C-1-01

A random-forest based method that can predict detailed enzyme functions and also identify specificity determining residues

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Keywords: Enzyme function, EC number, Random forests, Specificity determining residues

Determining enzyme functions is essential for understanding chemical reactions occurring in living cells. Although many prediction methods have been developed, it remains a significant challenge to predict enzyme functions at the fourth-digit level of the Enzyme Commission (EC) numbers [1]. A small number of mutations can often drastically change functional specificity of enzymes. Therefore, information about these specificity determining residues (SDRs) can potentially help discriminate detailed functions. However, because these residues must be identified by mutagenesis experiments, the available information is limited, and the lack of experimentally verified SDRs has hindered the development of detailed function prediction methods and computational identification of SDRs.

In this study, we developed EFPrf, a novel method for predicting enzyme functions at the fourth-digit level of EC numbers and identified a set of putative SDRs (rf-SDRs) by using a machine-learning technique known as random forests [2]. For each enzyme in each CATH homologous superfamily [3], binary predictors were constructed by random forests with full-length sequence similarities and the residue similarities for active sites, ligand binding sites and conserved sites as input attributes. From the most highly contributing attributes, we obtained the rf-SDRs. In a cross-validated benchmark assessment, EFPrf showed a prediction performance comparable to that of a related method currently available (precision=0.98, recall=0.89). The rf-SDRs included many residues, whose importance for specificity had been validated experimentally. The analysis of the rf-SDRs revealed both a general tendency that functionally diverged superfamilies tend to include more active site residues in their rf-SDRs than in less diverged suprefamilies, and more complicated relationships that the rf-SDRs strongly depend on the mechanisms of functional diversification in each superfamily.

[1] Webb E. C., NC-IUBMB, *Enzyme Nomenclature 1992, Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes.* Academic Press, 1992.
[2] Breiman L., Random Forests, *Machine Learning Journal*: 5-32, 2001.
[3] Orengo C. A., Michie A. D., Jones S., Jones D. T., Swindells M. B., Thornton J. M., CATH -a hierarchic classification of protein domain structures-, *Structure* 5: 1093-1108, 1997.

C-1-02

Large-Scale Fragment Molecular Orbital Calculations toward Evaluating the Drug Effects of AIDS Agents

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Keywords: HIV-1 protease, AIDS agent, drug effects, FMO calculation, quantum biology

In our continuous efforts to evaluate and predict the drug effects of currently approved HIV-1 protease inhibitors, we have carried out a large-scale fragment molecular orbital (FMO) calculation at FMO-MP2/6-31G level on the complexes of HIV-1 protease with five different peptidomimetic HIV-1 protease inhibitors such as Ritonavir (RTV), Indinavir (IDV), Nelfinavir (NFV), Darunavir (DRV), and Saquinavir (SQV) using ABINIT-MP/BioStation system [1-3]. In addition, we performed the analogous FMO calculations toward four different complexes of renin and pepsin with RTV and SQV to explore a relationship between the computed energies and the unwanted side effects of HIV-1 protease inhibitors. Renin and pepsin are aspartic proteases and exist in human kidney and stomach, respectively. It has been reported that RTV gives acute renal failure and severe gastrointestinal injury, while no renal toxicity is attributed to SQV.

FMO computations of five different inhibitor-bound HIV-1 protease complexes indicated that the calculated binding energy for the HIV-1 protease complexes can be correlated to the clinically measured pharmacokinetic parameters such as maximum drug concentration (Cmax) and area under the drug concentration-time curve (AUC) of the AIDS agents. We found that HIV-1 protease inhibitors having large values of Cmax and AUC such as RTV can strongly interact with HIV-1 protease, and that HIV-1 protease inhibitors with relatively large binding and interaction energies can be highly effective as an AIDS agent [1-3]. When a drug was administered, it reaches its peak level in the blood. A drug travels through the blood to tissues in the body, is then metabolized and removed from the blood. Cmax exhibits the highest drug level in the blood. AUC is universally regarded as a measure of the extent of drug absorption. Although the reasons for the correlation of the computed energy with Cmax and AUC are not clear, these two pharmacokinetic parameters may be useful as indications for drug effects of AIDS agents [3]. For the human protease complexes with RTV and SQV, the binding energy of RTV is larger than that of SQV. Furthermore, it was found that the interaction energy of RTV with the active site aspartic acid in both human proteases is large, while that of SQV with the active site is very small. We can safely say that the large interactions between the human proteases and RTV are implicated as a possible cause of renal dysfunction and severe gastrointestinal injury due to RTV inhibitor. Similar FMO4-MP2 calculations are under way to discuss the protease-inhibitor interactions in more detail.

[1] Yagi, Y., Iwasa, A., and Naoshima, Y., Large-Scale Quantum Chemical Calculation on the Complexes of HIV-1 Protease and Inhibitors: A Relationship of Calculation Energies with Drug Effects of Inhibitors, *Proceedings of International Conference on Modeling and Simulation Technology, JSST2011*, JSST, 51-57, 2011.
[2] Yagi, Y. and Naoshima, Y., Large-Scale Quantum Chemical Calculations for the Drug Effects of AIDS Agents, *Abstracts of Joint Conference on Informatics in Biology, Medicine and Pharmacology 2012*, C66_50, 2012
[3] Yagi, Y., Hattori, Y., and Naoshima, Y., Large-Scale Quantum Chemical Calculation on the Complexes of HIV-1 Protease and Inhibitors: A Relationship of Calculation Energies with Drug Effects of Inhibitors, *Trans. Japan Soc. for Simulation Tech.*, 4(2):41-50, 2012.

C-1-03

FMO calculations for nano-biotechnology

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Keywords: four-body corrected fragment molecular orbital (FMO4) method, solid, peptide

The fragment molecular orbital (FMO) method [1] has now been popular in the field of computational biochemistry and pharmachemistry, where proteins with a few hundred amino acid residues can be routinely computed within Kitaura's original two-body expansion (FMO2) of fragments. In Ref. [2], we developed the four-body extension (FMO4) in which the contribution from up to fragment tetramers are included. FMO4 provides better accuracy than does FMO2, and furthermore it makes the treatment of 3-dimensional cluster models of solids (with proper band-gap) tractable. The applicability of FMO4 to solids is promising to attack various important problems in the nano-biotechnology as an emerging field (e.g. fabrication of safer implants or protein-based chemical sensors). In this context, we [3] recently reported the FMO4 calculations to investigate the interaction between a designed peptide, with sequence of Arg1-Lys2-Leu3-Pro4-Asp5-Ala6 [4], and the silica surface modeled by a large cluster model including 257 silicon atoms (under an explicitly hydrated condition). The electron correlation effect was taken into account at the second-order perturbation (MP2) level with the Cholesky decomposition technique for acceleration. The importance of three charged residues (Arg1, Lys2 and Asp4) in the peptide-silica interaction was found, where the contributions from not only the electrostatic attraction but also the charge-transfer were addressed. The poster will resume the details of calculations and results in Ref. [3].Additionally, some preliminary data about ionic solids such as apatite and alkali halide will be presented as well.

[1] *The Fragment Molecular Orbital Method: Practical Applications to Large Molecular Systems*, edited by D. G. Fedorov, K. Kitaura (2009, Boca Raton, CRC press).
[2] "Development of the four-body corrected fragment molecular orbital (FMO4) method", T Nakano, Y. Mochizuki, K. Yamashita, C. Watanabe, K. Fukuzawa, K. Segawa, Y. Okiyama, T. Tsukamoto, S. Tanaka, Chem. Phys. Lett., 523 (2012) 128-133.
[3] "Modeling of peptide - silica interaction based on four-body corrected fragment molecular orbital (FMO4) calculations", Y. Okiyama, T. Tsukamoto, C. Watanabe, K. Fukuzawa, S. Tanaka, Y. Mochizuki, Chem. Phys. Lett., 566 (2013) 25-31.
[4] "A Hexapeptide Motif that Electrostatically Binds to the Surface of Titanium", K. Sano, K. Shiba, J. Amer. Chem. Soc. 125 (2003) 14234-24235.

C-1-04

A practical procedure to calculate intermolecular interactions including statistical information between a protein and a large ligand

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Keywords: Intermolecular interactions, Average structure, Water molecule, FMO, MD

We investigated the method to compute intermolecular interactions around the binding site in the system composed of the histone deacetylase SIRT2[1], a nonstandard cyclic peptide (S2iL5[2]) and solvent, especially in view of the influence of water molecules.

First, we performed 1 μs molecular dynamics (MD) simulation in the system and obtained an equilibrium time region corresponding to local fluctuations of SIRT2 from the MD trajectory. In this region, we selected the water molecules whose RMSF values were as small as those of SIRT2. These water molecules were considered to be less fluctuated and thus *stable*. In order to include the statistical information in one typical structure, we averaged the structures of the complex with the *stable* water molecules in the region and then optimized the obtained structure by the molecular mechanics method. Next, we investigated the conformation of the binding sites and calculated the intermolecular interaction energy by the fragment molecular orbital (FMO[3]) method. We found that the optimized structure reproduced the binding site that had been frequently caused in the trajectory although the structure without the stable water molecules did not. We also found that the interaction energy between SIRT2 and each residue of S2iL5 with the stable water molecules were different from that without the stable water molecules by about 20 kcal/mol in the maximum. This indicates that it is important for the intermolecular interaction by the quantum mechanical method to determine the structure of the system including the stable water molecules.

A series of techniques presented here is considered to be a practical procedure to include statistical information in the intermolecular interactions by quantum mechanical method, relating the quantum mechanical method and the classical MD simulation.

[1] Finnin, M.S., *et al.*, *Nature Structual Biology*, vol.8, num.7:621-625, 2001.
[2] Morimoto, J., Hayashi, Y., and Suga, H., Angew. *Chem. Int. Ed.*, 51:3423-3427, 2012.
[3] Kitaura, K., *et al.*, M., *Chem., Phys., Lett.*, 313:701-706, 1999.

C-1-05

Analysis of antibody-antigen interactions and prediction of their complex structures

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Keywords: Antibody-antigen interaction, Complex structure prediction, Drug design

A deeper understanding of antibody-antigen interactions is essential for the design of antibody drugs. To design an antibody drug having a high affinity with the target antigen, it is desirable to obtain an antibody structure in complex with its target antigen. Because it is generally not easy to determine protein complex structures by experimental methods, such as X-ray crystallography and NMR, model structures of protein complexes predicted by bioinformatics techniques are considered to be important for drug discovery.

Previous studies have found canonical conformations of antibody proteins, in particular, Complementarity-Determining Regions, based on the analyses of their sequences and structures [1,2]. In terms of antibody-antigen interactions, an antigen binding site on an antibody, paratope, and an antibody binding site on an antigen, epitope, have been analyzed and several epitope prediction methods have been developed [3].

In this study, we performed a careful analysis of antibody-antigen interactions from multiple perspectives, and developed a method for predicting paratopes and epitopes separately, towards accurate prediction of antibody-antigen complexes suitable for drug design. We are now developing a method for predicting antibody-antigen complex structures, where a probable combination of the predicted paratope and epitope sites is selected based on a scoring function constructed from our new observations, combined with the knowledge of antibody-antigen interactions elucidated previously.

[1] Chothia, C., and Lesk, A.M., Canonical Structures for the Hypervariable Regions of Immunoglobulins, *J Mol Biol*, 196:901-917, 1987.
[2] Shirai, H., Kidera, A., and Nakamura, H., H3-rules: identification of CDR-H3 structures in antibodies, *FEBS Letters*, 455:188-197, 1999.
[3] Andersen, P.H., Nielsen, M., and Lund, O., Prediction of residues in discontinuous B-cell epitopes using protein 3D structures, *Protein Science*, 15:2558-2567, 2006.

C-1-06

Screening and Design of Small Molecules that Reversibly Bind to Streptavidin

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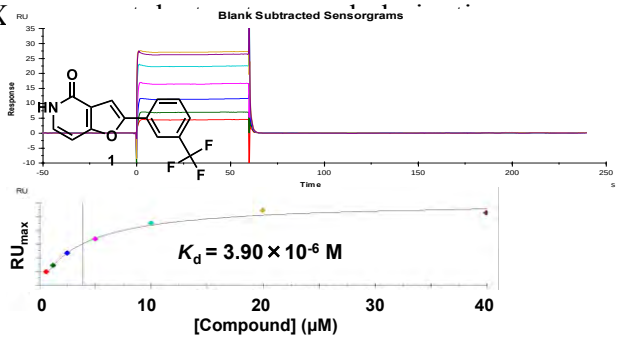
Keywords: Ligand docking, Protein-small molecule interaction

The interaction between streptavidin (from *Streptmyces avidinii*) and biotin is known as the strongest non-covalent interaction in nature (K_d = 10^{-13} M)[1]. Streptavidin-biotin system is used for many applications in biotechnology, such as affinity column chromatography (isolation), SPR sensor chip (immobilization) or fluorescence imaging (detection). However, for some applications, a few disadvantages of this system have been pointed out. One of the major limitations of this system is the almost irreversible binding under physiological conditions. To disrupt a streptavidin-biotin complex, it is necessary to denature the streptavidin using harsh condition.

In order to make the binding of biotin with streptavidin more easily reversible under mild conditions, much work has been done to modify streptavidin by genetic mutations. In contrast, another approach to develop biotin analogs with reduced affinity to streptavidin has been hardly reported, and the reported biotin analogs provide only partial reversibility. In this research, we screen a large chemical library to search for novel chemical scaffolds that bind to streptavidin, and aim to optimize the hit compounds based on information from X-ray crystal structure.

First, we performed the screening for avidin (from hen egg) which has identical tertiary structure with streptavidin. 166,480 compounds were screened by using fluorescence polarization, and we selected 773 compounds as primary hits. These compounds were subsequently screened for streptavidin, dose-dependently assayed, and analyzed by SPR (Surface Plasmon Resonance). Many biotin analogs were detected as hits, but we excluded these compounds. Finally, 6 compounds were selected as novel ligand scaffolds for streptavidin which exhibited fully reversible binding to the protein (K_d = 10^{-4}~10^{-6} M). We characterized the binding mode of compound 1 which showed the strongest affinity (Figure). Based on information from X-ray crystal structure analysis and synthesis, the amide moiety fixed in *cis* form was most likely to be essential for the binding. We synthesize further derivatives of compound 1 and optimizing the affinity to streptavidin. Then we will demonstrate the utility of the new reversible scaffold by applying it for affinity column chromatography.



[1] N. M. Green, *Adv. Protein Chem.*, 29:85-133, 1975.

C-1-07

Standard binding free energy calculation for theophylline-RNA aptamer system: alchemical transformation and metadynamics

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Keywords: Standard free energy, Alchemical transformation, Metadynamics

Binding affinity of theophylline to RNA apatamer (PDB *id*: 1EHT) is calculated using alchemical transformation, in which the interactions of the ligand with its surroundings are decreased to zero [1]. The parmbsc0 force field is used for describing potential functions of RNA to improve the accuracy of simulation [2]. A quadratic potential restraining the translation of the ligand is added to keep the relation to the standard state condition. The calculated binding free energy is -8.9 kcal/mol, which agrees well with the experimental value of -8.9 kcal/mol. This result indicates that experimental binding energy is well reproduced by simulations for alchemical route.

In order to realize the computer-aided drug design (CADD), understanding of the kinetics of association/dissociation phenomena is also necessary. Then, we perform metadynamics simulation to explore the free energy surface (FES) starting from the equilibrated binding geometry [3]. The distance between U23(C1') and TEP(C8) and the angle of G26(C6)-TEP(C2)-TEP(C8) are chosen as a set of collective variables (CVs) used here as shown (Figure 1). Metadynamics is a dynamics in the space of the CVs. In Figure 2, the FES shows a physical path for the ligand from the binding site to the external solution. A barrier from the binding site to the unbound state is estimated to be 13.5 kcal/mol while taking the experimental binding affinity into account. This value gives us information for recognizing the dissociation process of the ligand from the binding site and for ligand optimization.

It is shown that alchemical transformation and metadynamics simulation are powerful methods for exploring the properties of ligand in rational drug design.

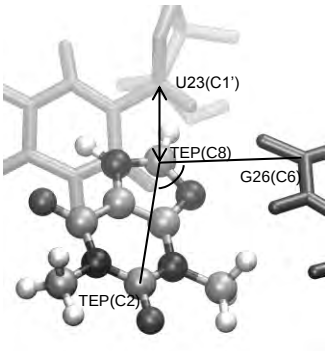

Figure 1. Theophylline-RNA binding geometry after equilibrium procedure. Particular atoms used are also shown.

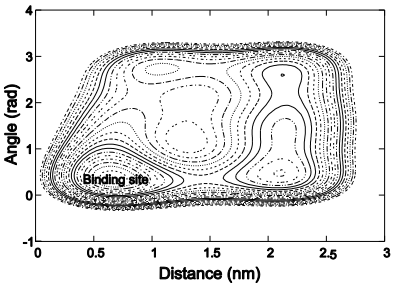

Figure 2. Free energy surface reconstructed using metadynamics as a function of the angle G26(C6)-TEP(C2)-TEP(C8) and of the distance U23(C1')-TEP(C8).

[1] S. Boresch, F. Tettinger, F. Leitgeb, and M. Karplus, *J. Phys. Chem. B*, 107: 9535-9551, 2003.
[2] A. Perez, I. Marchan, D. Svozil, J. Sponer, T. E. Cheatham III, C. A. Laughton, and M. Orozco, *Biophys. J.*, 92: 3817-3829, 2007.
[3] F. L. Gervasio, LA. Laio, and M. Parrinello, *J. Am. Chem. Soc.*, 127: 2600-2607, 2005.

C-1-08

Molecular Dynamics Simulation of Shiga Toxin

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

To develop a drug against infection by Shiga toxin (Stx)-producing *Escherichia coli* O157:H7 [1], this study investigates docking of Stx2 B subunit (Stx2 B) and a peptide neutralizer, using molecular dynamics simulations.
The used software is Generalized-Ensemble Molecular Biophysics (GEMB) [2]. The PDB code 1R4P is employed for the initial structure of Stx2 B, and the amino acid sequence of the neutralizer is MAPPPRRRRA. The number of residues of Stx2 B is 350, so that the required number of water molecules is estimated to be about 20,000. The force field is AMBER99SB for Stx2 B and TIP3P for water molecules.
Several simulations have shown that Arg's included in the neutralizer are attracted to Asp's and Glu's located at the binding sites of Stx2 B. In a representative result, among the amino acids in the sequence R9-R8-R7-R6-P5-P4-P3 of the neutralizer, R9 and R8 have electrostatic interactions with E15 and D16 of Stx2 B, respectively; R7 and P4 are thought to have small effects on the binding because they are directed towards the outside of Stx2 B from its inside; although R6 interacts with W33 through its alkyl part, this interaction is not electrostatic; P5 is located at the foot of R6 and not participating the binding; it is possible that P3 interacts with alkyl of R32, but the distance between them may be large. It can be suggested that two effects are important in simulations considering water molecules: screening of electrostatic interactions between basic and acidic residues; existence of hydrophobic residues in the neutralizer.

[1] Nishikawa, K., Watanabe, M., Kita, E., Igai, K., Omata, K., Yaffe, M.B., and Natori, Y., A multivalent peptide library approach identifies a novel Shiga toxin inhibitor that induces aberrant cellular transport of the toxin, *The FASEB Journal*, 20:2597-9, 2006.
[2] Okumura, H., Temperature and pressure denaturation of chignolin: Folding and unfolding simulation by multibaric-multithermal molecular dynamics method, *Proteins*, 80: 2397-2416, 2012.

C-1-09

Random Matrix Theory Analysis of Molecular Dynamics Simulation

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

We apply the random matrix theory [1,2,3] to analyze the time series data of motion of atoms of proteins which is produced by the molecular dynamics simulation [4]. We perform all-atom molecular dynamics simulation with solvent with the maximum duration of 1000ns. We study a data set with the different sampling-time intervals, 0.01fs, 0.1fs, 1fs, 10fs, 100fs, 1ps, and 10ps to observe the characteristic motion at each time scale. The variance-covariance matrices are constructed from the time series data. We calculate the probability density of the variance-covariance matrix, eigenvalue distribution, unfolded eigenvalue distribution of the nearest-neighbor and the next nearest-neighbor level spacings, inverse participation ratio, etc. They are the fundamental quantities which characterize the universality class in the random matrix theory. The results of the nearest-neighbor and the next nearest-neighbor level spacings agree well with the Gaussian orthogonal and Gaussian symplectic ensembles, respectively. On the other hand, the raw eigenvalue distribution has a crossover behavior between the universal and non-universal classes as a function of the sampling-time interval. Following the random matrix theory, we classify the dynamically-correlated sectors of the protein domain by analyzing the eigenvalues outside of the bulk and the inverse participation ratio, and decompose them into subsectors. Our method is an attempt to improve and refine the principal component analysis of protein domain [5]. As an example, we demonstrate our method in the bovine eye lens protein gamma-B (gamma-II)-crystallin, PDBID:4GCR to study the domain decomposition, and ubiquitin carboxy terminal hydrolase L1 bound to ubiquitin vinylmethylester, PDBID:3KW5 to study the protein-ligand and protein-protein dynamical interactions.

[1] E.P. Wigner, Proc. Cambridge Philos. Soc. 47, 790, 1951; F.J. Dyson, J. Math. Phys. 3, 140, 1962; F.J. Dyson and M.L. Mehta, J. Math. Phys. 4, 701, 1963; M.L. Mehta and F.J. Dyson, J. Math. Phys. 4, 713, 1963.
[2] L. Laloux, P. Cizeau, J.-P. Bouchaud, and M. Potters, Phys. Rev. Lett. 83, 1467, 1999; V. Plerou, P. Gopikrishnan, B. Rosenow, L.A.N. Amaral, and H.E. Stanley, Phys. Rev. Lett. 83, 1471, 1999; V. Plerou, P. Gopikrishnan, B. Rosenow, L.A.N. Amaral, T. Guhr and H.E. Stanley, Phys. Rev. E65, 066126, 2002.
[3] The first attempt to the protein is reported by R. Potestio, F. Caccioli, and P. Vivo, Phy. Rev. Lett. 103, 268101, 2009 by the solvable elastic network models. Here, we perform all-atom molecular dynamics simulation with water.
[4] M. Yamanaka, Journal of the Physical Society of Japan 82, 083801, 2013.
[5] For a review, see Akio Kitao, Normal Mode Analysis: Theory and Applications to Biological and Chemical Systems. Chapter 12: edited by Qiang Cui & Ivet Bahar, , Chapman & Hall/CRC, Boca Raton, FL, USA, 233-251, 2006, and references therein.

C-1-10

Binding Mechanism of KNI-272 with HIV-1 Protease

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Keywords: FMO, HIV-1 protease, neutron crystallography, structural water, X-ray crystallography

The complex of structure of HIV-1 protease (HIV-1 PR) with a transition-state analog inhibitor KNI-272 has been determined recently by both neutron and high-resolution X-ray crystallographies. As shown in Figure 1, the complex structure contains several structural water molecules, which can mediate hydrogen-bonding interactions between HIV-1 PR and KNI-272. [1] In this study, MM/MD and ONIOM (QM/MM) (M06-2X/6-31G*: Amber) calculations were carried out to quantitatively examine possible roles of these structural water molecules. After the ONIOM optimization, we performed the FMO-Inter Fragment Interaction Energy (FMO-IFIE: HF/6-31G*) analysis of KNI-272 with amino acid residues and structural water molecules, and examined the energitic contribution of each structural water molecule in the binding. Catalytic Asp25/25' residues have the largest contribution to the binding. Figure 2 shows FMO-IFIE together with the classical dispersion ones (E_{disp}). As can be seen in Figure 2, W1 (water molecule 1 shown in Figure 1), intervening between KNI-272 and Ile50/50' in HIV-1 PR, makes the most potent hydrogen-bonding interaction. W2 and W3 (water molecules 2 and 3) are also important for the binding of KNI-272 with between HIV-1 PR through hydrogen-bonding/electrostatic interactions. These results confirm that these structural water molecules inside HIV-1 PR play a decisive role in the binding of KNI-272 with HIV-1 PR, and suggest that it is very necessary to consider the "hydrogen-bonding/electrostatic network" among an inhibitor, HIV-1-PR and structural water molecules, when designing a new potent inhibitor of HIV-1 PR.

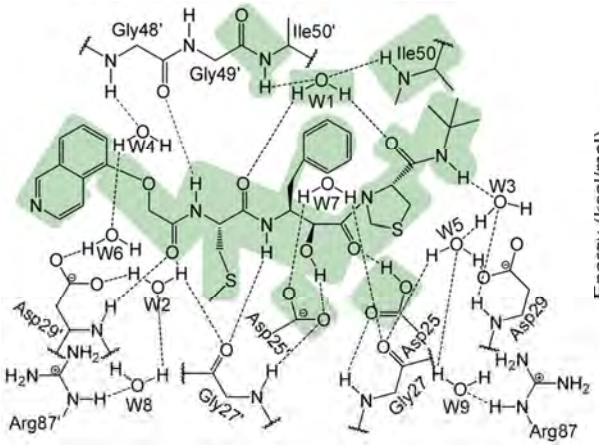

Figure 1. Complex of HIV-1 PR with KNI-272. Atoms in shadow areas are treated as the quantum region in the ONIOM optimization.

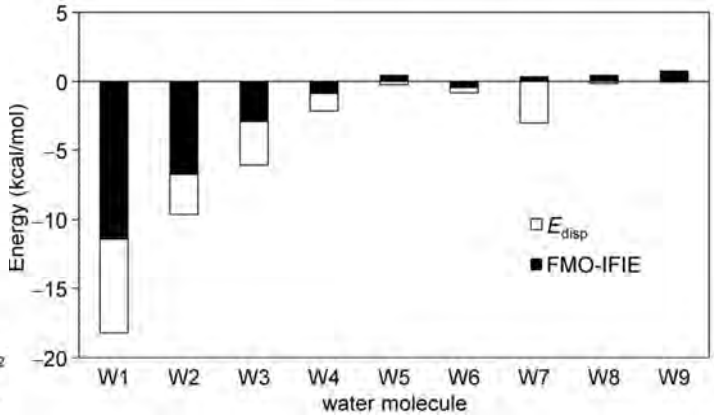

Figure 2. FMO-IFIE and E_{disp} of KNI-272 with structural waters in HIV-1 PR.

[1] M. Adachi *et al.*, *Proc. Natl. Acad. Sci.USA*, **2009**, *106*, 4641–4646.

LERE-QSAR Analysis of Binding of γ-Lactum Hydroxamic Acid Derivatives with Tumor Necrosis Factor-Alpha Converting Enzyme

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Keywords: LERE, ONIOM, Tumor necrosis factor-alpha converting enzyme

Tumor necrosis factor-alpha converting enzyme (TACE) is a metal enzyme that contains a zinc atom, and TACE converts TNF-alpha into an activated form by hydrolysis. Rheumatism arthritis and Crohn's disease are caused by overproduction of the activated form TNF-alpha. In this study, we examined the atomic and electronic mechanism underlying binding between TACE and hydroxamic acid derivatives, which have a γ-lactum ring [1] (Figure 1), using the ONIOM calculation and LERE (Linear Expression by Representative Energy terms)-QSAR procedure [2]. We constructed complex structures of TACE with each hydroxamic acid derivative using the MM/MD and ONIOM calculations. In the current study, we assumed that the observed overall free-energy change (ΔG_{obs}) in the complex formation can be expressed as a sum of the intrinsic interaction energy (ΔE_{bind}^{ONIOM}) and polar contribution of the solvation free-energy change (ΔG_{sol}^{pol}), and dispersion interaction energy change (E_{disp}), calculated with the ONIOM(HF/6-31G: Amber) mechanical embedding (ME) method, PB calculation, and MM calculation, respectively. The sum of these three representative energy terms is nicely linear with ΔG_{obs}, resulting in the following LERE-QSAR equation;

$$\Delta G_{obs} = 0.129\ [\Delta E_{bind}(\text{ONIOM/HF/ME}) + E_{disp} + \Delta G_{sol}^{pol}] + 16.1$$
$$n = 11,\ r = 0.912,\ s = 0.844\ \text{kcal/mol}$$

Figure 2 shows that there is a negative correlation between ΔE_{bind}(ONIOM/HF/ME) and ΔG_{sol}^{pol}, and that ΔG_{obs} parallels with E_{disp}. These results suggest that the variation of ΔG_{obs} among the derivatives is governed by E_{disp}. We also discuss a detailed binding mechanism by decomposing the binding energy obtained with the FMO calculation into individual contributions of amino acid residues.

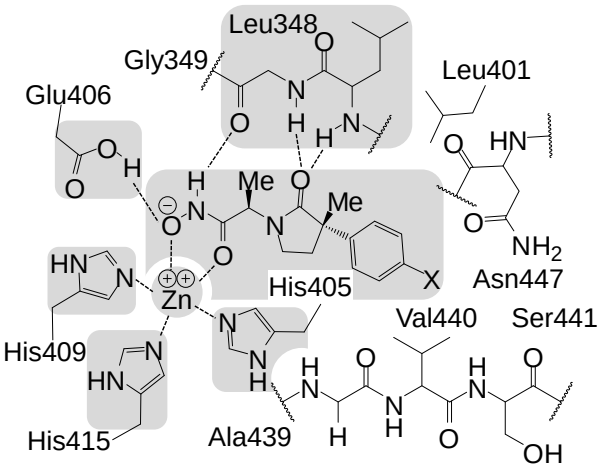

Figure 1. Schematic representation of a γ-lactum hydroxamic acid derivative bound in the TACE active site. Atoms in shadow areas are treated as the quantum region in the ONIOM calculation.

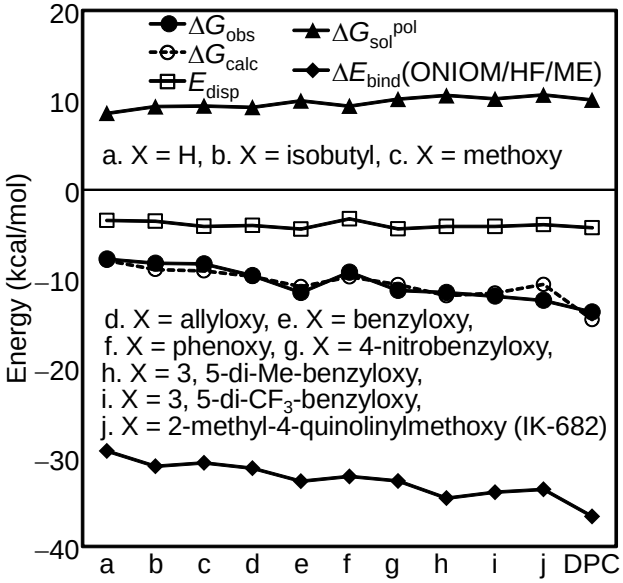

Figure 2. Variation in ΔG_{obs}, ΔG_{calc}, E_{disp}, ΔG_{sol}^{pol}, and ΔE_{bind}(ONIOM/HF/ME).

[1] J. J. W. Duan, *et al.*, *J. Med. Chem.* **2002**, *45*, 4954–4957.
[2] T. Yoshida, S. Hitaoka, A. Mashima, T. Sugimoto, H. Matoba, and H. Chuman, *J. Phys. Chem. B*, **2012**, *116*, 10283–10289.

Spatial distribution of QM based interaction energy between amino-acids and probe molecules

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Keywords: Quantum Chemical Calculation, Interaction energy, Molecular mechanics force field

Quantum chemical calculation and molecular dynamics simulation are used for prediction of the protein-ligand binding affinity [1]. First one is used as QM based single point calculation, which can deal with the molecular interactions accurately but have the problems in sampling. Second one is performed with MM force field, which has the problem in accuracy of interactions. Thus we have to use both QM based and MM based methods and select better one for adjusting the situation. For this purpose, we have to know how different between QM and MM for each situation. Current comparative studies between QM and MM, are limited on the potential minimum or along few directions. Thus, in this study, we evaluated interaction energies between amino acid residues and probe molecules with spatially comprehensive arrangement. We employed B97D/aug-cc-pVDZ method [2] because we confirmed this method provides only small deviation from higher level quantum chemical calculation (CCSD(T)/CBS) [3]. We compared QM based interaction energy and MM force field energy (MMFF94x) for each amino acid and different types of probe molecules. Figure 2 represents example of calculation for interaction energy PHE side chain and cation probe (NH_4^+). QM calculation provides spatially larger attractive region and deeper potential minimum. Comparison between various types of interaction will be discussed.

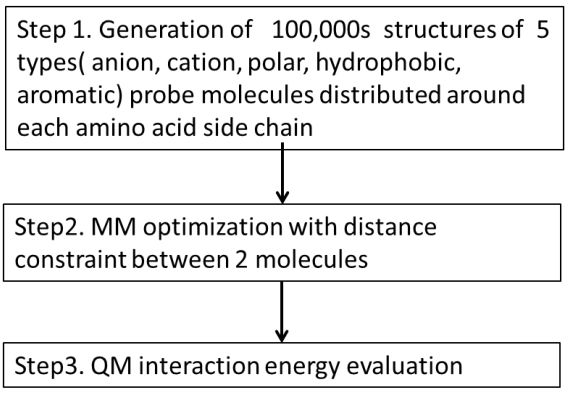

Figure1. Evaluation scheme of spatial distribution of QM based interation energy.

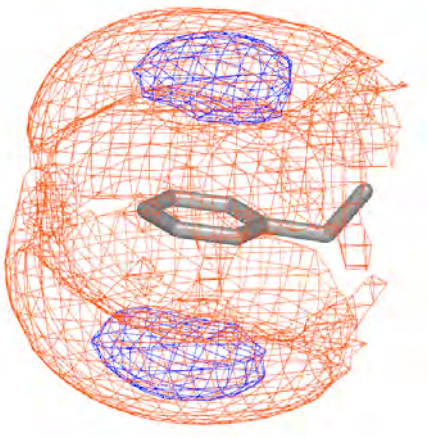

Figure2. Contour plot of interaction energy (Phe-NH_4^+) with QM(B97D:red) and MM(MMFF94x:blue).

[1] H. Watanabe et al. CBI Journal 10 (2010) 32-45.
[2] S. Grimme, J. Comput. Chem. 27, (2006), 1787-1799.
[3] The Benchmark Energy & Geometry Database (http://www.begdb.com/)

C-1-13

Estimation of Non-covalent Interactions with a New Efficient Dispersion Corrected HF Approach

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Keywords: BSSE, Dispersion, Hartree–Fock, LERE

An accurate estimation of binding interaction energy associated with complex formation of a ligand and protein is important for understanding the binding mechanism and rational drug design. Although the dispersion is one of the most important interaction as well as hydrogen-bonding and electrostatic ones, its evaluation based on molecular orbital methods is still not easy task. In this study, we examined our newly proposed dispersion correction for the Hartree–Fock method (HF-D) on several complexes of biologically relevant molecules.

We selected a set of eighteen small non-covalently bonded complexes: six dispersion-dominated (DISP), nine hydrogen-bonded (HB), and three other types of complexes (others). The geometry of each complex was fully optimized at the MP2/6-311++G(d,p) level. In our HF-D approach, the HF energy was corrected by introducing damped empirical dispersion energy term ($-f(R)C_6/R^6$) using a sigmoid-type function. We calculated interaction energies using HF-D and the counterpoise corrections were employed to reduce the basis set superposition errors (BSSE). The performance of HF-D were confirmed by comparing with post-HF methods (CCSD(T) and MP2) and several DFT based methods (M06-2X and Grimme's dispersion corrected B3LYP-D2 and B2PLYP-D).

Figure 1 shows the mean absolute error (MAE) of interaction energies estimated from the CCSD(T)/aug-cc-pVTZ reference values. The overall performance of HF-D is excellent; as expected, HF-D more accurately reproduces interaction energies for all three types of complexes than the conventional HF method. It should be noted that MAE of HF-D shows significantly smaller than that of more expensive MP2 in all complexes (MAE(overall) = 0.40 and 1.01 kcal/mol, respectively). Although M06-2X gives the best performance (MAE(overall) = 0.30) among the tested methods, HF-D is competitive with the M06-2X and other dispersion corrected B3LYP-D2 and B2PLYP-D. HF-D approach is probably effective and practical, compared with time-consuming post-HF and DFT methods to quantitatively evaluate the interaction energy of large molecular systems such as complex of a ligand with protein. We will also discuss the binding mechanism of a series of benzenesulfonamides with carbonic anhydrase using LERE-QSAR procedure [1] in which HF-D approach is introduced for estimation of binding interaction energy.

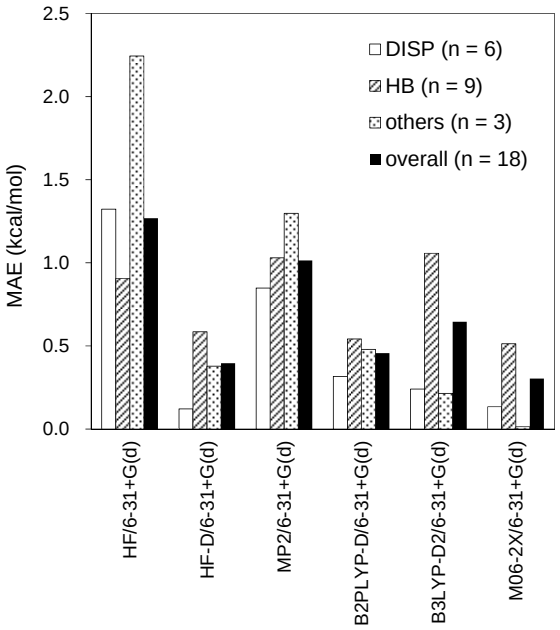

Figure 1. Comparison of the performance of HF-D and other methods for non-covalently bonded complexes.

[1] T. Yoshida, Y. Munei, S. Hitaoka, H. Chuman. *J. Chem. Inf. Model.*, **2010**, *50*, 850–860.

C-1-14

Refinement of Crystal Structures Using Partial Geometry Optimization and Electron Density Calculations Based on the Fragment Molecular Orbital Method.

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Keywords: Fragment molecular orbital (FMO) method, Protein-ligand interaction, Geometry optimization, Electron density

The *ab initio* fragment molecular orbital (FMO) method was applied to refine X-ray crystal structures of protein–ligand complex using ABINIT-MP/BioStation program package [1]. Partial geometry optimization surrounding a ligand and electron density calculations were performed at the MP2/6-31G* level for complex between human estrogen receptor α and 17β-estradiol (PDB ID: 3ERE) [2]. Members of hydrogen bond network were optimized: ligand, Glu353, Arg394, Phe404, His524 and a water molecule. In addition to the inter-fragment interaction energy (IFIE), the configuration analysis for fragment interaction (CAFI) was performed to analyze charge transfer interactions on the basis of optimal hydrogen bond network structures. Strong charge transfer interaction between the ligand and Glu353 was observed through a "shared proton" with long OH distance of ligand (1.07 Å) and short ligand - Glu353 hydrogen bond distance (1.42 Å). Such findings are consistent with the existence of considerable experimental electron density [3] between them. Three protonation states of His524 (HIE, HID, HIP) were also considered for the calculations. Based on the optimized structure, strength of interaction, and electron density map, we concluded His524 is most likely deprotonated HIE conformation.

[1] ABINIT-MP/BioStation download site; http://www.ciss.iis.u-tokyo.ac.jp/dl/index.php.
[2] Fukuzawa, K., Watanabe, N., Watanabe, C., Okiyama, Y., Tanaka, S., and Mochizuki, Y., in preparation.
[3] EDS electron density server (http://eds.bmc.uu.se/eds).

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C-1-15

FMO calculations with ABINIT-MP on K-computer

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Keywords: fragment molecular orbital (FMO) method, many-body expansion, Cholesky decomposition, massively parallel computing, K-computer

The K-computer, one of the fastest peta-scale supercomputers in the world, processes huge amounts of tasks at once with its massive parallelism and have started being utilized for drug discovery primarily based on the classical mechanical approaches.

On the other hand, fragment molecular orbital (FMO) method proposed by Kitaura *et al.* in 1999 has revealed the electronic properties of large biomolecular systems quantum-mechanically and has a great expectation for application to drug discovery. Especially, the four-body corrected FMO method (FMO4) [1], which is implemented to an FMO processing program, ABINIT-MP, enables us to divide a molecular system more finely with keeping good accuracy and analyze inter-fragment interactions with higher resolution favorable to the structure-based drug design (SBDD) [2]. While the number of tetrameric combinations of fragments needed for four-body correction voluminously increases as the whole system size, one can simultaneously complete all the tasks of processing the combinations in a moment by using the K-computer.

The Cholesky decomposition (CD) technique for two-electron repulsion integral approximation [3] is essential to accelerate the second-order Moeller-Plesset perturbation processes in FMO calculations by keeping the Cholesky basis on memory and executing the DGEMM operations. For saving memory and further acceleration, we introduce the CD approximation with one-center Cholesky basis (1C-CD) [4], which limits the atomic orbital pairs as the Cholesky basis to those on the same atoms. The computational times in Hartree-Fock processes are also reduced successfully by applying the 1C-CD approximation.

Details of our trials and applications with ABINIT-MP on the K-computer will be shown in the poster session.

Acknowledgment This research was supported by the grant for "Strategic Programs for Innovative Research" Field No. 4: Industrial Innovations from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT)'s "Development and Use of Advanced, High-Performance, General-Purpose Supercomputers Project."

References

[1] Nakano, T., *et al.*, *Chemical Physics Letters*, 523:128–133, 2012.
[2] Watanabe, C., *et al.*, *Journal of Molecular Graphics and Modelling*, 41:31–42, 2013.
[3] Okiyama, Y., *et al.*, *Chemical Physics Letters*, 490:41–45, 2010.
[4] Aquilante, F., *et al.*, *Journal of Chemical Physics*, 127:114107, 2007.

C-1-16

Molecular Simulation Analysis of Glucuronidation of UDP-glucuronosyltransferase 1A7 and 1A10

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Keywords: UDP-glucuronosyltransferase, Ligand docking, conjugation capacity

UDP-glucuronosyltransferase 1A (UGT1A) constitute an endoplasmic reticulum (ER) membrane-bound enzyme family whose members catalyze glucuronidation. We previously analyzed the bilirubin glucuronidation by UGT1A isoform 1 (UGT1A1) by means of (1) calculation of 3-D structure of mutant UGT1A1, (2) docking simulation of UGT1A1 and its coenzyme (UDPGA), (3) induced fit of UGT1A1-UDPGA complex, and (4) docking simulation of UGT1A1 and bilirubin. We found that the ratio of the docking when the hydroxyl group of bilirubin oriented toward UDPGA correlated with *in vitro* conjugation capacity report previously [1]. Based on this molecular mechanism that glucuronidation by UGT1A1 requires the hydroxyl group of ligand to locate toward the coenzyme [2], we reported the method to predict the conjugation capacity of UGT1A1 by our molecular simulation [3]. In this study we analyzed the glucuronidation of other UGT1A isoforms (UGT1A7 and UGT1A10) by our molecular simulation. As a result, we found that ratio of the binding of coenzyme to the enzyme in reactive orientation and the ratio of the ligand orientation whose hydroxyl group locate toward the coenzyme also correlated with *in vitro* conjugation capacity [4,5]. This result suggests that the conjugation capacity of other UGT1A isoforms is determined by the similar mechanism as UGT1A1.

[1] Yamamoto K. *et al.*, Contribution of two missense mutations (G71R and Y486D) of the bilirubin UDP glycosyltransferase (UGT1A1) gene to phenotypes of Gilbert's syndrome and Crigler-Najjar syndrome type II, *Biochim Biophys Acta* 1406:267-273, 1998.
[2] Takaoka Y. *et al.*, Ligand orientation governs conjugation capacity of UDP-glucuronosyltransferase 1A1, *Journal of Biochemistry*, 148(1):25-28, 2010.
[3] Takaoka Y. *et al.*, Method for predicting enzyme activity by computer simulation, PCT/JP2009/004286, 2009.
[4] Villeneuve L. *et al.*, Novel Functional Polymorphisms in the UGT1A7 and UGT1A9 Glucuronidating Enzymes in Caucasian and African-American Subjects and Their Impact on the Metabolism of 7-Ethyl-10-hydroxycamptothecin and Flavopiridol Anticancer Drugs, *The Journal of Pharmacology and experimental therapeutics*, 307(1):117-128, 2003.
[5] Dellinger R.W. *et al.*, Importance of UDP-glucuronosyltransferase 1A10 (UGT1A10) in the detoxification of polycyclic aromatic hydrocarbons: decreased glucuronidative activity of the UGT1A10^{139Lys} isoform, *Drug Metabolism and Disposition*, 34(6):943-949, 2006.

C-1-17

FMO-based cluster analysis for drug design by multi-dimensional scaling

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Keywords: Protein-ligand docking, Drug design, Fragment molecular orbital method

Recently, *in silico* drug design has attracted a great deal of general attention. On the basis of the fragment molecular orbital (FMO) method, a clustering method called VISCANA to analyze the pattern of interactions between receptor protein and ligand molecule was proposed [1], in which conventional, hierarchical clusterings for the inter-fragment interaction energies (IFIEs) were performed. Although this method is useful to understand the similarity of the interaction pattern, it does not tell us how and why the patterns are different between two clusters. In addition, while it can sort out the existing ligands and explain their experimental results, it cannot predict or design a novel ligand with favorable affinity and other appropriate properties. Here, we study how to cope with these difficulties by a multi-dimensional scaling (MDS) method for drug design. The MDS method, which is somewhat related to the self-organizing map (SOM) [2], is a method to construct a low-dimensional map from the knowledge of the distance between elements [3]. This method can help us understand why the difference takes place between the clusters of ligands, and can predict the binding characteristics of unknown ligands. We estimated the usefulness of this method for some examples.

[1] S. Amari, M. Aizawa, J. Zhang, K. Fukuzawa, Y. Mochizuki, Y. Iwasawa, K. Nakata, H. Chuman, T. Nakano, VISCANA: Visualized Cluster Analysis of Protein-Ligand Interaction Based on the ab Initio Fragment Molecular Orbital Method for Virtual Ligand Screening, *J. Chem. Inf. Model.* 46, 221-230 (2006).
[2] T. Kohonen, *Self-Organizing Maps*, 3rd ed., Springer (2000).
[3] W.S. Torgerson, *Theory and Methods of Scaling*, Wiley (1958).

C-1-18

Statistical analysis of 3D distribution of ligand atoms in Protein Data Bank using kernel density estimation

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Keywords: Protein-ligand complex, Protein Data Bank, Fragment Molecular Orbital method, Kernel density estimation

Scoring functions have been widely used to evaluate protein-ligand interaction in virtual screening. With increasing protein-ligand information in Protein Data Bank (PDB), various empirical and knowledge-based scoring functions are reported[1]. Previous studies of empirical scoring function, such as PMF score[2], used the structural information in PDB to train distance-dependent interaction energies between protein-ligand atom pairs. In this study, we analyzed the PDB data to estimate the 3D probability density distributions of ligand atoms for every grid points around amino acid residues, rather than to assess the interaction only considering the atom pair distance (Figure 1). Using kernel density estimation (KDE)[3], the probability distributions of ligand atoms around 20 amino acids were estimated for each of the atom types defined by PATTY[4]. As the result, the p-values were calculated for every grid points, and then compared to the score by force field calculation.

[1] Huang, S. Y., Grinter, S. Z., Zou, X., Scoring functions and their evaluation methods for protein-ligand docking: recent advances and future directions, *Phys. Chem. Chem. Phys.*, 12:12899-908, 2010.
[2] Muegge, I., Martin, Y. C., A General and Fast Scoring Function for Protein−Ligand Interactions: A Simplified Potential Approach, *J. Med. Chem.*, 42:791-804, 1999.
[3] Parzen, E., On estimation of a probability density function and mode, *Ann. Math. Stat.* 33:1065-1076, 1962.
[4] Bush, L. B., Sheridan, R. P., PATTY: A programmable atom type and language for automatic classification of atoms in molecular databases, *J. Chem. Inf. Comput. Sci.*, 33:756-762, 1993.

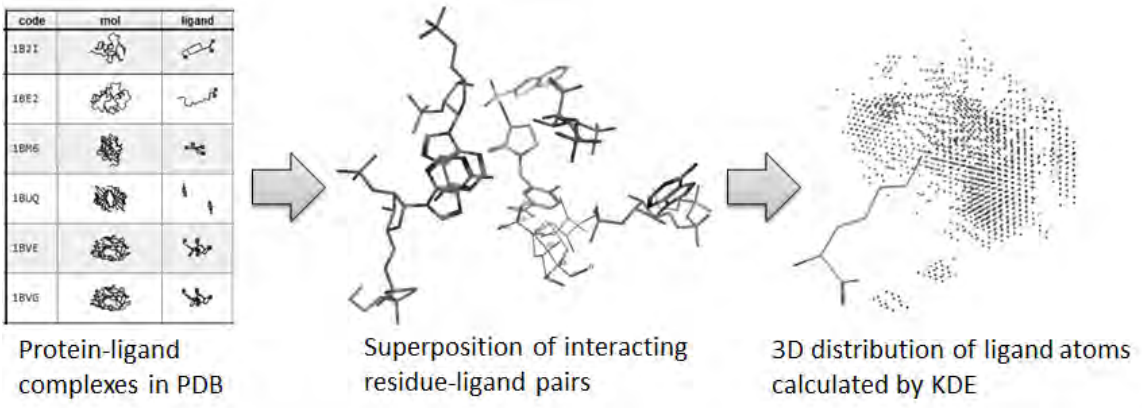

Figure 1. Brief procedure of the estimation of 3D distribution of ligand atoms.

C-1-19

FMO-based electron density analysis to protein structure refinement

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Keywords: Electron density, Refinement, Optimization, Fragment molecular orbital (FMO) method, ABINIT-MP, BioStation

Electron densities obtained by X-ray diffraction analyses for biomolecules determine the nuclear coordinates with some ambiguities in position of hydrogen atoms, protonation, conformation of amino acid residues, and orientation of ligand binding, and so on. Optimization using the classical molecular mechanics (MM) generally refines the coordinates to obtain the final structures, which are registered to PDB entry and used to understand functions of biomolecules. However, the MM optimization often fails to reproduce the distance of hydrogen bonding and such optimized structure has inadequate coordinates in the framework of quantum mechanics for example. For drug discovery using *ab initio* fragment molecular orbital (FMO) method [1], refinement of the structure determination is thus needed to evaluate protein-ligand interaction and reaction mechanism more properly. In this study, we examine an electron density analysis for the refinement by comparing between X-ray and FMO-based electron densities using Crambin protein.

The FMO-based electron densities are prepared as below: First, stating form the high-resolution (0.48Å) X-ray crystal structure of Crambin including hydrogen atoms (PDB-ID: 3NIR), multiple conformations are obtained by MM and QM optimizations: Next, FMO calculations of these conformations are carried out to generate FMO-based electron densities at MP2/6-31G* level by using a development version of BioStation [2] on K-computer. We here reveal position of hydrogen atoms, protonation, and conformation of amino acid by assessing the original X-ray electron density and the FMO-based ones each other. In addition, the same analysis is performed using the low-resolution (2.0Å) structure. Finally, we investigate how the optimized conformations based on low-resolution X-ray electron density, which are used in generally drug design, reproduce the original high-resolution X-ray electron density.

[1] "The Fragment Molecular Orbital Method: Practical Applications to Large Molecular Systems" ed D. G. Fedorov and K. Kitaura (Taylor & Francis/ CRC Press, Boca Raton, FL, 2009).
[2] The calculations and visualization of electron density are performed by a development version of BioStation (download site; http://www.ciss.iis.u-tokyo.ac.jp/dl/index.php.).

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Development of molecular modeling software for protein complex animations

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Keywords: Protein modeling, Protein Complex animation

For understanding functions of protein molecules the atomic coordinate data allows us to model the interacting protein complex. In addition to the experimental evidence for the molecular interactions, there are molecular dynamics studies for analyzing works of a protein complex. Since these techniques are the analyses by theoretical calculation based on a hypothesis of working mechanism, it is difficult to discover how the complex is actually working. Thus, the demand of making animated models of the protein complex has increased. Such tasks involving two or more existing software tools alternatively are usually not trivial. For these computational problems, we started a software development of the script editor to facilitate making animations of molecular models visualizing molecular simulations and the protein interactions. The implementation of this software was greatly supported by a graphical software development tool, Luxinia. It is also known as a game engine incorporating Open Dynamics Engine (ODE) for the physical simulation and the graphics library OpenGL by means of Lua, a quick scripting language. Our software development is ongoing for the molecular model arrangement functions required for creating animations, a time-line editor, and a simulation facility, while the function for the photorealistic rendering is omitted at first. Our development aims to clarify necessary functions required for making molecule animations for the researchers in molecular biology. The efficiency in improving and extending the software is reinforced by the scripting language that allows adding custom functions for individual researchers to animate protein molecules to fit their experimental results easily.

C-1-21

Prediction of Protein-ligand Binding Affinities Using Molecular Simulations

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Keywords: molecular dynamics simulation, protein-ligand binding affinity

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

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

[1] Nicolini, P.; Frezzato, D.; Gellini, C.; Bizzarri, M.; Chelli, R., Toward quantitative estimates of binding affinities for protein-ligand systems involving large inhibitor compounds: a steered molecular dynamics simulation route. *J Comput Chem* **2013,** 34, (18), 1561-76.

[2] Park, S.; Schulten, K., Calculating potentials of mean force from steered molecular dynamics simulations. *J Chem Phys* **2004,** 120, (13), 5946-61.

[3] Wriggers, W.; Schulten, K., Investigating a back door mechanism of actin phosphate release by steered molecular dynamics. *Proteins* **1999,** 35, (2), 262-73.

C-1-22

A proposal for peptide inhibitors to block the ligand-binding pocket of urokinase receptor: *ab initio* molecular simulations

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Keywords: peptide inhibitor, cancer invasion, urokinase-type plasminogen activator, urokinase receptor, fragments molecular orbital calculation, ligand docking, binding energy, specific interaction

1. Introduction

A variety of proteases were found to play important roles in cancer invasion and metastasis. In particular, binding of urokinase-type plasminogen activator (uPA) to uPA receptor (uPAR) existing on the surface of a cancer cell is considered as a trigger for cancer invasions. It is thus expected that the blocking of the binding can inhibit cancer invasion efficiently. Based on the X-ray crystal structure of uPA-uPAR complex, it was elucidated which amino acid residues of uPA are important for the binding, and several peptides having these residues were synthesized as potent inhibitors [1].

In our previous molecular simulations [2,3], the specific interactions between uPA and uPAR were investigated at an electronic level by *ab initio* fragment molecular orbital (FMO) calculations. The results are comparable to the experiment [1] and highlight some important residues of uPA, which have strong attractive interactions with uPAR. In the present study, we constructed novel peptides composed of the important residues and investigated their specific interactions to uPAR by the *ab initio* FMO calculations, with solvating water molecules considered explicitly.

2. Results and discussion

We first constructed 6 types of peptides composed of 5~10 residues and docked each of them to the uPA-binding domain of uPAR by a protein-ligand docking program. These docked structures were fully optimized in water by classical molecular mechanics method, and binding energy between the peptide and uPAR was investigated by the *ab initio* MP2/6-31G method in FMO. Figure 1 shows the optimized structure of the solvated complex of uPAR with our proposed peptide. This peptide has the largest binding energy to uPAR among our proposed peptides. In addition, it was elucidated that Glu36, Glu134 and Glu135 residues of uPAR have large contribution to the peptide binding. Based on the results simulated, we furthermore mutated some residues of the peptide in silico and searched for the novel peptide inhibitors having larger binding affinity to uPAR. Such peptides are expected to be more potent inhibitors for cancer invasions. The details of the results will be shown at the conference.

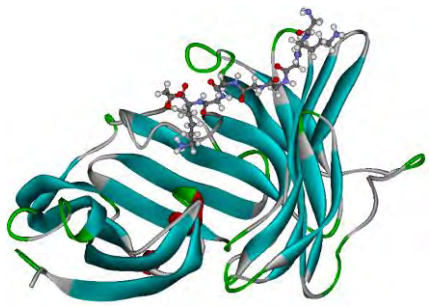

Fig.1 Our proposed peptide blocking the uPAR pocket

[1] C. Barinka, *et al.*, *J. Mol. Biol.*, 363(2006) 482.

[2] S. Tsuji, *et al.*, *J. Mol. Graph. Model.*, 29(2011) 975.

[3] T. Kasumi, *et al.*, *Molecular Simulations*, 2013, in press.

C-2-01

Protein-Ligand Docking Using Artificial Bee Colony Algorithm

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Keywords: Protein-ligand docking, Structure-based drug design, Artificial bee colony algorithm

Protein-ligand docking is one of the most significant issue in structure-based drug design (SBDD). Generally, this docking is considered as an optimization problem which specifies the energetically stable conformation of the ligand at the binding site. However, it is very difficult to identify the correct pose because of many optimization parameters with high correlation. In previous studies, it has been reported that popular docking programs (Glide[1], GOLD[2], FlexX[3], AutoDock[4], etc.) can identify the correct docking pose with an accuracy of only about 60%[5]. In this work, we attempted to apply Artificial Bee Colony algorithm[6] (ABC) to docking. ABC is an optimization algorithm based on the intelligent behavior of honey bee swarm, which has a higher global ability than other algorithms such as Simulated Annealing (SA), Genetic Algorithm (GA), Particle Swarm Optimization (PSO). The performance of the ABC for docking is evaluated for several protein-ligand complexes including some highly flexible ligands in comparison with other algorithms. The results reveal that the ABC might be more suitable for docking than others in particular for dealing with highly flexible ligands.

[1] Friesner, R. A., Banks, J. L., Murphy, R. B., et al., Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, *Journal of Computational Chemistry*, 47:1739-1749, 2004.
[2] Jones, G., Willett, P., Glen, R. C., et al., Development and validation of a genetic algorithm for flexible docking, *Journal of Molecular Biology*, 267:727-748, 1997.
[3] Rarey, M., Kramer, B., Lengauer, T., et al., A fast flexible docking method using an incremental construction algorithm, *Journal of Molecular Biology*, 261:470-489, 1996.
[4] Morris, G. M., Goodsell, D. S., Halliday, R. S., et al., Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, *Journal of Computational Chemistry*, 19:1639-1662, 1998.
[5] Plewczynski, Dariusz., Lazniewski, Michal., Augustyniak, Rafal., et al., Can We Trust Docking Results? Evaluation of Seven Commonly Used Programs on PDBbind Database, *Journal of Computational Chemistry*, 32:742-755, 2011.
[6] Karaboga, Dervis and Basturk, Bahriye, A powerful and efficient algorithm for numerical function optimization: artificial bee colony (ABC) algorithm, *Journal of Global Optimization*, 39:459-471, 2007.

C-2-02

A new approach for constructing CYP2C9 pKi estimation scheme with ligand-receptor interaction effects

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Keywords: CYP2C9 pKi, property estimation scheme, Field Point pharmacophores, QSAR

High quality Quantitative Structure Activity Relationship (QSAR) models are important in the drug discovery process. In general, it depends on the researcher's target whether a model is the best for them. Therefore, drug researchers sometimes need to construct their original estimation scheme. StarDrop[1] can provide the powerful tool for producing original high quality QSAR model[2]. We attempted to get a novel high quality CYP2C9 pKi model by using StarDrop. StarDrop can produce 330 descriptors which consist of a total of 321 SMARTS based descriptors and 9 whole molecule properties but also add user's original descriptors. It is our purpose to find a new approach for constructing CYP2C9 pKi estimation scheme with ligand-receptor interaction effects. For getting the descriptors including these effects, we used the Field Point technology[3] of Cresset[4]. Field points are the local extrema of the electrostatic, van der Waals and hydrophobic potentials of the molecule. They can be thought of as extended pharmacophores, with the advantages that their position is directly calculated from the molecule's physical properties. We will present how to get the descriptors of Field Point pharmacophores and the details of our original models.

[1] http://www.optibrium.com/
[2] *J. Comp.-Aided Mol. Desi.*, 2008, 22, 431-440
[3] *J. Chem. Inf. Model.*, 2006, 46, 665-676
[4] http://www.cresset-group.com/

C-2-03

Electronic Similarity of Molecules for Evaluation, Classification, and Discovery

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Keywords: Electronic structure calculation, Molecular Similarity, Electronic Similarity

Numerical expression of molecules is important for their quantitative description. By quantification, we can apply a variety of statistical methods to observe quantitative characteristics of molecules, which are expected to facilitate evaluating their values, classifying them, and discovering their derivatives. Intrinsic structures and properties of molecules are due to their electronic structures, it is natural to quantify molecules on the basis of electronic-structure calculations.

In this presentation, we suggest a method of defining molecular similarity by applying ab initio electronic-structure calculations. It will be shown that reasonable classification of molecules is possible for the trial set shown in Scheme 1. When a reference molecule is taken as lutein A, the carotenoid molecules are top-ranked as shown in Table 1. The present computational method is also applied to investigate bioisosterism of functional groups.

Scheme 1.

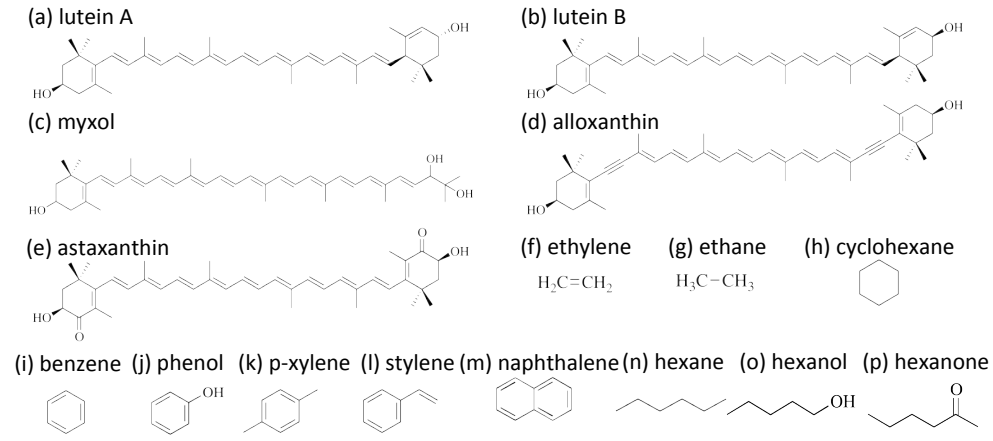

Table 1. Ranking of electronic similarity of molecules in Fig. 1.

Rank	Compd.	Similarity (%)	Rank	Compod.	Similarity (%)
1	lutein A	100.0	9	2-hexanone	31.5
2	lutein B	98.0	10	phenol	30.5
3	myxol	91.9	11	1-hexanol	29.7
4	alloxanthin	91.8	12	benzene	25.5
5	astaxanthin	77.9	13	hexane	24.9
6	p-xylene	37.7	14	ethane	20.4
7	naphthalene	37.3	15	ethylene	20.1
8	stylene	37.2	16	cyclohexane	19.2

C-2-04

Understanding polypharmacology and promiscuous chemotypes on LSKB

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Keywords: knowledge, Open Data Source, Polypharmacology, Molecular Framework, Workflow

Toward disease related target, it is important to classify the drug target(genes/proteins) based on its function, and the subtype has often different ligand recognition.
Knowledge of SAR and related studies is used for recognition of ligand structures ; In many cases, we try to find common core and/or scaffold from the analysis and use the information.
Bajorath et al., reported promiscuous chemotypes by analysis of polypharmacology between drugs and targets, using the chemical structures, scaffolds and molecular frameworks[3]. And also a series of compounds, which have common scaffold, is specific toward a target class in same case and they describe the benefit of activity cliffs for the understanding SAR[1,2].

In the poster, Life Science Knowledge Bank(LSKB)[4] is well-organized database system storing the binary relationship each combination of the terms (Gene/Protein/Disease/Tissue/chemical) mined from Medline and extracted information from various Open Data Sources. And the well-organized compound structure database is also available in LSKB; the chemical contents is built as non-redundant (NR)chemical structures from PubChem[5], ChEMBL[6], ZINC[7] and ligand structures collection taken from PDB protein-ligand complexes (where the information extracted from PDB[8]). And it associates LSKB knowledge dataset. In LSKB version 4.2, compound databases stores 70M NR compounds and, molecular frameworks associated with each compounds.
The latest release provides protein classification i.e., EC number, GPCR, Kinase. and registration function of interest gene set in private experimental data. We show you the benefit of combination, LSKB and workflow tool Pipeline Pilot[9] by several instances for understanding polypharmacology and SAR Matrix generation.

[1] Hu, Y. and Bajorath,J., *Polypharmacology Directed Compound Data Mining: Identification of Promiscuous Chemotypes with Different Activity Profiles and Comparison to Approved Drugs*, J. Chem. Inf. Model. 2010, 50, 2112?2118
[2] Hu, Y. and Bajorath,J., *Molecular Scaffolds with High Propensity to Form Multi-Target Activity Cliffs*, J. Chem. Inf. Model. 2010, 50, 500-510
[3] Bemis, G.W. and Murcko,M. A., *The Properties of Known Drugs. 1. Molecular Frameworks*, J. Med. Chem. 1996, 39, 2887-2893
[4] World Fusion Co., Ltd., 2-15-15 Nihonbashi Ningyocho, Chuo-ku, Tokyo 103-0013, Japan
[5] http://pubchem.ncbi.nlm.nih.gov/
[6] https://www.ebi.ac.uk/chembldb/
[7] http://zinc.docking.org/
[8] http://www.rcsb.org/pdb/home/home.do
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C-2-05

Ensemble Docking Simulation for β_2 Adrenergic Receptor Using Elastic Network Model

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Keywords: Elastic Network Model, Normal Mode Analysis, Ligand Docking, *In Silico Screening*

G protein-coupled receptors (GPCRs) are membrane proteins involved in signal transduction pathways, and so many of them are target molecules for drug discovery of various human diseases. Although more and more crystal structures of GPCRs have been determined with particular agonists and antagonists, it is now widely known that drug docking simulations based on such rigid crystal structures do not always succeed because of their very flexible structural features. Therefore, we adopted an ensemble docking method to take into account the protein flexibility in drug docking and screening.

In an ensemble docking method, each compound in the library including drug candidates is docked at the pockets of the individual receptor conformations, which were produced by molecular simulations. Molecular Dynamics (MD) simulation now becomes standard for producing putative different conformations of GPCRs, but the dynamic aspects of GPCRs can be captured by a much simpler model such as an elastic network model (ENM).

In this study, we generated the conformation polymorphism of β_2 adrenergic receptor by ENM with the successive Normal Mode Analysies (NMA), based on the X-ray crystal structure (PDBID: 2RH1). Various structual models were built by moving the atom positions following several representative modes, which were calculated by ENM-NMA. Then, docking simulations were made for those virtual models. The models based on the selected particular five modes showed the best scores for agonist and antagonist screenings. Compared to the initial crystal structure, virtual model provided 20% better screening efficiency.

C-2-06

Investigation of protein-ligand interactions

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Keywords: Molecular docking, Protein-ligand interaction

Finding therapeutic drugs is challenging matter. Many methodologies have been applied to this matter (*i.e.* drug design). In addition to the experimental techniques, various computational approaches are utilized at the different stages of drug design. In the early stage, compound libraries are screened in order to increase potential compounds. Due to select the compound from the huge number of compounds in relatively short time, molecular docking methods are routinely used. The molecular docking has already proven to be a successful virtual screening tool for several target proteins, but it is less reliable because the calculation of binding free energy between target protein and ligand is not so accurate. The problems with molecular docking have been widely mooted: protein dynamics and solvation effects are ignored, the energy functions are inaccurate, and so on. Therefore more effective computational method is necessary for rational drug design.

Molecular dynamics (MD) simulations can treat both proteins and ligands in a flexible manner, directly estimate the effect of explicit water molecules, and provide more accurate binding affinity, although their computational costs and times are significantly greater than those of molecular docking. We report an effective method for computational screening; this method is a combination of molecular docking and molecular dynamics simulations. The proposed method showed a higher and more stable enrichment performance than the molecular docking method used alone. We will use quantum chemical (QC) calculations as final filter of drug screening.

[1] Okimoto N. *et al*., PLoS Comput Biol 5(10): e1000528.

C-2-07

Predicting bioactivity of compound-drug target protein pairs using support vector regression models reflecting ligand efficiency

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Keywords: Support vector regression, G-protein coupled receptor, Protein kinase, Ion channel, ligand efficiency

Predicting bioactivity of compounds to drug target proteins using machine learning methods is one of the most intensively studied area in drug discovery and development. Although many previous machine learning studies have succeeded in predicting novel ligand-protein interactions with high performance, all of the previous studies to date have been heavily dependent on the simple use of raw bioactivity data of ligand potencies measured by IC50, EC50, Ki, and Kd deposited in databases. In our previous study [1], we have showed that, using support vector machines, binary classification models based on training data reflecting one of the representatives of ligand efficiency, Binding Efficiency Index (BEI) [2] can offer better performance in classifying active and inactive compound-protein pairs than models based on training data reflecting IC50 or Ki. In this study, we report that this result holds also when support vector regression (SVR) models are applied to bioactivity data.

Utilizing bioactivity data measured by IC50 in GPCRSARfari ver. 2, KinaseSARfari ver. 4, and ChEMBL 14 databases [3], we retrieved bioactivity data associated with G protein-coupled receptors, protein kinases, and ion channels and created four types of training data; IC50-based, pIC50-based, BEI-based, and Surface Efficiency Index (SEI)-based. Values of pIC50, BEI, and SEI were transferred from observed values of IC50 in the databases. The number of instances in the training data is shown in Table 1. To represent compound-protein pairs in the training data, three kinds of compound descriptors (MACCS, 2D descriptors in MOE, and OpenBabel FP2) and single protein descriptor (frequencies of dimmers of amino acid in protein sequence) were used. From GPCRSARfari ver. 3, KinaseSARfari ver. 5.01, and ChEMBL 15 databases, we collected newly added bioactivity data and used the data as validation data for evaluating the performance of the constructed SVR models. Objective comparisons of the performance of the SVR models showed that their prediction capabilities follow an order of SEI > BEI > pIC50 > IC50 as a whole. This result is independent of compound descriptors used and drug target protein families. The superiority of ligand efficiency-based SVR models may be partially attributed to distinct distribution patterns of pIC50s, BEIs, and SEIs, showing narrower range of BEIs than pIC50s and SEIs than BEIs.

[1] Sugaya, N., Training based on ligand efficiency improves prediction of bioactivities of ligands and drug target proteins in a machine learning approach, *Journal of Chemical Information and Modeling*, in press.
[2] Abad-Zapatero, C. and Metz, J. T., Ligand efficiency indices as guideposts for drug discovery, *Drug Discovery Today*, 10:464-469, 2005.
[3] Gaulton, A., *et al.*, ChEMBL: a large-scale bioactivity database for drug discovery, *Nucleic Acids Research*, 40:D1100-D1107, 2012.

C-2-08

CzeekD: Fragment-based *de novo* Drug Design System for Drug Discovery

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Keywords: FBDD, *de novo* drug design, PSO, CGBVS, RECAP

For early stages in drug development, it is a critical issue to find lead compounds with novel scaffolds. However, the set of all possible small organic molecules has been estimated to consist of more than 10^{60} compounds, it is difficult to figure out the best scaffolds from among the vast chemical space through experimental synthetic approaches.

We have developed a computational approach to efficiently generate lead compounds with novel scaffolds using fragment chemical library. The fragment-based drug *de novo* design system with a fast stochastic optimization algorithm, called “CzeekD”, has following functions:

- Generation of novel scaffolds in vast chemical space is achieved by combination of building blocks in fragment library.
- It is easy to synthesize the designed chemical structures because of using basic chemical reactions, i.e. RECAP rule.
- Chemical Genomics-Based Virtual Screening (CGBVS) is implemented as a scoring function, which can efficiently estimate the possibility of molecular interactions between the generated compounds and the target proteins.
- Particle Swarm Optimization (PSO) algorithm was newly developed to swiftly explore druggable molecular seeds and to efficiently find out diverse structures among the vast chemical space.

As a result of bioactivity evaluation of the designed compounds through organic synthesis and *in vitro* assay, we successfully identified novel active compounds with highly hit rate (20－40%) for GPCR proteins. This result suggested that CzeekD provides a powerful solution to guide medicinal chemists into the discovery of novel bioactive molecules.

[1] Hartenfeller M, Proschak E, Schüller A, and Schneider G., Concept of combinatorial de novo design of drug-like molecules by particle swarm optimization, *Chem Biol Drug Des*, 72:16-26. 2008.
[2] Lewell XQ, Judd DB, Watson SP, and Hann MM., RECAP–retrosynthetic combinatorial analysis procedure: a powerful new technique for identifying privileged molecular fragments with useful applications in combinatorial chemistry, *J Chem Inf Comput Sci*, 38:511-22, 1998.
[3] Yabuuchi H, Niijima S, Takematsu H, Ida T, Hirokawa T, Hara T, Ogawa T, Minowa Y, Tsujimoto G, and Okuno Y., Analysis of multiple compound-protein interactions reveals novel bioactive molecules, *Mol Syst Biol*, 7:1-12, 2011.
[4] J. Kennedy and R. C. Eberhart, Particle swarm optimization, *Proc. IEEE International Conference on Neural Networks*, 1942-1948, 1995.

C-2-09

Mixed Fragment-based screening of WNK1 inhibitor

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Keywords: Fragment screening, WNK1, SPR

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

Mobility shift assay, by which inhibitory activity for phosphorylations of WNK1 was estimated, was examined in order to select hit compounds out of 9,000 fragments. Then we selected compounds having structural similarity to the hit compounds, and their binding abilities, including those of hit compounds, to WNK1 were tested using SPR. The competition assay by SPR was carried out using a single or a mixture of the candidate compounds.

Figure 1 shows competition assay about combination of candidate compounds 1 (1,3-isoquinolinediol) and 2 [N-(4-methyl-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-b]pyrrol-6-yl)], and 3 (1,3-isoquinolinedione) and 4 (indirubin-3'-oxime). Compounds 1 and 2 were found to bind to the same site in WNK1 because the response of their mixture was almost the same as that of 2. In contrast, as shown in the right side of Figure 1, the response of the mixture of compounds 3 and 4 were the sum of those of the two. This clearly indicates compound 3 and 4 bind to the different sites in WNK1. The binding site of these compounds will be discussed using a docking study.

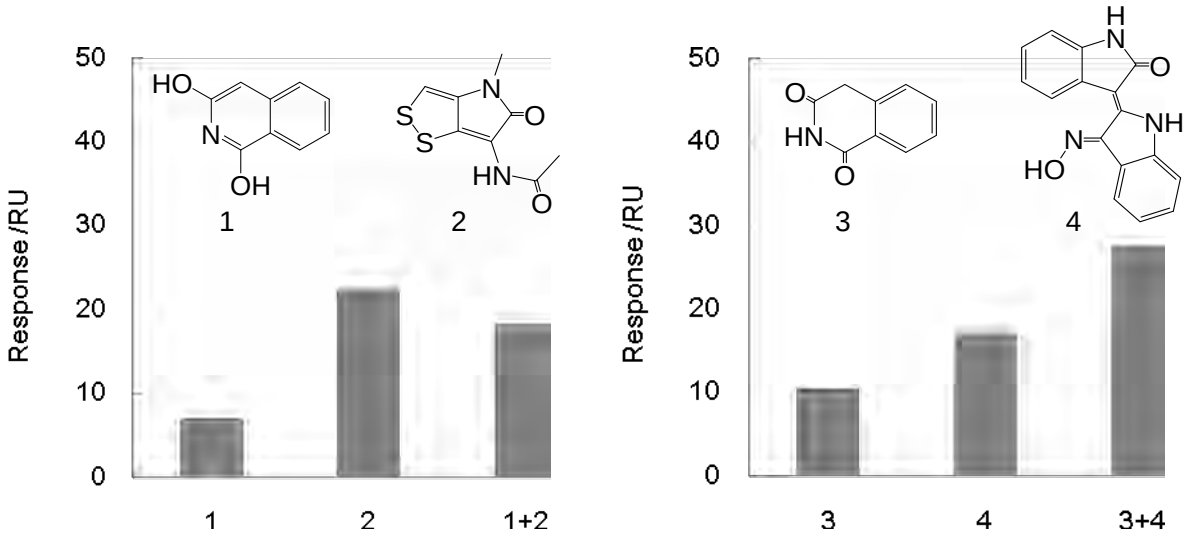

Fig. 1 Competition experiments using candidate compounds 1-4.

C-2-10

Identification of novel chemical agents with antibacterial activity against *Mycobacterium* by *in silico* structure-based drug screening

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Keywords: Antibiotics, Enoyl-acyl carrier protein reductase (InhA), *in silico* structure-base drug screening (SBDS), *Mycobacterium*, Protein-ligand interaction fingerprint analysis (PLIF)

The increasing prevalence of drug-resistant tuberculosis (TB) (i.e. multidrug-resistant TB: MDR-TB, extensively drug-resistant TB: XDR-TB), which is resistant to several effective antibiotics, presents a major global health threat [1]. The enoyl-acyl carrier protein reductase of *Mycobacterium tuberculosis* (InhA) is a key enzyme of the mycobacterial type Ⅱ fatty acid biosynthesis pathway (FAS-Ⅱ). In this pathway, the InhA plays major role in the production of mycolic acid and is known as a target protein for first-line drug isoniazid (INH), an effective antibiotics for TB chemotherapy [2].

In this study, we attempted to identify novel chemical compounds specifically targeting the InhA. We performed a hierarchical *in silico* SBDS [3,4] using InhA crystal structure data (PDB ID: 2H7I) and the virtual chemical library (ChemBridge, CA) including 154,118 chemicals. We then evaluated the antibiotic effects of the candidate chemicals, and we found two hits (KE3 and KE4), which were able to inhibit the growth of model mycobacteria strains (*M. vanbaalenii* and *M. smegmatis*). We also performed similarity analysis to identify five additional chemicals (KES1-KES5) with similar structures to the active chemicals from ChemBridge compound library, containing 461,383 compounds. The most potent inhibitors (KE4 and KES4) do not have any toxic effects in model intestinal bacteria (*E. coli* BL21 and JM109 strains) and mammalian cells (MDCK and SH-SY5Y cells). Moreover, we also confirmed that these chemicals directly inhibit the enzymatic activity of InhA.

In conclusion, the structural and experimental information regarding these novel chemical compounds is likely to be useful for the hit-to-lead optimization of new antibiotics for the treatment of TB. Furthermore, our screening methodology presented in this study could contribute to the further identification of novel hit compounds for other candidate medicinal drugs.

References

[1] Global Tuberculosis Control: WHO Report 2011, ISBN 978 92 4 156438 0
[2] H. Lu, *et al.*, *Acc. Chem. Res.* **2008** (41), 11-20.
[3] Y. Koseki, *et al.*, *Eur. J. Med. Chem.* **2013** (60), 333-339.
[4] T. Kinjo, *et al.*, *J. Chem. Inf. Model.* **2013** (53), 1200-1212.

C-2-11

Identification of novel potential antibiotics for *Staphylococcus* by Structure-Based Drug Screening

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Keywords: *in silico* Structure-Based Drug Screening (SBDS), *Staphylococcus*, Dihydrofolate reductase (DHFR), Protein Ligand Interaction Fingerprint (PLIF)

The emergence and spread of multidrug-resistant *Staphylococcus aureus* (*S. aureus*) increased the morbidity and mortality of the infected patients worldwide [1, 2]. Therefore, it is importance to develop new antibacterial drugs against the multidrug-resistance *S. aureus.* In this study, we attempted to identify novel potential antibiotics targeting *S. aureus* dihydrofolate reductase (saDHFR), which is an essential coenzyme for DNA replication.

We performed a hierarchical *in silico* Structure-Based Drug Screening (SBDS) [3] with crystal structure of saDHFR (PDB ID: 2W9G [4]) and virtual chemical compound library (154,118 chemical compounds, ChemBridge, SanDiego, CA). We identified five candidate chemical compounds predicted to have high binding affinity with active site of saDHFR. We then experimentally tested whether these candidate chemical compounds inhibit the growth of *Staphylococcus epidermidis* (*S. epidermidis*), a model bacteria strain. A chemical compound (KB1) exhibited growth inhibitory effect on *S. epidermidis*. Moreover, we found five KB1 analogs from a library including 461,383 chemical compounds (CemBridge, SanDiego, CA). Four KB1 analogs (KBS1-KBS4) inhibited the growth of *S. epidermidis*. The hit compounds (KB1 and KBS2-KBS4) in this study do not have toxic effects on model enterobacteria (*Escherichia coli* BL21 and JM109 strains) and mammalian cell (Madine-Darby Canine Kidney cell). Furthermore, we evaluated binding modes of hit chemical compounds using Protein Ligand Interaction Fingerprint (PLIF) analysis and Ligand Interaction (LI) analysis. Results of PLIF and LI analyses predicted that these hit chemical compounds are able to have inhibitory effects on growth of the resistance strains with mutated saDHFRs, similar to wild-type saDHFR.

In conclusion, structural information of four candidate chemical compounds identified in this study (KB1, KBS2-KBS4) will likely contribute to development of new drugs for *S. aureus*.

References

[1] Bai H., *et al.*, *PLoS One.*, **2012** (7), e29886.
[2] Iwase T., et al., Nature, **2010** (465), 346-349.
[3] T. Kinjo, *et al.*, *J. Chem. Inf. Model.* **2013** (53), 1200-1212.
[4] Holly H., *et al.*, *PROTEINS*, **2009** (76), 706-717.

C-2-12

Design of transfection reagents in RNAi therapeutics by chemoinformatics approach

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Keywords: Chemoinformatics, RNAi, Transfection reagent

RNAi is a natural biological process involving gene silencing or regulation with siRNA and expected to be applied in the therapeutics of gene disorders. The delivery of siRNA into the cell is demonstrated using cationic lipid. The lipid is called 'Transfection reagents' and the development of new reagents has recently advanced. Some relation exists between the chemical structures of the reagents and their properties, but it is not clear yet quantitatively and the development of new reagents depends on the experimental perception.

In this study we collected the data of the transfection reagents from the literature[123] and constructed statistical models between the structures of the reagents and their suppression efficiency of gene expression in order to predict the chemical structures of high efficient reagents in future.

[1] K. T. Love, et al., *PNAS*, **107**, 1864-1869, 2010
[2] K. P. Mahon, et al., *Bioconjugate Chem.*, **21**, 1448-1454, 2010
[3] A. Akinc, et al., *nature biotechnology*, **26**, 561-569, 2008

Toward Crowdsourcing Evaluation of Synthetic Accessibility

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Keywords: Synthetic accessibility, Crowdsourcing, Human computation

A rapid method for the assessment of synthetic accessibility for a vast number of chemical compounds is expected to bring about a breakthrough in the drug discovery. Although several computational methods have been proposed for estimating the synthetic accessibility [1-3], they do not meet the requirements of both quick computation time and high estimation accuracy. In the field of computer science, *crowdsourcing* has received considerable attention for being an efficient and scalable approach for solving complicated tasks that are difficult for computers but relatively easier for humans. Using crowdsourcing platforms such as Amazon Mechanical Turk [5], one can easily outsource various professional and non-professional tasks to a large group of people online. Jobs on crowdsourcing are typically decomposed into small and simple tasks that can be solved in parallel by different workers.

As the first step toward creating a crowdsourcing platform where the synthetic accessibility of a number of compounds is assessed by people with different levels of expertise and backgrounds, we investigate the consistency among experienced medicinal chemists in assessing synthetic accessibility. Unlike previous studies [2, 4], we prepared the following three compound groups: Already existing compounds, ones having substructures that are hard to be synthesized, and ones having novel substructures. We picked ten compounds from each group and asked the experienced medicinal chemists to rate the synthetic accessibility of each compound on a five-point scale within several minutes. Results show that there is a high correlation between the assessments given by a subset of the chemists.

[1] Baber, J. C., Feher, M.: Predicting Synthetic Accessibility: Application in Drug Discovery and Development. *Mini Reviews in Medicinal Chemistry*, 4:681-692, 2004.
[2] Boda, K., Seidel, T., Gasteiger, J.: Structure and Reaction based Evaluation of Synthetic Accessibility. *Journal of Computer-Aided Molecular Design*, 21:311-325, 2007.
[3] Ertl, P., Schuffenhauer, A.: Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions, *Journal of Cheminformatics*, 1:8, 2009.
[4] Takaoka, Y., Endo, Y., Yamanobe, S., Kakinuma, H., Okubo, T., Shimazaki, Y., Ota, T., Sumiya, S., Yoshikawa, K.: Development of a Method for Evaluating Drug-Likeness and Ease of Synthesis Using a Data Set in Which Compounds Are Assigned Scores Based on Chemists' Intuition. *Journal of chemical information and computer sciences*, 43:1269-1275, 2003.
[5] http://mturk.com

C-3-01

Identification of genes and pathways involved in DNA methylation in hepatocellular carcinoma

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Keywords: DNA methylation, Hepatocellular carcinoma, DNA microarray

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Despite major efforts to improve diagnosis and treatment of HCC, therapeutic options remain limited[1]. A role for DNA methylation in the regulation of gene expression was hypothesized many years ago. Recent genetic studies suggest that tumor suppressor genes (TSGs) are often silenced during carcinogenesis through epigenetic modification caused by metylation of promoter CpG island[2]. Therefore, detailed investigation of genes inactivated by DNA methylation in HCC is necessary for the development of novel treatment options for HCC.

In this study, we aimed to identify candidate genes commonly methylated in HCC. We treated eight HCC cell lines by the DNA demethylating agent 5-aza-2'-deoxycytidine (DAC). Comprehensive gene expression analysis revealed that 489 probe sets had more than two-fold up-regulated in at least six DAC-treated cell lines compared to the control cell lines. Moreover, Gene set enrichment analysis (GSEA) of DAC-treated cells suggested that down-regulation of immune response pathways were possibly induced by DNA methylation. Interestingly, chemokine (C-X-C motif) ligand 2 (CXCL2) was included in the 44 probe sets and is associated with immune response. Because immune surveillance is an important host system to inhibit carcinogenesis and thereby down-regulation of CXCL2 might cause immune escape of cancer cells, CXCL2 can be one of the key molecules for cancer progression by DNA methylation.

Our results suggest that these genes and pathways may play an important role in carcinogenesis and disease progress of HCC through inactivation by DNA methylation. Notably, the candidate gene CXCL2 might be an important molecule for further investigation of immune escape in HCC. In our validation studies, we showed that the expression of CXCL2 was down-regulated in HCC tissue as compared to non-tumor tissues. In addition, CXCL2 expression was significantly up-regulated by DAC treatment in HCC cell lines. This suggests that CXCL2 might be an important molecule for further investigation of aberrant methylation in HCC.

References

[1] Tischoff I, Tannapfe A. DNA methylation in hepatocellular carcinoma. *World J Gastroenterol*. 2008;**14**:1741-8.
[2] Partha M Das and Rakesh Singal. DNA methylation and cancer. *Journal of Clinical oncology*. 2004;**22**:4632-42.

C-3-02

^{1}H NMR-based metabolomics of plasma and dialysate from hemodialysis patients

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Keywords: Alanine, Creatinine, dialysate, Lactate, Pyruvate, TCA cycle,

^{1}H NMR observation of plasma and dialysate from hemodialysis (HD) patients was subjected for profiling of metabolites. Our previous work on ^{1}H NMR metabolomics of plasma from HD patients has revealed lactate increment after treatment.[1)] Lactate increment suggested impaired energy metabolisms in HD patients. Blood sample collection is limited in HD patients because of anemia. Then we used dialysate, which is non-invasive for patients and without limitations of collection. In analytical aspect, plasma samples were less reproducible because plasma is heterogeneous mixture of proteins, lipids, organic acids and other small metabolites. On the other hand, dialysate has good reproducibility for its composition of only small metabolites, and also ensures adequate quantification of metabolites by ^{1}H NMR. We have also verified dialysate as a surrogate for blood in measuring small metabolites during HD, by quantitative ^{1}H NMR. [2)]

In this study, 600 dialysate samples from 20 patients were collected in time course during HD sessions, and measured 1D single-pulse spectra by 600 MHz NMR (ECA, JEOL Ltd.) spectroscopy. The main metabolites were quantified by their peak integrations on the spectra. These concentrations in time course revealed to have unique pattern to patient in every HD session. The finding has potential information for future personalized therapy.

In all of patients, creatinine exhibited monotonous decay and valine showed plateau toward the end of the session. While patients derived from chronic-glomerular-nephrites exhibited significant increment of lactate together with alanine and pyruvate at the middle during HD sessions, which indicated rather amount of productions from the body.. HD treatments rapidly remove electrolytes, water, and small molecules including bioactive necessities and nutrients as well as uremic toxins from the blood. As the response to the HD stress, compensative reaction to maintain homeostasis may occur followed by their productions into blood. The increment of lactate, pyruvate and alanine suggested accelerated glycolysis and/or disturbances of metabolic pathway at the entrance to TCA cycle.

We will discuss the relation between metabolic profile in each patient and one's etiology, and the possible revisions of HD therapies.

1) Fujiwara M. *et al* Pattern recognition analysis for ^{1}H NMR spectra of plasma from hemodialysis patients. *Anal. Bioanal. Chem.* 2009, 394: 1655-1660

2) Ando I, Fujiwara M. *et al*, ^{1}H NMR Spectroscopic Quantification of Plasma Metabolites in Dialysate during Hemodialysis *Magn.Res. Med.Sci*. 2013, 12, No.2, 129-135

C-3-03

Epigenome-wide discovery of ovarian and breast cancer specific DNA methylation markers

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Keywords: *Next Gene Sequencing, Omics data, DNA Methylation, Epigenomics, Clinical Bioinformatics*

Breast and ovarian cancers pose huge and unsolved challenges to the medical profession. The EU FP7 consortium EpiFemCare develops blood tests based upon DNA methylation technology to facilitate early detection and prediction of therapeutic outcome of these cancers. In phase 1 of the project Infinium® HumanMethylation450 BeadChip technology is used to assess the methylation status of ~485'000 sites in cancer and control tissues. In parallel, Reduced Representation Bisulfite Sequencing (RRBS) is used to identify and confirm cancer specific methylated circulating DNA in matching serum samples. Using Genedata Expressionist® for Genomic Profiling, we have established an automated bioinformatics pipeline for the detection of cancer specific Differentially Methylated Regions (DMRs) that most likely fulfill the strict specificity criteria of a serum based test. The most promising DMRs are taken forward to clinical assay development (phase 2) and validation in thousands of serial samples from prospective clinical trials (phase 3).

C-3-04

Analysis of Human Immunodeficiency Virus Type 1 (HIV-1) CRF07_BC in China Using BEAST

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Keywords: HIV-1, Recombinant virus, Phylogeographic analysis

It has been reported that HIV-1 subtypes B' and C from Thailand and India, respectively, were introduced into Yunnan province of China in the late 1980s, via drug-trafficking routes and resulted in the initial outbreak of CRF07_BC virus among injection drug users in this region. Since then, this recombinant virus spread to many other regions and became one of main HIV-1 subtypes in mainland China nowadays. However, the contradictory results have been reported on the origin and transmission route of this virus. We conducted a comprehensive analysis to clarify this issue.

We retrieved the viral samples from more than one public databases and created five datasets: 1) *env* gene, 2) *gag* gene of subtype C origin, 3) *gag* gene of subtype B origin, 4) *pol* gene of subtype C origin and 5) *pol* gene of subtype B origin. These datasets included more locations (15 provinces), more sampling time points (1996-2011) and the longer genome regions of viral genes. Moreover, the most recent version of a Bayesian inference method for phylogeographic analysis was employed in the study.

The results showed that the origin of the CRF07_BC virus was Yunnan, but the transmission routes in China are quite complicated. The virus spread to other regions not only from Xinjiang but also from Yunnan, Sichuan etc. Our results suggest that it is not proper to simply conclude that the virus spread only from Xinjiang or Yunnan.

[1] Liu, J. and C. Zhang, Phylogeographic analyses reveal a crucial role of Xinjiang in HIV-1 CRF07_BC and HCV 3a transmissions in Asia. *PLoS One*, 6(8): p. e23347, 2011.
[2] Meng, Z., et al., A new migration map of HIV-1 CRF07_BC in China: analysis of sequences from 12 provinces over a decade. *PLoS One*, 7(12): p. e52373, 2012.

C-3-05

Computational Approach to Validate Long-range Chromatin Association between Estrogen Receptor Alpha Proteins and Candidate Genes of Breast Cancer MCF-7 within Different Human Reference Genomes

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Keywords: epigenetics, estrogen receptor alpha binding site, human reference genome sequence

To investigate mechanism of estrogen receptor alpha (ER-α) associated with gene expression, In particular, we studied computational long-range effects by mapping estrogen receptor alpha binding sites (ER-αBSs) on 37 breast cancer candidate genes with the aim to reveal the most associated genes along the transcription start sites. Then, we compared result of the computational long-range chromatin interaction analysis with pair-end tag (ChIA-PET) on different human reference genome sequences 17 (hg17), 18 (hg18) and 19 (hg19) [1, 2].

By obtaining numerical coordinates of ER-αBSs, we could see a more completely computational result with ChIA-PET workflow on hg19 by detecting more number of ER-αBSs associated with breast cancer candidate genes within the same range of the transcription start sites. By investigating ER-α on different human reference genome sequences, we could determine coordinates of ER-αBSs can be altered to detect different positions of ER-αBSs based on association with breast cancer candidate genes such GREB1 and SGK3. In this study, we obtained 3500, 3488 and 3501 ER-αBSs respectively from hg17, hg18 and hg19. We found common 3487 ER-αBSs between hg17 and hg18, common 3425 ER-αBSs between hg17 and hg19, and common 3426 ER-αBSs between hg18 and hg19. However, we only found 3412 common ER-αBSs among hg17, hg18 and hg19. Therefore, within our comparative study, we could detect different positions and number of ER-αBSs associated with candidate genes on a single map, as we had not expected so far. To specifically differentiate the coordinates of ER-αBSs generated by computational ChIA-PET workflows on hg17, hg18, and hg19, we also reported all different coordinates of ER-αBSs associated with detectable breast cancer candidate genes.

Within our comparative study, we found that ChIA-PET workflow really depends on human reference genomes and this would lead to detect different numbers of ER-αBSs; moreover, different positions of ER-αBSs from breast cancer candidate genes can also be confirmed within our research study.

[1] GuoLiang L, Han Y, Fabianus Hendiryan M, Stoyan V, Vinsensius V, Pramila Nuwantha A, Yusoff Bin M, Hong-Sain O, Wing-Kin S, et al: ChIA-PET tool for comprehensive chromatin interaction analysis with paired-end tag sequencing. Genome biology 2010, 11:R22.

[2] Vutha PHAV, Akihiko Konagaya. Comparison of computational approaches to estimate long-range chromatin interaction between human breast cancer candidate genes and estrogen receptor alpha proteins. Proceeding of InCob 2013 international conference; 2013, 20-22; Taicang, China.

C-3-06

Association of *CDKN2A/B*, *ADTRP* and *PDGFD* polymorphisms with coronary atherosclerosis in Japan

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Keywords: coronary artery disease (CAD), myocardial infraction (MI), pathology

Background: Genome-wide association studies have identified a series of susceptibility loci for coronary artery disease (CAD). Our study attempted to replicate the results for eight of these loci, *CDKN2A/B* (rs1333049), *ADTRP* (rs6903956), *PDGFD* (rs974819), *TCF21* (rs12190287), *COL4A1-A2* (rs4773144), *HHIPL1* (rs2895811), *ADAMTS7* (rs4380028), and *UBE2Z* (rs46522), in pathologically defined atherosclerosis of the coronary artery. Methods: Autopsy cases of elderly Japanese subjects were enrolled in the JG-SNP study (n=1536). Polymorphisms were genotyped, and their association with coronary stenosis index (CSI) and pathological myocardial infraction (MI) was investigated. Potential combinatorial effects of susceptibility loci were also investigated. Results: Among the eight loci tested, three gave a sign of positive association. *CDKN2A/B* showed the most robust association with CSI and MI (p=0.007 and OR=1.843, 95% CI 1.293-2.629, p=0.001, for CC+CG vs. GG). *ADTRP* showed association with CSI and MI, but the risk allele was opposite from the original report (p =0.008 and OR=1.652, 95% CI 1.027-2.656, p =0.038 for GG vs. AA+AG). *PDGFD* showed a suggestive association with CSI in females, but not in males (p =0.023). *CDKN2A/B*, and *ADTRP* were significantly associated with severity of CSI, in a case-control setting (top 75% vs. the rest: OR=1.683, 95% CI 1.219-2.323, p =0.002 for CC+CG vs. GG, OR=1.839, 95% CI 1.172-2.886, p =0.008 for GG vs. AA+AG, respectively). The cumulative risk allele counting of *CDKN2A/B*, *ADTRP*, and *PDGFD* indicated that increasing number of risk alleles associated with higher CSI (p <0.001). Conclusions: Our data confirms the association of *CDKN2A/B* with CAD, and suggests a different associated risk allele of *ADTRP*. *PDGFD* shows a gender specific association to CAD. The combination of multiple risk alleles may associate with higher risk of CAD.

[1] Schunkert, H., *et al*., Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease, *Nature Genetics*, 6;43(4):333-8, 2011.
[2] Peden, J.F., *et al*., A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease, *Nature Genetics*, 6;43(4):339-44, 2011.
[3] Wang, F., *et al*., Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population, *Nature Genetics*, 6;43(4):345-9, 2011.

C-3-07

PoSSuM Updates and Integration With ChEMBL For Application of Drug Reuse

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Keywords: Ligand binding site, Pocket similarity, Open drug discovery data, Drug reuse, Database

PoSSuM is a large-scale database for finding similar ligand-binding sites in proteins [1], which is freely available via our web-interface (http://possum.cbrc.jp/PoSSuM). As of Aug. 2013, PoSSuM stores over four millions of data points including both known small molecule-binding pockets and putative ones. Detection of structurally similar sites of known small-molecule drugs gives us useful information for rational drug design such as drug-reuse and off-target prediction. For the purpose, we have integrated PoSSuM pocket similarity data with ChEMBL open drug discovery data [2], and have developed a new function for supporting the user to retrieve potential binding pockets for a given small-molecule drug, in addition to a number of improved web interfaces.

First, we selected orally bioavailable small-molecule drugs from the release of ChEMBL16, and matched the compound structures with the latest PDB ligand structures using IUPAC standard InChIKey. In PoSSuM, 4,282 sites where 193 unique small-molecule drugs bound were found. Second, we annotated 3D protein-structures in PDB using the ChEMBL target information for drugs and drug candidates, and classified the protein structures based on its drug development stage. For this purpose, we extracted 7,547 protein-targets from ChEMBL16 and joined those to the FDA-approved drug targets from DrugBank [3]. The protein-targets were classified into the following classes: A) Approved drug targets, B) Hit-to-lead stage, C) Bioactive targets and D) Unknown. Then, we combined the drug target classification with PoSSuM pocket similarity data, and built a pipeline for automatically identifying potential new drug targets having a pocket similar to known small-molecule drug binding sites.

We identified in total 154,191 binding sites, from 10,255 protein structures, which are structurally similar to the focused 4,282 binding sites, covering 182 unique small-molecule drugs deposited in ChEMBL16. Users can retrieve all of the identified similar sites and can visualize 3D superpositoin between them on our new web-interface which will be available soon. We also conducted a preliminary study to compare between PDB-scale pocket similarity and genomic-scale bioactive data for surveying relations between the similarity score and binding affinities.

In this presentation, we will show new functions of the PoSSuM database, utility for structure-based drug design, and discuss the possibility and limitation for application of drug discovery purpose.

[1] Ito, J., *et al.*, *PoSSuM: a database of similar protein-ligand binding and putative pockets*, *Nucleic Acids Res*, **40**(Database issue):D541-D548. 2012.
[2] Gaulton, A., *et al.*, *ChEMBL: a large-scale bioactivity database for drug discovery*, *Nucleic Acids Res*, **40**(Database issue):D1100–D1107. 2012.
[3] Knox C., *et al.*, *DrugBank 3.0: a comprehensive resource for 'omics' research on drugs*, *Nucleic Acids Res*, **39**(Database issue):D1035-D1041. 2011.

C-3-08

Phasing haplotypes of HLA genes from Next Generation Sequencing data at individual level

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Keywords: Next Generation Sequencing, Human leukocyte antigen, Haplotype

Objective: Haplotype information of an individual is valuable for practical applications of personalized medicine, such as pharmacogenomics. For instance, haplotypes of HLA (human leukocyte antigen) genes, which are essential for immune functions, are reported to be associated with Stevens–Johnson syndrome (SJS), a drug induced severe adverse reaction of the skin. In order to facilitate pharmacogenomics application of NGS (Next Generation Sequencing) data, we employed these data to define haplotypes of HLA genes.

Method: Whole-Genome Sequencing (WGS) and Whole-Exome Sequencing (WES) data was collected from public database. Initially, we focused on HLA-B haplotypes, which are involved in SJS induced by Carbamazepine and Allopurinol. Fastq data was mapped against hg19 reference genome and HLA-B gene data was extracted. Genotypes and haplotypes of HLA-B were detected by applying the linkSNPs program [1]. Integrative Genomics Viewer (IGV) was used for visualizing short reads.

Results: Haplotype phasing results showed that, 22 haplotype clusters were found. Combined information from the short reads, all 7 exons of HLA-B gene can be phased into 4 major parts, with 16 possible haplotype combinations. Protein blast result of the 16 combinations above showed that 10 candidate haplotypes showed the highest scores. We further used haplotype frequency table, to rank the probability of haplotype combinations in the individual [2]. As a result, we inferred the most likely HLA-B gene haplotypes.

Conclusion: Haplotype information of HLA genes from an individual NGS data is an important issue in personalized pharmacogenomics. As a practical example, the method introduced in this study may be useful in estimation of adverse reaction risks of SJS.

[1] Sasaki E, Sugino RP, Innan H, The linkage method: a novel approach for SNP detection and haplotype reconstruction from a single diploid individual using next-generation sequence data, *Mol Biol Evol*, 30(9):2187-2196, 2013.

[2] Maiers M, Gragert L, Klitz W, High-resolution HLA alleles and haplotypes in the United States population, *Hum Immunol*, 68(9):779-788, 2007.

C-3-09

Dynamic evolution of forkhead transcription factors and changes in their DNA-binding sites

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Keywords: transcription factor binding site motif, protein–DNA interactions, multigene family

Forkhead box (Fox) proteins are transcription factors (TFs) related with various diseases. The Fox family, defined by a highly conserved winged helix DNA-binding domain (DBD), has diverged into dozens of subfamilies in animals, fungi, and related protists. We have used a combination of maximum likelihood phylogenetic inference and unbiased functional assays of DNA binding capacity to explore the evolution of DNA binding specificity within the Fox family. We present converging evidence that similar alternative sequence preferences have arisen repeatedly and independently in the course of Fox evolution. The vast majority of DNA binding specificity changes we observed are not explained by alterations in the known DNA-contacting amino acid residues conferring specificity for canonical Fox binding sites. Intriguingly, we have found Fox DBDs that retain the ability to bind very specifically to two completely distinct DNA sequence motifs. We propose an alternate specificity-determining mechanism whereby conformational rearrangements of the DBD broaden the spectrum of sequence motifs that a TF can recognize. DNA binding bispecificity suggests a new source of modularity and flexibility in gene regulation and may play an important role in the evolution of transcriptional regulatory networks [1]. In this conference, we will also discuss changes in Fox domain sequences and their binding sites by estimating ancestral sequences of several Fox subfamilies.

[1] Nakagawa S, Gisselbrecht SS, Rogers JM, Hartl DL, Bulyk ML., DNA-binding specificity changes in the evolution of forkhead transcription factors, *Proceedings of the National Academy of Sciences of the United States of America*,110(30):12349-12354, 2013.

C-3-10

Applications of an integrated data warehouse system to investigate complex biological systems

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Keywords: Data integration, Target prioritization, Data warehouse, Drug discovery

We have developed TargetMine, an integrated data warehouse for the purpose of early-stage drug discovery [1]. TargetMine is based on InterMine, a flexible and effective framework for combining biological data from various repositories, which enables navigating through diverse biological data within a single user-friendly interface. TargetMine incorporates a wide range of biological associations including pathways, Gene Ontology annotations, protein structures, chemical compounds and different types of biomolecular interactions. Furthermore, TargetMine includes multiple application programming interfaces, thereby permitting complex pipeline analysis. We have proposed several protocols and also implemented accessory tools to enable streamlined data analysis using TargetMine. These approaches have been effectively employed for target selection in protein-protein interaction (PPI) network-based analyses of Hepatitis C virus (HCV) pathogenesis [2,3,5] and lung tumorigenesis [4]. The academic version of TargetMine is freely available at http://targetmine.nibio.go.jp .

[1] Chen YA, Tripathi LP, Mizuguchi K (2011) TargetMine, an Integrated Data Warehouse for Candidate Gene Prioritisation and Target Discovery. PLoS ONE 6(3): e17844.
[2] Tripathi LP, *et al.* (2010) Network based analysis of hepatitis C virus Core and NS4B protein interactions. MolBiosyst 6: 2539-2553.
[3] Tripathi LP, *et al.* (2012) Proteomic analysis of hepatitis C virus (HCV) core protein transfection and host regulator PA28γ knockout in HCV pathogenesis: A network-nased study. J Proteome Res 11: 3664-3679.
[4] Ihara S, *et al.* (2012) Inhibitory roles of signal transducer and activator of transcription 3 in anti-tumor immunity during carcinogen induced lung tumorigenesis. Cancer Res.72, 2990-2999.
[5] Tripathi LP, *et al.* (2013) Understanding the Biological Context of NS5A-Host Interactions in HCV Infection: A Network-Based Approach. J Proteome Res.

C-3-11

Exploring Development Origin of Health and Disease (DOHaD) hypothesis employing publicly available data

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Keywords: Low birth-weight, Obesity

Background: DOHaD is a concept initially proposed by David Barker that environmental influences during fetal development may predispose the individual towards specific phenotype or diseases. Many epidemiological and animal studies have been undertaken to prove this hypothesis and study its molecular mechanism. However, the impact of this concept to public health is not fully described in Japan and other Asian countries.
Objective: As for a preliminary survey, we searched for clues that sustain DOHaD hypothesis, and find if there are correlation between low birth-weight and increasing trend of obesity in children and/or adult of Japan and other Asian countries.
Materials and Method: Publicly available data and literature search were employed. These include Japan`s school health data, Japan`s National Health and Nutrition Survey, WHO World Health Statistics data and other related databases. The correlation of the rate of low birth-weight during 1970-2000 with 5 years interval and the rate of obesity at their age of 10 years was analyzed by plotting the prevalence of obesity against prevalence of low birth-weight. Simple linear regression and Pearson`s correlation coefficient was used to determine the strength of correlation in variables.
Results: Among eight Asian countries surveyed, Japan and Korea were encountering a significant increasing trend of low birth-weight. We observed a tendency of positive correlation between rate of low birth-weight and rate of obesity at 10-year-old, but it was not statistically significant. ($p=0.15$)
Conclusion: The cause and outcomes of increasing rate of low birth-weight in Japan and other developed countries warrant further research studies. We are currently investigating birth-weight and other intrauterine factors that associate with DOHaD hypothetical outcomes among Japanese population.

[1] Barker, D.J.P., Developmental origins of adult health and disease, *Journal of Epidemiology and Community Health*, 58:114-115, 2004.
[2] Whitaker, R.C. and Dietz, W.H., Role of the prenatal environment in the development of obesity, *J Pediatr,* 132:768-76, 1998.
[3] Takimoto, H., Tetsuji, Y., Nobuo, Y. and Fukuoka, H., Increase in low-birth-weight infants in Japan and associated risk factors, 1980-2000, *J. Obstet. Gynaecol. Res.*, 31:314-322, 2005.
[4] http://www.mhlw.go.jp/bunya/kenkou/kenkou_eiyou_chousa.html
[5] http://www.e-stat.go.jp/SG1/estat/List.do

C-3-12

Correlation of annotation terms in heterogeneous databases and its application to Gene Set Enrichment Analysis (GSEA) and ontology construction

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Keywords: Gene Set Enrichment Analysis, Database, Annotation, Correlation, Ontology

Characterization of a given gene set, such as "gene set enrichment analysis (GSEA)" [1], has become an important task in omics era. In such analyses, annotations are used as if they were independent, despite that some annotations are correlated each other. To interpret complex multiple annotations, we comprehensively examined correlation among each annotation for human genes. We selected ten gene annotation (gene family, Gene Ontology, InterPro, KEGG pathway, protein-protein interaction, SCOP, SOSUI membrane protein prediction, OMIM, tissue specificity of gene expression, and subcellular localization) from the integrated human gene database, H-InvDB [2,3]. For all pairs of the terms, the correlations were evaluated using Fisher's exact (two-side) test with Bonferroni correction. As a result, we found 99,813 and 653 pairs with positive and negative correlation respectively. Many of the positive relationships were synonyms, such as "SCOP g.44.1.1 (RING finger)" and "IPR001841 (Zinc finger, RING-type)". We found other pairs with relevant but not apparent relationships, such as "GO:0006470, protein dephosphroylation" and "hsa04940: Type I diabetes mellitus". We also obtained negative relationships. Many of them seemed unlikely to co-occur in a gene and related to subcellular localization, such as extracellular and nuclear. Those information will help to refine predictive annotation, or perhaps include multifunctions of the genes. By integrating these annotation relationships together with other integrated databases, such as UniProt, we can re-evaluate complex annotations of GSEA results and produce a new summary report of the gene set, as well as a construction of an integrated ontology..

[1] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sc*i 102(43):15545-50, 2005
[2] Takeda J, Yamasaki C, Murakami K, Nagai Y, Sera M, Hara Y, Obi N, Habara T, Gojobori T, Imanishi T., H-InvDB in 2013: an omics study platform for human functional gene and transcript discovery. *Nucleic Acids Research* 41(Database issue): D915-9, 2013
[3] http://www.h-invitational.jp/

C-3-13

Analysis of abnormalities in gene expression and splicing patterns of spinocerebellar ataxia type 6 knockin mice using RNA-seq

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

Spinocerebellar ataxia (SCA) is a neurodegenerative disease characterized by a loss of motor coordination and balance capability. SCA is one of the polyglutamine (poly-Q) diseases including subtypes of SCAs and Huntington disease. Among them, SCA6 is late-onset autosomal dominant neurodegenerative disease caused by a poly-Q expansion in CACNA1A gene that encodes Cav2.1 voltage-gated calcium channel subunit. [1] Since there is no treatment for this congenital disease, the development of novel therapies is strongly needed. It has been reported that the poly-Q expansion causes Purkinje cell degeneration, but the detailed pathogenesis pathway is still unclear. Therefore, we developed a knockin mouse model that had a 118 CAG repeat tract (118Q) in CACNA1A and investigated abnormalities in gene expression and splicing patterns in this model mice. [2]

In this research, we aimed to analyze genes differentially expressed and spliced between cerebellar tissues of a wild-type mouse and an SCA6 model mouse. We obtein 1.6 hundred million reads (16 billion bases) from the wild-type and 1.5 hundred million reads(15.3 billion bases) from the SCA6 model mouse using HiSeq 2000 (Illumina).The TopHat package[3] was used for the alignment of the short reads to the mm10 reference genome, followed by Cufflinks[4] for transcript assembly RNA expression and alternative splicing analysis.We investigated several genes and pathways that might be associated with pathogenesis of SCA6 such as degeneration of Purkinje cells.

[1] Schöls L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. *Lancet Neurol.* 2004;3(5):291-304.

[2] Unno T., Wakamori M, Koike M, Uchiyama Y, Ishikawa K, Kubota H, Yoshida T, Sasakawa H, Peters C, Mizusawa H, Watase K. Development of Purkinje cell degeneration in a knockin mouse model reveals lysosomal involvement in the pathogenesis of SCA6. *Proc Natl Acad Sci U S A.* 2012;109(43):17693-8.

[3] Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 2009;25(9):1105-11

[4] Trapnell C, Williams BA, Pertea G, Mortazavi AM, Kwan G, van Baren MJ, Salzberg SL, Wold B, Pachter L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation *Nature Biotechnol* 2010;28 (5):511-5

C-3-14

Comprehensive transcriptome and proteome analysis revealed Wolfberry (*Lycium barbarum*) as a novel dietary intervention in optimal IBD management

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Keywords: Wolfberry, Transcriptomics, Proteomics, DSS induced colitic mice

Background and objectives
Wolfberry (WOL, *Lycium barbarum*), a traditional Chinese medicinal food has been reported to have antiaging, anticancer, health-promoting, and immune-boosting properties. We aimed to elucidate the anti-inflammatory molecular mechanisms of WOL in a dextran sodium sulphate (DSS) induced colitis model.

Methods
Seven-week-old male C57BL/6J mice were fed either 2% WOL or control diet for 1 week after which colitis was induced by administering 1.5% (w/v) DSS for 9 days and Disease Activity Index (DAI) was observed. Colonic mRNA and hepatic protein were extracted and subjected to microarray (Mouse Genome 230 2.0, Affymetrix) and quantitative iTRAQ proteome analysis respectively.

Results
WOL significantly suppressed colon length increase and DAI from Day 7 as compared to DSS group. Transcriptome and proteome analysis showed 131 altered genes and 227 altered proteins, respectively. Microarray and PCR evaluation showed that WOL supplementation significantly downregulated the expression of inflammatory cytokines-interleukin 6 (*IL6*) and matrix metallopeptidase 10 (*MMP10*); and haptoglobin (*HP*), a protein elevated in acute IBD. Expressions of genes downstream of *IL6*-- prostaglandin-endoperoxide synthase 2 (*PTGS2*); and *MMP10*-- intercellular adhesion molecule 1(*ICAM1*) and fibronectin 1(*FN1*) which are commonly elevated during inflammation were attenuated in WOL mice. In addition, proteome analysis revealed that serum amyloid A-1, a critical marker of inflammation which is regulated by *IL6* was decreased by WOL intervention.

Conclusion
These gene and protein expression alterations indicate that WOL modulates colonic inflammatory signaling and propose WOL as a novel dietary intervention in colitis prevention and therapy.

C-3-15

PathAct: a novel method for pathway analysis using gene expression profiles

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Keywords: DNA microarray, Gene expression profile, Pathway analysis

Gene expression profiling by microarray analysis provides a huge amount of biological information and has been widely used in biological and clinical research. We developed PathAct[1], a novel method for pathway analysis to investigate the biological and clinical implications of the gene expression profiles. The advantage of PathAct in comparison with the conventional pathway analysis methods is that it can estimate pathway activity levels for individual patient quantitatively in the form of a pathway-by-sample matrix using the median polish algorithm[2]. This matrix can be used for further analysis such as hierarchical clustering and other analysis methods.

To evaluate the feasibility of PathAct, comparison with frequently used gene-enrichment analysis methods was conducted using two public microarray datasets. The dataset #1 was that of breast cancer patients, and we investigated pathways associated with triple-negative breast cancer by PathAct, compared with those obtained by gene set enrichment analysis (GSEA)[3]. The dataset #2 was another breast cancer dataset with disease-free survival (DFS) of each patient. Contribution by each pathway to prognosis was investigated by our method as well as the Database for Annotation, Visualization and Integrated Discovery (DAVID)[4] analysis. In the dataset #1, four out of the six pathways that satisfied $p < 0.05$ and FDR < 0.30 by GSEA were also included in those obtained by the PathAct method. For the dataset #2, two pathways ("Cell Cycle" and "DNA replication") out of four pathways by PathAct were commonly identified by DAVID analysis.

Thus, we confirmed a good degree of agreement among PathAct and conventional methods. Moreover, several applications of further statistical analyses such as hierarchical cluster analysis by pathway activity, correlation analysis and survival analysis between pathways were conducted. Therefore, PathAct is a promising tool for pathway-level investigation and interpretation of the comprehensive gene expression data.

[1] Mogushi K, Tanaka H. PathAct: a novel method for pathway analysis using gene expression profiles. *Bioinformation*. 2013;9(8):394-400.

[2] Hoaglin DC, Mosteller F, Tukey JW, eds. *Understanding robust and exploratory data analysis*. Vol. 3. New York: Wiley, 1983.

[3] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A.* 2005;102(43):15545-50.

[4] Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 2003;4(5):P3.

C-3-16

RNA-Sequencing-Based Screening of Long Non-Coding RNAs Targeted by Steroid and Xenobiotic Receptor

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Keywords: long non-coding RNA, steroid and xenobiotic receptor, osteoblast, RNA-sequencing

Steroid and xenobiotic receptor, SXR, is a nuclear receptor that plays an important role in the detoxification of bile acids and chemical compounds [1]. We have previously showed that vitamin K_2-liganded SXR is a transcriptional regulator of bone marker genes [2,3]. Recent advance in genomic technology enables to identify numerous long non-coding RNAs (lncRNAs), which have no obvious coding capacities with their length >200 bp and widely transcribed in spatiotemporal specific manners [4]. While biological functions of some lncRNAs have been characterized, few lncRNAs have been identified in the context of nuclear receptor-regulated gene network.

Here we screened functional lncRNAs upregulated by SXR agonists in human osteoblastic MG63 cells overexpressing SXR, based on RNA-sequencing analysis. Among RefSeq transcripts with >1.5-fold up-regulation or < 0.67-fold down-regulation by either rifampicin or vitamin K_2 *versus* vehicle, ~10% were identified as NR non-coding transcripts and half of the NR transcripts were up-regulated by either SXR ligand (except transcripts down-regulated by the other ligand). Based on the recent genomic data, one third of the up-regulated NR transcripts were lncRNAs except non-coding variants of coding-genes. Interestingly, known lncRNA *HOTAIR* [5] was involved in the group of putative SXR target lncRNAs. We consider that this integrative screen of lncRNAs will reveal the RNA-mediated transcriptional regulation of SXR, and will provide useful information for the potential use of lncRNAs as new osteoblastic biomarkers and therapeutic targets for bone diseases such as osteoporosis.

[1] Sonoda, J., Rosenfeld, J.M., Xu, L., Evans, R.M., and Xie, W., A nuclear receptor-mediated xenobiotic response and its implication in drug metabolism and host protection, *Current Drug Metabolism*, 4:59-72, 2003.
[2] Tabb, M.M., et al., Vitamin K2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. *Journal of Biological Chemistry*, 278:43919-43927, 2003.
[3] Ichikawa, T., Horie-Inoue, K., Ikeda, K., Blumberg, B., and Inoue, S., Steroid and xenobiotic receptor SXR mediates vitamin K2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. *Journal of Biological Chemistry*, 281(25):16927-34, 2006.
[4] FANTOM consortium, The transcriptional landscape of the mammalian genome, *Science*, 309:1559-1563, 2005.
[5] Rinn, J. L., *et al.*, Functional demarcation of active and silent chromatin domains in human *HOX* loci by noncoding RNAs, *Cell*, 129:1311-1323, 2007.

C-3-17

The Inferring Method of the large scale regulatory network for omics studies

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Keywords: Inverse problem, S-system, System identification, Real-coded genetic algorithms

Recent advances in measurement techniques have provided a large number of time series data simultaneously. These data are important for understanding complex systems such as gene regulatory networks and metabolic pathways required to infer networks. We have proposed an efficient inferred method, the hybrid method, for such problems using S-system, the real-coded genetic algorism (RCGA) along with adaptive real-coded ensemble crossover (AREX) combined with the just generation gap (JGG) and the modified Powell method. At this present, we have succeeded in optimizing more than 270 real-valued parameters using this method. Although the hybrid method is powerful compared with conventional numerical optimization methods, it is still insufficient for inferring large networks with more than 10 components. In this study, we attempted to infer the 30 components network, which requires optimizing more than 900 parameters, by applying our improved inferring method.

[1] Savageau, M. A., *Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology*, Addison-Wesley, 1976.
[2] Akimoto, Y., Nagata, Y., Sakuma, J., Ono, I., Kobayashi, S., Proposal and Evaluation of Adaptive Real-coded Crossover AREX. *Journal of Japanese Society for Arti-ficial Intelligence*, 24(6): 446-458, 2009.
[3] Powell, M.J.D., An Iterative Method for Finding Stationary Values of a Function of Several Variables. *The Computer Journal*, 5: 147-151, 1962.
[4] Komori, A., Maki, Y., Nakatsui, M., Ono, I., and Okamoto, M., Efficient Numerical Optimization Algorithm Based on New Real-Coded Genetic Algorithm, AREX + JGG, and Application to the Inverse Problem in Systems Biology. *Applied Mathematics*, 3(10A): 1463-1470, 2012.

Development of an Ontology for Periodontitis

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Keywords: Periodontitis, Ontology, Multifactor disease, Systemic diseases, Biomedical process

Periodontitis is a multifactor inflammation disease which increases risks for many systemic diseases such as diabetes and cardiovascular disorders. Despite accumulation of biomedical literatures on periodontitis pathology, there has been no ontology for the biomedical concepts on the process of pathological progression and clinical treatments of periodontitis

We build an ontology for periodontitis by extracting conceptual terms from the articles retrieved from PubMed database with the keywords specific to molecular pathology of periodontitis. The ontology includes pathological processes in periodontitis specified by their activators, suppressors, locations and participants. In particular osteo-immune processes were specified in terms of relationships to systemic diseases.

We believe this ontology promotes bioinformatics analyses of data obtained from periodontal lesions

[1] Suzuki, A., Takai-Igarashi, T., Numabe, Y. and Tanaka, H., Development of a database and ontology for pathogenic pathways in periodontitis, *In Silico Biology*, 9:233-243, 2009.
[2] Grant, M.M., What do 'omic technologies have to offer periodontal clinical practice in the future?, *Journal of Periodontal Research*, 47:2-14, 2012.
[3] Harvey, A. and Bruno, G.L., Inflammatory mechanisms linking periodontal diseases to cardiovascular disease, *Journal of Periodontol*, 84:S51-S69, 2013.
[4] Gerard, J.L., Amy, L. and Frank A.S., Periodontal systemic associations: review of the evidence, *Journal of periodontal*, 84:S8-S19, 2013.
[5] Day-Richter, J., Harris, A.M., Haendel, M., The Gene Ontology OBO-Edit Working Group and Lewis, S., OBO-Edit-an ontology editor for biologists, *Bioinformatics*, 23:2198-2200, 2007

C-3-19

Improving the visual interpretation of experimental data by a more informative display of confidence intervals

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Keywords: Standard error, confidence interval, visual interpretation

The clear and effective communication of information is of paramount importance in science. Commonly, experimental results are depicted in figures, and the observed values (such as means, for example) are frequently accompanied by bars. These bars sometimes reflect standard error of the mean (SEM), standard deviation (SD), or a confidence interval (CI). Their visual interpretation, however, is far from being trivial, particularly in more complex study designs [1]. A clear and effective visual representation is therefore challenging [2,3]. Here, we discuss the involved problems and argue against displaying SEM bars because they easily invite misinterpretations. We recommend displaying adjusted confidence intervals in figures.

[1] Cumming G. and Finch S. (2005) Inference by eye: confidence intervals and how to read pictures of data. *American Psychologist* 60(2):170–180.

[2] Belia S., Fidler F., Williams J., Cumming G. (2005) Researcher misunderstand confidence intervals and standard error bars. *Psychological Methods* **10**(4):389–396.

[3] Estes W.K. (1997) On the communication of information by displays of standard errors and confidence intervals. *Psychological Bulletin & Review* **4**(3):330–341.

C-3-20

A comprehensive selection of single nucleotide polymorphisms to predict genetic risks of developing common chronic diseases in Japanese

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Keywords: SNPs, Database, Relative risk, Genetic susceptibility to disorders

Recently a number of susceptible loci for common chronic diseases and related disorders have been identified by genome-wide association studies (GWAS). Although the effect size of each SNP differs among different ethnics, abundant literatures allow us to assess each risk in a certain ethnic group. We here report a system to determine genetic risks of developing various disorders among Japanese people, using relevant allele frequencies and odds ratios of selected SNPs.

First we surveyed published papers for GWAS and smaller-scale studies conducted predominantly in Japan, and built a database of disease-susceptible SNPs, curating positions on the human genome, gene names, allele frequencies, odds ratios, p-values, and calculated relative risk to the general population. SNPs verified by replication studies, meta-analysis or multiple studies were preferentially chosen and used for calculating genetic risks. Linkage disequilibrium was considered to remove duplicate SNPs in a susceptible locus. As a result, 128 SNPs were selected for a total of 28 common diseases and related disorders. Among them 93 SNPs were from the results of GWAS with replication studies. Other 26 SNPs were from studies for more than 300 cases followed by replication or another studies, where statistical significance was found and p-values were less than 0.05. Remaining 9 SNPs were from smaller-scale studies with some reliability. Finally 1 to 14 SNPs per disorder were employed for risk calculation of these 28 disorders. Next, segregation ratios of each genotype and relative risks to the general population were calculated and integration of relative risks for each genotype was plotted.

The database with selected SNPs should be useful for predicting risks of developing disorders for Japanese individuals and are ready for the validation of the system.

C-4-01

Precise estimation of the contribution of CYP enzymes to overall metabolism from in vivo information: application of Cluster Newton Method

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Keywords: drug–drug interactions, pharmacokinetics, PBPK model, membrane transporters

Drug–drug interactions (DDIs) are a major cause of adverse drug reactions and subsequent withdrawal of new drugs from the market. For DDIs mediated by the inhibition of drug metabolisms, there are two key determinants of the degree of these interactions: inhibition constants (K_i) of coadministered drugs and fraction metabolized (f_m) by individual metabolic enzymes in the overall metabolism of substrate drugs.

In this study, we performed physiologically-based pharmacokinetic (PBPK) analyses of DDIs to determine "in vivo" f_m values. For the precise determination of f_m values, analyses of pharmacokinetic alterations in both parent drug and metabolites are essential. Since the pharmacokinetic analyses of metabolites are difficult with conventional parameter optimization methods owing to the lack of information on feasible initial parameters, we utilized Cluster Newton Method [1-2] to estimate solution spaces of pharmacokinetic parameters.

PBPK analyses were performed for the DDIs involving lansoprazole, lidocaine, or oxycodone, whose major clearance pathways are CYP2C19 and CYP3A4, CYP1A2 and CYP3A4, or CYP2D6 and CYP3A4, respectively. DDI data were retrieved from the University of Washington Metabolism and Transport drug interaction database (http://www.druginteractioninfo.org). As a result, f_m of each CYP enzyme was determined with small variations, only when we included metabolites' pharmacokinetic alterations in the analyses. Application of the proposed method may improve in vitro-in vivo extrapolations of f_m values, which can lead to the accurate preclinical prediction of DDIs involving the inhibition of metabolisms.

[1] Aoki, Y., *et al*., Cluster Newton Method for Sampling Multiple Solutions of an Underdetermined Inverse Problem: Parameter Identification for Pharmacokinetics, *NII Technical Report*, NII, NII-2011-002E, 2011.
[2] Yoshida, K., *et al*., Estimation of feasible solution space using Cluster Newton Method: application to pharmacokinetic analysis of irinotecan with physiologically-based pharmacokinetic models, *BMC Systems Biology*, 7(Suppl 2):S3, 2013.

C-4-02

Development of the Discriminant KY-methods for Toxicity Screening

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Keywords: KY-methods, Discriminant KY-methods, Toxicity prediction, In silico screening

The KY-methods are powerful data analysis methods which enables a perfect classification even if overlapping between classes of samples is very high and number of samples is quite large.

KY-methods have been developed on the premise that applied on toxicity prediction research field. The special features of the KY-methods are as follows. This approach can overcome various difficult data analysis conditions.

These are conditions which make extremely difficult to do a data analysis. For example, number of sample is very large, the spread of the sample space is large because structure diversity of compounds is quite large, and more overlapping between classes in the sample space is large.

On the toxicity prediction research fields, it have been almost impossible to achieve high classification value by simple application of multivariate analysis / pattern recognition methods by conventional approaches.

Basics of the KY-methods are the multi-step operations that repeat classification and sample re-construction.

Currently, three different discriminant analysis methods have been developed in accordance with the basics of the KY-methods. In this report, briefly discuss the current development status of the KY-methods.

[1] K-step Yard sampling (KY-methods); Kohtarou Yuta, US Patent NO.:US7725413 B2 May 25, 2010.
[2] http://insilicodata.com/pdf%20lists/2%20Model%20KY.pdf
[3] Kohtaro Yuta, Development of the “K-step Yard sampling method” and Apply to the ADME-T In Silico Screening, The 34-th SAR symposium , K06, Niigata, Japan, 2005.
[4] Kazuhiro Sato, etc., Skin sensitization study by a new qualitative structure-toxicity relationships (QSTR) approach: K-step Yard sampling (KY) methods, Journal of Oral Tissue Engin, 9(3):167-173, 2012.

C-4-03

Quantitative Structure-Pharmacokinetic Relationship (QSPkR) Analyses of Opioids

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Keywords: Opioid, QSPkR, QSAR, half-life, mean residence time, elimination rate constant

[Introduction] The prediction of the pharmacokinetic properties of opioids for which it is difficult to gain pharmacokinetic parameters, is useful in a variety of situations. In clinical practice, evaluating the mean residence time (MRT) and terminal half-lives ($t_{1/2}$) of active metabolites from opioids is necessary to understand the exact duration of activity. Pharmacokinetic knowledge is also important in the care of poisoned patients with compounds that are modified opioids structures such as designer drugs. Therefore, the construction of prediction models for MRT as well as $t_{1/2}$ was attempted with QSPkR analyses.

[Methods] After the construction of a dataset, consisting of opioids with pharmacokinetic parameters, a number of descriptors including physico-chemical, quantum-chemical and structural parameters were calculated from each optimized structure. A small number of parameters with importance for the prediction of MRT were selected and used for the construction of the MRT-prediction model by artificial neural network analyses.

[Results and Discussion] A pharmacokinetic dataset with 26 kinds of opioids such as morphine, phentanyl, and methadone was constructed. Remifentanyl was eliminated because of becoming an obvious outlier in the construction process of the MRT prediction model. LogP and Bond Information Content index (BIC) were the important parameters related to MRT. As a result of the feature selection, artificial neural network model for MRT prediction was constructed with two structural descriptors with high generalization capability verified by cross-validation and external validation. This model is applicable for estimating terminal $t_{1/2}$ because it was able to predict from MRT with a high accuracy in opioids.

C-4-04

Proposal of Toxicity Risk Index (TRI) for Warning Idiosyncratic Drug Toxicity

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Keywords: Pharmacokinetics, Toxicokinetics, Black box warning, Drug toxicity, Covalent binding, Idiosyncratic drug toxicity

Drug toxicity impedes not only its clinical use but also drug development. In the present study, a toxicity risk index (TRI), which is an index for warning idiosyncratic drug toxicity (IDT), was devised. The TRI of drugs was defined as a function of dose, pharmacokinetic parameters, and toxicokinetic data from covalent binding experiment. Twenty drugs classified into three categories by a report [1], were studied with TRI. The three categories were BBW (drugs with a block box warning for IDT), WNG (drugs without a black box warning but with a warning for IDT) and SAFE (drugs without any warning). The TRIs of drugs classified as SAFE were distinctly different from those classified as BBW. The TRI of SAFE drugs were lower than 0.456 (nmol/mg protein). In contrast, the TRI of the BBW drugs were higher than 1.10 (nmol/mg protein). These results warned us that a drug candidate, where the TRI is higher than 1.0 nmol/mg protein, should be categorized as a BBW drug. Thus, TRI may be useful for decision-making in drug development and its clinical use, and is proposed for warning IDT. Further study is now going on for obtaining a cut-off value with a statistical meaning.

[1] Nakayama, S., Atsumi, R., Takakusa, H., Kobayashi, Y., Kurihara, A., Nagai, Y., Nakai, D., Okazaki, O., A zone classification system for risk assessment of idiosyncratic drug toxicity using daily dose and covalent binding, *Drug Metabolism and Disposition*, 37: 1970-1977, 2009.

C-4-05

Improvements to the Cluster Newton Method for Underdetermined Inverse Problems - Parameter Identification for Pharmacokinetics-

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Keywords: Cluster Newton method; Underdetermined inverse problem; Beta distribution; Pharmacokinetics Electronic structure calculation, Ligand docking, Protein modeling

The Cluster Newton method (CN method) has proved to be very efficient at finding multiple solutions to underdetermined inverse problems. In the case of pharmacokinetics, underdetermined inverse problems are often given extra constraints to restrain the variety of solutions. In this paper, we propose an algorithm based on the two parameters of the Beta distribution to find families of solution near a solution of interest. This allows for a much greater control of the variety of solutions that can be obtained with the CN method. In addition, this algorithm facilitates the task of obtaining pharmacologically feasible parameters. Moreover, we also make some improvements to the original CN method including an adaptive margin of error for the perturbation of the target values and the use of an analytical Jacobian in the resolution of the forward problem.

[1] Aoki, Y., Hayami, K., De Sterck, H., and Konagaya, A., Cluster Newton method for sampling multiple solutions of an underdetermined inverse problem: Parameter identification for pharmacokinetics, *SIAM Journal on Scientific Computing* (accepted for publication) (Preliminary version available as *NII Technical Report*, NII-2011-002E, National Institute of Informatics, Tokyo, 2011, at http://www.nii.ac.jp/TechReports//11-002E.html.)

[2] Gaudreau, P., Hayami, K., Aoki, Y., Safoui, H., and Konagaya, A., Improvements to the cluster Newton method for an underdetermined inverse problem,(submitted, also to appear as a *NII Technical Report*).

C-5-01

New Tools for HT Engineering, Cloning & Expression of Multispecific Next-generation Antibodies

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Keywords: antibody screening, research workflow optimization, data management, automation, hit-selection, high-throughput, in-silico engineering/reformatting, humanization, database

Next-generation antibodies promise greater clinical efficacy and safety for a variety of severe human diseases. As a consequence, today's biotechs are faced with generating and testing vastly increased numbers of diverse alternative antibody molecule formats. A significant bottleneck is the establishment of high-throughput molecular biology and cloning processes required for systematically generating and validating DNA constructs to express the desired molecules. Here, we present a new workflow system for addressing the specific challenges for these antibody molecules produced in large-scale engineering campaigns. The diversity of new molecule formats (e.g. bi- and n-specifics: KinH BsIgG, Fab-scFv, IgG-scFv, Dual V domain IgG, etc.) and relevant cloning strategies pose workflow challenges that require tailored in silico cloning and automation tools for molecule design, DNA synthesis and verification; supporting expression and purification sample management; as well as an integrated processing of assays and analytics testing results. We present concrete examples of next-generation antibody workflows using the Genedata Biologics™ platform, including the high-throughput engineering of bispecifics.

Genedata Biologics™ is the first fully integrated workflow platform for comprehensively supporting the biologics R&D process, including that of next-generation antibodies. The focus of the platform lies on supporting complex laboratory workflows around molecule generation, with its built-in biologics-specific business logic, integrated data management and automation.

[END]

C-5-02

Installation of orthogonality to the interface that assembles two modular domains in the *Tetrahymena* group I ribozyme

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Keywords: Ribozyme, GNRA Tetraloop, Tetraloop Receptor, Kissing Loop

Two modular elements (P5abc and ΔP5) in the *Tetrahymena* group I ribozyme can be separated physically to generate a two-piece ribozyme derivative consisting of a separately prepared P5abc (P5 RNA) and the rest of the intron (ΔP5 RNA) [1]. Molecular recognition in the interface assembling P5 RNA and ΔP5 RNA is strong and specific, and the catalytic ability of the two-piece ribozyme is comparable to that of the parent unimolecular ribozyme [2].

In this study, we designed alternative tertiary interactions participating in the assembly of P5 and ΔP5. Using alternative interactions, orthogonality was successfully introduced into the P5/ΔP5 assembly interface. The artificial P5/ΔP5 assembly interface would expand the utility of the bimolecular complex as a structural platform for RNA nanotechnology and RNA synthetic biology [3].

[1] van der Horst, G., Christian, A., and Inoue, T., Reconstitution of a group I intron self-splicing reaction with an activator RNA, *Proc. Natl. Acad. Sci. USA.*, 88:184-188, 1991.
[2] Doherty, E.A., Herschlag, D., and Doudna, J.A., Assembly of an exceptionally stable RNA tertiary interface in a group I ribozyme, *Biochemistry*, 38:2982-2990, 1999.
[3] Tanaka, T., Furuta, H., and Ikawa, Y., A two-piece derivative of a group I intron RNA as a platform for designing self-assembling RNA templates to promote peptide ligation, *J. Nucleic Acids*, 2012, art. ID 305867, 2012.

C-5-03

Robust in vitro affinity maturation strategy based on interface-focused high-throughput mutational scanning

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Keywords: Protein engineering; Affinity maturation; Mutation scanning; High-throughput sequencing; Ribosome display

Abstract
Development of protein therapeutics or biosensors often requires in vitro affinity maturation. Here we report a robust affinity engineering strategy using a custom designed library. The strategy consists of two steps beginning with identification of beneficial single amino acid substitutions then combination. A high quality combinatorial library specifically customized to a given binding-interface can be rapidly designed by high-throughput mutational scanning of single substitution scanning libraries. When applied to the optimization of a model antibody Fab fragment, the strategy created a diverse panel of high affinity variants. The most potent variant achieved a 2110-fold affinity improvement to an equilibrium dissociation constant (Kd) of 3.45 pM with only 7 amino acid substitutions. The method should facilitate affinity engineering of a wide variety of protein-protein interactions due to its context-dependent library design strategy.

[1] Fujino,Y., Fujita, R., Wada, K., Fujishige, K., Kanamori, T., Hunt, L., Yoshihiro, S., Takuya, U., Robust in vitro affinity maturation strategy based on interface-focused high-throughput mutational scanning. Biochem. Biophys. Res. Commun. 428:395-400, 2012

C-5-04

In silico analysis of interactions between the ingredient molecules contained in cold medicines and HLA-A*02:06

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Keywords: adverse drug reactions, Stevens-Johnson syndrome, cold medicine, docking simulations, HLA-A*02:06

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe drug hypersensitivities with high mortality. Typical over-the-counter drugs of cold medicines are suggested to be causative. As multiple ingredients are generally contained in cold medicines, it is of particular interest to investigate which ingredients are responsible for SJS/TEN. However, experimental examination of causal relationships between SJS/TEN and a particular drug molecule is not straightforward. Significant association between HLA-A*02:06 and SJS/TEN with severe ocular surface complications has been observed in Japanese. We have undertaken *in silico* docking simulations between the HLA-A*02:06 molecule and various ingredients contained in cold medicines available in Japan. We use the composite risk index (CRI) that is the absolute value of the binding affinity multiplied by the daily dose to assess the potential risk of the adverse reactions. The drugs which have been recognized as causative drugs of SJS/TEN in Japan have revealed relatively high CRI and the association between SJS/TEN and HLA-A*02:06 has been qualitatively verified. The results have also shown that some drugs whose links to SJS/TEN have not been clinically recognized in Japan show the high CRI and suggested that attention should be paid to their adverse drug reactions.

Analysis of the Drug Prescribing Patterns of Physicians in Patients with both Cardiac and Renal Failure

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Keywords: Clinical data analysis, Physician prescribing patterns, Markov decision process

Cardiac and renal functions are physiologically related through interactions between systemic circulation regulation by the heart and extracellular fluid volume control by the kidney. Due to the increase in the number of patients with hypertension and/or diabetes, the incidence of coexistent cardiac failure and renal failure is increasing. However, evidence for the establishment of guidelines for treating patients with coexistent cardiac and renal failure is currently insufficient because patients with such complications are generally excluded from clinical trials. As a result, such patients are generally treated on an empirical basis.

In the present study, we analyzed clinical data to clarify the current medical treatment process in hospital. We evaluated the medical records of patients with cardiac failure from the database of The University of Tokyo Hospital. We modeled a series of treatments by physicians using the Markov decision process (MDP), wherein the laboratory data of the patient were employed as the state and the following drug prescription by the physician was employed as the action.

We identified the prescribing patterns of physicians according to the cardiac and renal state of the patients. Moreover, we also determined the overall trend of the subsequent state as a result of each prescription. Although most drugs were used under the recommended conditions according to the package inserts, these guidelines were not followed for certain other drugs. Only the present and subsequent states were considered in this study, but we are planning to perform further studies to identify more specific prescribing patterns according to the series of past states and changes in the trend of states.

[1] Bongartz, L.G., Cramer, M. J., Doevendans, P. A., Joles, J. A. and Braam, B., The severe cardiorenal syndrome: ‘Guyton revisited’, *Eur Heart J*, 26:11-17, 2005.
[2] Shlipak, M. G., Pharmacotherapy for heart failure in patients with renal insufficiency, *Ann Intern Med*, 138:917-924, 2003.
[3] Asoh, H., *et al.*, Modeling medical records of diabetes using Markov decision processes, *Proceedings of the 30th International Conference on Machine Learning*, ICML, 28, 2013.
[4] Bellman, R., A markovian decision process, *Journal of Mathematics and Mechanics*, 6:679-684, 1957.

C-5-06

Biophysical Approach of Fragment Screening to inhibit the heme transfer system of Pathogenic bacteria

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Keywords: Pathogenic bacteria, Heme transfer system, PPI, FBDD

Acquisition of iron from host sources is of vital importance to many pathogenic bacteria during the course of infection. The process of obtaining heme from the host by Gram-positive pathogens often involves binding of heme or hemoproteins by bacterial receptor proteins which then deliver the heme to a membrane transporter for translocation to the cytoplasm [1]. Interestingly, several pathogenic species of Gram-positive bacteria display surface proteins implicated in heme capture that contain more than one NEAT domain [2]. The heme capture protien has NTD, NEAT1, and NEAT2 domains. NTD has a hemoglobin-binding ability and both NEAT1 and NEAT2 has a heme-binding ability. However, their detail analysis and interaction among them is not done. We have studied the interaction with hemoglobin and its heme transfer system of Shr, and revealed that NTD can be a candidate of the target protein to inhibit the heme transfer.

Here we carried out the fragment-based drug screening to inhibit the interaction between hemoglobin and NTD. First hit screening of about 2000 compounds fragment library were conducted in surface plasmone resonance (SPR). After the 124 hit candidates were obtained in SPR screening, the competition assay of the candidates were carried out in SPR and we obtained 21 compounds that inhibit the interaction between hemoglobin and NTD. To validate the quality of the selected compounds in SPR, dose-dependent binding assay to NTD in SPR and thermal inhibition assay in isothermal titration calorimetry (ITC) were conducted [3]. In these hit validation, we obtained consequently some compounds that can inhibit specifically the PPI by binding to NTD.

[1] Nobles, C.L. and Maresso, A.W., The theft of host heme by Gram-positive pathogenic bacteria, *Metallomics*, 3:788-796, 2011.
[2] Andrade, M.A., Ciccarelli, F.D., Perez-Iratxeta, C. and Bork, P., NEAT: a domain duplicated in genes near the components of a putative Fe3+ siderophore transporter from Gram-positive pathogenic bacteria, *Genome Biology*, 3:research0047, 2002.
[3] Kobe, A., Caaveiro, J.M., Tashiro, S., Kajihara, D., Kikkawa, M., Mitani, T. and Tsumoto, K., Incorporation of rapid thermodynamic data in fragment-based drug discovery, *Journal of Medicinal Chemistry*, 56:2155-2159, 2013.

C-5-07

Inhibitor Screening of Capsular Polysaccharide Synthesizing Enzyme CapF from *Staphylococcus aureus*

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Keywords: FBDD, Enzyme inhibition, *Staphylococcus aureus*, SPR, ITC

The enzyme CapF is essential for synthesizing capsular polysaccharide of *Staphylococcus aureus*, and reduces its substrate by oxidizing NADPH [1,2]. Interestingly, the affinity of CapF for NADPH is approximately 100-hold higher than that for $NADP^+$. In addition, it has been reported that NAPDH binding to Ps3αHSD [3] or SDRvv [4] form helical structures, that have a similar function of the enzyme activity in CapF. Therfore, we had a hypothesis that the recognition ability of CapF is caused by its loop structure of CapF near the NADPH-binding site of one. Here, we performed a small-compoud screening to search a inhibitor for synthesizing capsular polysaccharide by regulating the significant recognition ability of CapF based on the hypothesis.

To find the specific binder to CapF, a hit screening based on a Fragment library (FBDD) is carried out using surface plasmon resonance and enzyme activity assay. We obtained some compounds in each screening assay. These compounds were characterized by the some assays such as does-dependent binding assays and calorimetric analyses.

[1] Miyafusa, T., Caaveiro, J.M., Tanaka, Y. and Tsumoto, K., Crystal structure of the enzyme CapF of *Staphylococcus aureus* reveals a unique architecture composed of two functional domains, *Biochemical Journal*, 443:671-680, 2012.

[2] Miyafusa, T., Caaveiro, J.M., Tanaka, Y., Tanner, M.E. and Tsumoto, K., Crystal structure of the capsular polysaccharide synthesizing protein CapE of *Staphylococcus aureus*, *Bioscience Reports*, 33:463-474, 2013.

[3] Buysschaert, G., Verstraete, K., Savvides, S.N. and Vergauwen, B., Structural and biochemical characterization of an atypical short-chain dehydrogenase/reductase reveals an unusual cofactor preference, *The FEBS Journal*, 280:1358-1370, 2013.

[4] Nakamura, S., Oda, M., Kataoka, S., Ueda, S., Uchiyama, S., Yoshida, T., Kobayashi, Y. and Ohkubo, T., Apo- and holo-structures of 3alpha-hydroxysteroid dehydrogenase from Pseudomonas sp. B-0831. Loop-helix transition induced by coenzyme binding. *The Journal of Medicinal Chemistry*, 281:31876-31884, 2006.

C-5-08

Functional analysis of Liposome binding peptide selected by cDNA display

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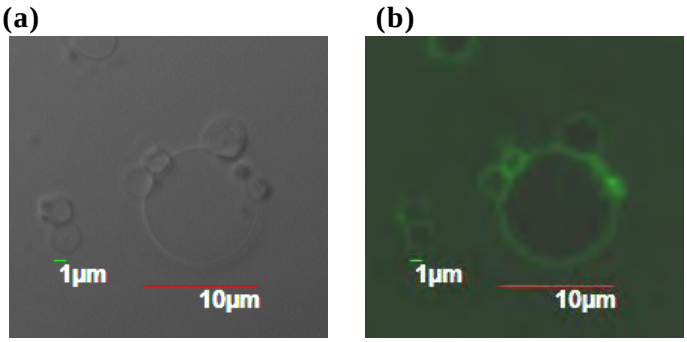
Keywords: mRNA/cDNA display, Liposome membrane-binding peptide, *In vitro* selection

cDNA display is a genotype-phenotype linking method that a peptide is conjugated with its encoded cDNA via a puromycin-linker using a cell-free translation system [1]. cDNA display enables to perform *in vitro* selection from a library of 10^{12} peptide molecules. We previously obtained some kinds of high-affinity peptide aptamers against interleukin-6 receptor by this method [2]. In this study, we tried in vitro selection to obtain peptides that bind with liposome which is artificial lipid bilayer from random peptide (30 residues in length) library. After six rounds of selection, the random sequences have been converged a unique consensus amino acids sequence. Then, we designed and synthesized a peptide modified a fluorescein at its N-terminus by chemical-synthesis and examined whether the peptide can interact with liposome membrane with a confocal laser scanning microscopy. Interestingly, the interaction between the peptide and liposome might depend on the method of preparing liposome. In this presentation, we will show the results and discuss about the interaction between the peptide and liposome in detail.

Fig.1 Interaction between peptides and liposome.

Giant unilamellar liposomes were prepared with dioleoylphosphatidylcholine (DOPC) and incubated peptides (6μM) with for 2h at 25℃.
(a)Differential interference contrast (DIC) image of liposomes.
(b) Fluorescence microscopy image of liposomes (with fluorescent dye labeled peptides). The image was taken from a confocal laser scanning microscopy.



[1] Mochizuki, Y., *et al*., *ACS Comb. Sci*., **13**, 478-485 (2011)
[2] Nemoto, N., *et al*., *Biochem Biophys Res Commun*., **421**, 129-133 (2012)

C-5-09

The CADU Alliance N3 Project

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Keywords: CBI, Quantum Computing, Open Courseware, Education of Genomics

The history of the Chem-Bio Informatics Society goes back to 1981. The term Chem-Bio Informatics (CBI) had been coined to cover various interdisciplinary areas for studying the effects of chemicals on biosystems at the molecular level. Since then, drug discovery has been the most attractive research area of the CBI, and over the years, it has been a force for the advancement and sophistication of computational methods, programming systems, databases, knowledge-bases, and arts of applying these methodologies and computational resources. Yet now it seems that this discipline has lost its vital energy to offer good new jobs, to attract investors, and to draw the attention of the general public.

We propose new frontiers, which should need new professionals as a workforce, and new partnerships for cultivating these frontiers in order to revitalize this discipline. First, we propose that since CBI consists of such interdisciplinary areas as natural sciences, informatics, and computing, it is natural to include physics here. Although information and computing had not been subjects of physics for a long time, the emerging new fields of quantum information and quantum computing have drastically changed this situation. It is time to consider, for example, computational chemistry simulation by quantum computers. Second, we propose to include applications of smart phones or tablets, and cloud services to CBI. Some examples were well discussed in several papers. Third, we propose to pay special attention to educating many professionals working in health and medical fields.

The last proposal must be considered most seriously, since such educational needs are urgent and tremendously important. It is urgent because very rapid advances of sequencing technology are drastically reducing the costs and time required for genetic and genomic testing so that such tests, which require some genomic competency, may be accepted in any service sectors in health and medical cares. This means that, not only basic researchers in clinical medicine, especially those who are dealing with cancer, but also many clinical professionals such as primary care physicians, nurses, pharmacists, nutritionists, and others must improve their competencies and literacy of genomics. There will be a need to recruit many bioinformaticians to take part in this task. Moreover such a career extension might open new chances for them. Open courseware based on the Internet would be a very strong tool for this purpose.

Right now we are proposing to implement some of these proposals in the context of activities of the CADU Alliance. We hope such efforts might contribute to revitalize this discipline.

C-5-10

Light-induced membrane potential regulates bone cell function; development of the light-responsive bone cells

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Keywords: ion channel, osteoblast, calcium

The PTH and VD_3 are not only osteogenic reagents but also major regulator for osteoclastogenesis. These molecules transiently increase bone resorption by increment of the expression level of RANKL, whereas their mechanism of RANKL-intracellular transportation (RANKL-iTP) remains unclear. The RANKL-iTP depends on lysosomal vesicles whose fusion is related to increment of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) following depolarizing membrane potential (MP). Thus, MP could regulate osteoblastic function and RANKL-iTP. So far little is known about the role of MP on bone cells, because it is hard to control temporal patterns of MP. Here, by novel system of light-responsive osteoblastic MP, we show that the transiently increasing $[Ca^{2+}]_i$ following depolarization regulates PTH- and VD_3- induced RANKL-iTP.

In membrane fraction of RANKL-GFP expressed MC3T3-E1 cell, PTH and VD_3 increase membrane-bound RANKL (mbRANKL) at 10 min (more than 300% increment compared to control), however those mRNA expression level were unchanged at same time. Confirming by time-lapse imaging, the addition of PTH and VD_3 translocated RANKL-GFP to cell membrane within 10 min. In previous reports, PTH and VD_3 transiently increase $[Ca^{2+}]_i$ following depolarization. The $[Ca^{2+}]_i$ spike was diminished by depolarization-activated calcium channel blocker (Diltiazem: Dil) or ER-dependent $[Ca^{2+}]_i$ blocker (Thapsigargin: Tg). The increment of mbRANKL by PTH and VD_3 was also decreased by Dil and Tg. These results showed that depolarization-dependent change of $[Ca^{2+}]_i$ affected PTH- and VD_3-induced RANKL-TPi.

To address whether depolarization controls RANKL-TPi, we developed a novel system of light-responsive osteoblastic MP by channel-rhodopsin wide receiver (ChWR). MPs in ChWR stably expressed cell were depolarized immediately (<20 msec) after light-stimulus (ΔMP: 16.3 ± 1.1 mV) and were returned to the pre-stimulus potential after the off-stimulus. Light induced depolarization increase $[Ca^{2+}]_i$ and mbRANKL at 10 min in similar to addition of PTH and VD_3. In co-culture of RAW267 and RANKL-GFP expressed MC3T3-E1 cell, TRAP activities were increased 4- fold by light stimulus and the development of mature osteoclasts was observed.

We showed that depolarizing MP is related to osteoblastic function. Our results indicates that RANKL-iTP is regulated by $[Ca^{2+}]_i$ following depolarization of MP. The mechanism of PTH- and VD_3-induced RANKL-iTP would have benefits for developing PTH- and VD_3-like medicine.

C-5-11

E. coli Host Engineering for Efficient Enzyme Discovery

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Keywords: *E. coli*, Ribosome, Functional Metagenomics

The bacterial ribosome consists of three rRNA molecules and 57 proteins and plays a crucial role in translating mRNA-encoded information into proteins. Because of the ribosome's structural and mechanistic complexity, it is believed that each ribosomal component coevolves to maintain its function. Unlike 5S rRNA, 16S and 23S rRNAs appear to lack mutational robustness, because they form the structural core of the ribosome. However, using *E. coli* Δ7 (null mutant of operons) as a host, we have recently shown that an active hybrid ribosome whose 16S rRNA has been specifically substituted with that from non–*E. coli* bacteria can be reconstituted *in vivo*.

To investigate the mutational robustness of 16S rRNA and the structural basis for its functionality, we used a metagenomic approach to screen for 16S rRNA genes that complement the growth of *E. coli* Δ7. Various functional 16S rRNA genes were obtained from the Gammaproteobacteria and Betaproteobacteria lineages (with minimal identity of ~80%) [1].

Based on this discovery, we investigated how ribosomal mutation affects gene expression. To this end, we used a series of reporter genes that are poorly expressed in native *E. coli* host. They were then introduced into mutant *E. coli* library that carry metagenomically retrieved 16S rRNA genes, and the expression levels were evaluated. As a result, some mutants expressed gene more efficiently than the native *E. coli* host. Thus ribosome engineering may be a feasible approach to solving the expression problem in metagenomic library screening, and should accelerate the enzyme discovery process [2,3].

[1] Kitahara, K., Yasutake, Y., and Miyazaki, K. Mutational robustness of 16S ribosomal RNA, shown by experimental horizontal gene transfer in *Escherichia coli*. *Proceedings of the National Academy of Sciences USA* 109:19220-19225, 2012
[2] Hosokawa-Okamoto, R., and Miyazaki, K. *Escherichia coli* host engineering for efficient metagenomic enzyme discovery. *in* "Metagenomics: Current Innovations and Future Trends" (ed. Diana Marco, pp. 241-252, Horizon Scientific Press), 2011
[3] Uchiyama, T., and Miyazaki, K. Functional metagenomics for enzyme discovery: challenges to efficient screening. *Current Opinion in Biotechnology* 20:616-622, 2009

C-5-12

Methylome diversification through changes in the sequence specificity of DNA methyltransferases

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Helicobacter pylori, a human gastric pathogen, has a large number of DNA methyltransferase genes with each strain carrying a unique repertoire. Previous genome comparison works suggested that these methyltransferases often change DNA sequence specificity through movement of amino-acid sequences in the target recognition domains between genes and within a gene (Domain Movement). By Single-Molecule Real-Time sequencing technology, we detected methylated DNA sites throughout several closely related genomes. Number of methylated sites varied between strains and had correlation with number of methyltransferase genes in each strain. We successfully deduced DNA sequence motifs for methylation and assigned each of them to a specific amino-acid sequence group of target recognition domains in the specificity determinant genes. Overall, the methylome turned out to be quite variable among the closely-related strains, although there are hypermethylated loci conserved in all the strains. Hypomethylated sites were also found on genomic island regions. As expected from their effects on gene expression, knockout of a specificity gene led to changes in the transcriptome. These results provide evidence for proposed mechanisms of sequence-specificity change in the DNA methyltransferases and lend support to the concept of epigenetics-driven adaptive evolution.

C-5-13

Site-specific Cellular Modification by Cre-incorporating Integrase-defective Retroviral Vectors

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Keywords: Integrase-defective retroviral vectors, Cre/*loxP,* Site-specific cellular modification

Retroviral vectors have been employed in various biotechnology fields such as gene therapy [1], generation of transgenic animals [2] and induced pluripotent stem cells (iPSCs) [3]. However, the nearly random integration property may cause problems such as insertional mutagenesis and gene silencing. Therefore, the development of targeted transgene integration system is desired for modification of retroviral vectors. We have achieved retroviral insertion of transgene into a pre-determined site of chromosome in CHO cells using integrase-defective retroviral vectors (IDRVs) and Cre-recombinase mediated cassette exchange (RMCE) [4]. In this case, founder cells were transfected with a Cre expression plasmid prior to retroviral transduction. To apply site-specific gene modification for hard-to-transfected cells, it is desired to encapsulate both gene of interest and Cre protein into the retroviral virion. In this study, we attempted to generate novel hybrid IDRVs, which can deliver both viral genome and enzymatically active Cre.

First, we designed a fusion protein-expression vector (pGPM-Cre), in which a Cre gene was inserted into the gag-pol precursor genes (pGPM). Cre-incorporating IDRV particles (Cre-IDRV) were produced by 293FT cells co-transfected using IDRV producing plasmids (pQMSCV/NE, pGPM and pVSV-G) and a pGPM-Cre plasmid. Western blot analysis revealed that Cre was incorporated into retroviral virions. A Cre-IDRV encoding marker genes (*ATG-deleted-Neo*^{r}*/IRES/EGFP*) flanked by wild-type and mutant loxP sites was infected into founder CHO cells (CHO/F17), in which a single copy of an expression unit containing a CMV promoter, an ATG codon and marker genes (*ATG-deleted-Hyg*^{r}*/IRES/DsRed*) flanked by corresponding *loxP* sites was introduced into the genome. We observed appearance of G418-resistant colonies expressing GFP. Moreover, site-specific integration of transgene was confirmed by PCR and sequence analyses. These results indicate that bioactive Cre protein was incorporated into virions and site-specific recombination between retroviral DNA derived from IDRV and cellular genome was mediated by Cre-RMCE. The Cre-IDRV system may expand the applicability of retroviral vectors.

[1] Qaism *et al*., Progress and prospects: Gene therapy for inherited immunodeficiencies. *Gene Therapy,* 16:1285-1291, 2009.
[2] Kamihira M *et al*., Production of chimeric monoclonal antibodies by genetically manipulated chickens. *Journal of Biotechnology*, 141:18-25, 2009.
[3] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126:663-676, 2006.
[4] Huang S *et al*., Cre recombinase-mediated site-specific modification of a cellular genome using an integrase-defective retroviral vector. *Biotechnology and Bioengineering*,107:717-729, 2010.

C-5-14

Construction of artificial sensor to regulate gene expression in the cyanobacterium *Synechocystis* sp. PCC 6803

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Keywords: Chimeric sensor; Environmental response; Ethylene; Histidine kinase

The cyanobacterium *Synechocystis* sp. PCC6803 is a model photosynthetic prokaryote, and is increasingly being recognized as a promising resource in the production of useful substances such as bioactive compounds [1] and biofuels [2], by genetic manipulations. Thus, it is inevitable that researchers will develop artificial systems to regulate gene expression.

Two-component signal transduction system is a major gene expression system in *Synechocystis*. One of the components of this system, a histidine kinase (Hik), includes two portions, a signal-input domain and a kinase domain, and linker domain between them. Substitution of the signal-input domain of a Hik with that from another Hik may alter the stimulus perceived by the former Hik to the later Hik [3].

In this study, we substituted the signal-input domain and the linker domain of SphS, a phosphate-deficient sensor, with those from ethylene sensors from *Arabidopsis thaliana*, i.e. ETR1, ETR2, ERS1, ERS2, and EIN4, to construct ethylene-responsible sensor. The activity of resulting five chimeric sensors was evaluated by the activity of alkaline phosphatase (AP), originally regulated by SphS.

Results showed that the cells carrying ERS1-SphS or ERS2-SphS did not expressed AP activity, whilst cells carrying ETR1-SphS, ETR2-SphS, or EIN4-SphS expressed AP activity under standard conditions. However, none of the chimeric constructs responded to ethylene treatment.

Then, we deleted 1 to 7 amino acid residues in the linker domain of ETR1-SphS to construct functional sensor. These linker-deleted mutants showed no responses to ethylene, but periodic changes in AP activity were shown corresponded to the number of deleted amino acids.

Currently, our experiments are focused on determining the suitable conditions under which the chimeric sensors will respond to ethylene in the cyanobacterial cells.

[1] Guerrero, F., Carbonell, V., Cossu, M., Correddu, D., Jones, PR., Ethylene Synthesis and Regulated Expression of Recombinant Protein in *Synechocystis* sp. PCC 6803, *Plos ONE*, 2012
[2] Schirmer, A., Rude, MA., Li, X., Popova, E., del Cardayre, SB., Microbial Biosynthesis of Alkanes, *Science*, 2010
[3] Shimura, Y., Shiraiwa, Y., Suzuki, I., Characterization of the Subdomains in the N-Terminal Region of Histidine Kinase Hik33 in the Cyanobacterium *Synechocystis* sp. PCC 6803, *Plant Cell Physiol.*, 2012

C-5-15

Development of an ON/OFF-transcriptional control system for plural genes to express sequentially

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Keywords: Sigma factor, transcription, stress response

The gene expression in either a bacterial cell or a eucaryotic cell is important bio-technology performed frequently. Generally expression is induced by adding an inducer, which is usually hard to be inactivated or removed, and depression after induction is difficult.

Sigma factors rule the DNA-binding specificity of RNA polymerase to the promoter, and are a conserved protein family among bacteria. The protein family of sigma factors is further divided into a subfamily, Extra-Cytoplasmic Function (ECF) sigma factors. The activity of ECF sigma factors is regulated by binding specifically to cognate anti-sigma factor proteins on a simple principle by protein-protein interaction. Anti-sigma factor proteins localize to the cytoplasmic membrane and response to various external and environmental stress.

ON/OFF control of gene expression is thought to be performed reversibly by some environmental stress (temperature, pH, the oxidation state, etc.). We have tried to use these plural sigma-anti-sigma control systems in order to control a lot of gene clusters consecutively.

In *Bacillus subtilis*, a Gram-positive sporulating soil bacterium, seven ECF sigma factors have been identified. The SigW protein is a well-studied ECF sigma factor in Bacillus subtilis. It is regulated by anti-sigma factor, RsiW. Their coding genes constitute an operon, whose transcription is induced in response to various cell-wall antibiotics, alkaline shock, and other stresses affecting cell-envelope homeostasis. SigM, is strongly involved in cell envelope integrity and regulated with YhdL and YhdK anti-sigma proteins. Expression and activity of SigM are elevated under acid, heat, salt, superoxide and cell envelope stresses.

We investigated whether the SigW and SigM could be used for ON/OFF control system by using pH exchange of medium used for *B. subtilis* cell culturing.

C-5-16

Which has a better chemotaxis controller, *E. coli* or *Paramecium?*

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Keywords: chemotaxis, *E. coli*, *Paramecium*, *control performance*

In this presentation, we briefly review our recent result on the performance of the internal controllers generating the chemotaxis [1]. We show two performance indeces to capture the chemotaxis. Based on them, the performance is evaluated for two controller models, which are of model organisms for the chemotaxis, *Escherichia coli* and *Paramecia*. It is disclosed that the coli-type controller achieves the chemotaxis fast but roughly, while the paramecium-type controller achieves it slow but precisely.

[1] S. Azuma, *et al.*, Performance Analysis of Chemotaxis Controllers, *52nd IEEE Conference on Decision and Control, to appear* (2013)

C-5-17

t-Riboregulator: Regulation of Nonsense Suppression by Modulating 3' Processing of Suppressor tRNA

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Keywords: Riboregulator, Suppressor tRNA, Nonsense suppression, tRNA processing

Recently, we rationally evolved in-vitro-transcribed, unmodified, and non-aminoacylated amber suppressor tRNAs toward higher suppression efficiency in wheat germ extract (WGE).[1] The artificial evolution was conducted in the following four steps: [1st] anticodon alteration of natural tRNAs; [2nd] chimerization of efficient suppressors in the first generation; [3rd] investigation and optimization of the effective parts in the second generation; [4th] combination of the optimized parts in the third generation. The suppression efficiency of the suppressor obtained in the last generation was approximately 60% (85% in the presence of an eRF1 aptamer), which was 2.4-fold higher than that of the best suppressor in the first generation.

In this study, we employed this highly evolved suppressor and an amber-mutated gene to evaluate tRNA processing, especially terminal (5' and 3') processing, in WGE. Specifically, we prepared various kinds of terminal-premature tRNAs based on the evolved suppressor and compared their suppression efficiencies to that of the mother tRNA. As a result, 3' processing including CCA addition was found to rapidly proceed in WGE in contrast to extremely slow 5' processing. However, 3' processing was effectively inhibited by distorting the tRNA structure with a distortion-inducing sequence in the 3' end. We therefore used the structure-distorted suppressor to develop a novel gene regulation system, named "t-riboregulator", wherein nonsense suppression (i.e., expression of full-length protein) is controlled by a modulator (nucleic acids) that restores the tRNA structure and the 3' processing activity of the structure-distorted suppressor. The t-riboregulator system is expected to be used as an alternative of or in combination with normal mRNA-based riboregulators or other gene regulators such as riboswitches to construct artificial gene circuits in synthetic biology.

[1] Ogawa, A., *et al*., Improvement of *in vitro*-transcribed amber suppressor tRNAs toward higher suppression efficiency in wheat germ extract, *Organic & Biomolecular Chemistry*, 9 : 8495-8503, 2011.

C-5-18

DNA Modification Enzymes Utilizing Sequence-Specificity of Zinc Finger Domains

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Keywords: DNA binding, DNA methylation, Gene editing

Artificial zinc finger proteins (ZFPs) consist of Cys_2-His_2-type modules composed of about 30 amino acids with a ββα structure that coordinates a zinc ion. ZFPs that recognize specific DNA target sequences can substitute for the binding domains of enzymes that act on DNA to create designer enzymes, such as nucleases, recombinases [1], and methylases [2], with programmable sequence specificity. Genome editing and modification by the enzymes could be applied for many fields of basic research and medicine. Some zinc finger nucleases (ZFN) are currently in human clinical trials that aim at therapeutic gene editing. In this presentation, ZFN pairs targeting promoter region of human telomerase reverse transcriptase (hTERT) were designed and constructed. Expressed zinc fingers were purified in vitro and the DNA binding properties were evaluated. Highly active zinc fingers were utilized for ZFN binding domain. The DNA cleavage of ZFNs was performed in vitro. Furthermore, the digestion of promoter region in the endogenous sequences was successfully confirmed. The telomerase activity in the cell treated by ZFN was reduced suggesting the digestion by ZFN affects to the expression of hTERT. There are few reports about the strategy with ZFN targeting the promoter region of the target gene. The present results give a new insight into the gene targeting and editing by sequence-specific nucleases. In addition, our current accomplishments on zinc finger enzymes, such as the zinc finger recombinase (ZFR) design and ZFN targeting replication sites of EB virus, will be presented.

[1] Nomura, W., Masuda, A., Ohba, K., Urabe, A., Ito, N., Ryo, A., Yamamoto, N., and Tamamura, H., Effects of DNA binding of zinc finger and linkers for domain fusion on catalytic activity of sequence-specific chimeric recombinases determined by a facile fluorescent system, *Biochemistry*, 51:1510-1517, 2012.
[2] Nomura, W., and Barbas, C. F., III, In vivo site-specific DNA methylation with a designed sequence-enabled DNA methylase, *J. Am. Chem. Soc.*, 129:8676-8677, 2007.

C-5-19

Discovery of a Potent and Selective FLT3 Kinase Inhibitor by Fragment Evolution

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Keywords: FLT3, acute myeloid leukemia, kinase inhibitor, fragment evolution

FLT3 (FMS-Like Tyrosine kinase 3), a member of class III receptor tyrosine kinase family, is a promising therapeutic target for acute myeloid leukemia (AML). Activating FLT3 mutations are found in approximately 30% of AML patients and associated to poor prognosis. To discover a novel FLT3 inhibitor, we performed a screening of our in-house fragment library. The screening hit was rapidly progressed to a potent and selective FLT3 inhibitor.

C-5-20

An approach to reconstruction of cell cycle oscillation of DnaA activity for replication initiation and transcription regulation

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Keywords: Chromosome replication, Cell cycle, *Escherichia coli*

In *Escherichia coli*, an initiation stage of chromosome replication is a main target to trigger cell cycle progression. DnaA initiator protein binds to chromosomal origin, *oriC*, leading to the initiation reaction. The DnaA activity is regulated by binding of ATP and ADP, and the cellular ratio of the ATP-bound form and ADP-form oscillates during cell cycle. The sliding clamp is a component of the replication machinery. Following replication initiation, the DNA-loaded sliding clamps accumulate behind replication forks [1]. The DNA-loaded clamp promotes hydrolysis of ATP bound on the active DnaA, yielding the inactive ADP-DnaA. Thus, the initiation reaction is regulated in a feedback manner. After completion of replication, the clamps are released from DNA and the feedback regulation of DnaA is withdrawn. Before the next round of replication initiation, the ratio of ATP-DnaA is re-accumulated. Chromosomal loci called DARS are involved in this re-accumulation by promoting a nucleotide exchange reaction from ADP-DnaA to ATP-DnaA. DnaA also serves as a regulator for transcription of several genes. In some of these genes, the transcription regulation activity of DnaA is known to be controlled by its ATP/ADP-binding. Thus, the oscillation cycle of DnaA activity seems to act globally for cell cycle progression. We present our approach to reconstruction of the oscillation cycle of DnaA activity by reproducing the dynamic behavior of the clamp.

[1] Su'etsugu, M. and J. Errington, J., The Replicase Sliding Clamp Dynamically Accumulates behind Progressing Replication Forks in *Bacillus subtilis* Cells, *Molecular Cell*, 41, 720-732, 2011

C-5-21

Photodynamic Effect of Single-walled Carbon Nanotubes and its potential for cancer phototherapy

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Keywords: Carbon nanotube, Photodynamic effect, Reactive oxygen species, Cancer cell killing

Single-walled carbon nanotubes (SWNTs) are known to be classified into two types, metallic and semiconducting ones (m-SWNTs, s-SWNTs), on the basis of their chiralities. SWNTs reveal photothermal (PTE) and photodynamic effects (PDE), which result in generation of heat and reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) and superoxide anion ($O_2^{\bullet -}$), respectively. While PTE of SWNTs has received much attention for cancer therapy, a recent report suggests that PDE of carbon nanotubes can be used for protein inactivation through photogeneration of superoxide anion[1]. In this study, we enriched m- and s-SWNTs by a gel chromatography and evaluated their PTE and PDE in detail. Under near-infrared laser irradiation, s-SWNTs generated both ROS much more efficiently than m-SWNTs, showing that s-SWNTs had higher PDE. For cell studies, s-SWNTs were successfully stabilized with a natural dispersant, high-density lipoprotein (HDL). HDL-stabilized s-SWNT did not show significant cytotoxicity, and caused photo killing of cancer cells through $^1O_2$ generation like other photosensitizers under near-infrared irradiation. This is the first example of observing cancer cell killing by photodynamic effect of SWNT.

[1] Joshi, A.; Punyani, S.; Bale, S. S.; Yang, H.; Tasciuc, T. B.; Kane, R. S. *Nat. Nanotechnol.* 2008, *3*, 41.

[2] Murakami, T.; Nakatsuji, H.; Inada, M.; Matoba, Y.; Umeyama, T.; Tsujimoto, M.; Isoda, S.; Hashida, M.; Imahori, H. *J. Am. Chem. Soc.* 2012, *134*, 17862.

C-5-22

Probabilistic Model Based Error Correction of Various Mutant Sequences Analyzed by the Single-Molecule Real-Time Sequencing

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Keywords: base call error; next generation sequencing; image restoration; quality score; quasispecies; sequence analysis; SMRT

To analyze the evolutionary dynamics of a mutant population in an evolutionary experiment, it is necessary to sequence a vast number of mutants by high-throughput sequencing technologies. Particularly, we focus on the single-molecule real-time (SMRT) sequencing technology [1], which enables rapid and parallel analysis of multikilobase sequences. However, the observed sequences as "circular consensus sequences (CCS)" obtained by the SMRT sequencing include many random errors of base call. Therefore, if the SMRT sequencing is applied to analysis of a heterogeneous population of various mutant sequences, it is necessary to discriminate between true bases as point mutations and random errors of base call in the observed sequences, and to subject the sequences to error-correction processes [2]. To address this issue, we have developed a novel method of error correction based on the Bayesian theory with the Potts model and a maximum a posteriori probability (MAP) estimation. The available information for error correction is (1) "quality scores" which are assigned to individual bases in the observed sequences [3] and (2) a spatial distribution of the observed sequences in sequence space [4]. The computer experiments of error correction of artificially generated sequences supported the effectiveness of our method, showing that 50-90 % of errors were removed. Interestingly, this method is analogous to a probabilistic model based method of image restoration developed in the field of information engineering [5].

[1] Eid J et al., Real-time DNA sequencing from single polymerase molecules. *Science*,**323**, 133-138, 2009.
[2] Zagordi O, Geyrhofer L, Roth V, Beerenwinkel N., Deep sequencing of a genetically heterogeneous sample: local haplotype reconstruction and read error correction. *J Comput Biol*.,**17**,417-428, 2010.
[3] Cock P.J., Fields C.J., Goto N, Heuer M.L. and Rice P.M., The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. *Nucleic Acids Research*, **38**, 1767–1771, 2010.
[4] Eigen M, Winkler-Oswatitsch R., Statistical geometry on sequence space. *Methods Enzymol.*, **183**, 505-530, 1990.
[5] Tanaka K. and Morita T., Cluster variation method and image restoration problem. *Physics Letters* A, **203**, 122-128, 1995.

C-5-23

Meta-synthetic metabolism: Identification of critical intermediates for synthetic amino acid derivative production

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Keywords: metabolic engineering, synthetic biology, database, chemoinformatics

The growing catalogue of known enzymes gives promise for creating new useful biological products by combining transgenes in vivo and engineering enzymes with novel catalytic capabilities [1, 2]. Amino acid production is part of the core metabolism of every free-living organism, but derivatives of the standard 20 amino acids are often produced as part of the 'secondary metabolism.' For example, many neurotransmitters are derivatives of standard amino acids, and therefore amino acid derivatives are rich targets for drug discovery. Toward engineering cell factories for the production of useful amino acid derivatives, we have explored the potential catalytic space of known enzymatic functions.

We began with the rich set of more than 11,000 amino acid-like compounds in the PubChem database [3], and using these as target molecules, calculated acceptable metabolic pathways for their synthesis from glucose. For this we used the annotated enzymes from the KEGG database [4], and identified reaction steps between compounds that are currently present in nature, along with enzymatic steps which might be easily engineered due to high chemical similarity between known and target compounds. We scored each reaction step by chemical similarity (with 1 being a known reaction in KEGG), and used these to rank the pathways for producing each of more than 3,000 compounds with identified synthetic pathways.

From the calculated synthetic pathways, we found several compounds which acted as 'critical intermediates' which participated in more than 15 reaction steps. These compounds act as intermediates in the synthesis of multiple identified amino acid derivatives. We also found many amino acid derivatives which could be synthesized by only a single reaction pathway, relying on a unique enzyme from KEGG for the critical reaction step. Along with these results, we present some of the interesting amino acids identified from the study, along with possible applications in industry.

[1] Nielsen, J., and Keasling, J.D., Synergies between synthetic biology and metabolic enginnering, Nature Biotechnology, Vol. 29:694-695, 2011.
[2] Lee, J.W., Na, D., Park, J.M., Lee, J., Chi, S., and Lee, S.Y., Systems metabolic engineering of microorganisms for natural and non-natural chemicals, Nature Chemical Biology, Vol. 8:536-546, 2012.
[3] http://pubchem.ncbi.nlm.nih.gov/
[4] http://www.genome.jp/kegg/

A Cellular Automaton Approach for Modeling Chemical Reactions in Protein Synthesis

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Keywords: Brownian Cellular Automaton, Protein Synthesis, Self-Reproduction

Self-reproduction is an important topic in the field of cellular automata (CAs), since the original CA model has been invented with the purpose to formalize biological self-reproduction [1]. Various self-reproducing machines on CAs have been proposed, such as universal constructors [1] and self-reproducing loops [2], resulting in the realization of simpler self-reproduction mechanisms [3,4]. However, there have hardly been investigations on self-reproduction that employs biologically realistic self-reproduction, such as chemical reactions in the protein synthesis.

This paper explores self-reproduction from a more biological point of view by modeling and simulating the chemical reactions in protein synthesis of biological systems. To this end we adopt a Brownian cellular automaton (BCA), which is a type of asynchronous CAs in which fluctuations drive operations. A BCA with an appropriately designed transition function can deal with both random processes, such as proteins flowing over an area, and sequential processes, such as synthesizing messenger RNAs [5]. There are four stages in the process of protein synthesis: the reproduction of DNA strands, the transcription from DNA strands to RNAs, the synthesis of messenger RNAs by a splicing process, and the synthesis of proteins. All these processes are modeled in this paper through the use of BCAs.

[1] Banks, E. R., Universality in cellular automata, *IEEE 11th Annual Symposium on Switching and Automata Theory*, 194–215, 1970.
[2] Langton, C. G., Self-reproduction in cellular automata, *Physica D*, 10:135–144, 1984.
[3] Serizawa, T., 3-state Neumann neighbor cellular automata capable of constructing selfreproducing machine, *IEICE Transaction Japan*, J69:653–660, 1986.
[4] Reggia, J. A., Armentrout, S. L., Chou, H.-H., and Peng, Y., Simple systems that exhibit self-directed replication, *Science*, 259:1282–1287, 1993.
[5] Peper, F., Lee, J., and Isokawa, T., Brownian Cellular Automata, *Journal of Cellular Automata*, 5:185–206, 2010.

Dynamical control of protein concentration using synthetic two-component system

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Keywords: two-component system, system modeling, dynamical control of protein concentration

To control cells using external input is useful for understanding biological function. For the analysis of the function, adding a time-invariant perturbation to a component is effective, but adding a time-variant perturbation is also effective. Dynamical control of protein concentration using external input (e.g. inducer or light) have been studied recently[1][2]. For dynamical control, a control system that converts external input into output like protein is required, and mathematical model of the system is desirable because of time dilation and noise. These studies are recently developed and it is necessary to increase variety of control systems. By using Cph8/OmpR two-component system that responds to a red-light stimulus and activate transcription of target gene, we controlled protein concentration tracking a desired value with mathematical model.

Cph8/OmpR two-component system has Cph8 as sensor protein and OmpR as regulator protein. Cph8 was synthesized by photoreceptor protein to be able to respond red light in E.coli[3]. In this study, Cph8/OmpR two-component system activates GFP expression under dark condition and can't activate it under red light condition.

Since the signal transduction mechanism of Cph8 is unknown, we constructed some mathematical models based on a few assumptions. In the process of parameter identification, we used time evolution of output (protein concentration) in response to dynamical switching of the input light. Finally, we dynamically controlled GFP concentration by changing input light pulse cycle. Mathematical model of the system is used to determine the optimum input. We show that Cph8/OmpR two-component system can be used for dynamical control of protein concentration. These studies would be useful for understanding biological function.

[1] Milias-Argeitis A, Summers S, Stewart-Ornstein J, Zuleta I, Pincus D, EI-Samad H, Khammash M, In silico feedback for in vivo regulation of a gene expression circuit, *nature biotechnology*, 29:1114-1116, 2011.

[2] Uhlendorf J, Miermont A, Delaveau T, Charvin G, Fages F, Bottani S, Batt G, Hersen P, Long-term model predictive control of gene expression at the population and single-cell levels, *Proc Natl Acad Sci U S A*, 109(35):14271-14276, 2012.

[3] Levskaya A, Chevalier AA, Tabor JJ, Simpson ZB, Lavery LA, Levy M, Davidson EA, Scouras A, Ellington AD, Marcotte EM, Voigt CA, Synthetic biology: engineering Escherichia coli to see light, Nature, 438(7067):441-442, 2005.

C-5-26

Synthetic cellular membrane systems

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Keywords: soft interface, cell membrane, liposome, photo-control, nano-particles

In living systems, bio-molecules self-assemble in a predetermined fashion under specific conditions into highly organized supramolecular structures with specific functions. The constructive and synthetic approach to biological systems is an important and interesting challenge that spans across several disciplines, including physics, chemistry and biology. A closed two-dimensional surface consisting of a lipid bilayer is an essential compartment of living organisms. We have developed a synthetic membrane system that exhibits cellular functions [1].

We utilized a synthetic photosensitive amphiphile to achieve the photo-based manipulation of the opening and closing of membranes through reversible transitions between sphere and disk structures. The manipulation is based on the photo-switching of the membrane line tension, as deduced from the fluctuation of the membrane edge. Furthermore, we demonstrated the controllable capture and release of a targeted object into and from a membrane vesicle. This successful photo-manipulation of mesoscopic membrane structures in a noncontact and reversible manner may see wider application, such as in micro-reactors and smart drug-delivery systems (SDDS).

We then investigated the association of nano-particles (NPs) on a model membrane surface [3]. We found that lateral heterogeneity in the membrane mediates the partitioning of NPs in a size-dependent manner: small particles with a diameter of ≤200 nm were localized in an ordered phase, whereas large particles preferred a fluidic disordered phase. In terms of the membrane elastic energy, we present a physical model that explains this localization preference of NPs. These findings may lead to a better understanding of the basic mechanisms that underlie the association of nano-materials within a cell surface. These results help us to design proto-cell systems that take advantage of the membrane thermodynamic characteristics to recognize contacting objects.

[1] Hamada, T. & Yoshikawa, K., Cell-sized liposomes and droplets: real-world modeling of living cells, *Materials*, 5:2292-2305, 2012.
[2] Hamada, T. et al., Membrane disc and sphere: controllable mesoscopic structures for the capture and release of a targeted object, *J. Am. Chem. Soc.*, 132:10528-10532, 2010.
[3] Hamada, T. et al., Size-dependent partitioning of nano/micro-particles mediated by membrane lateral heterogeneity, *J. Am. Chem. Soc.*, 134:13990−13996, 2012.

C-5-27

Molecular Evolution of a TALE Protein to Change DNA Binding Manner

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Keywords: Protein-DNA interaction, Molecular evolution, Synthetic biology

Artificial DNA binding proteins binding to desired DNA sequences are useful to control transcription of various genes and/or gene editing. So, they can be powerful tools for synthetic biology. Transcription activator-like effectors (TALEs) are sequence-specific DNA binding proteins secreted by the bacterial pathogen *Xanthomonas*. Their DNA binding specificity is determined by a series of tandem repeats of typically 34 highly conserved amino acids, containing repeat variable di-residues (RVDs) at positions 12 and 13, that define the base preferences of a repeat. Because of the simple one-to-one base recognition by each repeat, TALEs can be readily designed to target desired DNA sequences just by replacing the RVDs. Though TALEs have the target sequence versatility determined by tandem repeats regions, almost all TALE binding sites are preceded by a highly conserved 5’ terminal T nucleotide. N-terminal noncanonical repeat (-1st repeat) has been thought to interact specifically with the 5’-T. However, the details of the base recognition mode are still unclear. In this study, we substituted amino acids that correspond to the residues of the adjoining loops of the -1st repeat and verified the contributions to the 5’-T recognition. In addition, we performed directed evolution of the -1st repeat to bind to non 5’-T sequences using a bacterial 1-hybrid assay.

We introduced a point mutation in a tryptophan residue at the adjoining loop of the -1st repeat of dHax3. The DNA bindings of the mutated dHax3s were examined by luciferase reporter assays in HeLa cells. Only the substitutant with tyrosine showed a comparable activity to wild type. A significant decrease in the luciferase activity was observed when the tryptophan residue was substituted with each of the other 18 amino acids. In addition, all of the mutants showed a DNA binding preference to 5’-T. This result suggests that tryptophan and tyrosine play an important role in the DNA recognition by the -1st repeat, but the recognition of the 5’-T appears not to be so simple as the canonical repeat domains. Therefore, we randomized the four amino acid residues in the loop region and intended to change the recognition pattern of the 5’-terminal nucleotide. Through a bacterial one-hybrid screening using a reporter vector with a dHax3 binding site starting from 5’-C, we obtained highly conserved amino acid sequences of the loop region. The selected dHax3 mutant showed significantly strong DNA binding activity to the binding sites starting from non 5’-T. This result directly indicates the importance of the -1st repeat on the recognition of the 5’-terminal nucleotide.

C-5-28

A Magnetically Triggered Gene Expression System Mediated by Heating of Nanoparticles

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Keywords: Inducible gene expression, Transcriptional amplification, Magnetite nanoparticles, Heat shock protein promoter, Tetracycline-responsive transactivator

Gene therapy promises a wide range of applications in medicine, including cancer treatment. Similar to chemotherapy, the therapeutic efficacy depends on the dosage (gene expression level) and timing. Thus, controlled gene expression is essential for gene therapy. Synthetic biology focuses on designing artificial gene expression systems. Numerous gene circuits have been developed to control gene expression. Among them, the tetracycline (Tet)-inducible system [1] and heat shock protein promoter system [2] have been used as effective inducible gene expression systems. Using a synthetic biological approach, we have previously constructed a heat-inducible transgene expression system incorporating a transcriptional positive feedback loop mediated by Tet-inducible system that enhances heat-induced gene expression [3].

Remote activation of target cells to trigger specific gene expression *in vivo* can provide a useful research tool and potential means to control gene expression in clinical settings [4]. For this purpose, nanotechnology is becoming increasingly important in medicine. Magnetite nanoparticles have been used for drug delivery systems and cancer diagnosis as contrast-enhancement agents in magnetic resonance imaging. Furthermore, magnetite (Fe_3O_4) particles absorb energy and generate heat in response to an alternating magnetic field (AMF) [5]. Thus, heat generation by AMF exposure can be a potent tool as a switch to induce target gene expression. Here, we combined synthetic biology with nanotechnology to convert a local heating signal using magnetite nanoparticles and AMF exposure into high-level gene expression at a specific site. We investigated the *in vivo* feasibility of this approach for cancer gene therapy using a tumor xenograft model.

[1] Stieger, K., Belbellaa, B., Le, Guiner. C., Moullier, P., and Rolling, F., In vivo gene regulation using tetracycline-regulatable systems, *Advanced Drug Delivery Reviews*, 61:527-541, 2009.
[2] Huang, Q., Hu, J. K., Lohr, F., Zhang, L., Braun, R., Lanzen, J., Little, J. B., Dewhirst, M. W., and Li, C. Y., Heat-induced gene expression as a novel targeted cancer gene therapy strategy, *Cancer Research*, 60:3435-3439, 2000.
[3] Yamaguchi, M., Ito, A., Okamoto, N., Kawabe, Y., and Kamihira, M., Heat-inducible transgene expression system incorporating a positive feedback loop of transcriptional amplification for hyperthermia-induced gene therapy, *Journal of Bioscience and Bioengineering*, 114:460-465, 2012.
[4] Stanley, S. A., Gagner, J. E., Damanpour, S., Yoshida, M., Dordick, J. S., and Friedman, J. M., Radio-wave heating of iron oxide nanoparticles can regulate plasma glucose in mice, *Science*, 336:604-608, 2012.
[5] Gazeau, F., Lévy, M., and Wilhelm, C., Optimizing magnetic nanoparticle design for nanothermotherapy, *Nanomedicine (Lond)*, 3:831-844, 2008.

C-5-29

A combinatorial library of enzymes for anthocyanin biosynthesis toward designing of an artificial anthocyanin operon

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Keywords: operon, synthetic biology, anthocyanin, OGAB

Many of biosynthetic pathways for useful chemicals of plant have been tried to transplant in bacterial cell for the purpose of increase production speed and quantity. Construction of a heterologous metabolic pathway in a bacterial cell is, however, still difficult as the number of required enzyme is getting bigger. To investigate a method for construction of metabolic pathway, we render a biosynthetic pathway of plant specific pigment, anthocyanin as a model. In our former work, a series of 9 enzymes required for production of peralgonidin, an anthocyanin compounds, in *Escherichia coli* were assembled in a plasmid as polycistronic operon form. However, any anthocyanin was detected in an *E. coli* harboring the operon, although production of naringenin, an intermediate metabolite, was observed. This implies that design for the artificial operon is necessary to produce peralgonidin. We then focus on relevant four enzymes required for peralgonidin synthesis from naringenin. Since, in an artificial operon profile of expression level of the genes is expected as monotonical decrease from promoter [1, 2], we thus tried *in vitro* production of peralgonidin with different amount of the enzymes mimicking possible 24 (=4!) operons to find out optimal 4-enzyme ratio for production. As a result, specific combinations showed significant production of peralgonidin. Now we are making several artificial operons including four enzyme genes with optimal gene orders according to the enzyme combinations by Ordered Gene Assembly in *Bacillus subtilis* (OGAB) method [3].

[1] Nishizaki, T., Tsuge, K., Itaya, M., Doi, N., and Yanagawa, H., Metabolic engineering of carotenoid biosynthesis in *Escherichia coli* by ordered gene assembly in *Bacillus subtilis*. *Appl. Environ. Microbiol*. 73:1355-1361, 2007.
[2] Hiroe, A., Tsuge, K., Nomura, C.T., Itaya, M., and Tsuge, T., Rearrangement of gene order in the *phaCAB* operon leads to effective production of ultrahigh-molecular-weight poly[(R)-3-hydroxybutyrate] in genetically engineered *Escherichia coli*, *Appl. Environ. Microbiol.* 78:3177-3184, 2012.
[3] Tsuge, K., Matsui, K., and Itaya, M., One step assembly of multiple DNA fragments with a designed order and orientation in *Bacillus subtilis* plasmid. *Nucleic Acids Res.* 31:e133, 2003.

C-5-30

Mathematical modeling and theoretical analysis for the quantitative control of the target gene expression of synthetic genetic circuit

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Keywords: synthetic genetic circuit, synthetic biology,

In recent years, synthetic genetic circuits have been developed as a gene expression regulatory system in expectation of biotechnological applications. For practical applications of them, quantitative control of target genes expression is necessary. For this purpose, various parameters, such as promoter or RBS strength and copy number of genes, should be adjusted properly according to characteristics of the circuit. Development of mathematical modeling on the basis of the experimental results *in vivo* is important for the efficient and deliberate construction of desired circuits. In this study, we designed a simple inducible switch which is composed of two gene expression modules ($P_L$tetO_1::*gene1* and $P_L$lacO_1::*tetR* (repressor) *gene2*) and a constitutive source of LacI repressor (P*lacI*^{q}::*lacI*). The switching of expression from *gene1* to *gene2* should be induced by addition of IPTG.

At first, we experimentally evaluated promoter strength of the two P_L promoters by monitoring expression level of GFP in *Escherichia coli*. Next, we built two stochastic models of each module and fitted their parameters to the results of the *in vivo* experiment. The desired switching behavior was realized by integrating two stochastic models above. Furthermore, the expression level of *gene1* and *gene2* showed a positive and a negative correlation with the increase in the copy number of P*lacI*^{q}::*lacI*, respectively. Based on these results, we constructed the genetic switch and examine its dynamic behavior *in vivo*. As the results, we obtained the desired switching and observed the predicted correlation between the copy number of *lacI* and target genes expression. For quantitative control of target genes expression, other parameters such as promoter strength or RBS strength can be adjusted in future work.

[1] Callura, J.M., Cantor, C.R., Collins, J.J., Genetic switchboard for synthetic biology applications, *Proc. Natl. Acad. Sci. USA.,* 10, 5850-5855, 2012.
[2] Zhang, F., Carothers, J.M., Keasling, J.D., Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids, *Nat. Biotechnol*., 25, 354-359, 2012.

C-5-31

Synthetic analysis of the effect of parameter balance on phenotypic stabilities in a synthetic mutual inhibitory network

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Keywords: mutual inhibition, phenotypic stability, synthetic gene circuit

Mutual inhibitory network, in which two genes inhibit each other, appears in high frequency in various organisms from bacteria to mammalian: for example, Nanog and Gata6 in embryo [1]. Cells carrying the mutual inhibitory network are known to exhibit either of two distinct phenotypes where one gene is expressed a lot while the other is slightly when parameters such as the expression rate constants of the two genes are balanced [2]. However, quantitative knowledge about the effect of parameter balance on the stability of each phenotype is not experimentally investigated well. In this work, we analyzed the effect of the parameter balance to phenotypic stabilities in a synthetic mutual inhibitory network constituting of CIts and LacI, based on P_{luxlac} toggle [3]. The net rate constants of the CIts expression in our synthetic mutual inhibitory network can be tuned by the addition of an inducer N-acyl homoserine lactone (AHL). We measured the phenotypic ratios, determined by the switching rates and growth rates of each phenotype, at stationary state under various AHL concentrations, and analyzed the relationship between the switching rate corresponding to the phenotypic stabilities and the balance of the promoter strength. Our results and approach would be a basis of an experimental system to estimate and adjust switching rates and phenotypic ratios in mutual inhibitory networks of interest.

[1] Zhou, J. X., and Huang, S., Understanding gene circuits at cell-fate branch points for rational cell reprogramming, *Trends in Genetics*, 27:55-62,2011.

[2] Gardner, T. S., Cantor, C. R., and Collins, J. J., Construction of a genetic toggle switch in *Escherichia coli*, *Nature*, 403:339-342, 2000.

[3] Sekine, R., Yamamura, M., Ayukawa, S., Ishimatsu, K., Akama, S., Takinoue, M., Hagiya, M., and Kiga, D., Tunable synthetic phenotypic diversification on Waddington's landscape through autonomous signaling, *Proceedings of National Academy of Sciences U. S. A*, 108:17969-17973, 2011.

C-5-32

Theophylline-dependent Riboswitch as a Useful Genetic Tool for Synthetic Biology in Cyanobacteria

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Keywords: Synthetic biology, Theophylline riboswitch, Translational regulation, Cyanobacteria

Cyanobacteria are favorable cell factories for the production of renewable biofuels and valuable chemicals because of their ability to capture solar energy. However, biotechnology for cyanobacteria still lags behind conventional model species such as *Escherichia coli* or baker's yeast. In this work, we employed engineered riboswitches to control translational initiation of target genes in cyanobacterium *Synechococcus elongates* PCC 7942. A firefly luciferase reporter assay revealed that three theophylline riboswitches performed well in *Synechococcus*. Among the riboswitches, the best one exhibited very low leaky expression of luciferase and dose-dependent ON/OFF regulation of protein expression by theophylline. The maximum magnitude of the induction versus basal level was ~190-fold. We also adopted this riboswitch to another gene regulation system, in which expression of the circadian clock *kaiC* gene product is controlled in a theophylline dose-dependent manner. The result demonstrated that the adequately adjusted expression level of KaiC restored complete circadian rhythm in the *kaiC*-deficient arrhythmic mutant. These suggest that the theophylline-dependent riboswitch system has great promise as a useful genetic tool for synthetic biology in cyanobacteria.

[1] Nakahira, Y., Ogawa, A., Asano, H., Oyama, T., and Tozawa, Y., Theophylline-dependent riboswitch as a novel genetic Tool for strict regulation of protein expression in cyanobacterium *Synechococcus elongatus* PCC 7942, *Plant Cell Physiology*, in press, 2013.

C-5-33

Automatic Design with Frequency Characteristics for Synthetic Gene Oscillators

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Keywords: synthetic gene circuit, oscillator, design automation

Oscillation is an essential phenomenon in biological systems. Many activities and life maintenance of organisms are supported by that phenomenon. For this reason, a deeper understanding about oscillation in biological system is important in biology. In synthetic biology, synthetic gene circuit is used as a tool to understand the biological system [1]. In order to get a deeper understanding of biological systems, the more complex and larger gene circuits are required. As increasing the number of components, however, difficulty of design becomes higher exponentially. It is difficult to design in human trial and error or ad-hockery. Therefore, Automatic design approaches are important [2].

We propose an automatic design method for synthetic gene oscillators. In previous research related to automation of design, an evaluation is calculated by error of protein concentration in only time domain. However, that evaluation function might make inadequate landscape to optimize an oscillate solution because it' sensitivity for a shift of phase is too strong. The proposed method is also to use the frequency characteristic in the evaluation function. That function can make more appropriate landscape to search oscillators. Moreover we propose two-stage design method. In the first stage, it optimizes the network structure by frequency characteristics. In the second stage, it optimizes parameters by error in time domain. As a result of performance examinations, it was shown that the probability to find an optimal solution by proposed method is higher than previous methods.

[1] Elowitz, M. Lim, W.A., Build life to understand it, Nature, 2010.
[2] Macdonald J.T., Barnes C., Kitney R.I., Freemont P.S., Stan G.B., Computational design approaches and tools for synthetic biology, Integrative Biology, 2011.

C-5-34

RNA-protein complexes for designing functional nanostructured molecules

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Keywords: RNA-protein complex, nanostructure, synthetic biology

Nanostructures consisting of DNA provide promising frameworks for designing nanomachines with defined shapes and sizes. However, the DNA based structures have problems when applied *in vivo*. To avoid such problems including genomic integration or immune responses, several approaches including episomal technologies have been practiced without sufficient success.

RNA-protein complexes (RNP) can also be employed to design and construct nanostructures. They have characteristic features as follows. (1) RNA can be designed by using a simple Watson-Crick base pairings like DNA nanostructure and naturally occurring tertiary interactions, (2) proteins can be exploited for reinforcement of the RNA structure and to give a function to the RNP, and (3) RNA-protein structures have potential to be produced and assembled *in vivo*. We have designed triangular shaped RNP [1].

In this study, we have further exploited the potential use of the RNPs with defined nanostructures. Various shapes (i. e. square or zigzag) of the RNP have been designed, constructed and visualized by HS-AFM. Several functional RNA-binding proteins have been successfully attached to the RNAs with defined structures to improve their physiological functions. Thus, the RNA-protein complexes are potentially useful for designing novel nanostructures linking the structures to the functions.

[1] Ohno, H.; Kobayashi, T.; Kabata, R.; Endo, K.; Iwasa, T.; Yoshimura, S. H.; Takeyasu, K.; Inoue, T. & Saito, H. (2011), 'Synthetic RNA-protein complex shaped like an equilateral triangle.', *Nat Nanotechnol* **6**(2), 116--120.

C-5-35

Construction of a computational platform for metabolic pathway design

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Keywords: metabolic engineering, synthetic biology, chemoinformatics

Computational design of metabolic pathways has become an essential and complementary approach to identify metabolic pathways for the production of chemical products. In order to find the metabolic pathways of value, it is primarily necessary to utilize comprehensive chemical and enzymatic information to ensure a wide range of the enzymatic reaction, and obtain pathway candidates including putative compounds and enzymatic reactions. We have developed an efficient algorithm and a computational tool for the design of extensive metabolic pathways.

The design tool begins the search for metabolic pathways with the initial and last compounds as queries. In the result viewer, the candidate list of metabolic pathways is presented with ranking scores, and the changes in the chemical structures in the metabolic pathways can be checked at a glance. The putative candidates for reactions and compounds are displayed with scores based on the comparison of structural similarities. The design tool provides a user with various design options including the number of reactions, reaction steps and so on. Any chemical and reaction information can be also applied to the design tool, which will be a powerful tool to identify unknown metabolic pathways of chemical products.

C-5-36

Highly parallel and sensitive method for analyzing gene expression kinetics

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Keywords: synthetic gene circuits, absolute quantification, multiplex measurement

Synthetic biology is a bottom-up approach to a deeper understanding of biological phenomena by building synthetic gene circuits and studying their kinetics in cells. Analysis of kinetics of intricate synthetic gene circuits with multiple components plays a crucial role in elucidation of biomolecular networks and in their bioengineering applications. Since synthetic gene circuits are integrations of chemical reactions, it is eagerly expected to develop a method for monitoring temporal changes of gene expression not in terms of relative amounts of transcription products but in terms of their absolute amounts. Moreover, in the case of analyzing synthetic gene circuits introduced into living cells, a large number of originally-existing internal genes in the cells may interfere with external genes introduced from the synthetic circuits. Therefore, a highly multiplex method that can measure both the internal and external genes is required for investigating behaviors of such synthetic circuits.

Here, we describe a highly parallel and sensitive method for analyzing gene expression kinetics by measuring the absolute amounts of their transcripts without reverse transcription processes[1]. The method determines the absolute mRNA amounts by quantifying well-designed DNA tag sequences called DNA-Coded Numbers (DCNs) [2] generated by photo-chemical ligation of pairs of two chemically synthesized DNA oligonucleotides that specifically bind to the target mRNA. The converted DCNs are then amplified by PCR and finally detected on microarrays. The conversion to DCNs also provides advantages such as the use of target-independent microarrays and no need for sample labeling. The method was validated by using chemically synthesized RNA samples of known quantities and total RNA samples prepared from mouse liver and retina. The absolute amounts of 43-144 mRNA species were reproducibly quantified in parallel with high sensitivity (24-60 zmol) from a small amount of total RNA samples (20 ng) in 7 hours.

[1] Yokomori, M., Gotoh, O., Fujimoto, K., Suyama, A. submitted.

[2] Gotoh, O., Murakami, Y., Suyama, A. Multiplex cDNA quantification method that facilitates the standardization of gene expression data. *Nucleic Acids Res.* **39**, e70 (2011)

C-5-37

A development of molecular sensor that delivers environmental information to inside of vesicle-based molecular robots

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Keywords: DNA, lipid, vesicle

In the molecular robotics project, a planned molecular robot that is based on a vesicle or a gel as a body consists of molecular sensors, molecular computers, and molecular actuators. These components are communicated with single-stranded DNAs each other. We covered a development of molecular sensor that was a conjugated molecule between DNA strands and lipid.[1] This sensor molecule located on a surface of vesicle could hybridize with a complementary DNA/RNA strand that represented an environmental information. The hybridization caused a shape change of the molecular sensor, consequently a single-stranded DNA was released into an inner water pool of vesicle. The released DNA was a trigger for molecular computers encapsulated in the vesicle.

[1] P. Barton, C. A. Hunter, T. J. Potter, S. J. Webb, and N. H. Williams *Angew. Chem. Int. Ed*. **2002**, *41*, 3878-3881.

DNA computer-based rational construction of artificial genetic circuits in cell model vesicles containing cell-free protein synthesis system

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In the field of synthetic biology, the construction of artificial genetic circuits is aimed for understanding natural biological systems and designing new biological parts. However, the rational construction of artificial genetic circuits large enough to be of practical value is not easy at all. No effective method for the rational construction has yet been developed. Here, we report a DNA computer-based approach to the construction of artificial genetic circuits, which may have the potential for the rational construction of large artificial genetic circuits of practical value. In this study, a simple artificial genetic circuit was constructed based on a framework of RTRACS, which is a modular DNA computing system comprised of modules communicating with each other by using input and output RNA strands (Phys. Rev. E, 78:041921, 2008). The simple circuit receives input RNA for two inputs and produces output mRNA coding green fluorescent protein (GFP) as a consequence of the AND operation on the two inputs (Fig. 1). The constructed circuit was encapsulated into a Giant Unilamellar Vesicle (GUV), which is an artificially-prepared phospholipid-bilayer vesicle and has been recognized as a model to study biological cells. Expression of GFP gene controlled by input RNA strands was observed by green fluorescence emitted from GFP translated (synthesized) from the output mRNA using a cell-free protein synthesis system in a GUV. It was confirmed that the genetic circuit worked successfully in a GUV (Fig. 2). The present work would be the first small but important step toward the rational construction of more sophisticated artificial genetic circuits of practical value working in cell model vesicles.

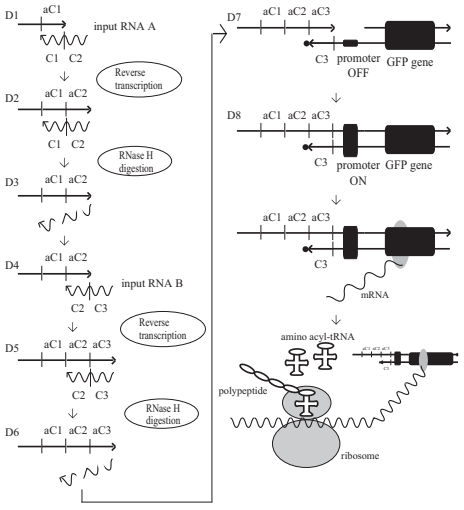

図 1　Reaction scheme of constructed module.

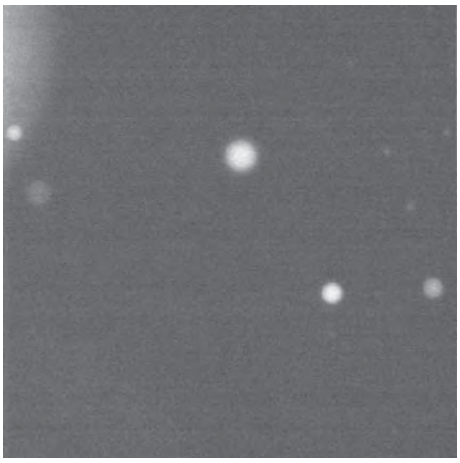

図 2　FM observation of GFP-expressed GUVs.

New Bottom-up Construction Strategy of Biomolecular Circuits Based on Dynamic Robustness Measure

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Keywords: Synthetic biology, Robustness analysis, Bistability

We analyze the biomolecular switchable memory system created by a bottom-up construction strategy [1] to reveal the reason to the successful synthesis. The system is represented by a feedback form. Utilizing the robust bifurcation analysis method for feedback systems [2], which evaluates the robustness against both of dynamic and parametric perturbations, we can guarantee the validity of the bottom-up construction. Lastly, we propose a novel construction strategy for system-level biomolecular circuits by assembling core modules with high dynamic robustness.

[1] Padirac A., Fujii T., and Rondelez Y., *Proceedings of the National Academy of Sciences*, 109:E3212-E3220, 2012.
[2] Inoue M., Imura J.-I., Kashima K., Aihara K., *International Journal of Bifurcation and Chaos*, 23, 2013 (in press).

iGEM 2013 Team Tokyo Tech Project: Mutant Ninja Coli

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Keywords: Synthetic biology, iGEM, genetic circuit

International Genetically Engineered Machine competition (iGEM) is an international synthetic biology competition in which undergraduate teams from around the world compete one another. The participating teams design and construct the original genetic circuits by assembling various gene parts inside living cells during summer. The teams perform the presentation about their work on the Jamboree held at MIT. Our Tokyo Tech iGEM team has been participating iGEM since 2006 and getting bright results such as the acquisition of gold medal and track prizes.

In this year, we proposed to create E. coli that mimics some of the characteristics of ‘ninja’ warrior-spies. A ninja must receive and pass on correct information at all times. A mistake will be fatal. We have designed an artificial genetic circuit that avoids crosstalk between two signals used in bacterial cell-cell communication. Ninjas are also known for their star-shaped ‘shuriken’ throwing knives. Our E. coli ninja has a similar weapon, an M13 phage which is released to infect other E. coli, injecting plasmid DNA into them. Moreover, ninjas must harmonize with the natural environment, so that their relationship with nature is very important. Plant hormones help plants to grow efficiently, and we attempted to construct a circuit that synthesizes two plant hormones depending on the soil environment.

C-5-41

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

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

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

[1] Miyazono Y., Hayashi M., Karagiannis P., Harada Y., Tadakuma H. Strain through the neck linker ensures processive runs: a DNA-kinesin hybrid nanomachine study. EMBO J. 29:93-106. 2010.

C-5-42

Heat-induced Morphological Transformation of Supramolecular Nanostructures

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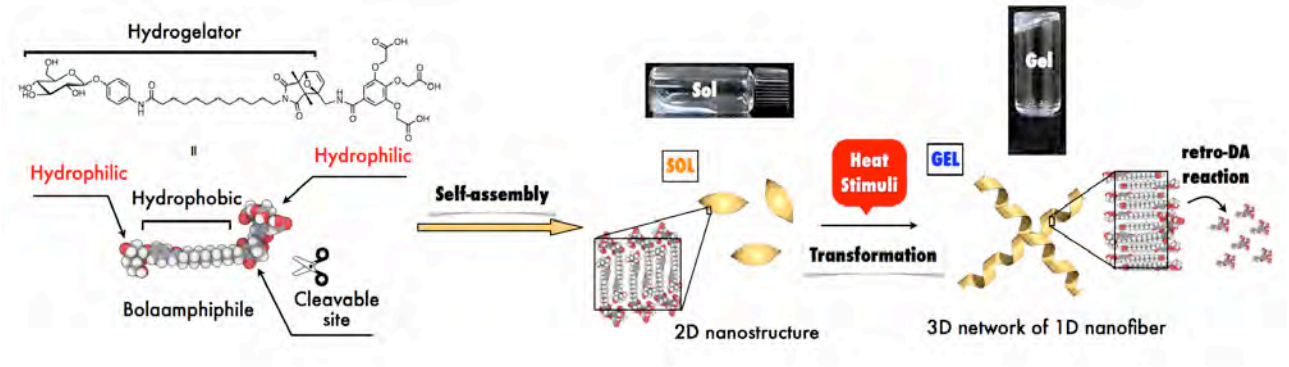
Keywords: Supramolecular nanostructure, Stimuli-response, Hydrogel

Self-assembly is utilized increasingly in many disciplines and is one of the few practical strategy for creating nanostructure. Supramolecular hydrogel is a macroscopic material consisting of self-assembled nanostructures (3D network of mostly 1D nanofibers). The supramolecular hydrogel has recently attracted much attention because of their mild fabrication process, biocompatibility, and dynamic functions such as stimuli-responsiveness

To install stimuli-responsive functions in the supramolecular nanostructures and hydrogels, modulation of noncovalent interactions among component self-assembling molecules has been widely employed. By contrast, rearrangement of covalent bonds in the component molecules has not yet been explored to a significant extent. We believe that incorporation of a chemical reaction site into the component molecules may open up a new avenue for exploring unprecedented stimuli-responsive supramolecular materials, which is impossible to realize only by modulation of noncovalent interactions. Here we present heat-responsive supramolecular hydrogels on the basis of self-assembling molecules bearing a chemical reaction site.

We designed a bolaamphiphile as a self-assembling precursor molecule of a hydrogelator as shown in Figure; the bolaamphiphile is constructed from a hydrogelator bearing a dienophile (maleimide) and a water-soluble diene (furan) via Diels-Alder (DA) reaction. We demonstrated that heating facilitated molecular conversion from the bolaamphiphile to the hydrogelator by means of a retro-DA reaction, which triggered a drastic morphological transformation from 2D nanosheets to a 3D network of 1D nanofibers, giving rise to a unique heat-set supramolecular hydrogel[1]. Heat stimuli required in the present system (60 ºC, 5 h) would be too harsh for bio-applications, whereas the present rational molecular design could allow us to decrease or tune the heat stimuli by using the other diene-dienophile pair [2] and to extend to the other structural motif such as vesicles and nanotubes.



[1] Ikeda, M., Ochi, R., Kurita, Y.-s., Pochan, D. J., Hamachi, I., *Chemistry –A European Journal*, 215:403-410, 1990.
[2] Ochi, R., Nishida, T., Ikeda, M., Hamachi, I., to be submitted.

C-5-43

Towards an Automatic Recognition of DNA Nanostructures on AFM images

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Keywords: DNA nanostructure, AFM image recognition, curvature scale space method, convexity-concavity detection, open robot technology middleware

With the technology of Atomic Force Microscope (AFM), we can directly observe DNA nanostructures in high resolution. An AFM image of DNA nanostructures contains a variety of shapes including single DNA nanostructures, overlapped DNA nanostructures and a lot of noise shapes. Automatic recognition and classification of DNA nanostructures are strongly demanded to reduce human efforts in AFM image analysis.

In this research, automatic-recognition of single DNA nanostructures in AFM images based on the information of their outer contours has been proved. The information about the outer contour of a DNA nanostructure includes not only peak points in the curvature scale space (CSS) images, but also the convexity-concavity of the outer contour. Our prototype system of DNA nanostructure recognition demonstrates that the CSS information is effective for the classification of open and closed forms of DNA origami pliers [1,2].

The prototype system is developed on robot technology middleware (OpenRTM-aist) [3] to integrate multiple components written in different languages running on multiple operating systems such as linux and windows. The prototype system currently consists of two components named “GetNanoDNASize” and “RecognizeNanoDNA”. The former component is a preprocessing system for picking DNA nanostructures in AFM image focusing on the lengths and areas of DNA nanostructures. The latter component classifies DNA nanostructures based on CSS and convexity-concavity information as well as the lengths and the areas of DNA nanostructures.

[1] A. Kuzuya, Y. Sakai, T. Yamazaki, Y. Xu, and M. Komiyama. *Nanomechanical DNA origami 'single-molecule beacons' directly imaged by atomic force microscopy*. Nature Communications, Vol. 2, DOI: 10.1038/ncomms1452, 2011.
[2] T. Yamazaki, Y. Aiba, K. Yasuda, Y. Sakai, Y. Yamanaka, A. Kuzuya, Y. Ohya, and M. Komiyama. *Clear-cut observation of PNA invasion using nanomechanical DNA origami devices.* Chemical Communications, Vol. 48, Pp. 11361-11363, 2012.
[3] http://www.openrtm.org/

Simple Local-information-based Self-optimizing Algorithms in Grid Networks

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Keywords: Distributed algorithm, Self-optimization, Grid network, Shortest path construction, BFS tree construction

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

For this reason, it is natural to apply distributed algorithms (i.e., algorithms for distributed systems) to molecular robotics. Many distributed algorithms are proposed for many problems in literature. However, since such distributed algorithms are designed for computers to achieve the highest performance, the algorithms are complex and implementing them in molecular devices is difficult. To apply distributed algorithms to molecular robotics, it is necessary to develop simple distributed algorithms.

In this poster, we focus on grid networks, and introduce simple distributed algorithms for the shortest path construction [2] and the BFS (bread-first-search) tree construction. The shortest path is useful to transmit a signal from the source to the destination quickly, and the BFS tree is useful to transmit a signal from the source to all devices quickly. The algorithm for the shortest path construction (resp., the BFS tree construction) constructs the shortest path (resp., the BFS tree) from any initial path (resp., tree) in a self-optimizing manner. Both algorithms are simple: the algorithm for the shortest path construction uses only three rules and that for the BFS tree construction uses only four rules. These rules locally update a path or a tree with preserving the connectivity and can be executed asynchronously.

While the characteristics of the above distributed algorithms are suitable for molecular robots, it still requires some researches to implement them in molecular robots because the capability of current molecular devices is limited. As future work, we intend establishing a theoretical model for molecular robotics and developing distributed algorithms on the model.

[1] Murata, S., Konagaya, A., Kobayashi, S., Saito, H., and Hagiya, M., Molecular robotics: A new paradigm for artifacts, *New Generation Computing,* 31, 27-45, 2013.

[2] Takatsu, S., Ooshita, F., Kakugawa, H., and Masuzawa, T., Zigzag: Local-information-based self-optimizing routing in virtual grid networks, *Proc. of the 33rd International Conference on Distributed Computing Systems,* 2013.

A Study on pH-Responsive Photosensitizers for Potential Applications in Molecular Robotics

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Keywords: Molecular robotics, Photosensitizer, Organic synthesis

Molecular robotics has emerged as a novel research area that aims to rationally design and implement dynamic artificial systems consisting of self-assembled molecules [1]. In order to realize robot-like molecular systems which correctly respond to outer stimuli, several classes of molecular devices are necessary including sensors, actuators, and compartments. With respect to sensors, a number of fluorescent probes were developed in our laboratory in the last decade that were designed on the principle of photoinduced electron transfer (PeT) [2-3], Förster resonance energy transfer (FRET) [4], and so on. However, these fluorescent sensors are not suitable for use in molecular robotics because it is difficult to convert their output signal (i.e. fluorescence emission) to the next chemical reaction. Hence, we are focusing on the development of a novel sensor system that is based on activatable photosensitizers [5].

As a proof of principle, we herein designed and synthesized a novel pH-responsive photosensitizer using 2I-BODIPY [6]. In this presentation, we will present the photophysical properties of the compound and discuss future directions.

[1] Murata, S., Konagaya, A., Kobayashi, S., Saito, H., and Hagiya, M., Molecular Robotics: A New Paradigm for Artifacts, *New Generation Computing*, 31:27-45, 2013.
[2] Terai, T., Urano, Y., Izumi, S., Kojima, H., and Nagano, T., A practical strategy to create near-infrared luminescent probes: conversion from fluorescein-based sensors, *Chem. Commun.*, 48:2840-2842, 2012.
[3] Terai, T., Kikuchi, K., Urano, Y., Kojima, H., and Nagano, T., A long-lived luminescent probe to sensitively detect arylamine *N*-acetyltransferase (NAT) activity of cells, *Chem. Commun.*, 48:2234-2236, 2012.
[4] Myochin, T., Hanaoka, K., Komatsu, T., Terai, T., and Nagano, T., Design Strategy for a Near-Infrared Fluorescence Probe for Matrix Metalloproteinase Utilizing Highly Cell Permeable Boron Dipyrromethene, *J. Am. Chem. Soc.*, 134:13730-13737, 2012.
[5] Yogo, T., Urano, Y., Kamiya, M., Sano, K., and Nagano, T., Development of enzyme-activated photosensitizer based on intramolecular electron transfer, *Bioorg. Med. Chem. Lett.*, 20:4320-4323, 2010.
[6] Yogo, T., Urano, Y., Ishitsuka, Y., Maniwa, F., and Nagano, Y., Highly Efficient and Photostable Photosensitizer Based on BODIPY Chromophore, *J. Am. Chem. Soc.*, 127:12162-12163, 2005.

C-5-46

Acceleration of DNA strand exchange reaction by cationic comb-type copolymers

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

DNA strand exchange reaction (SER) between double-stranded DNA(dsDNA) and its complementary single-stranded DNA(ssDNA) is a key reaction in biotechnological applications(1) There are many reports describing DNA nanomachines triggered by the SER. Highly programed SER has been exploited to build DNA logic gate (2). In these application, the dsDNAs generally have sticky end as known as toehold in order to control rate of the SER because of very slow rate of SER between dsDNA having blunt end and ssDNA. In nature, recombination proteins promote the SER even in DNA having blunt end. However, the applications of the proteins to DNA biotechnology based on the SER are limited by the instability and resource ineffectiveness of the proteins. An artificial agent showing the activity of the proteins has been desired for the application of the DNA biotechnology including molecular robotics.

We have previously demonstrated that cationic comb type copolymers consisting of polycation backbone and abundant hydrophilic graft chain influence kinetics and thermodynamics of nucleic acid hybrid under physiologically relevant condition. For example, poly(L-lysine)-*graft*-dextran (PLL-*g*-Dex) copolymer significantly accelerates DNA hybridization over 200-folds(3). Moreover, the copolymer markedly accelerated the SER by 4-5 orders of magnitude (4), while stabilizing dsDNA DNA (5). However, the stabilization of dsDNA is considered to be principally unfavorable for acceleration of the SER, because partial dissociation of dsDNA is required for an initial step of SER. In this study, we focused on urea which is a well-known destabilizing agent for dsDNA, we prepared cationic comb type copolymers partially modified with urea (ureido) groups, poly(allylamine-*co*-allylurea)-*graft*-dextran. The copolymers showed higher acceleration effect of SER than unmodified copolymer.

[1]Zhang, D.Y. and Seelig, G. (2011) Dynamic DNA nanotechnology using strand-displacement reactions. *Nat Chem*, **3**, 103-113.
[2]Qian, L. and Winfree, E. (2011) Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades. *Science*, **332**, 1196-1201.
[3]Wu, L., Shimada, N., Kano, A. and Maruyama, A. (2008) Poly(l-lysine)-graft-dextran copolymer accelerates DNA hybridization by two orders. *Soft Matter.*, **4**, 744-747.
[4]Kim, W.J., Akaike, T. and Maruyama, A. (2002) DNA Strand Exchange Stimulated by Spontaneous Complex Formation with Cationic Comb-Type Copolymer. *Journal of the American Chemical Society*, **124**, 12676-12677.
[5]Maruyama, A., Katoh, M., Ishihara, T. and Akaike, T. (1997) Comb-Type Polycations Effectively Stabilize DNA Triplex. *Bioconjugate Chemistry*, **8**, 3-6.

C-5-47

A study on controller structure of biochemical reaction networks

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Keywords: controller structure, frequency response analysis, biochemical reaction networks

Many mathematical models of intracellular signal transduction systems have been developed toward model-based analysis [1]. However, since the models are typically complex and nonlinear, it is still a difficult task to analyze them [2]. In this poster, we address a fundamental study on what characteristics the biochemical reaction networks have if the system is considered to be a controller.

[1] Nakakuki, T., *et al.*, Ligand-specific c-Fos expression emerges from the spatiotemporal control of ErbB network dynamics, 141, 5, 884-896, *CELL*, 2010.
[2] Nakakuki, T. *et al.*, Methods for the Analysis of Intracellular Signal Transduction Systems (In J. Wu, Ed., Biomedical Engineering and Cognitive Neuroscience for Healthcare: Interdisciplinary Applications), Medical Infor-mation Science Reference (an imprint of IGI Global), 2012.

Toward Spatial and Temporal Gel-Sol Transition of Hydrogel Driven by DNA Hybridization Reaction

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Keywords: Molecular robotics, DNA hydrogel, Polyacrylamide gel, DNA computing,

One of the goals of molecular robotics is synthesizing a hydrogel whose state transition can be driven by DNA devices [1]. One possible demonstration of the hydrogel is a system where gel state gradually propagates in one direction. For example, gel is gradually formed in a capillary from one end, where some signal is put, to the other end. We propose a candidate implementation of such hydrogel and discuss preliminary experimental results.

Gel-sol transition of hydrogel has potential applications such as drug release, chemical sensor, and micro fluidics. Recently, various gel-sol transitions of DNA-functional hydrogel have been demonstrated, which make use of DNA hybridization reactions [2]. One of the hydrogels is made of polyacrylamide that is cross-linked by complementary DNA strands, which can solate by adding an additional DNA strand that displaces the linker DNA [3]. Synthesizing this kind of DNA gel is very costly because high concentration of DNA is required for gelation. However, another hydrogel fully made of DNA using enzymatic reaction does not require high concentration of DNA because polymerization begins with small concentration of primer DNA [4].

We show the spatial and temporal solation of DNA cross-linked hydrogel, which is achieved by the diffusion of displacement strand. To propagate a gel state in a sol solution, we need to implement a mechanism that only the sol near the boundary of a gel turns into a new gel. Our idea to achieve such mechanism is releasing the primer of polymerization by the product of the polymerization. More complex control of gel-sol transition may be possible in the future by combining with DNA state transition devices such as logic gates.

[1] Murata, S., Konagaya, A., Kobayashi, S., Saito, H., and Hagiya, M., Molecular Robotics: A New Paradigm for Artifacts, *New Generation Computing*, 31:27-45, 2013.
[2] Liu, J., Oligonucleotide-functionalized hydrogels as stimuli responsive materials and biosensors, *Soft Matter*, 7:6757-6767, 2011.
[3] Lin, D. C., Yurke, B., Langrana, N. A., Mechanical Properties of a Reversible, DNA-Crosslinked Polyacrylamide Hydrogel, *Journal of Biomechanical Engineering*, 126:104--110, 2004
[4] Lee, J. B., Peng, S., Yang, D., Roh, Y. H., Funabashi, H., Park, N., Rice, E. J., Chen, L., Long, R. Wu, M., Luo, D., A mechanical metamaterial made from a DNA hydrogel, *Nature Nanotechnology*, 7:816--820, 2012

Science Communication in DNA nano-engineering

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Keywords: Molecular Robotics, DNA nano-engineering, learning process, science communication

DNA nano-engineering is a new field of technology that gives the foundation of Molecular Robotics. Most of people know the word “DNA”, however, for them DNA is regarded as a molecule that contains genetic information. In order to understand the concept of DNA nano-engineering, structural and functional aspects of DNA as a nano-material is crucially important. Understanding properties of DNA is usually difficult for layman because it requires many background knowledge in biology, chemistry and physics. The purpose of this research is to develop an aid that helps general people understand the properties of DNA. We need to make the understanding process as interesting as we can, to let learners self-motivated. As a consequence, it will increase the number of people interested in DNA nano-engineering.

As a first step of this attempt, we have made a movie to explain basic principles of DNA nano-engineering. We also have conducted experiments with visitors who came to our open laboratory. By using questionnaire we measured the level of understanding and interest. Some analysis of the result will be reported in the poster. As the next step, we adopt gamification method [1] to improve the effectiveness of the learning process.

[1] Jane MacGonical, “Reality Is Broken : Why Games Make Us Better and How They Can Change the World”, Penguin Books (2011)

Spatio-temporal change of BZ gel

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Keywords: Volume phase-transition of gel, the Belousov-Zhabotinsky reaction, Non-equilibrium conditions

The Belovsov-Zhabotinsky reaction is well-known as one of the oscillatory chemical reactions to understand rhythmic phenomena under non-equilibrium conditions including living matter and living cell. On the other hand, living body is made from various biopolymers. In particular, motor proteins (myosin, kinesin) and their scaffolds (actin, microtubule) generate motion under the hydrolysis of adenosine triphosphate (ATP), a kind of non-equilibrium conditions. Here, we present a theoretical scenario for the BZ gel, which is a gel containing the BZ reaction field, based on free-energy arguments of volume-phase transition of cross-linked polymer gel and the Oregonator (2 parameter version), a simple model of the BZ reaction.

The essence is that polymer chain elongates in good solvent, while it collapses in poor solvent, where the BZ reaction switches the solvent quality (good or poor solvents) spontaneously. Addition to this, we roughly assume that the volume of polymer gel reflect the size of a sub-chain, which is the short polymer chain between cross-linking points. Free energy of the gel, F_{gel}, can be described as [1]

$$F_{gel} \sim (3/2\,N_{sub})\left(\alpha^2+\alpha^{-2}\right)+C*\alpha^{-6}/N_{sub}-\ln\left[1-\lambda/(N_{sub}^{-1/2}\alpha^{-2})\right] \quad (1)$$

where N_{sub} is the length of a sub-chain, α is the normalized size of a polymer chain ($\alpha \sim 0$; collapse, $\alpha \sim 1$; elongate), $-C^*$ is the solvent quality (smaller: good solvent, larger: poo solvent), the aspect ratio of a sub-chain λ. The BZ reaction changes $-C^*$, which is obtained from the Oregonator. The Oregonator is

$$\varepsilon\frac{dx}{dt} = x(1-x) - fz\frac{x-q}{x+q} \quad (2)$$

$$\frac{dz}{dt} = x - z \quad (3)$$

where x, y, z are the concentrations of chemical species, and $1 - z = -C^*$ although we skip the explanation of the parameters. From the calculations of the Oregonator, we obtain oscillating $-C^*$. We thus succeed to describe the oscillatory volume change of the BZ gel. Based on this scenario, we succeeded to explain other experimental results of polymer system under the BZ reaction.

[1] Mayama, H., Nakai, T., Takushi, E., Tsujii, K., and Yoshikawa, K., Marked differences in volume phase-transitions between gel and single molecule in DNA, *J. Chem. Phys.*, 127:034901, 2007.

Construction of Giant Vesicle Containing Microspheres at High Volume Fraction and Its Transformation

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Keywords: Giant Vesicle, Microspheres, Phospholipid, Fluorescence microscopy

Giant vesicles (GVs), which are closed lipid bilayer compartments with cellular size, have much drawn attention as a container of cell model. In the cytoplasm, biological macromolecules occupy a sizeable fraction of the total volume of the media (typically 20–30 vol%), and the effects of this "crowded" state are important for their functions.[1] Crowding significantly influences two physicochemical features of the macromolecules: (i) thermodynamics stabilization to increases the free volume of the macromolecules, and (ii) changes in the mobility of the macromolecules. For the purpose of quantitatively understanding these features, GVs containing microspheres serve as valuable experimental model systems.[2]

Here our purpose is the preparation of GV containing microspheres at a variety of volume fractions and the clarification of the crowding effect for the GV transformation. Because the water-in-oil emulsion centrifugation method[4] is fast and easy for encapsulation of soft matter particles inside of GVs, we utilized it herein to encapsulate 1-μm microspheres with volume fractions in the range of 0–45 vol%.[5] GVs containing polymers or colloidal particles exhibit a unique transformation from an oblate (or prolate) spheroid to linked spheres after external stimuli such as osmolality change or electrical stimulation.[3,6] These findings are thought to be typical examples of the crowding effect for GVs concerning about the thermodynamics of the membrane bending energy and the entropic action of encapsulated particles. Here we found that GVs encapsulating 1-μm microspheres with a specific volume fraction transform from sphere to polygonal structure transiently after osmolality change. The fluorescence microscopy observation on GV membrane proved that this transient state associated with the tubular membrane protrusion apart from the GV. As far as we know, the polygonal structure of GVs is a characteristic shape only if GV contains polymerizable proteins such as actin or microtubules.[7] Although the mechanism of the transient polygonal structure of the GV is still veiled, the current finding can provide us a clue to clarify the crowding effect for the GV in a non-equilibrium state.

[1] Minton, A. P. and Ellis, R. J., *Nature*, 425: 27-28, 2003.
[2] Dinsmore, A. D., Wong, D. T., Nelson, P., and Yodh, A. G., *Physical Review Letters*, 80: 409-412, 1998.
[3] Natsume, Y., Pravaz, O., Yoshida, H., and Imai, M., *Soft Matter*, 6: 5359-5366, 2010.
[4] Pautot, S., Frisken, B. J., and Weitz, D.A., *Langmuir*, 19: 2870-2879, 2003.
[5] Natsume, Y., and Toyota, T., *Chemistry Letters*, 42: 295-297, 2013.
[6] Terasawa, H., Nishimura, K., Suzuki, H., Matsuura, T., and Yomo, T., *Proceedings of the National Academy of Sciences of the U. S. A.*, 109: 5942-5947, 2012.
[7] Miyata, H., and Hotani, H., *Proceedings of the National Academy of Sciences of the U. S. A.*, 89: 11547-11551, 1992.

Reversible Gel-Sol Transition of DNA Gel

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Keywords: Molecular Robotics, DNA Gel, Photo-cross linker

DNA gel have been attracted much attention for its programmable functionality. We propose a DNA gel made of DNA motifs with designed base sequences. The motif is a cross shaped DNA junction with a self-complementary sticky end and a photo-linking artificial base at each arm. Under controlled temperature with UV irradiation, we have been succeeded in making the sol-gel state transition in repetitive manner. The physical properties of DNA gel such as the swelling degree and diffusion coefficient is measured in both sol or gel state of the DNA. This data will be used to design slime-type molecular robot.

[1] Soong Ho Um, Jong Bum Lee, Nokyoung Park, Sang Yeon Kwon, Christopher C. Umbach and Dan Luo, Enzyme-catalysed assembly of DNA hydrogel . *Nature Materials* 5, 797-801 (2006)
[2] Yongzheng Xing, Enjun Cheng, Yang Yang,Ping Chen, Tao Zhang,Yawei Sun, Zhongqiang Yang, Dongsheng Liu, Self-Assembled DNA Hydrogels with Designable Thermal and Enzymatic Responsiveness. *Advanced Materials* 23, 1117–1121 (2011)
[3] Yoshinaga Yoshimura and Kenzo Fujimoto, Ultrafast Reversible Photo-Cross-Linking Reaction: Toward in Situ DNA Manipulation. *Org. Lett.*, 10 (15), 3227–3230 (2008)
[4] Satoshi Murata, Akihiko Konagaya, Satoshi Kobayashi, Hirohide Saito, Masami Hagiya, Molecular Robotics: A New Pardigm for Artifacts, New Generation Computing, 31, 27-45 (2013)

Smart Lenses Created with Transparent Shape Memory Gels

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Keywords: Gel, Shape memory, Transparent, Lens, Medical, Health and care

Gel is a dispersion system consisting of a solid three-dimensional network as the continuous phase and a liquid (dispersing medium) within a solid as the discontinuous phase. Gel exhibits no flow and behave like solids when in the steady state due to the three-dimensional cross-linked internal network structure, which may result from chemical bonds (chemical gels) or physical bonds (physical gels), as well as crystallites or other junctions. Virtually any fluid can be used as an extender including water (hydrogels), organic solvent (organogel), and air (aerogel). Since 2001, high-strength gels like topological gel, nanocomposite hydrogel, tetra-PEG gel, double-network gel were developed [1-3]. In our group, one novel hydrogel [4,5] is developed. The hydrogel has the property of shape memory, and is great transparent and flexible (Fig. 1). We named it TF-SMG. In this paper, we report the development of smart lenses (Fig. 2) with this excellent gel material of TF-SMG.

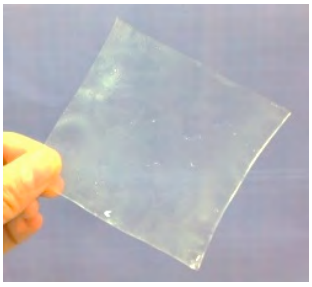

Fig.1 TF-SMG

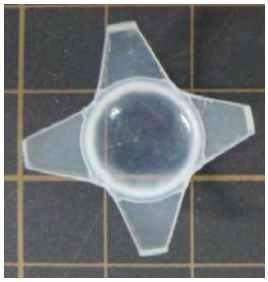

Fig. 2 Smart lens

Acknowledgments

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[1] Okumura, Y., and Ito, K., *Advanced Materials*, 13(7): 485-487, 2001.
[2] Haraguchi, K., and Takeshita, T., *Advanced Materials*, 14(16): 1120-1124, 2002.
[3] Gong, J. P., Katsuyama, Y., Kurokawa, T., Osada, Y., *Advanced Materials*, 15(14): 1155-1158, 2003.
[4] Amano, Y., Hidema, R., Gong, J., Furukawa, H., *Chemisty Letter*, 41: 1029-1031, 2012.
[5] Harada, S., Hidema, R., Gong, J., Furukawa, H., *Chemisty Letter*, 41: 1047-1049, 2012.

Chain-Reactive Molecular Releasing System

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Keywords: International Biomolecular Design Competition (BIOMOD), Molecular releasing system, liposome, DNA nanostructure

BIOMOD is a design competition where undergraduate students compete to master control of biomolecules on the nanometer scale [1]. Students execute projects during the summer and then gather in Boston in November to present their work and win awards.

This year, Team Sendai [2] aims at creating a novel chain-reactive molecular releasing system by using liposome as a container. In the proposed system, chain reactive collapse of liposomes will be triggered by absorption of DNA nanostructure onto the surface of liposome. By designing appropriate DNA-liposome interaction mechanism, we try to realize quantitative control of drug release over time.

[1] BIOMOD2013 Website
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[2] BIOMOD2013 Team Sendai Wiki
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C-5-55

Dynamic biomolecular computing system for artificial genetic network

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Keywords: DNA computing, Logic gate, Genetic network

RTRACS (Reverse-transcription and TRanscription-based Autonomous Computing System) is a modular biomolecular computing system composed of DNA, RNA and enzymes, whose mechanism is based on retroviral replication [1, 2]. The modularity of RTRACS allows various simple modules to be combined together by using output RNA of a module as input for successive modules to create systems capable of sophisticated computational operations. When the output RNA of the module is assigned to mRNA, RTRACS is available for artificial genetic network with cell-free protein synthesis.

RTRACS can basically perform computation to process time-dependent input data by employing modules capable of dynamically changing output RNA depending on input RNA. However, logic gate modules previously developed [2, 3] do not operate dynamically because output RNA production continues indefinitely once the logical operation is complete. Here, we report a new AND gate module that can operate dynamically in response to input RNA data. The dynamic operation was achieved by two additional degradation reactions, one for the newly synthesized DNA strands and the other for the remaining input and output RNA strands. These degradation reactions return the AND gate module to the original state when the input RNA strands are no longer present. Since the degradation reactions are effective for all kinds of RTRACS modules, the present method can be applied to other RTRACS modules to make them succeed in operating dynamically.

[1] N. Nitta and A. Suyama., Autonomous biomolecular computer modeled after retroviral replication. *Lect.Notes Comput. Sc.,* 2943, 203-212 (2004).
[2] M. Takinoue, D. Kiga, K. Shohda, and A. Suyama., Experiments and simulation models of a basic computation element of an autonomous molecular computing system. *Phys. Rev. E,* 78:041921 (2008).
[3] A. Kan, K. Shohda, and A. Suyama., A DNA-based molecular logic gate capable of a broad class of logical operations. *Lect. Notes Comput. Sc.,* 7433, 86–97 (2012).

C-5-56

Towards Persistent Molecular Computers for Molecular Robots

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Keywords: Molecular computing, DNA computing, Molecular robotics

Although a large number of models for molecular computers have been proposed and many of them have actually been implemented typically by using DNA molecules with or without modifications, most of those models are restricted in the sense that computational resources are consumed by each instance of computation and cannot be reused iteratively. Models based on strand displacement of DNA, such as that of seesaw gates by Qian et al. [1], are not exceptions. On the other hand, the idea of molecular robots is now commonly accepted [2], and computers for molecular robots are expected to be reusable and sustainable. The model of dynamical systems by Rondelez et al. is one of few such models [3]. We envision that molecular computers for molecular robots should have the following properties. They 1) receive input from the environment, 2) adapt to the environment by solving some optimization (or learning) problems, and finally 3) save the result of computation (solutions to the problems) for handling the next input from the environment.

In this ongoing and preliminary work, we take the k-SAT problem as an optimization problem whose clauses model input from the environment. The molecular computer solves the problem, and re-solves it when its clauses are modified or new ones are added. As in the work by Sakamoto et al. [4], each clause of an instance of the k-SAT problem is represented by a computational unit. While one literal is chosen from a clause in advance in [4], a computational unit in this work takes one of the literals in its corresponding clause as a state, and changes the state for solving or re-solving the problem.

In this poster, we propose and compare three molecular implementations of the above abstract scheme. 1) Implementation by a molecular complex whose conformational changes realize progression of state transitions by alternating irradiation of two kinds of light [5]. 2) Implementation by polymerase extension and digestion by nicking enzymes, which can be considered as an extension of DWPCR [6]. 3) Implementation by a dynamical system of Montagne et al. [3].

[1] Qian et al.: Neural network computation with DNA strand displacement cascades, Nature 475:368, 2011.
[2] Molecular Robotics: http://www.molecular-robotics.org/en/
[3] Montagne et al.: Programming an in vitro DNA oscillator using a molecular networking strategy, Molecular Systems Biology 7:466, 2011.
[4] Sakamoto et al.: Molecular computation by DNA hairpin formation, Science 288:1223, 2000.
[5] Nishioka et al.: A photon-fueled DNA nanodevice that contains two different photoswitches, Angewandte Chemie International Edition, 51:1165, 2012.
[6] Rose et al.: Displacement Whiplash PCR: Optimized Architecture and Experimental Validation, Lecture Notes in Computer Science 4287:393, 2006.

A Computing Model for Biochemical Reactions

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Keywords: Models of biochemical reactions, Reaction automata, Turing computability

Recent developments on the theory of reaction automata ([4,5,6]) are surveyed. In these years, Ehrenfeucht and Rozenberg have introduced a formal model, called reaction systems ([2]), for investigating interactions between biochemical reactions. Reaction systems provide a formal framework suited for investigating at an abstract level the way of biochemical functioning. Meanwhile, the notion of a multiset has frequently appeared and been investigated in many areas of computer science and related fields ([1,7]) because of its usefulness. In fact, identifying a volume of compound chemicals as a multiset makes it possible that a multiset rewriting provides a natural way to express chemical reactions. Combining two notions of a reaction system and of a multiset rewriting, we have introduced reaction automata as computing devices for accepting string languages ([4]), in order to model and analyze the behaviors of biochemical reactions in the computational framework.

The notion of reaction automata is an extension of reaction systems in which reaction rules are defined by triples consisting of reactants, inhibitors, and products. A computation process of a reaction automaton is performed in such a way that each time receiving one symbol from an input string, a reaction automaton changes its memory (multiset) by applying reaction rules to the multiset. Since reaction automata are a computing model based on multiset rewriting that accepts string languages, their modeling capability for chemical reactions can be investigated in terms of the theory of computation ([3]).

It is shown that reaction automata are computationally Turing universal. Further, Computational complexity issues for some classes of reaction automata are explored in comparison to language classes in the Chomsky hierarchy.

[1] C.Calude, Gh. Paun, G. Rozenberg and A. Salomaa (Eds.), *Multiset Processing*, LNCS 2235, Springer, 2001.
[2] A. Ehrenfeucht, G. Rozenberg, Reaction systems, *Fundamenta Informaticae* vol.75, pp.263-280, 2007.
[3] J.E. Hopcroft, T. Motwani, J.D. Ullman, *Introduction to automata theory, language and computation* - 2nd ed, Addison-Wesley, 2003.
[4] F. Okubo, On the Computational Power of Reaction Automata Working in Sequential Manner, *4th Workshop on Non-Classical Models for Automata and Applications*, book@ocg.at series 290, pp.149-164, Österreichische Computer Gesellschaft, 2012.
[5] F. Okubo, S. Kobayashi, T. Yokomori, Reaction Automata, *Theoretical Computer Science*, 429, pp.247-257, 2012.
[6] F. Okubo, S. Kobayashi, T. Yokomori, On the Properties of Language Classes Defined by Bounded Reaction Automata, *Theoretical Computer Science*, 454, pp.206-221, 2012.
[7] Y. Suzuki, Y. Fujiwara, J. Takabayashi, H. Tanaka, Artificial Life Applications of a Class of P Systems, in [1], pp.299-346, 2001.

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Progress in the Enumeration Approach to Computing Equilibrium of Interacting Nucleic Acid Strands

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Keywords: Nucleic Acid Strand Interaction, Equilibrium, Molecular Robotics, DNA Computing

DNA is one of the most promising material for constructing logical circuits, memory, intelligent devices equipped in molecular robots([1]). The problem of designing nucleic acid strands to avoid unwanted structures is difficult since they can form tremendously many number of structures and thus we need much of computation time for the analysis. Nucleic acid strand interaction analysis at equilibrium has been tackled by Dirks, et al.([2]),who have nicely extended Mackaskill's partition function computation algorithm for a single RNA molecule ([3]) to the case of multiple strands, and furthermore succeeded in computing the equilibrium state of interacting RNA molecules by using convex programming after computing partition functions of all strand complexes. The author introduced a new general approach to computing chemical equilibrium based on graph theory and optimization theory([4][5]). Distinguished feature of the method was that it only used convex programming but not dynamic programming. Essential point of the method based on the idea of enumerating all the structures in a reaction system by using a graph. This method was applied also to nucleic acid strands interaction, where we considered only linear secondary structures([6]). In this poster presentation, we will present further progress of this approach to the analysis of interacting nucleic acid strands.

[1] S. Murata, A. Konagaya, S. Kobayashi, H. Saito, M. Hagiya, Molecular Robotics: A New Paradigm for Artifacts, New Generation Computing, 31 (2013), 27-45, Ohmsha, Ltd. and Springer.
[2] R.M. Dirks and J.S. Bois and J.M. Schaeffer and E. Winfree and N.A. Pierce, Thermodynamic Analysis of Interacting Nucleic Acid Strands, SIAM Review, 49, 65-88, 2007.
[3] J.S. McCaskill, The Equilibrium Partition Function and Base Pair Binding Probabilities for RNA Secondary Structure, Biopolymers, 29, 1105-1119, 1990.
[4] S. Kobayashi, A New Approach to Computing Equilibrium State of Combinatorial Hybridization Reaction Systems, in Proceedings of Workshop on Computing and Communications from Biological Systems: Theory and Applications, CD-ROM, paper2376, 2007.
[5] S. Kobayashi, Enumeration approach to computing chemical equilibria, Theoretical Computer Science, 499, 51-88, 2013.
[6] Satoshi Kobayashi and Takaya Kawakami, Enumeration Approach to the Analysis of Interacting Nucleic Acid Strands, in Biomolecular Information Processing --- From Logic Systems to Smart Sensors and Actulators, Chap.12, pp.225-244, 2012.

Modeling and Simulation of self oscillating gel - toward a molecular gel robot

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

The self-oscillating gel is one of the interesting applications of the gel with the swelling-shrinking dynamics. The self-oscillating gel is the gel which pulsates spontaneously itself using the dynamics of BZ reaction inside the gel, and it is now developing as materials for actuators or soft robots. To use self-oscillating gel as these materials, we need much precise design of functions and properties, and encounter much more difficulties. For the purpose of the precise designing of the self-oscillating gel, we make the model for self-oscillating gel, and performing these simulations.

In the dynamics of self-oscillating gel, swelling and shrinking dynamics occur repeatedly, and in these dynamics the solvents and the polymers moves inside the gel. To model the self-oscillating gel, we must treat both dynamics of solvents and polymers.

In this study, the model of self-oscillating gel based on dissipative particle dynamics (DPD) [1] method is constructed on OCTA system[2], which is the multi-scale simulation system for soft materials in the meso-scale. The swelling-shrinking dynamics was represented by changing the interaction parameter between polymer and solvent particles. In the simulations, the frequency of the changing the interaction parameter can be controlled, and with changing its frequency, the structure and the dynamics of gel can be observed. In the slower frequency case, gel can take almost equilibrium structure at each time and the change of size of gel in the swelling-shrinking cycle is much larger, however in the faster case, the movement of solvents and polymers cannot follows along the change of the interaction parameter and the delay of phase of the swelling-shrinking dynamics occurs. Noted that our model follows the previous theoretical model of gel, stress-diffusion coupling model [3], which can be applicable to the many kinds of dynamics of gel widely.

Our model is also applied to the peristaltic motion of gel. This is one of the models of worm or soft molecular robot. Our simulations indicate that our worm-like gel moves with pulsating. Our model indicates that our gel model is swimming in the solvent using the power of the BZ reaction. Detail of our simulation results and some movies will be shown in the session.

References

[1] R.D. Groot and P.B. Warren, J. Chem. Phys. 107, 4423 (1997)
[2] http://octa.jp
[3] T.Yamaue, T. Taniguchi, M. Doi, AIP Conf., Proc. 519, 584 (2000)

C-5-60

Numerical studies of protein-induced shape changes of liposomes

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Keywords: Lipid bilayer membranes, cytoskeleton, numerical calculations, molecular robotics

Liposomes are closed vesicles formed by lipid bilayer membranes. They have been well studied as simplified models of biological membranes, and are also considered as a possible component for molecular robots. When cytoskeletal proteins, such as actin and tubulin, are polymerized inside liposomes, elongating cytoskeletal filaments push the membrane and deform it. As a result, liposomes change their morphology into a lemon shape, flattened shape, spoon shape, or a shape having long membrane projections. Other proteins, such as talin, induce large holes in the membrane, and liposomes become cup-like shapes. We have analyzed these phenomena using a membrane elasticity model, and showed that the numerical calculations reproduce most of these shape changes. Only a few piconewtons of cytoskeletal forces are needed for large membrane deformations, but actual liposomal morphologies are determined also by the membrane properties, arrangement of cytoskeletal filaments, and pressure difference between inside and outside of the membrane. We expect that the methods used in the calculations may also be useful for designing the molecular robots.

C-5-61

Robustness Criteria of Hybridization of double-stranded DNA Sequences

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Keywords: Electronic structure calculation, Ligand docking, Protein modeling

Predicting the behavior of DNA molecules in vitro is a fundamental issue on DNA computing and related areas, but is also known to be quite difficult. Computational simulation is one of the most reasonable approaches for the prediction, which enables us to circumvent money/time-consuming biochemical experiments, and several simulation models have been proposed indeed. However, simulation approaches are still time-consuming in some scenario; for example, in order to design DNA sequences that hybridize in some preferable way, standard local search algorithms need to refer thousands of simulation results, which is actually impossible to complete it in reasonable computational time. In this paper, we propose simple criteria that are useful to roughly predict the behavior of DNA sequence hybridization. By using this criteria, we can determine whether a given DNA sequence *s* and its complement sequence *s'* form the perfect double stranded structures quickly, or slowly. The proposed criteria do not require computational simulations and can be easily computed. We conducted computational experiments that simulate hybridization of double-stranded DNA sequences under a probabilistic simulation model proposed in [1], whose validity is shown via comparison with biochemical experiments. The simulation results show that the proposed criteria are promising to predict the hybridization speed of double-stranded DNA sequences.

[1] Masashi Shiozaki, Hirotaka Ono, Kunihiko Sadakane, Masafumi Yamashita: A Probabilistic Model of the DNA Conformational Change. Proceedings of DNA 2006, Lecture Notes in Computer Science, 4287 274-285, 2006.

C-5-62

Recapitulation of the hepatic function using *in vitro* liver model from murine ES/iPS cells

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Keywords: Embryonic stem cell, Liver, Metabolism, Cell polarity, Urea cycle

Polygonal and multipolar hepatocytes in the liver are surrounded by sinusoids, bile canaliculus, and adjacent hepatocytes. It is only in the context of hepatic tissue architecture that hepatocytes can express their specific and multiple functions. Hepatocyte polarity exerts a major influence on the physiology of the cell. Recently, we established a unique system of in vitro liver model derived from murine ES/iPS cells, *i.e.*, IVL$^{mES/iPS}$ [1, 2]. The IVL$^{mES/iPS}$, consisting of not only hepatocytes, but also endothelial networks, together with cardiac mesoderm differentiation, was induced after the embryoid body formation.

To confirm cellular polarities of the IVL$^{mES/iPS}$, first, dichlorofluorescein diacetate (CDFDA) was added into the IVL$^{mES/iPS}$. In liver, CDFDA is incorporated into hepatocytes via OATP2 which expresses at apical side, and afterward CDFDA was hydrolyzed by cytoplasmic esterase to green fluorescent CDF, and which is excreted to bile canaliculus via MRP2. CDF was observed to be accumulated at the boundary of the cells in the IVL$^{mES/iPS}$, but not in primary hepatocyte culture. Second, we tried to activate urea cycle by addition of L-ornithine in the IVL$^{mES/iPS}$ or liver perfusion system. Urea production increased and ammonia decreased in a dose-dependent manner with respect to the amount of L-ornithine both in the IVL$^{mES/iPS}$ and the liver perfusion system, but not in primary hepatocyte culture [3].

Here, we demonstrated that architectural and functional properties in the IVL$^{mES/iPS}$ were quite similar to those in the liver perfusion system, but different from those in the culture of primary hepatocytes. The IVL$^{mES/iPS}$ has great promise to be useful for drug metabolism and pharmacokinetics in liver as an alternative to animal experiments.

[1] Ogawa, S., Tagawa, Y., Kamiyoshi, A., Suzuki, A., Nakayama, J., Hashikura, Y., Miyagawa, S., Crucial roles of mesodermal cell lineages in a murine embryonic stem cell-derived in vitro liver organogenesis system, *Stem Cells*, 23:903-913, 2005.

[2] Tamai, M., Yamashita, A., Tagawa, Y., Mitochondrial development of the in vitro hepatic organogenesis model with simultaneous cardiac mesoderm differentiation from murine induced pluripotent stem cells, *Journal of Bioscience and Bioengineering*, 112:495-500, 2011.

[3] Tamai, M., Aoki, M., Nishimura, A., Morishita, K., Tagawa, Y., *In vitro* recapitulation of the urea cycle using murine embryonic stem cell-derived *in vitro* liver model, *Amino Acids, in press*.

A Design Method of Artificial Genetic Circuits on Effective Search of These Logical Structures

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Keywords: Synthetic biology, Artificial genetic circuit, Logical modeling, Dynamic modeling

In the context of synthetic biology, artificial genetic circuits are designed in the following way: after setting a biological target phenomenon to be investigated, reaction parameter estimations among related molecules are conducted based on the dynamic analyses with mathematical models, finally, a system of biological reactions is developed with these molecules in vivo or in vitro. It is desired to develop an effective method to select the suitable circuits for realizing the target phenomenon, because not a few models are possible as candidates for the target phenomenon.

We propose a new procedure to effectively design a mathematical model by the following two steps. The first step is the process creating possible structures of the mathematical by a logical technique. The second step is the process creating dynamic models in two ways; a system of differential equations for the analysis of dynamics in the model and a model of hybrid functional Petri net as a common platform for knowledge sharing between biologists and computer scientists.

[1] Gardner, T.S., Cantor, C.R., Collins, J.J., Construction of genetic toggle switch in Escherichia coli, Nature 403:339-342, 2000.
[2] Fauré, A., Thieffry, D., Logical modeling of cell cycle control in eukaryotes: a comparative study, Molecular Biosystems, 5:1769-81, 2009.
[3] Cell Illustrator: http://www.cellillustrator.com/home

C-5-64

Identification of Key Regulators in Glycogen Utilization in *E. coli* Based on the Simulations from a Hybrid Functional Petri Net Model

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Keywords: metabolic pathway; glycogen; hybrid functional Petri net; PTS; HPr and EIIAGlc proteins; gene regulation

Background: Glycogen and glucose are two sugar sources available during the lag phase of E. coli, but the mechanism that regulates their utilization is still unclear.

Methods: Attempting to unveil the relationship between glucose and glycogen, we propose an integrated hybrid functional Petri net (HFPN) model including glycolysis, PTS, glycogen metabolic pathway, and their internal regulatory systems.

Results and Conclusions: By comparing known biological results to this model, basic necessary regulatory mechanism for utilizing glucose and glycogen were identified as a feedback circuit in which HPr and EIIAGlc play key roles. Based on this regulatory HFPN model, we discuss the process of glycogen utilization in E. coli in the context of a systematic understanding of carbohydrate metabolism.