Unique Segment Pairs (USPs), the sequences that make a perfect match only with their complementary strands. USPs were selected by a program "T-GUSP" which we wrote in C. T-GUSP mainly consisted of two modules, the first module select the set of USPs by overlapping adequate number of Seeds (typically 7 nucleotides in length) and the second assign the USPs according to the design. The set of USPs were evaluated by the free energy using dynamic programming algorithm incorporated into T-GUSP. All structures were assembled by one pot annealing where equivalent molecule of short and long strands were mixed in annealing buffer and cooled from 96 deg. to room temperature over the course of 24 hours. The successful building of structures was confirmed by gel electrophoresis and thermal melting profiles.

3J0936 Purification of ferritin with magnetic nanoparticles

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Apo ferritin is a spherical protein with a diameter of 13 nm and internal cavity of 7 nm, in which the protein forms a ferrihydrite core. Apo ferritin is also known to form cores of other metal compounds in the cavity such as cobalt, nickel, or chromium in vitro. Here we report synthesis of magnetite (Fe₃O₄) nanoparticles in the apo-ferritin cavity and separation methodology of apo ferritin and apo ferritin with magnetite cores. A recombinant apo ferritin cloned from cDNA of the L-subunit of horse liver apo ferritin was used for experiment. The synthesis method was in principle followed by Meldrum et.al. (Science 257, 522-523, 1992). A solution is apo ferritin in AMPSO buffer (pH 8.5) was incubated in argon gas atmosphere at 60°C and ferrous ammonium sulfate dissolved in deaerated water was added gradually. According to a transmission electron microscopy, core formation was examined to be about 50 %. To select the ferritin with magnetite core, we have utilized magnets and 10 ml of milli-Q water was gently applied. After incubated this solution at 60°C for 2 hr, magnets with ferritin was washed by 100 ml of fresh milli-Q water. After draining this solution, ferritin absorbed to the magnets was collected by washing the magnets with small amount of water. As a result, we succeeded to concentrate magnetite-ferritin up to 80 %. We are going to improve the synthesis and purification method to achieve 100 % core formation.

3J0948 生体反応過程を再構成する人工ナノ空間を模した様々なサイズ・元素のヤマス粒子とカップの作製

Fabrication of Janus Particles and Cups Having Various Sizes and Elements as Artificial Nano-Space to Reconstruct Biomolecular Reactions

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Janus particles are composed of two chemically distinct hemispheres. These particles can be employed as sensors, or as model systems to study self-assembly phenomena. Production of various Janus particles is therefore fundamental, and we propose a method for the fabrication of Janus particles and Janus cups having various sizes and elements by a simple procedure. Janus particles and cups were fabricated as follows. Polystyrene spheres (30-1000 nm in diameter) were used as casts for the production, and placed on substrates. Diameters of the spheres were processed into desired sizes by plasma etching, and metals were deposited on the spheres with desired thickness by thermal evaporation. Janus particles whose half was composed of deposited metal and the other was polystyrene were then fabricated. For the production of Janus cups, two different metals were sequentially deposited and the polystyrenes were removed by UV- ozone oxidation. By using this method, more than 250 kinds of Janus particles or cups were fabricated.

For a practical application, DNA was immobilized onto the inside of Au-Ni double layered Janus cup as a model. Small gold nano-particle on which complementary DNA was immobilized was reacted with the cup, and both surface-selective immobilization of DNA onto the inside of Janus cup and specific hybridization reaction at the inside of the cup were confirmed with FE-SEM observation. These results indicate that fabricated Janus particles and cups can be used as artificial nano-space for biomolecular reactions.

3J1012 G タンパク質共役受容体に対するペプチドリガンド探索を目指した高感度オンチップセルソーターの開発

Development of effective on-chip cell sorting system for mammalian G-protein coupled receptor ligand screening

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G-protein coupled receptors (GPCRs) are involved in many different cellular processes, such as neurotransmission and cell growth, and are one of the most important drug targets. We aim to develop a novel drug screening system for GPCRs, using a combination of our on-chip cell sorting system and the yeast *Saccharomyces cerevisiae* cells expressing GFP in response to ligand stimulation. For efficient sorting of the yeast cells, we have developed a cell sorting device based on three-dimensional focusing technique.

The sorting device was fabricated by polydimethylsiloxane (PDMS) casting using a SU-8 mold. The device has one sample inlet and two carrier inlets. The sample stream is sheathed in both horizontal and vertical direction by the carrier flows. Consequently, the sample stream is focused to the center of channel. Upon detection of fluorescent target particles, the focused samples are sorted by a heat-induced sol-gel transition of thermo-reversible gelation polymer (MebiolGel).

Using this device, we have demonstrated separation and collection of fluorescent microbeads with high degree of purity (more than 80%) and recovery (more than 70%). We also succeeded in sorting and recovery of the yeast cells expressing GFP in response to ligand stimulation with an accuracy of 82%. Now, we are trying to apply the device to ligand screening for GPCRs.

3J1024 非平衡な人工細胞モデルのためのマイクロ流体技術と DNA ナノテクノロジー

Microfluidics and DNA nanotechnology toward the construction of nonequilibrium artificial cells

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In this presentation, I introduce microfluidic technology and DNA nanotechnology toward the construction of nonequilibrium artificial cells.

In recent years, microfluidic technology such as biochemical reactions in micrometer-sized reactors based on water-in-oil (W/O) microdroplets and giant liposomes have been attracted much attention as a powerful method to realize cell-like tiny compartmentalized reaction systems [1]. In addition, DNA nanotechnology has succeeded in developing programmable and highly-controlled autonomous reaction systems and autonomous molecular machines using DNA, RNA, and protein molecules. These technologies are expected to create nonequilibrium artificial cells that have autonomous reaction dynamics (nonequilibrium open systems are essential for sustainable autonomous reaction dynamics [2]).

Here, I demonstrate some biophysical tools for the construction of artificial cells based on microfluidics and DNA nanotechnology [3,4] and plan to discuss the possibility to create nonequilibrium artificial cells.

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3J1036 温度感受性ポリマーを使ったマイクロ流体ソーティングシステムによる蛍光性ナノ粒子の分離

Separation of fluorescent nanoparticles with microfluidic sorting system using a thermoreversible gelation polymer

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The fluorescence-activated cell sorter (FACS) is a well-established method for analyzing cells based on their sizes and fluorescence emission. However, nanometer-sized particles, such as organelles, viruses, or supramolecules are difficult to separate with conventional FACS. Here, a microfluidic device for analyzing and sorting nanometer-sized particles has been developed. We demonstrated the sorting of fluorescent nanoparticles, quantum dots (Qdots), for a proof-of-concept. To achieve sensitive detection, the sample flow was hydrodynamically sheathed and effectively excited with a focused laser beam. Flow control was performed by a sol-gel transition of a thermoreversible gelation polymer (TGP). The PDMS chip used in this system has Y-shaped microchannels with one inlet and two outlets. A solution of fluorescent targets