Ken-ichi Sano¹, Ryuzo Kawamura¹, Taiki Tominaga¹, Naoko Oda¹, Kuniharu Iijiro^{1,2}, Yoshihito Osada¹, (¹RIKEN, ASI; ²RIES, Hokkaido Univ.)

We are aiming to create a 'bio-hydrogel' having biological functions, such as recirculating and self-repairing. We prepared herein F-actin hydrogel by covalent cross-linking with bi-functionalized PEG. We first evaluated the mechanical properties of the actin hydrogel by an oscillatory rheometry. It was found that the actin hydrogel (0.5 wt% actin concentration) has a storage modulus (G') about several kPa and a loss modulus (G'') about 10-20 Pa. This actin hydrogel showed reversible gel-sol transition by actin polymerization and depolymerization. Then we added oscillatory shear to this actin hydrogel by changing a strain from 10 to 1000%. When oscillatory strain is applied more than 20 %, the G' of the actin hydrogel immediately decreased three to five orders of magnitudes, indicating that the hydrogel transferred to a sol state under a large shear strain. When the large shear strain was removed, the values of G' were returned to 80 % within several minutes. In contrast, F-actin network without cross-linking were reported to be irreversibly weakened their storage modulus. Electron micrographs of cross-linked actin under a shear by pipetting appeared that filamentous actin structures were disassembled into oligomers or less, whereas the F-actin structures still maintained without cross-linking. These results suggest that the self-repairing of actin gel can rapidly occurred polymerization of disassembled actin perhaps by cross-link induced cooperative depolymerization of F-actin.

1P157 筋収縮系自励振動現象 (SPOC) の生理的意義
1D1520 Physiological significance of spontaneous sarcomeric oscillations (SPOC) in cardiac beat

Norio Fukuda¹, Takako Terui², Fuyu Kobirumaki¹, Takahiro Serizawa³, Togo Shimozaawa⁴, Shin'ichi Ishiwata³, Satoshi Kurihara¹, (¹Department of Cell Physiology, The Jikei University School of Medicine; ²Department of Anesthesiology, The Jikei University School of Medicine; ³Department of Physics, Waseda University; ⁴Department of Physics, Tokyo University)

Cardiac sarcomeres exhibit spontaneous oscillations (SPOC) under intermediate activating conditions. We have reported that the period of sarcomeric oscillations in skinned myocardium correlates with that of resting heart rate in various animal species (2005, 2006). In the present study, we analyzed sarcomeric oscillations in isolated single cardiomyocytes of the rat, by using Qdots conjugated with anti- α -actinin antibody for clear visualization of the Z-lines. First, we measured the period and amplitude of SPOC at various sarcomere lengths (SLs) in skinned cardiomyocytes, and found that the amplitude of oscillations was inversely related to SL. We also conducted a SL measurement in intact cardiomyocytes at various stimulation frequencies, after transfection of Qdots into the cells. At low frequencies (e.g., 1 Hz), the shortening and relengthening of the sarcomere during a contraction cycle simply reflected the changes in $[Ca^{2+}]_i$. However, an increase in stimulation frequency to the physiological level (3-5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in the waveform being similar to what was observed during SPOC. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres may contribute to the regulation of cardiac beat *in vivo*. We are now performing real-time single sarcomere imaging in the beating heart of the rat.

1P158 ウサギ骨格筋筋原線維におけるクロスブリッジ周期性と破断力の顕微計測
1D1535 Microscopic measurement of periodic rigor cross-bridge formation and their unbinding force in rabbit skeletal myofibril.

Madoka Suzuki¹, Shin'ichi Ishiwata^{1,2}, (¹Waseda Bioscience Research Institute in Singapore, Waseda University; ²Department of Physics, Faculty of Science and Engineering, Waseda University)

Single molecule studies previously reported that the thin (actin) filament sterically regulates the myosin binding sites, called target zones. But it has been established that the periods of long helices of the thin filament and the thick (myosin II) filament are mismatched to each other, i.e., 36 nm and 42 nm, respectively, so that the question has been raised how the target zone is distributed, i.e., periodical or unperiodical. EM images and X-ray diffraction patterns of muscle fiber have provided the models on the interaction between the thick and the thin filaments, but direct measurements in live muscle are missing. Therefore, here we directly observed and measured the binding of single actin filament with myosin II on the A-band in skeletal muscle of higher vertebrates under optical microscope. Single bead-tailed actin filament was manipulated by optical tweezers, and the unbinding events of rigor cross-bridges due to the external force were detected. We found that the cross-bridge formation occurred periodically, and frequently observed the large gaps, which were attributable to the mismatch between the helical pitches of the two kinds of filaments. The distribution of the unbinding force had two peaks. The weaker and stronger peaks probably correspond to single-headed and double-headed binding, respectively.

1P159 二枚貝以外の軟体動物のキャッチ機構
Catch mechanism in mollusks other than bivalves

Akira Yamada, Maki Yoshio, Hiroaki Kojima, (KARC, NICT)

Some muscles of bivalves (class Bivalvia) such as the smooth part of the adductor and the byssus retractor are known as "catch muscles" that can maintain high passive tension for long periods with little energy expenditure after their active contraction. The state of this high passive tension is called "catch" and it has been hypothesized that some special "catch mechanism" exists for the maintenance of the tension. We have developed an *in vitro* catch assay in which the catch state is reconstituted by using proteins purified from bivalve catch muscles. With this assay, we have revealed that the catch tension is due to binding of actin to myosin filaments which can be directly visualized under a light microscope. We have also found that twitchin, a connectin/titin-related protein associated with myosin filaments, is an essential key component for the catch. It was originally found in a nematode *Caenorhabditis elegans*, and was later found widely in many invertebrates. This raises the question whether animals having twitchin other than bivalves also have the catch mechanism. To answer this, we prepared synthetic thick filaments containing myosin and twitchin from the foot of a spiral shell *Monodonta labio* (class Gastropoda) and a chiton *Liolophura japonica* (class Polyplacophora), and performed the *in vitro* catch assay. We found that the catch mechanism, at least at the molecular level, exists also in these mollusks. The results suggest that the catch mechanism is distributed more widely in Animal Kingdom than we know.

1P160 複数の接触配置のアクチン 2 分子における高分子込み合い効果ポテンシャル
Calculated potential map for two G-actin molecules in contact with relative orientations through macromolecular crowding effect

takuma mizobe¹, masayuki irisa², (¹Kyushu Institute of Technology Graduate School of Computer Science and Systems Engineering; ²Kyushu Institute of Technology)

Potential of mean force between two G-actin molecules is important for studying a filament formation of actin molecules. We calculated potentials of mean force through macromolecular crowding effect between two G-actin molecules with a few relative orientations by using extended scaled particle theory (XSPT) in the previous meeting.

In this study, we have systematically calculated the potentials of mean force between two G-actin molecules in contact with 210 relative orientations in order to make a potential map for the molecules. The sites chosen for contact locations are 4, thereby 210 orientations are not enough to investigate a dimer formation, which is a first stage of an actin filament formation. However, this calculation can be extended to more orientations with a high-performance computer in a straight-forward way. The theoretical calculations of the potentials with an infinite number of crowding agents, actin molecules themselves in this work, require less computer time than numerical calculations with a finite number of crowding agents.

1P161 バクテリアのプロトン駆動型べん毛モーター固定子複合体 MotA/B の局在化の分子機構
Localization mechanism of the MotA/B complex to be the stator of the proton-driven bacterial flagellar motor

Yusuke Morimoto¹, Shuichi Nakamura¹, Nobunori Kami-ike¹, Keiichi Namba¹, Tohru Minamino^{1,2}, (¹Graduate School of Frontier Biosciences, Osaka University; ²PRESTO, JST)

The flagellar motor of *Salmonella enterica* is powered by proton motive force (PMF) across the cytoplasmic membrane. MotA and MotB form a transmembrane proton channel that acts as the stator of the motor to couple proton flow through the channel to torque generation. The C-terminal periplasmic domain of MotB is proposed to play an important role in targeting and stable anchoring of the stators to their binding sites of the motor. However, it remains unclear where their binding-sites are. Here, we constructed *Salmonella* strains expressing GFP-MotB and MotA-mCherry and investigated their subcellular localization by fluorescence microscopy. The D33N mutation in MotB, which interferes with the proton flow, did not affect the assembly of GFP-MotB into the motor, indicating that the proton conductivity is not required for stator assembly. Over-expression of MotA markedly inhibited wild-type motility, and it was due to the reduction in the number of functional stators. In agreement with this, MotA-mCherry was observed to co-localize with GFP-FlhG even in the absence of MotB. These results suggest that MotA alone can bind to the stator binding sites of the flagellar motor.

1P162 Research for sodium-driven flagellar hook-basal bodies of *Vibrio alginolyticus* with Zernike phase-contrast cryo-electron tomography