(where pF is a phosphotyrosine and X is any amino acids). CRK have been considered to be indispensable for malignant features of human cancers, and it is important to develop novel inhibitors of CRK. Mediated protein-protein interactions for new cancer therapies. In this work, we performed docking based screening of small peptides against the CRK SH2.

Conventional docking programs have been developed to design small molecules and cannot be applied for peptide design because of their following characteristics. (1) Peptides have many rotatable bonds, which makes difficult to predict binding mode in a peptide-protein complex. (2) Peptides also have more polar functional moieties than small molecules and no proper scoring functional available for peptide docking. Scoring of docking poses using MM (molecular mechanics) is a useful approach to estimate binding affinities of peptides to their target protein. But the problem of inaccuracy of docking poses still remains.

In this work, we developed our docking program using MM and the generalized born (GB) solvent. For large computational requirements of MM and GB, we accelerated our program using GPU. After docking, a MM-PBSA rescoring was conducted to estimate binding affinities. Furthermore, we performed additional conformational search of peptides in unbound states to take account of ligand’s reorganization effects. Our methods showed high performances in discrimination of known binding sequences.

**2PT141** タンパク質 (DHFR) における周囲環境が引き起こす運動性の変化に関する理想的な模倣

**Theoretical study of change of motility by local mutation in DHFR**

**Tomo Matsubara,** Masashi Fuji, Hiraku Nishimori, Akinori Awasu (Dept. of math and Life Sci, Hiroshima Univ)

The dihydrofolate reductase (DHFR) is composed of some secondary structures and fluctuating loops called Met20 loop, CD loop, FG loop, GH loop. The recent experimental study indicates that the dynamical properties encoded by such molecular structure provide the dominant contribution to the function of DHFR, where a small mutation of CD loop which does not give structural variation of the molecule and far from the reaction site (around Met20 loop) gives a drastic change in intra-molecular global motion and its reaction activity. In the present study, we perform the normal mode analysis of an all-atom model of DHFR and discuss the general features of the relations among the local and global motions of such molecules.

We employ the elastic network model as all-atom model of protein dynamics. In this model, each atom is described as beads and each pair of beads connected by virtual bonds with the natural length that equals to the native distance between the corresponding atoms. We obtained the atomic coordinates from Protein Data Bank (PDB code: 1x2r, 1x6x) to construct the structure of DHFR. By the normal mode analysis of this model, we investigate the correlation of motility among characteristic local structures and discuss the mechanism of the suppression of the reaction activity by the minor local mutation observed in the experiment.

**2PT142** Theoretical evaluation of structural stability of the active site of T1 lipase: cation-π vs. water-π interactions

**Atsushi Nakamura,** Jiyoung Kang, Masaru Tateno (Grad. Sch. Sci., Univ. Hiroji)

Crystallographic data obtained by Matsumura, H., et al. (Osaka Univ.) suggested that the sphere electron density that was found in the active site of T1 lipase is corresponding to the interaction of Na+ and the aromatic ring of a Phe residue (i.e., cation-π interaction). However, it was also argued that a water molecule can interact with π electrons (i.e., water-π interaction). Thus, experimental approaches could not dismiss the possible presence of water instead of Na+ in the active site of T1 lipase. We theoretically evaluated these two types of interactions, employing molecular dynamics (MD) simulations.

The current force fields cannot estimate the cation-π interaction correctly, requiring *ab initio* quantum mechanical calculations. So, we developed a novel efficient scheme to calculate the potential energy of cation-π interaction, with the high accuracy at the CcSD(T) level and the low calculation cost comparable to the standard force field. Our MD calculations showed that a large enthalpy gain of the Na+–π interaction can preserve the catalytic core structure. In contrast, water-π interaction seriously induced the structural instability. Thus, the possible presence of water may be excluded [1-2]. Our novel scheme is currently the only way to perform long-time MD simulations involving cation-π interaction, with reasonable calculation cost. In the session, the functional roles of cation-π interactions in T1 lipase will be discussed.

[1] JCTC 7 (2011) 2593

---

**2PT143** 分子動力学シミュレーションによる緑色蛍光タンパク質の切断位置と蛍光発現の研究

**Research of Split Point and Fluorescent Recovery of Green Fluorescent Protein by Molecular Dynamics Simulation**

**Masahiro Ito** 1,2, Shoji Takada, 2 Takaezi Ozawa 2 (1 Dep. Chem., Sch. Sci., Univ. Tokyo, 2 Dep. Bio, Grad. Sch. Sci., Kyoto Univ.)

Split Green Fluorescent Protein (Split GFP) is a useful tool for investigating protein-protein interaction in vivo. Each fragment of Split GFP are reconstruct chromatophore by binding and recover fluorescence. However, less is known about the mechanism of folding and reconstituting of Split GFP. In particular, the reason why fluorescence depends on the position of split point. To reveal this, we performed molecular dynamics simulation of Split GFP, Coarse-Grain simulation and All-Atom simulation. From Coarse-Grain simulation of Split GFP, here we report three points about Split GFP Folding. First, N-terminal fragment in Split GFP is folding earlier than C-terminal at all events. Second, the folding of C-terminal fragment and whole GFP are at the same time. Finally, the stability of N-terminal fragment is related to the speed of folding of whole Split GFP. From these data we devised a model of Folding of Split GFP that first N-terminal fragment becomes core structure of GFP, and then it will covered by C-terminal fragment. From All-Atom simulation, we evaluate the stability of Split GFP of each split point and show the difference of split point in loop regions or β-strand regions. These results provide a new perspective on split protein folding.

**2PT144** SlyD融合による人工三本葉ペプチドの可変性の試み

**Solubilization approaches of artificial three-stranded beta-helices by fusing SlyD protein**

**Kaname Nishijo** 1, Shuji Kanamaru 2, Fumio Arisaka 1 (1 Dept of LifeScience, Grad Sch of Biosci&Bioeng, Tokyo Institute of Technology, 2 Dept of Bioengineering, Grad Sch of Biosci&Bioeng, Tokyo Institute of Technology)

The C-terminal domain of gsp of bacteriophage T4 has a unique three-stranded parallel beta-helix and forms a triangular prism. The amino acid sequence of this domain has the characteristic 12 repeats of 8 amino acids VXGXXXXX. We aim to create artificial triple beta-helix proteins whose length is controlled by tandemly connecting the building block of 6 repeats of VXGXXXXX (VXXGh).

In our previous studies of tandemly connected (VXGh), by genetic engineering, we were able to create and purify the artificial triple beta-helix which was expected to consist of one or two building blocks. However, large amounts of larger oligomers as well as the dimer of beta-helix were formed. Those larger oligomers are formed by association the artificial three-stranded beta-helix. In order to avoid the dimer formation of the beta-helix, we fused 300SlyD at the N-terminus of the three-stranded beta-helix. SlyD is known to have the ability to solubilize fused proteins. Sedimentation velocity analysis of the purified proteins indicated that the fusion product was still the dimer of the beta-helix. In addition, size exclusion chromatography of the products indicated the presence of partially unfolded beta-helices. Thus we concluded that the dimerization of the beta-helix spontaneously occurred at the N-terminus of the beta-helices which is partially unfolded.

**2PT145** Fluorescence Titration for finding the binding sites of Peptide Aptamers on Calmodulin

**Yasodha Manandhar,** Wei Wang, Takamori Uzawa, Yoshihiro Ito, Toshihiro Ajigaki (RIKEN, Advanced Science Institute)

Aptamers are in vitro selected nucleic acids or peptides which specifically bind to various target molecules. Although aptamers modified with a fluorescent molecule are often used for diagnoses to signal out its target binding, such modifications sometimes interfere the specific binding. To avoid this we previously selected signaling peptide aptamers from a library of random sequenced peptides including a non-natural fluorescent amino acid, 7-Nitro-2, 3-benzoxadiazole modified amino phenyldalanine (NBD-aa). We choose a multifunctional intermediate messenger protein, calmodulin (CaM), as a target. CaM transduces calcium signals by binding calcium ions on its two approximately symmetrical globular C and N domains. We found that one of the selected peptides, C5 (YWDKDKI*G**G**NBD-aa) has a strong binding affinity (Kd= 2) with the C domain of CaM. However the binding sites of the other selected peptides have not been clarified yet. Thus motivated here we investigated the binding sites of those peptides from the titrations with the CaM-