The problem was cured only partially in spite of lots of efforts. We decided to look for better bacterial sources, and isolated 27 strains living in unusual environments like hot springs. Those strains show interesting features on growth temperature. While usual thermophilic bacteria like PS3 grow only at high temperatures like 70 degree but do not at usual temperatures like 37 degree, some of our bacteria grow at both temperatures. Considering a limited temperature range for stability of proteins and that we crystallize proteins at much lower temperatures, our new strains may be suited to structural study of ATP synthase.

We have purified ATP synthase from 27 such strains and to them applied crystallization screening test using conditions including the best ones for PS3 ATP synthase. Four of the 27 strains showed much better results than for (old days') PS3. However, we realized recently that this applies only to the early stage of screening. All of 4 have failed to produce genuine crystals so far. We are now examining crystallization conditions in combination with examination of their preparation methods.

2P196 分子モーターのミクロなイベント特性をマクロな観察量から統計的に定量する：計測分解能以上の情報を統計解析により抽出する
Quantitative determination of properties of a molecular motor from statistical analysis of experimentally observable macroscopic data.

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Molecular motors (MM) develop unidirectional sliding (rotary) movement with stochastic processes (SPs). Single molecule nano measurements have revealed those SPs and shown that the unidirectional (forward) movements are generated by biased stepping motion. It is very important to assess in detail properties of stepping events (movements) of MMs in the elementary processes. However, nano measurements have some constraints (difficulties), such as low ATP concentrations, insufficiency of temporal (spatial) resolution, measurement skills, etc. In order to overcome these constraints (difficulties), we have developed mathematical method to estimate microscopic (micro-) properties of MM statistically from only macroscopic (macro-) data. In this paper, we propose and test the statistical method (SM) which allows us to quantitatively determine micro- (nano-) properties of MM from experimentally observable macro-data (sliding distance, variance) without direct nano-measurements. Here, we use coarse-grained model for formulation of the SM. In the model, stepping event is formulated with three micro-parameters, size, forward probability, and dwell time of stepping event. To test the SM, we have used computer simulation, and succeed to quantitatively determine the micro-properties of stepping event from macro-data. We are trying to apply the SM to experimentally observed data. This method can be very powerful tool to quantitative determination of mechanical properties of MMs beyond the spatial- and/or temporal- resolution of measurement system.

2P197 回転分子モーター F₁-ATPase の触媒部位へ結合した蛍光性ヌクレオチドの位置と向きの測定
Localization and Orientation of Single Fluorescent Nucleotides Bound to the Catalytic Sites in Rotary Motor F₁-ATPase

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F₁-ATPase is an ATP-driven rotary molecular motor in which a central γ subunit rotates against hexagonally arranged subunits $\alpha_3\beta_3$. Three catalytic sites locate in α - β interfaces, mainly hosted by a β subunit, and they bind ATP sequentially and hydrolyze it to generate the rotation of γ . This sequential-binding process is initially proposed by Boyer as the binding change mechanism. To visualize the process directly at the single-molecule level, we localized single fluorescent ATP (Cy3-ATP) bound to each catalytic site in F₁ during the rotation. The microscope was placed in a temperature-controlled room which kept the change in temperature within ≤ 0.1 °C/hour without forced air flow. The drifting displacement of the sample stage was reduced to <10 nm/hour for x - and y -axis, and <50 nm/hour for z -axis. Cy3-ATPs fixed on a glass surface were localized with a spatial accuracy of ± 3 nm (SD) by fitting their fluorescent spots with Gaussian distribution. With 60 nM Cy3-ATP and 60 nM unlabeled ATP, we have observed three localizations of the bound Cy3-ATP corresponding to every 120° angle of γ . For further position accuracy of the localization, we are improving the system: heat source that causes the drifting of the sample stage is eliminated, and the numerical aperture of the collected light is controlled to reduce errors in the fitting. Furthermore, to see an environment in the catalytic site that binds a nucleotide in intermediate

angle, we estimated the three-dimensional orientation of bound Cy3-ATP by the defocused image of the fluorophore.

2P198 Unbinding force of cytoplasmic dynein.

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Dynein is a molecular motor that moves on microtubules using the chemical energy of ATP hydrolysis. Cytoplasmic dynein play roles in positioning the Golgi complex and other organelles in cells, movement of chromosomes, and positioning the mitotic spindles during mitosis. Force generating by a dynein molecule, thus, is one of the important factors for understanding its molecular properties. Recently, our group showed that the maximum force of cytoplasmic dynein is 7-8 pN, while others showed it is 1-2 pN. To address this controversial issue, we measured unbinding force, which is the rupture force of dynein-microtubule complex, of recombinant cytoplasmic dynein. We used truncated C-terminal motor domain, thus we can eliminate a possible effect of tail region and/or accessory proteins on the motor activity of dynein. The maximum unbinding force was about 10pN, suggesting that a motor domain alone can produce higher stall force.

2P199 F₁-ATPase の β サブユニット C 末領域のトルク伝達における役割
Robustness of torque-transmission between β and γ of F₁-ATPase

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F₁-ATPase is a rotary molecular motor which the ATP binding on β subunit leads the inward swing motion of the C-terminal domain of β toward γ , which is then converted into the γ rotation. Therefore, the directly contacting loop of this domain with γ , including a highly conserved sequence 'DELSEED', is thought to have a critical role in torque-transmission. A recent finding that an axle-less F₁-ATPase still shows unidirectional rotation revealed the importance of contact between β C terminus and γ on torque-transmission (Furuike *et al.* 2008, *Science*), whereas mutagenesis works elucidated that slight modification of this region does not affect on the activity of F₁ (Hara *et al.* 2000, *JBC*, Mnatsakanyan *et al.* 2009, *JBC*). With attempt to explore the role of β C terminus as a whole cluster, we have carried out extensive Glycine substitution around DELSEED-loop (residue 380-401) to completely destroy the specific interactions and also structural rigidity. Surprisingly, this mutant was still active in ATPase and rotation. The average rotary velocity slightly decreased and the value of torque decreased by half of wild-type. Even β C terminus does not form any structure, F₁ could still show unidirectional rotation, means torque-transmission has occurred properly. Based on these findings, we concluded that the torque-transmission between β and γ is not mediated by any specific electrostatic or steric interaction and it is designed to be highly robust against perturbation.

2P200 Acto-S1 により加水分解を受ける ATP 水溶液の誘電緩和分光法による連続測定
Sequential measurement of ATP aqueous solution hydrolyzed by acto-S1 by dielectric relaxation spectroscopy

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Microwave dielectric spectra of ATP aqueous solution hydrolyzed by acto-S1 were sequentially measured by network analyzer (Agilent 8364B+85070E+Ecal system) in the range from 0.2 to 26 GHz with scanning time of 20 s. Actin and myosin (papain S1) were taken from chicken pectoral muscle. The solution (S1 6.45 mg/mL, KCl 50 mM, MgCl₂ 2mM, Hepes 2 mM pH 7.4, NaN₃ 0.01 wt%) was introduced in a thermo-controlled glass cell and kept at 20.00°C. Sequential dielectric spectra were taken every 1 minute. After 5 spectra Na2ATP of 0.2 M was injected to the cell to become 5 mM. The temperature was kept within 0.01°C. The solution pH changed from 7.4 to 6.4 during the measurement, where the ATPase activity of S1 was 0.041 s⁻¹ and 0.052 s⁻¹, respectively. From each spectrum after injection the dielectric spectrum of the spherical region containing a solute with surrounding water was calculated by Wagner mixture theory, and was linearly decomposed into three Debye relaxation functions and a bulk water component. The hydration boundary was determined by no bulk condition. The standard errors of the final 5 spectra for 1 ~ 26 GHz were below 0.015. As a result both the ATP-rich and the ATP-consumed spectra have 19.3~20.2 GHz component A (hyper-mobile water) and 13.1 ~ 13.8 GHz component B (constrained water). It was observed that A-component decreased by hydrolysis whereas B-component increased. The measurement was still low in S/N, and should be improved.