2P145 神経細胞の軸索輸送におけるキネシンとダイニンの数の測定：揺らぎの定理の応用**Measuring the numbers of kinesin and dynein on neuronal cargo transport by using the fluctuation theorem****Kumiko Hayashi**¹, Yasushi Okada² (¹Sch. Eng., Tohoku Univ., ²QBiC, RIKEN)

We observed organelles, which are transported by kinesin and dynein along microtubules in neurons, by using fluorescence microscopy. We investigated non-equilibrium fluctuation in the constant drag motion of the organelle's center position. We estimated the drag force acting on the organelle exerted by the motors using the fluctuation theorem, a theorem for entropy production that has been applied to measure forces of biomotors. We found that the distribution of the drag force, which was calculated from the drag force of each organelle, had several peaks. The values at these peaks may correspond to forces exerted cooperatively by multiple motors. The number of motors attached to an organelle is discussed based on the drag force distributions.

2P146 Observing RecBCD Translocation along Individual Chi-Containing Gapped DNA**Cinya Chung**, Hung-Wen Li (*Department of Chemistry, National Taiwan University*)

The *E. coli* RecBCD initiates the repair of double-strand DNA breaks (DSB). Translocating and recognizing chi site (crossover hotspot instigator, 5'-GCTGGTGG-3') induces a conformation change that enables the enzyme to preserve the 3'-to-5' single-stranded DNA for later RecA assembly. RecBCD is composed of three subunits, RecB (3'-to-5' helicase), RecD (5'-to-3' helicase), and RecC, which is believed to be responsible for chi recognition. Here we use a single-molecule tethered particle motion technique to directly monitor the translocation of RecBCD along chi-contained DNA. Using bead-labeled enzymes, we monitored the RecBCD translocation along DNA by measuring the gradual decrease in the bead Brownian motion as the enzyme moves along the DNA towards the surface.

2P147 Dynamical energy landscape theory for the force-generation process in actomyosin motor**Qing Miao Nie**^{1,2,3}, Masaki Sasai¹, Tomoki P. Terada¹ (¹Dept. of Comp. Sci. Eng., Nagoya Univ., ²Institute for Molecular Science, ³Dept. of Applied Physics, Zhejiang Univ. of Tech.)

The mechanism of the myosin II motor is still under debate. To resolve the controversy between the lever-arm model and the biased Brownian motion model, we investigate the roles of conformational and nucleotide state changes by using a coarse-grained model of actomyosin. The free energy landscape is calculated for different conformational and nucleotide states in the force generation process, and kinetic transitions among states are simulated by stochastic jumps among landscapes. We found that the free energy landscape is much affected by both the conformational change of myosin and change in electrostatic interactions between actin and myosin, and the jumps among these landscapes explain how the lever-arm and the biased Brownian motion contribute to force generation.

**2P148 マイコプラズマ Gli349 タンパク質の構造ダイナミクス解析
Structure and dynamics of the gliding protein Gli349 from
*Mycoplasma mobile*****Junichi Inatomi**¹, Yuuki Hayashi¹, Munehito Arai^{1,2} (¹Dept. Life Sci., Univ. Tokyo, ²PRESTO, JST)

Mycoplasma mobile clings to a cell surface and glides over it using a plausible leg protein Gli349 through its large conformational changes. Previous studies suggest that Gli349 is composed of 18 repeats of ~100 residues. However, detailed structure and dynamics of the protein remain unclear. One approach to solve this problem is to study the structure and dynamics of separate fragments corresponding to the repeats of Gli349 and then reconstruct its overall structure. To this end, we have synthesized the genes corresponding to the repeats J, K, and L and a hinge h2 separately. These regions are considered to be essential for the conformational changes of Gli349. Structural characterization of these regions will be presented at the meeting.

2P149 Gene manipulation of gliding bacterium, *Mycoplasma mobile***Isil Tulum**, Atsuko Uenoyama, Makoto Miyata (*Osaka City University*)

Mycoplasma mobile, a pathogenic bacterium, glides on solid surfaces with a unique mechanism, not related to known bacterial motility systems or conventional motor proteins. The gliding machinery is composed of at least three huge surface proteins and an ATPase, and potentially more than ten cytoskeletal proteins. To elucidate this mechanism, first we constructed a transformation system for *M. mobile*, by optimizing parameters such as electroporation conditions, recovery time and reduction of film-spot. We will present localization of at least two gliding proteins by EYFP tagging, replacement of gliding proteins by modified ones, and the discussion about the updated working model for gliding mechanism.

**2P150 単一糖鎖上でのマイコプラズマの滑走と結合
Gliding and binding of mycoplasma on uniform oligosaccharide****Taioshi Kasai**, Tasuku Hamaguchi, Makoto Miyata (*Grad. Sch. Sci., Univ. Osaka City*)

Mycoplasmas, pathogenic bacteria bind to solid surface and glide to one direction. Previously, we showed that mycoplasma cells catch sialylated oligosaccharides in gliding, but mycoplasma gliding on uniform oligosaccharide has never been observed. In the present study, we analyzed the gliding and binding of mycoplasma to 53 uniform oligosaccharides fixed to a solid surface, and found that the gliding speed relate inversely to the affinity of mycoplasma for sialylated oligosaccharide. The recognition of mycoplasma foot for sialylated oligosaccharide also has been suggested as follows. (i) The reducing terminal Neu5Ac is tightly recognized in an occluded position. (ii) The second and third sugars are loosely recognized. (iii) The third sugar is recognized from one side.