tendency to move along the edges of micropatterns. Here, we will report detailed analyses on *M. mobile* movements and collisions between *M. mobile* cells. Then, the results obtained from the analysis will be implemented into the simulation, expecting to achieve more accuracy.

2TA5-06 相同組換えタンパク質 RecA を用いた新規オプティカルマッピング 2P-276 グ法の開発

Development of novel DNA optical mapping method using homologous recombination protein, RecA

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We report a novel method for a simple-and-rapid optical mapping of DNA. Optical mapping is a powerful method to locate unique sites on a large, even genomic-size, DNA. Conventionally, a denaturing of a target DNA is done first, followed by a label with fluorescence-labeled ssDNA probe. The reaction is usually slow and needs one night because the labeling competes with rehybridization of denatured DNA itself. To develop an efficient method, we used homologous recombination protein RecA, a natural-born sequence explorer. In homologous recombination, an ssDNA tail is enzymatically generated from one of a set of homologous DNA molecules. Then, RecA forms helical polymer along the ssDNA. The RecA-ssDNA complex binds to another homologous DNA and locates the sequence homologous to that of the ssDNA. We applied this searching reaction of RecA to optical mapping. A fluorescent DNA probe, consisting of ssDNA (80 mer), Q-dot and RecA, was made and introduced into a flow cell, in which target DNA (48.5 kbp) had been electrically stretched and fixed with a microelectrode system. As a result, fluorescent probes were found to be at unique sites of the stretched DNA. The sequences at the sites were homologous to that of the ssDNA used for the probe. The results were caused by RecA because probe was uniformly distributed along stretched DNA without ATP, known requirement for RecA to search homology. Moreover, no bound fluorescent spot was found without RecA. In summary, RecA can be used to map target sites toward high-throughput optical mapping.

2TA5-07 マイクロ流体デバイスを用いた 8 並列型ソーター 8-Parallel Bioparticle Sorter with 3-Dimensional PDMS Chip

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On-chip sorter is expected to be one of the attractive methods to enrich bioparticles in the field of organelle proteomics, because the sorter separates fluorescently labeled bioparticles with sensitive detection. However, it requires a lot of time to collect enough amounts of bioparticles for following analysis. Therefore, we are developing a parallel sorter for rapid collection of bioparticles such as mitochondria. The sorting mechanism is based on the sol-gel transition of thermoreversible gelation polymer (TGP). The mixture of bioparticles and TGP solution is introduced into a chip which has a microchannel branching into a collection and waste channel. Target particles are sorted into the collection channel with the sol-gel transition of TGP induced by local heating by an infrared laser. We previously reported that this sorting system worked in a 4-parallel channel chip and the recovery ratio of target particles was 40%. This paper reports that the performance of parallel sorting has been greatly improved by installing sheath flow. We have integrated 8 sheath-flow sorters, in which a sample stream is sheathed by two buffer streams to focus the sample stream, into a single chip. Each sorter integrated in the chip successfully sorted fluorescent microspheres and *Escherichia coli* cells expressing fluorescent proteins with 10 - 20 ms of flow switching time and more than 80% of recovery ratio.

2TA5-08 異種ビーズを隣接させた状態で観察可能なダイナミックマイクロ 2P-277 アレイ

Dynamic Microarray Technology for observation of adjacent different beads

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A bio-hybrid microfluidic systems is an attractive technology in biophysical research; this system can realize automatic and high-throughput analysis of biochemical reaction. In this field, it is highly needed to handle a large number of small particles or living cells simultaneously for cellomics studies and drug screening. We have developed a dynamic microarray that allows us to transport and immobilize particles such as cells or microbeads during experiments. Using this method, it is possible to reduce the necessary amount of samples due to the mechanism to make them certainly trapped at each spot. It has been, however, difficult to array more than two different cell-sized samples. In this study, we demonstrated a new strategy to trap two adjacent different beads trapped in one spot and to observe. The channel consists of trapping and bypass flow channels,

so that it has advantages in the point that only one bead is trapped in the trapping spot and the following beads will advance to the bypass. By combining a pair of channels, we can array two different types of 100 μ m-sized beads in a line without mutual interference. In addition, we succeeded in the scale-down of the channel's width and trapping 15 μ m-sized beads. We eventually expect that this method to play important roles in observing cell-cell interactions or imaging the molecular diffusion from closed system to the other in the future.

2TA5-09 細胞ビルドアップ型ウエットナノロボティクスの構築と機能発 現—長期間室温で駆動する昆虫細胞シートの開発—

Construction and Function Emergence of Cellular Wet NanoRobotics-Development of Long-term and Room Temperature Operable Insect Cell Sheet-

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Here we propose insect heart muscle cell sheets fabrication in order to effectively utilize the cells beating as a driving force of a novel bioactuator. A micro bioactuator utilized a mammalian heart muscle cell sheet was previously reported. But, the actuator requires precise environmental control to maintain the sheet contraction. Therefore, we focused on an insect cell as an alternative driving source, which is generally robust over a range of culture conditions compared to a mammalian cell. In this study, we fabricated insect cell sheets using temperature-responsive culture surfaces for a novel bioactuator. Insect cells dissociated from dorsal vessel tissues which are autonomously beating by trypsin at 4 °C for 24 h were contracting more than 2 weeks. Insect cell sheets detached from temperature-responsive culture surfaces by reducing temperature. The insect cell sheets would be utilized as a driving component of an environmentally-robust and autonomous bio-hybrid robotic system.

2TA5-10 bistable な DNA で実装する温度バンドパスフィルタ 2P-278 Thermal band pass filter implemented with a bistable DNA

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DNA is a useful material for the bottom-up construction of nano-machines, due to its natural compliance with a simple Watson-Crick base-pairing rule, by which complementary sequences in one or more DNA strands hybridize with specificity. The motion of nano-machines is executed by DNA structural changes, generated in response to external stimuli. In addition to stepwise hybridization directed by the addition of short DNA strands, various physical properties of DNA, such as, the B-Z transition of a helix triggered by an ionic strength change, and the duplex-i-motif transition by a pH change have been introduced as engines to drive nano-machines. In the present study, we report that a bistable DNA molecule, which can fold into two different hairpin structures, could be programmed through sequence design to function as a thermal band pass filter for regulating biochemical reactions. The gross system behavior of the formation of the dominant intramolecular structures of the bistable DNA was modeled by coupling the isolated equilibrium models governing the formation of each of the independent hairpins, which compete with each other. For an appropriately-designed bistable DNA, a peculiar behavior as a nano-machine possessing the ability to sense thermal variations and form a target hairpin structure within a limited temperature range was predicted. The predicted thermal response was validated via fluorescence measurements with a dual fluorophore-labelled DNA.

2TP1-01 アジ化物イオン結合酸化型チトクロム酸化酵素の精密構造解析 2P-073 Refined structural analysis of azide ion bound cytochrome c oxidase in the oxidized state

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Cytochrome c oxidase (CcO) is a membrane protein complex in the mitochondrial and bacterial respiratory chain. CcO catalyzes the oxygen reduction to water at the heme-copper metal site (heme a_3 -Cu $_B$ site). Several small molecules, CO, NO, CN $^-$, N $_3^-$, etc., inhibit catalytic activity of CcO by binding to the heme-copper site. Therefore, these molecules are used as a probe to understand a property of the catalytic site and the function of CcO. We have analyzed these ligand-bound structures in the reduced and oxidized states of CcO by the X-ray crystallography. In this study, bovine CcO crystal in the oxidized state was treated by sodium azide, which resulted in small absorption change at the α -band, and the N $_3^-$ -bound structure was determined at 1.8 Å resolution. The electron density map revealed bound N $_3^-$ at the heme-copper site. The N $_3^-$ -binding caused slight movement of the heme plane toward the phenol group of Tyr244, which covalently links to the imidazole group of the copper ligand His240. This sliding manner of the heme is similar to that previously observed on the CN $^-$ binding to the heme-copper site in the reduced state. In the N $_3^-$ -bound oxidized structure, the