N. et al., JCB: 353, 2004), protein concentrations, and diffusion constants (Takahashi, K. et al. PNAS: 2473, 2010).

We reconstituted the ERK phosphorylations and dephosphorylations in *E. coli* cells, in which concentrations of ERK, MEK and MKP were independently controllable using extracellular chemicals. The attained protein concentrations were estimated by fluorescence intensities of Cerulean (a cyan fluorescent protein variant), Venus (a yellow fluorescent protein variant), and mCherry (a red fluorescent protein variant) each fused on ERK, MEK and MKP, respectively. The phospho-ERK concentration was assayed by antibody staining at individual cells. Culturing *E. coli* cells under different conditions, we could change the concentrations of MEK and MKP by a factor of IPTG-aTc independently. On average, ERK phosphorylations depended on the ratio of MEK to MKP expressions with higher phosphorylations under higher expressions of MEK. However, there were large fluctuations in the phosphorylation levels in individual cells. We are planning to evaluate the characteristics of ERK phosphorylation by comparing with computational analysis.

3P345 バクテリア再構成デバイスの検討

Attempt to reconstitute a bacterium in the micro fluidic device.

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Bacteria have functions that are very useful to us. Their functions include, for example, the production of antibiotics, the production of ethanol and butanol, and the fixation of carbon. These functions are resulted through mutual interactions between proteins inside the bacteria. However, these functions consist of extremely complex interactions, and consequently, it is difficult to understand them altogether. If the components and protein interactions inside bacteria can be directly manipulated, that might help understand these functional expression systems. Therefore, we studied a device made by PDMS that produce liposomes which can release the internal contents of bacteria into a microspace, cover them once again with a lipid bilayer membrane containing the components of the cell membrane, and virtually preserve the intracellular environment. We have confirmed the formation of liposomes inside the device, but lipid bilayer membrane formation had a low rate and a low efficiency. In addition, we started developing electrodes, which induce fusion between lipid bilayer membranes and bacterial protoplasts inside the device. To evaluate the electrodes, we carried out fusion of bacterial protoplast and have looked a fusion of bacterial protoplasts among themselves. Moreover, we have also developed a culture device aimed at culturing the formed liposomes separately from the other. In this presentation, we will introduce the structure and the functions of the device.

3P346 ラティス構造による物語ダイナミクスの表現 Expressing story dynamics with lattice structures

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This research is an attempt to model dynamics of stories quantitatively. By using rough set theory and lattice theory, we can construct lattice structures that are based on subjects and their attributes in a given scene. To acquire a lattice, we have designated a fixed window as a source of information and slid that window down the story steps. Each story step is an action or an event in a story. The lattices are analyzed according to their complementarity and distributivity. Complementarity is a complement existence rate ($1 \geq C$) and distributivity is a complement possession rate ($1 \leq D$). Complementarity and distributivity is plotted against the story steps. The complementarity decreases whenever there is a character with attributes that covers the other characters' attributes. The distributivity increases when the attribute overlap occurs spo-

radically. Changes in complementarity and distributivity tell us how character relations evolve in a story.

3P347 イオンモビリティと衝突誘起解離によるカルボニックアンヒドラーゼ 2 のフォールディング解析 Folding analysis of carbonic anhydrase2 using ion mobility and subsequent collision-induced dissociation

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[Introduction] The study of folding analysis of proteins has been performed by using ESI that can directly transfer ions from solution to gas phase with only limited structural distortion. Recently, ion mobility spectrometry (IMS) has been used for folding analysis of proteins because it can distinguish the molecular size and/or shapes such as folded and unfolded states even among the same m/z values. In this study, we applied CID method to the peaks separated with IMS. The IMS driftgrams of carbonic anhydrase2 (CA2) under several solvent conditions were obtained for same m/z ions. The folding states of CA2 in solution are discussed on the basis of the IMS/CID patterns of the protein. [Preliminary data] In IMS measurements at pH3.6 and pH5.0, doublet peaks (A and B) were found in the driftgram extracted from some charge number. This means there are two different folding states of CA2 ion, because the folded proteins are considered to be more compact than the unfolded proteins. In each IMS/CID spectrum obtained from the separated peaks A and B, the abundance of the product ion y67 was plotted against charge number, and the most abundant product ion of the peak A with short drift time was y67 (4+), while that of the peak B with long drift time was y67 (5+). This charge number shift suggests that the IMS/CID spectra can be related to the gas-phase folding states of CA2 ion.

3P348 基質の結合によるタンパク質コンフォメーション変化におけるイオンモビリティ測定 Using Ion Mobility to Measure Changes in Protein Structure upon

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Electrospray is an ionization technique which can efficiently generate large biological gas-phase ions. Transfer of non-covalently associated protein-protein complexes from solution to the gas phase generally results in the retaining their native folded structure. Additional biological structural determination techniques can be used to gain insight into protein structure, such as NMR and X-ray crystallography. Both of which have the capability of providing a 3-dimensional structure. Here we present a method providing rapid determination of substrates binding to the protein BCL-XL and also the ability to measure change in shape, as a consequence of substrate binding. The gas phase shape to the protein, in the presence and absence of the substrate is compared to and demonstrated to be consistent to the solution phase NMR structure. BCL-XL was buffer exchanged into an aqueous solution of 100mM ammonium acetate, to a final working concentration of 1.0μM. The peptide substrates BAD and BAK were added in stoichiometric amounts. SF6 was used as the trap/transfer gas. Nitrogen was used as the ion mobility gas. All samples were introduced into the SYNAPT HDMS system using a nanoflow Z-Spray ion source. It would appear that BCL-XL, in the gas phase exists as a mixture of folded and unstructured protein. This is consistent to what has been observed by solution NMR (PDB 1LXL) where a large portion of the protein is unresolved due to its unstructured nature. The CCS for charge states +9 and +8, showing a single ATD, can be measured accurately.