oscillated when two semi-batch reactors were interacted. The objectives of this study are to evaluate conditions causing the color cycle oscillation between the reactors and to study its oscillation mechanism.

The BR reaction solutions in different semi-batch reactors were mixed with a micro-tube pump. Flow rates, a reactor number, an initial amount of the solution, and an interval length were changed in order to clarify the mechanism of the color cycle oscillation.

As a result, though the color cycle oscillation was observed in case of the two semi-batch reactors, it was never observed in case of the three semi-batch reactors. When the three semi-batch reactors were employed, they were finally synchronized. The color cycle oscillation may depend on a flow rate, an initial amount of the solutions, and an interval length between reactors.

1P-253 反応複製系のエネルギー論

Energetics of a reproducing protocell with chemical reaction process

Yohei Kondo (1), Kunihiro Kaneko (1) ((1) *The University of Tokyo*)

Living systems are energy transducer in the sense that they get energy from environment for their maintenance and reproduction. They have potentiality for growth and reproduction, and in this sense, are distinguishable from conventional chemical engine to transducer energy that has been analyzed by thermodynamics. Here we take a simple cell system consisting of chemical reaction processes, with which external resources are flowed in, and transformed to other chemicals with which both the number of molecules and cell volume grow, and study the energetics for cell growth. In equilibrium condition with external environment, the internal reaction also reaches the equilibrium without cell growth. On the other hand, under flow of resources by nonequilibrium condition, the system can maintain an out-of-equilibrium condition for steady growth, depending on the internal reaction process. Through thermodynamic analysis, we analyze the ratios of energies consumed for growth and maintenance to the supplied energy, to elucidate their dependence on the resource flux to the cell system. By taking several types of models with reaction networks, we study the dynamics of the cellular state, and reveal its relationship with the above ratios of energy transduction.

1P-254 フラクタル様マイクロ流路を用いた生化学反応計測システム

Fractal-shaped micro channel system for kinetic analysis of biochemical reaction

Katsuki Hirata (1), Tetsuo Ichii (2), Hiroaki Suzuki (1), Tomoaki MaTsuura (1), Tetsuya Yomo (1) ((1) *Osaka university*; (2) *ERATO, JST*)

Microfluidic systems have been widely used to investigate the chemical and biochemical reactions in a small volume. They are designed to meet the time scale and detection scheme for specific reactions to be analyzed. However, time scale of the reaction dynamics may vary over a wide range depending on the type of reaction and concentrations used.

Here, we present the microchannel with fractal shape design is advantageous for measurement of the biochemical reaction with various time-scales. In this configuration, reaction at the discrete time periods is monitored at a single observation point. Moreover, timescale of the measurement can be changed by choosing the node of the fractal design. As a model reaction, we employed simple enzymatic reaction (enzyme β -galactosidase hydrolyzes the fluorogenic substrate resorufin β -D-galactopyranoside to liberate red fluorescent product). Aqueous solution containing each component (enzyme and substrate) was introduced by syringe pumps, and merged to initiate the reaction. The mixture was then encapsulated in water-in-oil emulsion to avoid the diffusion along the flow path. Time course measured at all nodes provides the detailed information of reaction kinetics, which gave the correct Michaelis constant of this reaction system. With the present system, measurement of variable biochemical reaction will become possible, including the stochastic behavior of single enzyme molecule by further miniaturizing the droplet size.

1P-255 蛍光偏光法を用いる新規 SNP 検出法の開発

Development of a Novel SNP Detection Method Using Fluorescence Polarization

Masafumi Shimizu (1), Hiromi Arai (1), Makoto Tsuruoka (1) ((1) *School of Bioscience and Biotechnology, Tokyo University of Technology*)

It is known that single nucleotide polymorphism (SNP) is one of the factors that determine individual genetic features. For the detection of SNP, a simple and high-throughput analysis method using a small quantity of sample is desirable. In recent years, many SNP analysis methods have been reported, however, these methods are not very suitable for high-throughput analysis because they require extremely time consuming processes. Here, we focused on fluorescence polarization analysis (FPA) as a candidate for single nucleotide polymorphism (SNP) detection method. In FPA, a fluorescently labeled molecule is excited with a polarized light, and the emitted fluorescence is depolarized due to the rotational Brownian motion of the labeled molecule. The degree of depolarization is strongly affected by the effective volume of the molecule. FPA is considered to be

better suited to high-throughput SNP detection than electrophoresis or solid phase methods.

In this research, we investigated the potential of FPA as a method for detection of SNP. We monitored the hybridization between DNAs with optimizing salt conditions for the hybridization and the length of target DNAs. Furthermore, for the target DNAs, which contain one base replacement in the sequence where the probe hybridizes, the features of the hybridization process were monitored with fluorescence polarization and compared with those for the non-mismatch target. We successfully detected a SNP using synthesized DNA as a target DNA with FPA. In the presentation, we will discuss the feasibility of SNP detection for the DNA, which is extracted from human cell and amplified using asymmetric PCR.

1P-256 定常流中の大腸菌速度の大腸菌サイズ依存性の理論的導出

The theoretical evaluation of the dependency of the velocity of *Escherichia coli* on their size in the stationary flow

Takeshi Miyakawa (1), Kitao Fujiwara (1), Kyouhei Takahashi (1), Ryota Morikawa (1), Masako Takasu (1) ((1) *Tokyo University of Pharmacy and Life Sciences*)

Background

Ca²⁺ ion is important for the cell-division of *Escherichia coli*. The size variation (related to the cell-division) dependent on the calcium concentration was observed with the fluorescence correlation spectroscopy (FCS) by Fujiwara *et al.* In order to analyse this result in detail, they measured the velocity of the spherical polystyrene bead with different size, which depends on their radius in the stationary flow.

Theoretical Method

Based on Fujiwara *et al.*'s experiments, we evaluate theoretically the dependency of the velocity of *E. coli* on their size using the following approximations.

We regard the form of *E. coli* as the prolate spheroid. The rotation of *E. coli* is neglected. We consider only the viscous resistance because of the slowness of the stationary flow.

In these approximations we can derive the terminal velocity of *E. coli* under the condition that three forces, the buoyancy, the gravity and the viscous resistance, are balanced.

We evaluate the dependency of the velocity of the spherical polystyrene bead in the same manner as of *E. coli*.

Conclusion

Comparing the results in the case of the spherical polystyrene bead, we can estimate the major radius and the minor radius of *E. coli* from their velocity in the stationary flow.

1P-257 原子間力顕微鏡による β ラクトグロブリン-抗 β ラクトグロブリン抗体に対する温度の影響の計測

Evaluation of temperature effect on interaction between beta-lactoglobulin and anti-beta-lactoglobulin antibody by AFM

Jun'ichi Wakayama (1), Shigeru Sugiyama (1) ((1) *National Food Research Institute, NARO*)

Intermolecular recognition such as interaction between antibody and antigen plays a crucial role in biological system. The interaction can be characterized by an energy landscape consisting of potentially energetic barriers along a reaction coordinate. Dynamic force spectroscopy (DFS), which consists of measurements of the binding forces between a molecule of interest and a complementary molecule at different loading rates by use of atomic force microscopy (AFM), is useful tool to investigate the energy landscape of the interaction at the single molecule level. We have previously reported the measurements of the binding forces between anti-beta-lactoglobulin antibody and beta-lactoglobulin at different temperatures and various loading rates using AFM. In the present study, we investigate the effect of temperature on the energy landscape of the interaction between the antibody and the antigen using previous results. The unbind rate at zero, the energy landscape roughness and the poison of energy barrier of the interaction, which are parameter of the energy landscape, were determined using Bell-Evans (Bell, 1978; Evans and Ritchie, 1977) and Dudko (Dudko *et al.*, 2007) model. Based on the results, we will report the temperature effect on the energy landscape of the interaction between the antibody and the antigen. This work is supported by Food Nanotechnology Project, MAFF.

1P-258 リン脂質膜で囲まれたマイクロメートルスケールの空間に閉じ込められた長鎖 DNA 分子の高次構造変化

Conformational transition on a long DNA molecule confined in a micrometer-scale space surrounded by a phospholipid membrane

Ayako Kato (1), Takahiro Sakaue (2), Akihiko Tsuji (3), Kenichi Yoshikawa (4) ((1) *Grad. Sch. Pharm. Sci., Josai Univ.*; (2) *Dept. Phys., Grad. Sch. Sci., Kyushu Univ.*; (3) *Spatio-Temporal Order ICORP, JST*; (4) *Dept. Phys., Grad. Sch. Sci., Kyoto Univ.*; *Spatio-Temporal Order ICORP, JST*)

It has been established that a long DNA molecule (>20–30kbp) exhibits a conformational transition from a coiled state to a highly folded state under the presence of condensing agents such as polyamines. In a cell, DNA is placed in a

micrometer-scale space surrounded by phospholipid membranes. To study the conformational behavior of a long DNA molecule within such a confined space, we encapsulated T4DNAs labeled with fluorescent dyes in a cell-sized microdroplet (20–60 μm) covered with a phospholipid membrane, and the distribution and conformation of DNAs within the droplet was investigated under fluorescence microscopy.

We previously reported that T4 DNA is adsorbed onto the inner-surface of droplet composed of DOPE in the presence of Mg^{2+} , and under the presence of spermine, the adsorption accompanies the conformational transition of the DNA molecule from a folded state in the bulk solution to an elongated coil conformation on the membrane surface.

To make clear the characteristics and driving forces of these phenomena, we observed the distribution and conformation of DNAs at various concentrations of Mg^{2+} and spermine to build a phase diagram. Also, we investigated the droplet-size dependence on the distribution of DNAs, and found that DNAs trapped in large droplets (>100 μm) tend to not be adsorbed onto the membrane surface. A thermodynamic analysis on these experimental results shows that the translational entropy loss of a DNA molecule caused by adsorption is a key factor for the phenomena under the micrometer-scale confinement.

1P-259 バクテリア細胞レベルでの細胞内 ATP イメージング

Single cell imaging of ATP level inside bacteria

Keisuke Tomiyama (1), Masahiro Nakano (1), Hiromi Imamura (2), Hiroyuki Noji (1) ((1) the institute scientific and industrial research, osaka university: (2) Japan Science and Technology Agency)

ATP is one of the most important molecules in cell, functioning as a universal energy currency. However, it is unclear how ATP concentration inside bacteria changes during growth or affected by surrounding environments at single cell level. We have developed the FRET-based ATP indicators, called ATeam. ATeam indicators consist of CFP, ϵ -subunit of $\text{F}_1\text{-ATPase}$ and YFP. FRET between CFP and YFP changes, when ATP binds to ϵ -subunit. By expressing ATeam in cells and measuring the YFP/CFP fluorescence ratio, intracellular ATP can be estimated without disrupting the cells. However, we found that the ATeam indicators used for eukaryotic cells ($K_d = 1.2$ and 3.3mM) were inadequate for detecting ATP inside *E.coli*, because it showed very low FRET. So we constructed new ATeam, which has a K_d of $\sim 100\ \mu\text{M}$, by using a mutant of ϵ -subunit derived from *Bacillus PS3*. The new ATeam showed enough FRET signal, indicating that ATP concentration of *E.coli* was much lower than that of eukaryotic cells. This is consistent with the data obtained from bulk measurement using firefly luciferase. However, it still remains possibilities that unmaturing ATeam protein included inside cells affects FRET signal. Therefore, we are now developing ATP indicator that is not sensitive to the maturation.

1P-260 ナノ蛍光金クラスターの開発と生体分子計測への応用

Preparation of Highly Fluorescent Au Nanoclusters and Application for Biomolecular Imaging

Shin-ichi Tanaka (1), Dharmendra Tiwari (2), Takashi Jin (2), Yasushi Inoue (1) ((1) Graduate school of Frontier Biosciences, Osaka University: (2) Immunology Frontier Research Center, Osaka University)

Although molecular nanoprobe such as organic dye, fluorescent protein and semiconductor quantum dot have been often utilized in live-cell imaging and single-molecular studies, their application is seriously limited due to several shortcomings such as poor photostability, cytotoxicity, steric hindrance and so on. To address these issues, we have developed for the first time, the fluorogenic Au Nanoclusters (NC) consisting of several to tens of atoms and having a size below 2 nm. Particularly, Au NC has nontoxicity, which makes it well suited for biomolecular imaging. To synthesize Au NC, we used polyamidoamine (PAMAM) dendrimer as a template, because this dendrimer can encapsulate Au ion and can produce Au NC with mono-dispersed size. By mixing PAMAM dendrimer and HAuCl_4 into ultrapure water, Au ions were encapsulated and reduced within the dendrimer. This reaction mixture was incubated for 1 week with continuous stirring until the formation of Au NC completed. In order to remove large particles, ultracentrifugation was performed. The resulting Au NC solution was colorless. The excitation and emission maxima of Au NC at 370 and 450 nm were measured, respectively. Finally, we stained the cell membrane of HeLa cell with Au NC and performed fluorescence microscopy. We have observed fluorescence from Au NC in the cell membrane. More importantly, we have noted that Au NC retained its fluorescence during long-term observation. These results provide a good starting point to apply Au NC to single-molecular bioimaging.

1P-261 蛍光蛋白質を用いた張力感受性プローブの開発

Developments of the force sensitive fluorescent probe using fluorescent proteins.

Tomonobu Watanabe (1), Keiko Yoshizawa (1), Yanagida Toshio (1) ((1) WPI, Immunology Frontier Research Center, Osaka University)

Fluorescent probes have been a powerful tool for biological research during the past decade, because we can obtain the information about the localization of the target proteins in living cells and/or tissues. However, there is a serious problem emerged in the localization of the proteins does not directly indicate its function. Hence, we have challenged to overcome this big problem.

We are focusing on developing the force sensitive GFP (Green Fluorescent Protein) valiance. GFP is a spontaneously fluorescent protein isolated from coelenterates, such as the *Pacific jellyfish*, *Aequoria victori*, etc. The other fluorescent probes have been developed to make more intense and stable, however, GFP is still major fluorescent probes because of its simple and quick preparation for labeling. Amongst GFP valiant, YFP (Yellow Fluorescent Protein) has a unique chromophore, which consists of the chromophore of GFP and a phenol ring closely approached each other. We assumed that the emission spectrum of YFP was shifted toward blue when the phenol ring moved far from the chromophore by loading extra-force.

We found that the insertion of Gly residues between 144 and 145 affected the emission spectrum of YFP. The number of the insertion (1 to 12) was related to the degree of the spectral blue shift. The spectral shift was also observed in the circular permutations. However, spectral shifts of the emission of GFP were not observed while the excitation spectrum was affected. Thus, it is thought that a minor structural change caused the spectral shift of the emission of YFP. Now we are trying to characterize the force-spectrum properties of YFP at single molecular levels by using 3D magnet nanometry system combining with a fluorescent spectral microscope. In this meeting, we will discuss about our research progress and so far.

1P-262 バイオリジカルシステムに最適化した三次元ナノスコープ 3D nanoscopy optimized for biological systems

Singo Fukui (1), Keiko Yosizawa (2), Tomonobu Watanabe (2), Tosio Yanagida (1) ((1) Graduate School of Frontier Biosciences, Osaka University: (2) Immunology Frontier Research Center, Osaka University)

Cells usually change their shape during diverse motile processes. One example is Cytoskeletal structural reorganization, which normally involves actin and is fundamental for cellular motility and contractile processes. The reorganization is often done by two actin cytoskeletal architectures, filopodia and lamellipodia. Filopodia, the architecture of interest here, are slender projections that protrude from the leading edge of migrating cells and play an important role in a wide range of cell motility such as cancer cell migration and neuronal path finding. While many studies have examined the role and structure of filopodia, the mechanism of its assembly has not been clarified. Here, we focused on the structure of actin networks inside the filopodia using electron microscopy and a modified version of 3D STORM (Stochastic Optical Reconstruction Microscopy) leading to optimized and automated tracking of the three dimensional position of proteins. By using our 3D STORM, we could observe the structure of actin bundle with an accuracy of 10 nm on the x-y plane and 40 nm in the z direction. In this meeting, we will discuss our method, which is performed with Qdot, in detail.

1P-263 量子ドットを用いたベータアミロイドペプチド凝集のイメージング

Imaging of amyloid-beta peptide aggregation in vitro and in vivo by a quantum dot-based nanoprobe

Kiyotaka Tokuraku (1), Meg Marquardt (2), Tsuneya Ikezu (2) ((1) Miyakonojo National College of Technology: (2) University of Nebraska Medical Center)

Neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and prion diseases are characterized by misfolded protein aggregates, termed amyloids. However, the exact mechanism of amyloid aggregation and its links to multiple disease pathogenesis are not fully understood. In this presentation, we will report the preparation of fluorescent semiconductor nanocrystal (quantum dot; QD)-labeled amyloid-beta (A β) peptide (QDA β) and a number of advanced applications in vitro and in vivo using this nanoprobe. The QDA β retained A β oligomer-forming ability, and the sizes of these oligomers could be estimated from the relative fluorescence intensities. Both QDA β coaggregation with intact A β 42 and insertion into fibrils were detected by fluorescence microscopy. The coaggregation process was observed by real-time 3D imaging (4D-imaging) using Swept-field confocal microscopy. The time course of A β aggregation in the 4D image showed a typical sigmoid curve: the lag-time and the saturation time were 1.5 h and 12 h, respectively. Inhibition of coaggregation using an anti-A β antibody was observed on a microscopic scale, suggesting that this method can be applied to high-throughput drug screening systems. Additionally, lysosomal accumulation in microglia and binding on neuronal surface of aggregated QDA β were successfully observed. These data demonstrate that QDA β is a novel nanoprobe for studying A β aggregation in multiple modalities, and may be applicable for high-throughput drug screening systems.

1P-264 VSFG 検出赤外超解像顕微鏡を用いた生きた細胞の赤外イメージ