1N1524 変位拡大機構を用いた広範囲高速 AFM スキャナーの開発**Development of a wide-view high-speed AFM scanner with displacement magnification mechanism**

Toshihide Kobashi, Takayuki Uchihashi, Toshio Ando (*Department of Mathematics and Physics, Graduate School of Natural Science and Technology, Kanazawa University*)

High-speed AFM has recently made it possible to directly visualize dynamic structural changes of protein molecules under physiological conditions, which accelerates our understanding of the functional mechanism of proteins. In the current setup, the imaging area is, however, limited to $\sim 6 \mu\text{m} \times 6 \mu\text{m}$ because of a trade-off between the scan speed and the scan size. This narrow range does not allow applying HS-AFM to the dynamic observation of live cells. To overcome this limitation, we attempted to expand the maximum possible scan size without sacrificing the scan speed by adopting a displacement magnification mechanism and a feedforward active damping technique. We succeeded in increasing the maximum displacement up to $\sim 44 \mu\text{m} \times 47 \mu\text{m}$, whereas the reduced resonant frequency of the scanner was compensated by a digital feedforward control technique (an inverse compensation method). As a result, a frame rate of 1 frame/s was achieved with 100 scan lines for such a wide area, which will enable us to observe dynamic phenomena in live cells.

1N1536 蛍光顕微鏡複合型高速原子間力顕微鏡の開発**Development of high-speed AFM combined with fluorescence microscopy**

Shingo Fukuda, Takayuki Uchihashi, Toshio Ando (*Department of Mathematics and Physics, Grad School of Natural Science and Technology Kanazawa Univ.*)

High-speed atomic force microscopy (HS-AFM) is now coming of age. It allows directly observing dynamic processes of biological molecules under physiological conditions, and hence, facilitates our understanding of how biological molecules operate to function [1, 2]. For simple systems consisting of 2-3 spices of biological molecules, the obtained images are easily interpreted. However, for more complex systems, the identification of visualized molecules is often difficult. In contrast, such identification is much easier in fluorescence microscopy, although fluorophore-labeled molecules themselves are invisible in the observations. Moreover, small molecules hardly seen by AFM can be visualized by fluorescence microscopy. Thus, a HS-AFM system combined with a fluorescence microscope would be a more powerful tool for biological research. To materialize such a combined system, we have been developing a tip-scan type of HS-AFM system implemented with a device for optical tracking of a scanning cantilever. In this study, we further developed devices including an optical beam deflection detector with an infrared laser and a small cantilever holder compatible with the optical tracking system. Using the combined system, we are now attempting to simultaneously record HS-AFM and fluorescence images using test samples.

[1]N.Kodera, T. Ando, et al., *Nature*, 468, 72-76 (2010)

[2]M. Shibata, H. Yamashita, T. Uchihashi, et al., *Nature Nanotech.* 5, 208-212 (2010)

1N1548 高速 AFM による天然変性タンパク質 PQBP-1, MeCP2, FliK P79 の直接観察**Direct visualization of intrinsically disordered proteins PQBP-1, MeCP2, and FliK using high-speed atomic force microscopy**

Tetsuya Mori¹, Noriyuki Kodera², Shino Mizuno³, Shin-Ichi Aizawa³, Mineyuki Mizuguchi⁴, Heather Szerlong⁵, Mary Porter-Goff⁵, Jeffrey C. Hansen⁵, Toshio Ando^{1,2} (¹*School of Math. & Phys.*, ²*Bio-AFM Frontier Research Center, College of Sci. & Eng., Kanazawa Univ.*, ³*Dipt. of Life Sci., Pref. Univ. of Hiroshima*, ⁴*Fac. of Pharm. Sci., Univ. of Toyama*, ⁵*Dept. Biochem. & Mol. Biol., Colorado State Univ.*)

Functional proteins without unique 3D structures are highly abundant in nature. These intrinsically disordered proteins (IDPs) are involved in a number of crucial biological functions (Uversky & Dunker, BBA, 2010). We previously reported that high-speed atomic force microscopy (HS-AFM) can directly visualize the thin and flexible structure of ID regions (Miyagi et al., *ChemPhysChem*, 2008). To explore potential general natures of ID regions, we here performed HS-AFM observations of the three IDPs, PQBP-1, MeCP2, and FliK. PQBP-1 is a nuclear protein that interacts with disease proteins containing expanded polyglutamine repeats. HS-AFM revealed that PQBP-1 consists of a

long unstructured chain and a small folded core, consistent with the NMR study (Takahashi et al., BBA, 2009). Observations of some deletion mutants further fortified the deduced structure, allowing us to estimate the mechanical properties of the ID region. MeCP2 is a 53-kDa nuclear protein that binds to methylated DNA and its mutation leads to Rett syndrome. HS-AFM visualized a long ID region, a part of which interestingly showed repetitive transitions between ordered and disordered states. FliK has been suggested to be a molecular ruler determining the flagellar hook length. HS-AFM showed a structure with two globular domains connected by a flexible linker. The molecular length is $\sim 25 \text{ nm}$, much shorter than the length of the hook and rod ($\sim 90 \text{ nm}$). HS-AFM facilitates identification and characterization of ID regions in IDPs.

1N1612 核内を運動する粒子の軌跡解析**P80 Analysis of trajectory of moving particles in nuclei**

Soyomi Uchibori^{1,2}, Maiko Kuramochi^{1,2}, Emiko Kobayashi¹, Kaoru Katoh^{1,2} (¹*Biomed.Res.Inst. AIST*, ²*Grad.Sch.of Life & Enviromental Sci.*)

Cell nucleus plays important roles in gene expression. The expression process should require dynamic change in architectural framework of nuclear structures and transport of many kinds of molecules. Thereby, many researchers microinjected fluorescently labeled molecules into the nucleus and showed high mobility in the nucleoplasm. Recently, advance in fluorescently tagged proteins lead to more precise analysis of dynamics of nucleoplasm. However, not so many studies were reported in the living unstained nucleus.

We, therefore, developed pupil projection apodized phase contrast (PPAPC) microscopy, (external phase contrast with special frequency filtering). The PPAPC revealed many moving particles in living cell nucleus, which were unable to observe with conventional phase contrast microscopy. We reported that G1/S/G2 phase can be recognized by the moving pattern of the intranuclear moving particles.

Here, to know what is intranuclear moving particles, we interactively observed identical nucleus with optical (PPAPC) and electron microscopy. The interactive observation of optical and electron microscope showed that 10-12 sequential images of electron micrographs corresponds to a single image of nucleus with PPAPC. The identified structures will be clearly shown in the presentation.

Moreover, we tracked the moving particles and analyzed the trajectory. Motile mechanisms of the particle were discussed in the presentation.

1N1624 高輝度量子ドットを用いたマウス耳内血管の非侵襲イメージング**Imaging of mouse ear blood vessel under non-invasive conditions with high-fluorescence quantum dots**

Kenji Kikushima, Sayaka Kita, Hideo Higuchi (*Department of Physics, School of Science, The University of Tokyo*)

Quantum dots (QDs) show good photostability and strong fluorescence compared to conventional organic dyes and fluorescent proteins. However, they show irregular blinking which hinder observation continuously for long time. We created QD aggregation to overcome this drawback and to manufacture brighter fluorescence conjugate, in order to observe motility of the fluorophore under non-invasive conditions.

QDs formed aggregation after quick freezing in the liquid N_2 . This QD aggregations were purified under the existence of PEG6000, and were separated in size by centrifugation and gel filtration. The purified QDs glowed continuously in time and bright enough for imaging under non-invasive conditions. We were actually able to observe the QDs on the mouse ear from its opposite side.

To visualize QDs flowing in the mouse ear blood vessel under non-invasive conditions, high concentration (over 50 nM in total blood) of QDs were needed, by spinning disc laser confocal microscopy. However, NH_2 -coated QDs tended to attach to wall of the blood vessel non-specifically, causing interference with normal blood flow. By using PEG-coated QDs, we could visualize blood vessel without blocking the blood flow. Moreover, aggregated PEG-coated QDs were observed by video-rate in mouse ear under non-invasive conditions.

1N1636 量子ロッドを用いたミオシン V のステップと 3 次元方位の同時計測**Simultaneous observation of myosin V three-dimensional orientation and linear steps**

Masashi Ohmachi¹, Yasunori Komori², Atsuko Iwane¹, Fumihiko Fujii¹, Toshio Yanagida¹ (¹*Grad. Sch. Osaka Univ.*, ²*Grad. Sch. Univ.Tokyo*)

Single molecule fluorescence techniques have become increasingly useful for observing the dynamic properties of single molecules including spatial orientation. Because molecules move in three dimensions, knowing their three-dimensional orientation would more accurately describe their dynamics. However, most methods for this purpose require a large number of photons, a relatively long observation time, and cumbersome analysis, limiting their biological application. Here we describe a new microscopy system for simultaneously measuring the three-dimensional orientation and position of a fluorescent probe based on the principal of polarization analysis proposed by Fourkas. This method requires only that one collect fluorescence counts at four different polarizations followed by relatively simple mathematical analysis. To show the effectiveness of this technique, we observed the axial rotation of actin filaments sliding over myosin molecules fixed onto a glass surface by measuring the polarization of individual rhodamine phalloidin fluorophores sparsely bound to the filaments. Further, we applied quantum rods (Q rods), which exhibit linearly polarized emissions, to study the molecular dynamics, as Q rods allow for better accuracy when simultaneously measuring position and three-dimensional orientation. We also measured Q rods bound to the stalk of single myosin V molecules to investigate rotation around their own axis. This new microscopy system has particular applicability for a wide range of dynamic biological processes.

1N1648 圧力感受性蛍光蛋白質の開発と細胞内圧力計測

P81 Intracellular pressure measurement by using pressure sensitive yellow fluorescent protein

Tomonobu Watanabe^{1,2}, Katsumi Imada³, Keiko Yoshizawa¹, Masayoshi Nishiyama⁴, Fumiyoshi Abe⁵, Chiaki Kato⁶, Toshio Yanagida^{1,2,3} (¹RIKEN, Quantitative Biology Center, ²Osaka University, Immunology Frontier Research Center, ³Department of Frontier Biosciences of Frontier Biosciences, Osaka University, ⁴Graduate School of Science, Kyoto University, ⁵College of Science and Engineering Aoyama Gakuin University, ⁶Japan Agency for Marine-Earth Science and Technology)

Fluorescent protein-based indicators for intracellular-environment such as pH, Ca²⁺ concentration and force, is a promising technique to investigate the dynamics of the states of living cells. However, there have been no fluorescent proteins reported that can detect intercellular pressure to date, while pressure is one of important factors in biology. We have developed a novel pressure indicative yellow fluorescent protein (YFP) by inserting several glycines into it. In response to changes in external pressure, a spectrum shift and an intensity change of YFP was observed, indicating applicability of the present YFP for measuring the intercellular pressure on a live specimen. By using the pressure dependency of the intensity of the present YFP, we have succeeded in detecting the pressure change in a cell when actin filaments were disrupted. This study shows that our mutation enhanced unknown properties of YFP that may allow us to measure the intercellular pressure. Our pressure sensitive fluorescent proteins are a first, and have tremendous potential for the study of phenomena that heavily involve external pressure.

13:00~17:00 Q会場/ Room Q

光生物_視覚・光受容 1

Photobiology: Vision & Photoreception 1

1Q1312 理論計算によるルミロドプシンの構造解析

A theoretical study on the structure of lumirhodopsin

Motoshi Kamiya, Shigehiko Hayashi (*Grad. Sch. Sci., Kyoto Univ.*)

Rhodopsin undergoes a series of structural changes initiated by the photo-isomerization of its chromophore, 11-cis retinal. The isomerized chromophore, all-trans retinal, is highly distorted just after the photo-isomerization. In the early stage of the photo-induced structural changes of rhodopsin, the distorted all-trans retinal is relaxed to the unstrained conformation through the structural rearrangement of the surrounding protein moiety and the strain of the retinal is believed to be fully released in lumirhodopsin or metarhodopsin-I. The purpose of this study is to elucidate the structure of lumirhodopsin and probable pathways toward it.

We first performed classical molecular dynamics calculations (MD) of rhodopsin in POPC membrane. After the equilibration, the chromophore is isomerized by changing dihedral potential function to mimic the process of the photo-isomerization. We then perform steered MD calculations, which moderately accelerate the structural change of protein, to find pathways toward lumirhodopsin. In this calculation, β -ionone ring moves toward helix IV. The

planarity of the retinal is increased significantly by this structural change, although there still remains distortions at C6-C12 region. We will compare the structures using normal mode analyses and excitation energy and discuss about the structure of lumirhodopsin.

1Q1324 Activation mechanism of rhodopsin elucidated by molecular dynamics simulations

Fuyuki Sakai, Shoji Takada (*Dept. Biophysics, Div. Biol. Graduate School of Science Kyoto University*)

Rhodopsin, one of G protein-coupled receptors, consists of opsin, apoprotein, and a covalently bound chromophore, 11-cis-retinal. Activation of rhodopsin is triggered by photo absorption followed by cis-trans isomerization of its retinal. After the initiation, deprotonation of retinal Schiff base causes transmembrane helix 6 (TM6) motion, and rhodopsin becomes active conformation (Meta II). From the augmentation of biochemical and biophysical studies by recent high-resolution 3D structures, its activation intermediates can now be interpreted. Yet, the last part of activation mechanism is still unclear because the active conformation structure with all-trans-retinal (Meta II) had not been solved. For the active state of rhodopsin, recently two crystallographic models were reported, which contained agonist all-trans-retinal in completely different orientations each other; the normal and the flipped orientations. Here, we report all atom molecular dynamics simulation results for the active form of rhodopsin with various protonation states and mutations, finding that the flipped retinal orientation is the more stable form in Meta II state. The retinal can flip nearly 180 degree in a few hundred nanoseconds in the binding pocket of the active form of rhodopsin. The stability of the flipped form is due to the proton transfer from Glu113 to Schiff base. In addition, the process of activation will be analyzed.

1Q1336 X線小角散乱によるタコロドプシンの研究

Small-angle X-ray scattering (SAXS) of octopus rhodopsin

Shingo Watanabe¹, Teruaki Onai², Mitsuhiro Hirai², Tatsuo Iwasa^{1,3} (¹CEDAR, Muroran IT, ²Grad. Sch. Eng., Gunma Univ., ³Grad. Sch. Eng., Muroran IT)

Photo-activated rhodopsin couples with G-protein, triggers an enzymatic cascade and finally results in the depolarization of the octopus photoreceptor cell. In the present study, we aimed to study the conformational change of octopus rhodopsin depend on light and interaction with G-protein using SAXS. As the first step of the work, we measured SAXS of octopus rhodopsin. The microvillar membrane (MV) was obtained from octopus retina by sucrose floatation and divided into two layers (lower layer, LL and upper layer, UL) by density gradient centrifugation using percoll. SAXS measurements of MV solubilized with 1% n-Dodecyl- β -D-maltoside (DDM) were done by using the SAXS spectrometer installed at BL10C beam port of the synchrotron radiation source (PF) at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. We measured four kinds of samples (MV: LL and UL, and DDM-solubilized rhodopsin of LL and UL) and obtained intense WAXS signal enough to analyze. The gyration radii (Rg) of MV samples were estimated as 119 Å (UL) and 108 Å (LL), respectively. The Rg of DDM-solubilized rhodopsin was 104 Å (UL) and 107 Å (LL), respectively. Theoretical scattering profiles of bovine rhodopsin and metarhodopsin were estimated using their crystal structural data. The theoretical scattering pattern of bovine metarhodopsin was different from that of rhodopsin in the large angle region, suggesting that the light-induced conformational change of octopus rhodopsin could be investigated by SAXS.

1Q1348 赤外分光法による活性化ロドプシンと α サブユニットのC末端ペプチドとの相互作用解析

FTIR Study of the Interaction between Activated Rhodopsin and the C-Terminal Peptide of Transducin α -Subunit

Daiki Kawata, Kota Katayama, Hideki Kandori (*Nagoya Inst. Of Technol.*)

Rhodopsin (Rh) is a heptahelical family A G-protein coupled receptor (GPCR) responsible for twilight vision. Photoisomerization of the retinal chromophore initiates concerted movements of transmembrane helices to create a binding and activation site for a G-protein transducin (Gt). Crystal structures of several states opened understanding of the molecular mechanism in atomic details, and light-induced difference FTIR spectroscopy is a powerful tool for this aim. Although complex formation between Rh and Gt was not observed in hydrated films [1], we recently reported that Rh can interact with hydroxylamine in