motor system at sufficiently high resolution. The observation must be done addressing the structural, dynamical and energetical aspects simultaneously. We here attempted such observation by an *in silico* single molecule experiment, and studied the actin-based and microtubule-based motor systems. By analyzing the intermolecular interaction between the motor protein (myosin, kinesin, and dynein) and the track protein, we show that a Brownian ratchet mechanism is inherent in these motor systems (the study for dynein is in progress). As for actomyosin, we scrutinized the observed ratchet-like energy landscape by perturbing the intermolecular interaction, and found good agreements with wet experiments. Our results illuminate the substantial contribution of Brownian ratchet mechanism to the force-generating mechanism of molecular motors.

2P-119 ゲルゾリンC末端部位を使った細胞質アクチンのアフィニティ精製
Purification of Cytoplasmic Actin by the Affinity Chromatography Using C-Terminal Half of Gelsolin

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Actin filaments in living non-muscle cells undergo continuous dynamic turnover and remodeling. These processes involve polymerization, depolymerization, severing, capping, and branching of actin filaments through the interaction with a vast array of actin binding proteins. Cytoplasmic actin had previously been purified by the affinity chromatography using the immobilized DNase-I, which binds to G-actin with high affinity ($K(d) = 0.05$ nM). After being eluted from a DNase-I column, actin had to be exposed to high concentrations of a denaturant, such as 10 M formamide or 3 M guanidine-hydrochloride. We have previously reported the method of the cytosolic actin purification, based on the affinity chromatography using a carboxyl-terminal half of gelsolin (G4-6), which is an actin filament severing and capping protein, without the use of a denaturant (Ohki et al, (2009) *Biochem Biophys Res Commun.*, 383, 146-150). To test whether purified actins were functional, they were evaluated by measuring the rate of polymerization and the filament length, and by characterizing the interaction with myosin V. Cytoplasmic actin from HeLa cells and the recombinant beta-actin purified by G4-6 column chromatography are both able to polymerize similarly to skeletal muscle actin and the cytoplasmic actin purified by DNase-I. No significant differences in the average sliding velocity and its distribution were observed among the three cytoplasmic actins, and all the three were only 10% slower than rabbit skeletal actin. We report here the modifications and new applications of this method.

2P-120 タンパク質間粗視化力場のアクトミオシン結合過程への応用
Application of an inter-protein coarse-grained force field to binding process of actomyosin

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Protein-protein interaction is important for many biological processes. Especially, when molecular motors fulfill their functions, they sometimes form multi-subunit complex or move through alternate binding and unbinding processes. Therefore, it is important to model inter-protein interaction appropriately in order to reveal working mechanism of molecular motors. Coarse-grained model is often used to analyze working mechanism of molecular motors for computational efficiency, but modeling of inter-protein interaction sometimes becomes difficult problem. In this study, we apply a sequence dependent inter-protein coarse-grained force field (each amino acid is coarse-grained to one bead) that considers electrostatic interaction between charged residues and sequence dependent contact interaction [Kim and Hummer, *JMB* (2008)] to a molecular motor, actomyosin. Myosin is known to take detached, weakly bound and strongly bound state to its rail protein, actin, during ATP hydrolysis cycle, and the force is thought to be generated during the weakly-to-strongly transition. These binding states should be coupled to nucleotide dependent conformational changes of myosin such as open-close motion of the actin-binding cleft and rearrangement of surface loops like loop II. We will discuss the effect of the myosin conformational changes on the binding process.

2P-121 ミオシンVの運動性におけるトロポミオシンの役割
Role of tropomyosin in the motility of myosin V along an actin filament

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Myosin V is a processive motor, which transports organelles along an actin filament to the barbed-end in the cells. Details in the walking manner of myosin V on the undecorated actin filament have been revealed by the single-molecule experiments. Several studies have shown that in cells myosin V is able to move on the networks of actin filaments, which might be modulated by the accessory proteins stabilizing the filaments or cross-linking between filaments. Tropomyosin, one of those accessory proteins, binds along the side of actin

filaments. In muscle, tropomyosin plays a crucial role in modulating the interaction between actin filament and myosin II. On the other hand, tropomyosin, which involves in stabilizing actin filaments and/or probably determining the filament length, might also modulate the interaction between the actin and myosins working in non-muscle cells. Although the effects of tropomyosin on the motility of myosin II have been well characterized, there is still no information on the effect of tropomyosin on the motility of non-muscle myosins including myosin V. Here, we have examined the motility of myosin V along the filament decorated with tropomyosins (and troponin), through the gliding velocity of those filaments in an *in vitro* motility assay and the direct measurements of run length, velocity, and stall force of single myosin V molecules using TIRF and optical tweezers.

2P-122 理論的立場から見たミオシン分子モーターにおける歪みセンサの役割
The role of the strain sensor in myosin motor molecules from theoretical point of view

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A recent single-molecule experiment has shown that the strong binding by myosin heads to actin is greatly (around 30-fold) accelerated when backward strain is applied to weakly-bound heads during actin search (Iwaki et al., *Nature Chemical Biology*, Vol. 5, pp. 403-405, 2009). It means that there exists a strain sensor which biases the Brownian movement of myosin by changing the binding rate, depending on the direction of the strain force on the myosin. Thanks to the strain sensor, myosin can extract one-directional movement by biasing the Brownian motion, whose bias energy is of the order of the thermal noise, which is beyond our intuition. In order to understand such counter-intuitive experimental result, we need the theoretical investigation. In this report, I investigate chemically driven ratchet model with such strain-dependent asymmetric catch mechanism, and discuss the role of the strain sensor in myosin motor molecules from theoretical point of view.

2P-123 シンプルな光学系で高時間・高空間分解能を実現する暗視野顕微鏡の開発
Simple Total Internal Reflection Dark-field Microscopy with Nanometer spatial precision and Microsecond Temporal Resolution

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Molecular motors, such as kinesin, myosin and F_1 -ATPase, are involved in many important cellular processes. These motor proteins showed a nanometer scale stepwise movements in microsecond to millisecond time scales. So far, some methods have been developed to measure these small and fast movements with high spatial and temporal resolution. However, these methods must require relatively complicated experimental system. Here, to measure these small and fast movements, we have developed the simple dark-field imaging system using an objective-type evanescent illumination, which can selectively illuminate in a thin region adjacent to a glass-water interface and yield images having high signal-to-noise ratios. Only using the customized mirror in place of the dichroic mirror in the conventional objective-type total internal reflection fluorescence microscopy (TIRFM) optics, nanometer spatial precision and microsecond temporal resolution are accomplished simultaneously. This system was applied to study the rotary mechanism of F_1 -ATPase. We have successfully visualized the fluctuation of the gold nanoparticle attached to the γ -subunit during catalytic dwell and the stepping motion during torque generation with 9.1 μ s temporal resolution. Because of the simplicity of the optics, this method will be applicable in various biophysical studies with high spatial and temporal resolution in vitro and also in living cells.

2P-124 新規3次元配向計測顕微鏡の開発
Development of new microscopy for determining three dimensional orientations of single molecules

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Single molecule fluorescence techniques are increasingly important to observe the dynamic properties of single molecules. One such important dynamic property is the single molecule's orientation. To determine orientation of single molecules, many laboratories have attempted various methods. Although orientation can be measured in the plane perpendicular to the axis of observation, it is necessary to measure three dimensional orientations of proteins since proteins move three dimensionally in solution. However, most methods developed for three dimensional orientation observation require a large number of photons, a relatively long observation time, and cumbersome analysis. Here we developed new microscopy for determining three dimensional orientations based on the principal of polarization analysis proposed by Fourkas. This method requires only that one collect fluorescence counts from a single molecule at three different