3TP1-04 真性粘菌変形体のチューブ構造に保存される細胞運動の履歴

3P-240 Locomotive history of *Physarum* plasmodium recorded in its tubular structure

Tomohiro Shirakawa (1), Yukio-Pegio Gunji (2), Yoshihiro Miyake (1) ((1) Department of Computational Intelligence and Systems Science, Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology; (2) Department of Earth and Planetary Sciences, Graduate School of Science, Tokyo Institute of Technology)

The plasmodium of *Physarum polycephalum* is a very large unicellular and multinuclear organism. Despite its very large size, whole cell body of the plasmodium behaves cooperatively, maintaining the integrated unity as a single cell. For instance, all the parts of the plasmodium remain connected by cellular tubes, and the arrangement of tubes is so highly effective to allow the plasmodium to be used in graph theoretical studies. Recently, such an aspect of the organism is attracting a lot of attention in the fields such as mathematical biology, information science, and systems science.

We found in the previous study that the morphology of each sub-region of plasmodial tubular network tends to be homologous. In other words, the morphological properties of the local sub-networks are spatially shared throughout the cell body. We tested in this study whether such an effect is also present temporally. In our experiment, we provided narrow paths which are several centimeters in length, on agar plate. The plasmodium traveled along with the path, and then entered a plane without any obstacles. We observed the motility of the plasmodium on the obstacle-free plane, and found that the motility depends on the arrangement of its tubular structure left in the path. Since the tubes are formed in the track of locomotion, the results thus indicates that the organism leave the tubular structure as a record of its planar exploration, using the tubes as a "memory device".

3TP1-05 バクテリアのセンシングノイズと走化性

Bacterial chemotaxis is enhanced by nonadaptive fluctuation in sensory system

Masatoshi Nishikawa (1), Tatsuo Shibata (2) ((1) Hiroshima University, CREST: (2) Hiroshima University, CREST, PREST)

Adaptation is the essential property in the sensory system. It enables to respond over the wide range of the signal, in the presence of the background stimulus. In the case of bacterial chemotaxis, adaptation makes chemotaxis range of bacterium over the four order of chemoattractant concentration. However, recent experiment demonstrated that the sensory system generates the large fluctuation. Furthermore our numerical study finds that the receptor activity fluctuates depending on the environmental attractant concentration.

We constructed a two state receptor model and calculated the fluctuation strength in detail. It is found that ligand sensitivity of the fluctuation is a consequence of the competition between the parallel depletion paths of receptor activity, in which are the enzymatic modification and ligand dependent transition. Such fluctuation affects the bacterial behavior. In the spontaneous motion, run duration shows the ligand dependent change. In the absence of the ligand, it has the heavy tail in the distribution, whereas it is exponentially distributed in the presence of enough ligand. This change affects the mobility, measured as the effective diffusion constant, in long time. It is decreasing as the increase of the environmental ligand. Furthermore, the behavioral fluctuation also enhances the chemotactic velocity. In long time scale, the sensory fluctuation also improves the aggregation property. Our finding supposes that the sensory fluctuation holds the environmental information, enhancing the bacterial motility.

3TP1-06 生体分子のバタン表面での挙動をシミュレートする

Computational Simulation of the Behaviors of Biological Macromolecules on Patterned Surfaces

Dan Zhu (1), Bo Zhao (1), Chun Mao (1), Xiaohua Huang (1), Masaru Matsuo (2) ((1) Jiangsu Key Laboratory of Biofunctional Materials, College of Chemistry and Environmental Science, Nanjing Normal University; (2) Nara Women's University)

The computational simulation on the dynamic behaviors of bio-macromolecules or their composite form on the patterned surfaces, such as Si (100), has been done by the DMol calculation of Materials Studio. The mechanism of the adsorption or the adhesive has been studied, as well as the orientation of the molecules on the surface. The topological morphology and the electronic arrangement of the material surface affect the dynamic behavior of the bio-molecules.

3TP2-01 金属置換した紅色光合成細菌反応中心の赤外分光研究

3P-225 FTIR study of metal-replaced bacterial photosynthetic reaction center.

Akihito Watanabe (1), Tastyu Iwata (1), Mark L. Paddock (2), Melvin Y. Okamura (2), Hideki Kandori (1) ((1) Department of Frontier Materials, Nagoya Institute of Technology; (2) Department of Physics, University of California, San Diego.)

The bacterial photosynthetic reaction center (RC) from *Rhodobacter sphaeroides* contains the primary (Q_A) and secondary (Q_B) quinones, which are interconnected via a specific His-Fe-His bridge (Q_A : His-M219: Fe^{2+} : His-L190: Q_B). In the Q^-/Q difference FTIR spectra of WT RC, broad positive bands appear in the 2800-

2400 cm^{-1} region. This indicates the appearance of strongly hydrogen-bonded X-H groups (X=O, N), but we recently showed that the bands do not originate from water O-H stretches [1]. A candidate is the N-H stretching vibration of His ligating Fe^{2+} . The present FTIR study shows the bands shifting upon ^{15}N -labeling supporting this proposal. We further studied the structure by use of metal-replaced RCs. We prepared RCs containing Zn^{2+} , Cd^{2+} , and Pb^{2+} in place Fe^{2+} and compared the Q_A^-/Q_A and Q_B^-/Q_B difference FTIR spectra. These replacements do not seriously affect the reaction. The obtained spectra were mostly very similar. However, we found negative bands at 3338 and 3303 cm^{-1} for Q_A^-/Q_A and Q_B^-/Q_B , respectively, with altered frequencies dependent on the coordinated metal ion. We thus concluded that these bands originate from His-M219 and His-L190 in the charge neutral state (Q_A and Q_B respectively). Upon formation of the Q^- state by photo-induced electron transfer, the N-H stretching vibrations of His appear at the frequencies down-shifted by $>500\text{ cm}^{-1}$. The interaction mechanism of the active center will be discussed on the basis of the present FTIR results.

[1] Iwata et al. Biochemistry 48, 1220 (2009).

3TP2-02 好熱性ラン藻の D1:3 は恒常的に発現している D1:1 よりも構造的に安定な光化学系 II 複合体を構成する

The substitution of D1:3 for D1:1 in Photosystem II of *Thermosynechococcus elongatus* results in a structurally more stable complex.

Miwa Sugiyama (1), Sayo Harada (2), Eri Iwai (2), Takashi Manabe (2), Hidenori Hayashi (1), Alain Boussac (3) ((1) Cell-Free Science and Technology Research Center, Ehime University; (2) Department of Chemistry, Ehime University; (3) iBiTec-S, CEA Saclay, France)

Photosystem II (PSII) the enzyme responsible for photosynthetic oxygen evolution. It is composed of 17 membrane-spanning proteins and 3 extrinsic proteins. Most of the cofactors concerning the electron transport locate on D1 protein. D1 is known as the protein which has the faster turn over in part as a consequence of photoinhibition processes. The thermophilic cyanobacterium *Thermosynechococcus elongatus* has 3 homologues D1 genes in its genome. The respective amino acid sequences of the three D1 variants are not identical. For instance, D1:3 and D1:1 differ by 21 amino acid residues. The D1:1 gene is constitutively expressed whereas the expression of D1:3 is induced by high light irradiation. We found that the oxygen-evolving activity of purified PSII composed of D1:3 is ~ 1.8 times higher than that in PSII with D1:1. Little is known on the role of the small subunits in PSII and their link with the PSII disassembly/assembly process occurring during the D1 turn over. In this work, we analysed the role of PsbJ in D1:1 strain and in D1:3. Δ PsbJ/D1:1-PSII decreased the oxygen-evolving activity by 70%, whereas it has no effect in Δ PsbJ/D1:3-PSII. MALDI TOF/MS analysis demonstrated that the Δ PsbJ/D1:1-PSII complex lacked not only PsbJ but also PsbM and Cyt *c*550, whereas the Δ PsbJ/D1:3-PSII lacked only PsbJ. Moreover, 50% of Δ PsbJ/D1:1 PSII complexes were monomer, whereas Δ PsbJ/D1:3-PSII complexes were dimeric form. These results will be discussed in view of the α helices interactions.

3TP2-03 シリカメソ多孔体細孔中に導入した好熱性シアノバクテリア光化学系 II コア複合体の機能

Function of photosystem II core complexes of a thermophilic cyanobacterium introduced into silica mesoporous materials

Tomoyasu Noji (1), Chihiro Kamidaki (1), Keisuke Kawakami (2), Jian-Ren Shen (2), Tsutomu Kajino (3), Yoshiaki Fukushima (3), Takeshi Sekitoh (4), Shigeru Itoh (1) ((1) Division of Material Science, Graduate School of Science, Nagoya University; (2) Graduate School of Natural Science and Technology, Okayama University; (3) Toyota Central R&D Labs. Inc.; (4) TOYOTA MOTOR CORPORATION)

Silica mesoporous materials has nano size pore in large quantity, and are an inorganic materials. We so far succeeded in introducing some membrane proteins of photosynthesis into silica mesoporous materials. LHII isolated from thermophilic purple photosynthetic bacterium *T. tepidum* and PSI from *T. elongatus* maintained activity and increased thermal stability after introduced into silica mesoporous materials. We introduced the photosynthetic photosystem II (PSII) pigment-protein complex into silica mesoporous materials. The dimer complex of PSII core complexes (756 kDa) were purified from a thermophilic cyanobacterium *Thermosynechococcus vulcanus*. The adsorption to silica mesoporous materials partially decreased the oxygen evolution activity without significant effects on the pigment assembly and the protein structure based on the absorption and fluorescence spectrum. The high thermal stability of oxygen evolution reaction was maintained inside silica. We study the effects of pore size too. PSII adsorbed to silica can be used as a new material for the artificial reactions.

3TP2-04 Ca^{2+} を除去した Mn クラスターの S_0 および高酸化状態の ESR Modified S_0 -state and higher oxidized state EPR signal in Ca^{2+} -depleted Mn-cluster.

Momoko Kuriyaki (1), Hiroyuki Mino (1) ((1) Graduate School of Science, Nagoya University)

O₂ evolving is carried out by Mn-cluster in Photosystem(PS) II. In the enzyme cycle a Mn-cluster goes through five sequential redox states denoted S_n, where n varies from 0 to 4, upon the absorption of four successive photons. In the S₄ state two molecules of water are oxidized, the S₀-state is regenerated, and O₂ is released. By low-pH treatment, one Ca ion per PS II is selectively released concurrently with the loss of the capability of O₂ evolution which is restored by exogenously added Ca. The S₀ and S₂ states are paramagnetic with S=1/2, giving rise to a broad EPR multiline signal that can be detected at low temperature. Low-pH treatment leads to the formation of an S₂ state that is characterized by EPR multiline signal with distinctly altered multiline patterns. In order to investigate the structural modification of Ca²⁺-depleted Mn-cluster, we measured S₀-state and higher oxidized state EPR signal in Ca²⁺-depleted PS II. The S₀-enriched untreated membranes were prepared by 3 flash illumination of the S₁-enriched membranes after Y_D[•] radical was reduced chemically. The low-pH treatment has performed in S₀-enriched membranes. We detected modified S₀ multiline signal similar to that observed in untreated S₀ multiline signal. By addition of Ca²⁺ ion, normal S₀ multiline signal was appeared. Based on these observations, we will discuss the modified structure in Ca²⁺-depleted PS II.

3TP2-05 Photosystem II の蛋白質環境がどのようにしてクロロフィルペア P680 の強力な酸化力を引き出しているか

How photosynthetic reaction centers control oxidation power in chlorophyll pairs P680 in Photosystem II

Hiroshi Ishikita (1) ((1) *Kyoto University, Career-Path Promotion Unit for Young Life Scientists*)

At the heart of photosynthetic reaction centers (RCs) are pairs of chlorophyll *a* (Chl*a*), P700 in photosystem I (PSI) and P680 in photosystem II (PSII) of cyanobacteria, algae, or plants, and a pair of bacteriochlorophyll *a* (BChl*a*), P870 in purple bacterial RCs (PbRCs). These pairs differ greatly in their redox potentials for one-electron oxidation, *E*_m. For P680, *E*_m is 1,100-1,200 mV, but for P700 and P870, *E*_m is only 500 mV. Calculations with the linearized Poisson-Boltzmann equation reproduce these measured *E*_m differences successfully. Analyzing the origin for these differences, we found as major factors in PSII the unique Mn₄Ca cluster (relative to PSI and PbRC), the position of P680 close to the luminal edge of transmembrane alpha-helix d (relative to PSI), local variations in the cd loop (relative to PbRC), and the intrinsically higher *E*_m of Chl*a* compared with BChl*a* (relative to PbRC).

3TP3-01 DNA—ヘリカーゼ相互作用の1分子観察

3P-253 Single—molecule observation of DNA-helicase interaction

Hiroaki Yokota (1), Yuko Chujo (2), Emi Nishimoto (3), Yoshie Harada (3) ((1) *iCeMS, Kyoto Univ.*; *PRESTO, JST*; (2) *Dept. of Frontier Sci., Univ. of Tokyo*; (3) *iCeMS, Kyoto Univ.*)

Escherichia coli UvrD is a superfamily 1 DNA helicase which plays a crucial role in nucleotide excision repair and methyl-directed mismatch repair. There is a general consensus that the enzyme unwinds a double-stranded DNA (dsDNA) from a 3' end single-stranded DNA (ssDNA) tail, a gap, or a nick and translocates along ssDNA in a 3'-5' direction. However, conflicting models for the detailed unwinding mechanism have been proposed.

To understand the unwinding and translocation mechanisms of the helicase in more detail using single-molecule fluorescence microscopy, we have been carried out fluorescence labeling of the helicase with a couple of fluorophores i.e. fluorescent dye, quantum dot or fluorescent bead and making improvements to our single-molecule force-fluorescence microscope in which single-molecule fluorescence imaging and single-molecule DNA manipulation by magnetic tweezers can be simultaneously performed.

We clearly visualized the binding modes of the helicase to various DNA substrates (18-nt ssDNA, 18-bp dsDNA, 18-bp dsDNA with a 20-nt 3' ssDNA tail, 4.7-kbp dsDNA and 4.7-knt ssDNA) immobilized on a PEGylated silicate coverslip, demonstrating that the helicase has low affinity with the 18-nt ssDNA and blunt dsDNA but high affinity with ss/ds DNA junction and that multiple helicases can attach to the 4.7-knt ssDNA¹. We then determined the number of the helicase interacting with DNA (18-bp dsDNA with various 3' ssDNA tail lengths (≥ 12 nt)) using a Cy5 labeled Cys-Ala mutant of the helicase, revealing that oligomeric form is the active form of the helicase and that dynamic interaction between them is necessary for efficient DNA unwinding.

1. Yokota *et al. Chem. Lett.* **38** 308-309 (2009).

3TP3-02 II型 DNA トポイソメラーゼの手品のような反応を直接みる

3P-261 Direct Observation of DNA Unlinking Magic by a Type-II DNA Topoisomerase.

Katsunori Yogo (1), Taisaku Ogawa (1), Saki Obata (1), Gen Nakajima (1), Junpei Suzuki (1), Kazuhiko Jr. Kinosita (1) ((1) *Department of Physics, Waseda University*)

Type-II DNA topoisomerases (topoII) are molecular machines that play key roles in the maintenance of DNA topology in cells. They control the degree of supercoiling of DNA and unlink the catenanes that arise during replication or recombination. Lack of their activities during cell division ultimately causes cell

death. TopoII unlinks DNA catenanes in an ATP-dependent manner, by catalyzing the transport of one DNA segment to the other side of a second DNA segment through a transient double-stranded break in the second segment. The work of topoII would seem like that of a magician who fascinates the audience.

We have developed a system that enables us to observe directly this unlinking magic under an optical microscope with fluorescently labeled DNA substrates. A pair of single molecules of DNA fixed on a chamber surface is mechanically braided with dual optical tweezers. Advantages of this method against bulk assay are that we can 1) observe unlinking reaction by a single molecule of topoII, 2) control the geometry of and the tension in the DNA braid in real time, and 3) observe the reaction repeatedly for the same DNA substrates. We have observed a DNA braid a few micrometer long is unlinked in one burst, in several tenths of a second after a waiting time typically a few minutes long. Apparently a single-molecule of topoII can unlink a braid of DNA in a processive fashion. We have also examined the dependence of unlinking rate of topoII on the DNA geometry and tension. Chiral preference for left- or right-handed DNA braid is also studied.

3TP3-03 細胞内 mRNA の長時間観察を可能にする蛍光核酸プローブ

3P-262 Long-term monitoring of intracellular mRNA by hybridization-sensitive fluorescent nucleotide probe

Takeshi Kubota (1), Shuji Ikeda (1), Hiroyuki Yanagisawa (1), Mizue Yuki (1), Akimitsu Okamoto (1) ((1) *ASI, RIKEN*)

We recently developed a doubly thiazole orange-labeled probe to achieve high fluorescence intensity for a hybrid with the target DNA or RNA, and effective quenching for a single-strand state of the probe. Emission from the probe was controlled by interdyne excitonic interaction. Here we evolved the probe for long-term live cell imaging. HPLC analysis showed that newly designed probes with a 2'-O-MeRNA structure had high resistance against exo- or endo- nucleases, which is in contrast to the low resistance of the former DNA probes. These modified probes retained the function on the on-off switching of fluorescence emission in sensitive response to RNA recognition. For live cell imaging, the probe was introduced using lipofectamine 2000 reagent, and observed for 18 hours in culturing condition. The probe fluorescence was detected mainly from nuclei, and translocation of fluorescence to the cytoplasm region was also observed. Cell life events, for example, migration and division of probe loaded cells, were also observed in a long-term monitoring and were similar to those observed for normal cells. Therefore, the probe showed no severe cytotoxicity. We concluded that it will be a practical tool for effective monitoring of the spatiotemporal characteristics of RNAs in living cells.

3TP3-04 軸対称偏光素子を用いた GFP1 分子発光双極子モーメントの3次元計測

Three dimensional measurement of emission dipole orientation of single GFP molecules by the use of axisymmetric polarizers

Tomomi Tani (1), Mami Nomura (4), Kenta Saito (2), Takeharu Nagai (1) ((1) *Research Institute for Electronic Science*; (2) *Nikon Imaging Center, Hokkaido University*; (3) *JST*; (4) *Graduate School of Frontier Sciences, University of Tokyo*)

Fluorescent single molecule imaging is one of the potential approaches to monitor structural dynamics and the function simultaneously. We employ this approach to monitor the structural dynamics of TrkA during its activation process. TrkA is a founding member of neurotrophin receptors that possess a single transmembrane domain and tyrosine kinase domain at its cytoplasmic region. Upon binding to its ligand, nerve growth factor (NGF), receptor forms a homo-dimer and the following activation of kinase domains occurs through the auto/trans phosphorylation of kinase domains. This phosphorylation is thought to be accompanied with the dynamic structural changes as has been seen in the crystal structural analysis of phosphorylated and non-phosphorylated forms of insulin receptor's kinase domain (Hubbard, 1997). We have been trying to detect this structural change of TrkA by monitoring the orientation of GFP that is connected to the cytoplasmic domain of the receptors. Defocused single molecule orientation imaging is suitable for immobilized proteins, however, for membrane proteins, fast two-dimensional and rotational diffusions makes it difficult to capture the clear defocused single molecule images. Here we present a novel method to detect the components of emission dipole moment in 3D by using a pair of polarizers which have radial and azimuthal transmission axes. Fluorescence from single GFP molecule is decomposed into polarization component parallel to the optical axis (z axis) and that perpendicular to the optical axis (x and y axes) in a dual-view apparatus. We could acquire the 3D orientation information of GFP dipole with the sampling rate as fast as 30 ms.

3TP3-05 光学顕微鏡（対物外アポダイズド位相差）と電子顕微鏡のインタラクティブ観察による、細胞核内部を運動する構造の同定

3P-260 Interactive observation of optical and electron microscopy revealed moving particles in the nucleus.

Kaoru Katoh (1), Ayako Kojima (1), Emiko Kobayashi (2), Kazunori Kawasaki (2) ((1) *Neurosci. Res. Inst, AIST*; (2) *Cell Eng. Res. Inst., AIST*)