Bio molecular machines generally express macroscopic outputs (MOs) via cyclical and/or multiple microscopic elementary events (MEEs). For example, some bio molecular motors develop sliding or rotating movement (as MO) via cyclical unitary stepping events (as MEE). It is important to reveal such MEEs for understanding working mechanism of bio machines. However, such MEEs are often difficult to observe directly, while expressed MOs are often easy to observe. Therefore, a method that enables us to model MEEs and to estimate their characteristics (size of unitary step, stepping ratio, rate constants, etc. for molecular motors) from "easily observable" MOs, would be a powerful tool to overcome difficulties of direct observation and help understanding working mechanism of bio nano machines. We propose widely suitable method and procedure to model elementary events, and to estimate their characteristic in this study. We theoretically formulated statistical relation between MEEs and MOs for general motion and propose a estimation method (procedure) of characteristics of MEEs with non-linear fitting of "observable" MOs.

As a result of our tests for application, our method showed its potential to determine model of MEEs and their characteristics successfully. Our method and procedure proposed in this study can be a powerful tool to tackle more detail characteristics of bio molecular machines, and to improve apparent spatial and time resolutions of measurement system by using statistical analysis.

1PS051 Photo-control of microtubule flexibility using photochromic nucleotide analogue entrapped within GTP binding site of tubulin

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Azobenzene is a photochromic molecule that undergoes rapid and reversible isomerization between the cis- and trans-form in response to ultraviolet (UV) and visible (VIS) light irradiation, respectively. Previously, we have incorporated azobenzene derivative into functional region of ATP driven motor protein and succeeded to control microtubule dependent ATPase activity reversibly by photo-irradiation. The results suggested that the photo-control system using photochromic molecules might be applicable to other functional biomolecules. It is known that tubulin utilize GTP to polymerize and the GTP is entrapped within the site of tubulin. Interestingly, it was demonstrated the structure of the microtubules composed of GTP analogue GMP-PCP is more flexible than that composed regular GTP. In the present study, we have employed photochromic nucleotide analogue, Phenylazobenzoyl-iminoethyl-Tri-Phosphate (PABITP) in order to photo-control flexibility of microtubules. The microtubules composed of PABITP showed much more flexible shape change during gliding on in-vitro motility assay than that composed of regular GTP. The effect of photoisomerization of photochromic PABITP entrapped within the site of microtubules induced by UV and VIS light irradiation for flexibility of microtubules was examined.

1PS052 ヌクレオチドを利用する生体分子機械へのフォトクロミックヌクレオチドアナログの応用

Possible application of photochromic nucleotide analogue to the nucleotide required bio-molecular machines

Takeshi Itaba, Shinsaku Maruta (Div. of Bioinfo., Grad. Sch. of Eng., Soka Univ.)

Previously, we have synthesized photochromic nucleotide analogues Phenylazobenzoyl-iminoethyl-Tri-Phosphate (PABITP) that change its structure reversibly by light irradiation in order to photo-regulate the function of myosin II. PABITP was hydrolyzed by skeletal muscle myosin and induced dissociation of acto-myosin. X-ray small angle solution scattering analysis of myosin interacting with PABITP showed that the radius of gyration values of S1·PABITP(trans) and S1·PABITP(cis) are identical to those of S1·ATP and S1·ADP respectively. The results suggested that the photo-isomerization of the PABITP induce conformational change of myosin head, which may reflect energy transduction. In this study, we examined the possible application of PABITP to other nucleotide required bio-molecular machines, smooth muscle myosin, myosin V, conventional kinesin, Kif18A, Eg5, tubulin and small G-protein Ras. PABITP was hydrolyzed by these kinesin and myosin ATPase. PABITP induced microtubule gliding on conventional kinesin and polymerization of tubulin to microtubules. We also examined the effect of reversible photoisomerization of PABITP for the nucleotide required bio-molecular machines.

1PT001 タンパク質ドッキング過程における溶媒和自由エネルギー変化 Solvation free energy in protein docking process

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Hydration water is believed to play an important role in the process of protein functional expression, for example in protein structural change, or in folding, or in the ligand binding process. The series of intense studies on the water hydration has been done through last several decades. It showed the characteristic chemical/physical parameters, that is, low diffusion constant, vectorial or anisotropic motion, and protein dynamics controlled by water motion. These results suggest a strong correlation mechanisms between water and protein dynamics.

Recently, we have established a methodology to estimate the excess chemical potential or the solvation free energy in the use of trajectory data from MD simulation. In the case of a single globular protein, the protein structural changes caused $\sim 10^3$ kcal/mol solvation free energy change which strongly correlated with the density of the first hydration and the second hydration shell water.

In this study, under the motivation to investigate the solvation effect in the course of protein docking process, we calculate the solvation free energy of Barnase-barstar complex. From MD trajectory performed on each configuration, a solvation free energy was estimated by means of the classical MD simulation and the energy representation method. We investigated the correlation between the solvation free energy depending on the distance and the configuration among two protein, the hydration water density. These results suggest that the hydration water mediates the protein docking process.

1PT002 ミオシン周囲の水の密度揺らぎ

Density fluctuation of water around myosin

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Association and dissociation between myosin and actin are essential for force generation of the actomyosin molecular motor. However, the origin of the intermolecular interaction involved in these processes is complicated due to the coexistence of the electrostatic and hydrophobic interactions. In the previous report, by conducting an extensive explicit-water all-atom molecular dynamics (MD) simulation, we showed that the ATP-induced dissociation of myosin from the actin filament is likely to be caused by the allosteric response of myosin in the actin-binding loops (such as loop 2) and in the residues on the actin-binding surface, which are electrostatically-controlled by the ATP charge. We also found that the water density around the actin-binding surface slightly increases upon ATP-binding (the orientation of water is also affected by the ATP binding). In this study, we further investigated the hydration state of myosin from the viewpoint of water density fluctuation around the myosin surface, because the water fluctuation is closely related to the hydrophobic interaction (Acharya, et al. 2010). We found that some regions with large water-density fluctuation coincide with the actin-binding hydrophobic regions, and mobile regions including surface loops generally exhibit large water-density fluctuations. By placing hydrophobic small probes in the system, we directly examine whether those regions with large water-density fluctuation actually cause intermolecular attraction.

1PT003 中性子散乱によるF-アクチン周辺水分子のダイナミクス研究

Dynamics of Water around F-actin in Solution Studied by Neutron Scattering

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Proteins are constantly fluctuating under the influence of surrounding aqueous environment. These thermal fluctuations, or dynamics, of the protein are indispensable for the structural changes that make the protein function. Ultimate understanding of the protein functions thus requires understanding of the dynamics of the proteins including the hydration water. We have been investigating the dynamics of F-actin. How the dynamics of water around F-actin affects the internal dynamics of F-actin is particularly interesting because the water around F-actin shows unusual behavior such that it has higher mobility than bulk water (the hyper-mobile water). Here we employed quasi-elastic neutron scattering (QENS) to characterize the dynamics of the hydration water as well as the internal dynamics of F-actin. The QENS spectra of the concentrated solutions of F-actin in D₂O and H₂O were measured with the instrument IN5 in the Institut Laue-Langevin, Grenoble, France. The spectra arising from the dynamics of the hydration water and those from the internal dynamics of F-actin were extracted from the obtained spectra. The dynamics of the hydration water around the protein in the "solution" samples is characterized

for the first time here, and it is shown that translational diffusion constant of water around F-actin has a similar value to that of the bulk water ($2.8 \times 10^{-5} \text{ cm}^2/\text{s}$) but longer residence time (3.3 ps compared to 0.6 ps of the bulk water). The results of the detailed analysis will be presented.

1PT004 Mean Exit Time Analysis about Water Molecules around Membrane Surfaces

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The structures and dynamics of water molecules in the hydration layer of membranes are known to be different from those in bulk. In order to investigate the diffusivity around the surface of the membrane, we performed molecular dynamics simulations of a 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) bilayer and water molecules at temperatures ranging from 250-350 K. The diffusion coefficient cannot properly characterize the diffusivity of water molecules on membrane surfaces because water molecules do not remain attached to the surface for all time. To characterize translational motion of the water molecules near membrane surfaces, we introduce the concept of mean exit time and show that translational motions of water molecules around the surface of the membrane are slower than those in bulk. Furthermore, we find that the distribution of trapping times of water molecules on membrane surfaces obeys a power law distribution, and the power-law exponent depends on temperature. These results imply that power-law trapings enhance the viscosity of water molecules around the surface of membranes.

1PT005 アルギニンによるカフェ酸の可溶化とそのダイナミクス Solubilization and Its Dynamics of Caffeic Acid Induced by Arginine

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Poor aqueous solubility of low molecular weight drug substances hampers their broader applications. We have shown that arginine increases the solubility of small organic compounds as well as proteins. Here we examined the molecular mechanism of arginine on the solubility of caffeic acid that also has a low aqueous solubility. The solubility of caffeic acid increased with increasing pH in the aqueous solutions. At a given pH, arginine further enhanced its solubility. Ratio of the solubility of caffeic acid in arginine solution to that in water was almost constant over the wide range of pH. The interaction between two caffeic acid molecules in the presence or absence of arginine was investigated by molecular dynamic simulation. Our simulation showed arginine decreases binding free energy between the caffeic acid molecules, which suggested the increased solubility of caffeic acid by arginine was attributable to stabilization of the dissociated state.

1PT006 Single molecule analysis for the energy conversion of actomyosin in the presence of osmolyte

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Actomyosin is a biological molecular machine of nanoscopic size that converts the chemical free energy of ATP into mechanical work. Recent single molecule measurements enabled us to detect the mechanical motion at a phenomenological level. However, the energy transduction at the substantial level is still unsolved. It is expected that the majority of free energy of ATP to be used is originated from hydration and dehydration of surrounding water molecules. Here, we modulated the hydration of actomyosin during an energy transduction using osmolyte, Sucrose. From a viewpoint of osmotic pressure, the pressure at 0.5-1 M sucrose is comparable with that in cell. In a cell, various biological molecules exist and is crowding. This is far from conventional in vitro environment. Our experiment would teach us to predict the energy transduction in a cell.

1PT007 低分子周囲の水のダイナミクスおよび電荷計算：MD および QM 計算

Calculation of dynamics and charges of water around small solute molecules: MD and QM calculations

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The physico-chemical properties during enzymatic reaction such as ATP hydrolysis are affected by solvent conditions, where the solvent ions and water around biomolecules seem to play important roles. To elucidate the properties, classical MD simulations of water molecules around several types of solutes (poly-phosphates, AMP, and monovalent ions) were done with several water models. The hydrating water molecules around mono-phosphate and di-phosphate molecules with a moderate negative charge showed increased mobility of water compared with bulk water and the results were consistent with the experimental results of the dielectric relaxation spectroscopy although no HMW was observed around tri-phosphate molecules where both the SPCE and TIP5P models were tried. As well as the classical MD, the QM calculations of water around phosphate molecules and other polar and non-polar small molecules were done with Gaussian software. In case of the tri-phosphate molecules (H5P3O10 ~ P3O10) with various protonated states (i.e., the net charge of the molecule varies from neutral to -5 e), the calculated absolute charge of water oxygen atom was smaller as the solute charge became negative and the tendency was not changed in several calculation conditions such as different system size, basis function and chemical model. The details of other QM results will be shown in the meeting.

1PT008 力場パラメタが溶媒和ダイナミクスに及ぼす影響の探索 Investigation of force field parameter effect on solvation dynamics

Yoshito Kondo, Takuya Takahashi (*College of Life Sciences, Ritsumeikan Univ.*)

Ions and water are important players in most of the biological phenomena. The anomalous behavior of water around ions would indirectly affect dynamics of biomacromolecules. In addition, hyper-mobile water (HMW) which has higher dielectric relaxation frequency than bulk water is observed around most of the solutes called water structure breakers. NMR experiments also show the existence of water molecules whose translational self-diffusion coefficient is larger than that of pure water around the structure breaker solutes. In our previous studies, molecular dynamics (MD) simulations of water molecules were done around monatomic ion solutes by using conventional water models (TIP3P, TIP4P, TIP5P, SCP etc.). Then, the self-diffusion coefficients and rotational relaxation times in all simulations were calculated to examine the motility change of water molecules due to the solute molecule. As the HMW was not reproduced with the conventional water models, we developed the modified TIP5P water model which force field parameters were optimized with quantum mechanical calculations. After that, we could partly reproduce the HMW. In this study, we tried a variety of parameters of force field to find the most suitable one that reproduces the dynamics of water more quantitatively. The mobility change of water molecules in ionic solutions by the conditions of the appropriate force field parameters from the analysis results will be shown.

1PT009 蛋白質水和の物理的特性. IV. 水和サイト動力学の定量的解析

Physical Properties of Protein Hydration. IV. Quantitative Analysis of Hydration-Site Dynamics

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[Background and Objective] In a series of studies on protein hydration, we have newly defined the hydration site (HS) of proteins to report on static and dynamic properties of HSs and detailed molecular mechanism of the dynamics of internal hydration water (HW). These studies showed that HSs of a protein are classified into Single HSs (SHS) and Clustered HSs (CHS) composed of one and more than one polar groups (PGs) respectively. They confirmed that, in any of SHSs, external CHSs and internal CHSs, HWs are very dynamic entity. Quite different from that given by X-ray crystallography, the real view of HWs is far from static. The aim of this study is to characterize quantitatively dynamics of the HW of lysozyme (Lzm).

[Method and Results] Performing MD simulation on an aqueous solution of Lzm, we obtained data on the fluctuation of the hydration state of Lzm. Analysis of the data led to the conclusion as follows: (1) Fluctuations of the hydration state of all the SHSs relax very rapidly within a few tens of ps. (2) In CHSs, HWs H-bonding to their respective PGs fluctuate in mutually correlated manner. Temporal fluctuations of HWs in the largest internal CHS, 'Hg', can be