- 3P348 オンチップ1細胞システムを用いたマクロファージの食食ダイナミクスの測定
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Phagocytosis dynamics after a series of antigen stimulation in macrophages by on-chip single cell cultivation system
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Macrophage and Dendritic cells are major components of the phagocyte system and are professional antigen presenting cells. However, it is still hard to explain the variability of responses caused by a lack of knowledge of the modulation mechanism of the immune response of single macrophages against multiple antigen stimulations. We analyzed the processing information time of phagocytosis isolated single macrophage used multiple step-by-step zymosan stimulations with the fully controlled on-chip system. Macrophage cells showed different processing toward multiple zymosan stimulations: single stimulation, same times double stimulation, delay time double stimulation, angle different double stimulation and multiple angle and numbers stimulation. We previously showed that second stimulation that is delayed long enough might be neglected until the completion of the first phagocytic process under the double stimulations. However, this delay did not depend on angle stimulation. We found time sequences of processing mechanism that occurs in phagocytosis under the multiple angles step-by-step zymosan stimulations. When from one to four stimulated multiple angles, macrophage phagocytosed numbers of zymosan form one to four. We could show one of the mechanisms of complex signal processing that occurs in phagocytosis when there are multiple stimulations to macrophages.

- 3P350 マイクロ流体チップを用いたオルガネラソーティング
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Organelle Sorting with a Microfluidic Chip
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Separation of organelles to identify their components is required to characterize their functions. On chip sorter would be one of powerful methods for separating organelles because the sorter separates biomolecules according to their fluorescence signals independent of specific characters such as size, density, molecular weight, and isoelectric point. This paper reports a fluorescence-activated organelle sorting system with a polydimethylsiloxan (PDMS) chip. This system uses thermoreversible gelation polymer to control the flow by laser-induced heating, and three-dimensional sheath flow to focus the sample flow containing fluorescence targets. Upon detection of a fluorescence signal, the flow resistance changes at a branch point by sol-gel transition to realize high speed flow switching. The switching speed was estimated to be 5 ms and the sorting accuracy was 92.5 %. Mitochondria of HeLa cells were fluorescently labeled by Mito Tracker, and the cells were homogenized. The homogenate was centrifuged and the supernatant was collected. The 1.5 millions mitochondria of the supernatant were successfully separated by this system during 60 hours. The sorted fractions were analyzed using two-dimensional (2D) gel electrophoresis. The 2D gel maps obtained from the collection fraction and waste fraction separated over 50 distinct spots as visualized by Sypro Ruby. We are analyzing the difference of these two maps. This sorter will be a useful method to purify biomolecules.

- 3P349 オンチップ1細胞培養システムを用いた大腸菌の1次元運動解析
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One-dimensional motility analysis of *Escherichia coli* cells using on-chip single-cell cultivation system
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Phenotypic diversity in a clonal population of bacteria has attracted a lot of attention as "non-genetic variability". We focused on motility of *Escherichia coli* cells and to investigate their variability quantitatively, we used on-chip single cell cultivation system and one-dimensional motility microchamber array plate. The advantages of this system are continuous cultivation of an isolated cell under a constant cultural condition and immediate exchange of medium at any time. One-dimensional motility chamber is microfabricated narrow grooves, height 1μm and width about 1.5μm. *E.coli* cells, which are ordinarily 2μm or more in length and width about 1μm, were not able to rotate horizontally in the chamber, so we could obtain identification of their traveling direction and orientation of both ends of the cell. First we observed cell motility under steady conditions. As a result of analyses on swimming velocity and appearance of flagellar, we confirmed that back-and-forth motion of cells in the chamber was equivalent to run-and-tumbling motion in free space. We measured running time between each turns, and found its frequency distribution was exponentially and bilaterally symmetric. Each cell showed each different value of mean running time and the value was size-independent. Second we added 1mM aspartic acid and observed a process of adaptation at a single cell level. Now we are investigating traveling direction in the presence of attractants and the probability of inheritance of these motility characteristics to daughter cells.

- 3P351 腸炎ビブリオ菌の三型分泌装置複合体の可溶化
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Solubilization of Type III Secretion Structural Proteins in Search of Needle Structures of *Vibrio parahaemolyticus*
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Vibrio parahaemolyticus is a world-wide cause of food-borne gastroenteritis. This pathogenic bacterium has two sets of genes for type III secretion system, whose attribution to pathogenicity has been tested and discussed. Recent progress in visualization of the TTSS 'injectisomes' of *E. coli*, *Shigella* and *Salmonella* species revealed their needle-like structure (NS) embedded in bacterial membranes. As for *V. parahaemolyticus*, however, it has not yet been successful in observing the actual apparatuses of either secretion system. Our goal of this study is to isolate the 'injectisomes' of each TTS system for structural observation and determination of the components. The purification strategies were revised with referring to those of the needles and flagella of other species, and then the samples were to be observed under TEM. At this moment, we have not confirmed the structure of the injectisome; however, by western blotting the outer-membrane component of the TTS machinery was detected in the detergent-soluble fraction that can be precipitated by high-speed centrifugation. We are about to identify the other major components of this fraction using mass spectroscopy. We have also constructed GFP-fusion protein of a cytoplasmic component of the injectisome to visualize a single machinery embeded in the membrane. So far, several dots have been observed about the cell periphery with uniform GFP gleaming in the cytoplasm, and this localization was disrupted when using the secretion mutant.