3C1112 好冷性酵素内での化学反応における構造揺らぎの理論的研究**Theoretical Study of Structural Flexibility upon a Chemical Reaction in a Psychrophilic Enzyme**Takahiro Kosugi, Shigehiko Hayashi (*Department of Chemistry, Graduate School of Science, Kyoto University*)

It is known that some enzymes exhibit peculiar temperature dependence for the catalytic activity. It is often considered that the temperature dependence of catalytic activity is related to structural flexibility of the enzyme. However, it was only indirectly researched and is still not clear how the flexibility affects the catalytic activity. Psychrophilic enzymes have been researched as the one of best examples to understand the relationship between temperature dependence and structure flexibility. We researched the role of structure flexibility for catalytic activity in a psychrophilic α -amylase. The enzymes catalyze the hydrolysis of starch, glycogen, and related polysaccharides by cleaving internal α -1,4-glycosidic bonds. It is reported that the reaction would be divided into two stages, α -glycosidic bond cleaving and addition of a water molecule. In this work, we developed QM/MM Reweighting Free Energy SCF (QM/MM-RWFE-SCF) that improved QM/MM free energy with mean field approximation to be applicable for large and anisotropic systems such as enzymes. With this new method, the electronic structures and geometries in the active sites were optimized on free energy surface, which includes the structural flexibility of enzyme, and the first step of chemical reaction in a psychrophilic α -amylase was researched. As a result, it was shown that the structural flexibility of enzyme was important for the chemical reaction in the enzyme.

3C1124 タンパク質 (DHFR) における局所変異が与える大域的揺らぎの変化に関する理論的考察**Theoretical study of modulation of global fluctuation by local mutation in DHFR**Daiki Kaita, Akinori Awazu, Hiraku Nishimori (*Dept. of Mathematical and Life Sciences, Hiroshima University*)

The dihydrofolate reductase (DHFR) is composed of some domains like CD loop (or Met20 loop), FG loop and Active site. The recent experimental study indicates that the relations between the structure and dynamics are of central importance on the catalyzed reaction of DHFR, where a small mutation of the minor local structure gives a drastic change in intra-molecular global motion and its reaction activity. In the present study, we perform the simulation of a coarse-grained 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 coarse-grained model of protein dynamics. In this model, each amino acid is described as a beads and each pair of beads connected by virtual bonds with the natural length that equals to the native distance between the corresponding amino acids. We refer PDB (code: 1rx1,2,3,4,5,6) to construct the structure of DHFR. By the Brownian dynamics simulation and the normal mode analysis of this model, we investigate the correlation of fluctuations among characteristic local structures and discuss the mechanism of the suppression of the reaction activity by the minor local mutation observed in the experiment.

3C1136 Inconsistency in the Structure-Function Relationship of a Typical Allosteric Protein: What Do We Call T and R?Antonio Tsuneshige¹, Yusuke Tajiri², Kenji Kanaori², Yuki Yashiro¹, Hajime Nishihara¹ (¹*Hosei University*, ²*Kyoto Institute of Technology*)

By chemically modifying the intradimeric $\alpha 1\beta 1$ subunit interface of tetrameric hemoglobin, we have obtained a derivative that shows a markedly reduced affinity for oxygen and normal response to pH. At pH 7.4 and in the presence of 0.1M chloride, the reduced affinity for oxygen of this derivative was comparable to that of normal Hb in the presence of strong allosteric heterotropic effectors, such as inositol hexaphosphate (IHP) and bezafibrate (BZF). The EPR spectrum of the nitrosyl derivative in the absence of effectors showed a hyperfine structure around g=2 typical of a derivative in the low-affinity conformation that is obtained for native Hb only in the presence of strong allosteric effectors. Isothermal titration calorimetry showed that the dimerization of this derivative was slightly hampered. This fact was consistent with its T-conformation character. In contrast, 1H-NMR measurements showed strikingly opposite results. Both the O₂-ligated and CO-ligated forms of this derivative exhibited spectra typical of the R-conformation, i.e., the absence of the typical T-makers around 11 and 14 ppm even in the presence of IHP and

BZF, whereas the unligated form of this derivative showed the typical T-makers. These NMR results are diametrically opposed and entirely inconsistent with the functional and structural characterization of the derivative obtained previously. This derivative represents a case in which there is a complete divorce between "structural T" and "functional T."

3C1148 New implementation of the reaction path determination in the QM/MM methodJaewoon Jung¹, Suyong Re², Sugita Yuji¹, Seiichiro Ten-no³ (¹*RIKEN AICS*, ²*RIKEN*, ³*Kobe University*)

We implement a new method of minimum energy path determination within the GHQ QM/MM framework. Our method is based on the constraint optimization with the locally updated planes method and includes three new techniques for enhancing the stability and effectiveness of the calculation: (1) An improved tangent estimation of the reaction path by using only the neighboring image with higher energy, (2) an energy-weighted reparametrization of each image using a cubic spline interpolation scheme before updating local tangents, and (3) the removal of constraints for translation and rotation. For the efficient optimization, we use a microiteration optimization scheme with a second order optimization for the QM region and first order optimization for the environment. We test our implementation on the isomerization of an alanine dipeptide, conversion of chorismate to prehenate, and the catalytic subunit of cAMP-dependent protein kinase (PKA). Unlike the previous constrained optimization scheme, our method could find a reliable reaction path of the alanine isomerization and chemical reaction with an enzyme environment. Moreover, our scheme needs less number of energy/gradient calculation with good parallel efficiency than other chain-of-states method.

13:10~16:10 C会場 / Room C

分子遺伝・遺伝情報制御、発生・分化、放射線生物 / 活性酸素

Molecular genetics & Gene expression, Development & Differentiation, Radiobiology & Active oxygen

3C1322 協調的な細胞運動と自己組織化する集合シグナルとの相互依存性**Relation between collective cell migration and self-organization of chemoattractant waves**Akihiko Nakajima¹, Shigenori Hirose², Daisuke Taniguchi¹, Gad Shaulsky², Adam Kuspa², Satoshi Sawai^{1,3} (¹*Department of Basic Science, Graduate School of Arts and Sciences, University of Tokyo*, ²*Baylor College of Medicine*, ³*Research Center for Complex Systems Biology, University of Tokyo*)

Development of Dictyostelium discoideum is largely dictated by chemoattractant cAMP that self-organize as periodic waves that direct collective cellular movement. The similarity between wave patterns of cAMP and those typically observed in BZ-reaction points to the fact that as a first approximation, formation of waves can be depicted as simple reaction-diffusion process. However, different wave patterns such as meandering and multi-arm spirals often appear during cell aggregation, which suggests that there is a mutual feedback between the chemical field and cell migration. Here, we investigate phase dependence between the cycles of the cAMP wave propagation and cell migratory behaviors. With the aid of a FRET-based cAMP reporter, we performed in-vivo imaging of the spatio-temporal oscillations of cytosolic cAMP level during the aggregation stage. We demonstrate that the periodicity of the wave propagation switches from slow (6-9 min) to fast (3-4 min) oscillation and that this transition coincided with the onset of directional cell migration. In the deletion mutants of the cell adhesion molecules tgrB1 and tgrC1, we found that the fast frequency cAMP wave propagation takes place for an extended period of time and that waves become unstable. This instability resulted in the dispersion of aggregated cells. These results indicate that the observed change of wave frequency works as a signal for the onset of aggregation and that the cell-cell contact mediates the stabilization of cAMP wave propagation.

3C1334 分化・未分化状態間の揺らぎを検出するために単層培養したマウス ES 細胞のタイムラプスイメージング**Time-lapse imaging of mES cell monolayer cultured for detection of fluctuation between undifferentiated and differentiated states**Atsushi Maruyama¹, Kiyoshi Oonuma¹, Yuichi Wakamoto² (¹*TRI, Nagaoka University of Technology*, ²*Grad. Sch. Arts and Sci., Univ. Tokyo*)