecular robotics[2]. In order to improve DNA-Origami design, automatic classification of AFM image objects is highly demanded[3]. In this study, we apply a Deep Learning system (Convolutional Neural Network) to classify smiley marks from other DNA origamis such as stars and hearts (Fig1).

Our Deep Learning system has achieved 90% accuracy after training with regards to the classification of smiley marks and star shapes..

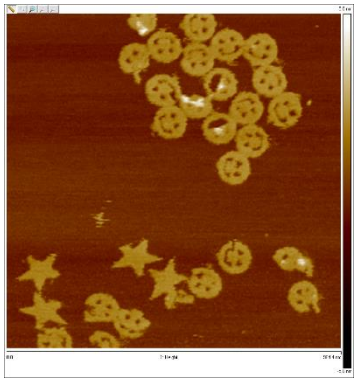

Fig1 AFM image (smiley and star)

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P5-25

Structural basis of kinase selectivity of pyrrolo pyrimidine inhibitors between HCK and FLT3

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Keywords: X-ray crystallography, kinase selectivity, SAR

Hematopoietic cell kinase (HCK) and Fms-like tyrosine kinase 3 (FLT3) are highly implicated in acute myeloid leukemia (AML). For example, upregulated expression is reported for the HCK kinase in leukemic stem cells [1], and approximately 30% of AML patients are known to possess constitutive-active mutations in the FLT3 kinase [2]. Previously, we have discovered potent HCK inhibitors by in silico screen together with a high-throughput enzyme inhibition assay. In addition, we have solved several crystal structures of HCK with these inhibitors [3].

On the hypothesis that a dual inhibitor of HCK and FLT3 can be a better medicine for AML, we have selected two pyrrolo-pyrimidine compounds which displayed inhibitory effect against FLT3 in addition to HCK, and solved co-crystal structures of FLT3 with these compounds. Both compounds bound to the inactive state of FLT3 with the activation loop stabilized in the closed conformation. By combining structure determination with structure-activity relationship analysis (SAR), we were able to determine the binding modes of these compounds for both HCK and FLT3, which can explain their inhibitory potency. These findings can contribute to further development of a dual inhibitor of HCK and FLT3, including many AML-associated FLT3 mutants.

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Nanoscale Investigation of mRNA Localization and Dynamics in Stress Granules

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Keywords: mRNA, Stress granules, Super-resolution fluorescence microscopy, Single particle tracking

mRNA serves as a key molecule for the gene expression in cells, conveying the genetic information from DNA to the ribosomes. To control the quality of this process, eukaryotic mRNA is regulated in various steps, including modification, localization, translation and degradation. In response to the environmental stresses, cytoplasmic mRNAs and proteins dynamically gather to form granular structures called stress granules (SGs). Although it is identified that the main components of SGs are mRNAs stalled in translation initiation, translation initiation factors and RNA binding proteins, the localization and dynamics of them remain unclear.

In this study, we investigated the spatio-temporal regulation of mRNAs in mammalian SGs at the nanometer scale by combination of super-resolution fluorescence microscopy and single particle tracking technique. Fluorescently labeled antisense oligonucleotides are attached to the target mRNAs to image the endogenous mRNAs.

Super-resolution microscopy revealed that mRNAs inside SGs distribute in heterogeneous manner, in which they form both high and low concentration domains. Based on the clustering analysis on high concentration domains, we found that the number of clusters increase with a rise in SGs size while they maintain the same size (~70 nm in diameter). Single particle tracking of endogenous mRNAs inside SGs showed that the mobility of mRNAs is decreased inside SGs compared to in the cytosol. Interestingly, a part of observed mRNAs was freely diffusive, suggesting that the dynamics of mRNAs inside SGs is also heterogeneous as we confirmed in the localization analysis. To investigate the relationship between the localization and dynamics of mRNAs inside SGs, we combined the above two techniques in living cells. As a result, we observed mRNAs anchored to the nanoscale clusters.

Based on these investigations, we hypothesize that SGs are not just random aggregations but are well-organized structures that contain the high concentration domains with low mobility and the low concentration domains with high mobility.

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Control of DNA hybridization by Electric fields

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Keywords: Electronic structure calculation, Ligand docking, Protein modeling

Control of the DNA hybridization is important for realizing molecular mechanisms and artifacts based on DNA. Electric-driven melting of DNA fixed to conductive surfaces is a promising method because the melting requires short time; however, in such electrochemical experiments there are factors such as pH variability and Joule heating that may affect the DNA dissociation. To enable pure electric control, we need to exclude those factors.

Here, we developed an experimental setup to electrically control the hybridization of DNA nanostructures to DNA hydrogels, while suppressing changes of pH and temperature. The DNA gel, which was incorporated in a capillary tube, was placed between platinum electrodes connected to a power source. When applying a strong electric-field, the nanostructure dissociates from the gel and diffuses. Re-association to the gel was also possible by applying an inverse weaker electric-field. This repeatable hybridization control may be used for realizing reusable DNA devices and computers.

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Controlling diffusion coefficient in gelatinous medium by using DNA strand displacement

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Keywords: Pattern formation, Reaction-diffusion mechanism, Programmable chemical reaction

Pattern formation, which is ubiquitous in chemistry and biology, is modeled by reaction-diffusion mechanism. Based on such models, it has been tried to synthesize artificial patterns by using DNA nanotechnology. In this context, it is possible to program the reaction network by designing DNA sequences, while controlling the diffusion coefficient requires to change the molecular weights and the medium in general.

Here, we propose a new method to control the diffusion coefficient of a “target” DNA molecule in a gel medium by using an additional DNA strand called “competitor”. Target” is complementary to “template” DNA which is immobilized to a hydrogel. Upon the addition of “competitor”, the “competitor” binds to “template” via a short region called toehold so that the “target” is displaced. Eventually, the diffusion coefficient of the “target” increases by the competition of hybridization to the “template” between “target” and “competitor”.

We found that the diffusion coefficient of the “target” can increase up to 3 folds by changing the concentration of the “competitor”, ranging from ~7.5 $\mu m^2/s$ to ~12 $\mu m^2/s$.

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Simulation of DNA Nanostructure Self-assembly by using Game Engines

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Keywords: Physical Engines, Molecular Simulation, DNA Nanotechnology, Molecular Robotics

Simulation of self-assembly process of DNA nanostructure is important for estimating the molecular dynamic properties. However, the simulation is difficult due to the enormous number of atoms and the dynamic process. Here, we proposed a method to simulate the self-assembly of DNA nanostructure utilizing game engines for optimizing the design of dynamic 3D molecules.

We developed coarse grained models to simulate the self-assembly processes of two different molecules. The first model corresponds to a DNA origami monomer to control the size of multimers based on a Vernier mechanism. The second model corresponds to a DNA monomer which forms a membrane.

We implemented the models as a molecular dynamics software utilizing the game engine [1]. Typical game engine consists of physical engines, objects handling, user interfaces and event generation. We successfully simulated the self-assembly process and analyzed the time evolution. Compared to the conventional method such as all-atom simulation, the proposed software enables us to estimate the molecular dynamic properties of DNA nanostructure more efficiently.

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P5-30

Membrane-like structure made of DNA motifs

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Keywords: DNA membrane, DNA motif, DNA structure, Molecular Robotics, Lipid bilayer

In nature, lipid bilayer forms a membrane structure that serves as a continuous barrier between cells and their surroundings. Similarly, in the field of molecular robotics, a vesicle made of lipid bilayer is commonly used as a container encapsulating molecular circuits and integrating other devices [1,2].

Here, we propose a new membrane structure assembled with well-designed DNA motifs. This DNA membrane consists of 3 kinds of DNA motifs (double-Φ motifs) with different base sequences at their four connecting parts. These motifs connect with each other by the hybridization of connecting parts to form a membrane structure. Moreover, melting temperature (25 ± 1℃) of the connecting parts is expected to give liquidity of the membrane like a lipid bilayer under room temperature.

The membrane made of DNA is supposed to be more useful for molecular robotics compared to lipid bilayer due to the programmability of DNA sequence. In the poster presentation, we will discuss the experimental results of the DNA membrane.

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