distinct computational methods, MD simulation and 3D-RISM theory. The B-form of DNA is suitable for the evaluation of calculated hydrations because a well-defined hydration pattern known as water spine appears in the minor groove of particular sequences. Both methods are promising for obtaining hydration properties, but until now there have been no thorough comparisons of the 3D distributions of hydrating water. Our rigorous comparison showed that MD and 3D-RISM provide essentially similar hydration patterns when there is sufficient sampling time for MD and a sufficient number of conformations to describe molecular flexibility for 3D-RISM. This suggests that these two computational methods can be used to complement one another when evaluating the reliability of the calculated hydration patterns.

3P-105 水の集団運動の解析およびそれを通じた溶質分子と水分子の動力学的相互作用の解明

Analysis of cooperative modes of water and understandings of dynamical interaction between water and solute molecules through it

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Water is thought to play an important role in dynamics of solute molecules such as proteins because proteins cannot exhibit their function without water. However, the question of how water molecules dynamically assist proteins to exhibits their function still remains unsolved. In order to address the question, we develop a method to extract cooperative dynamics of water molecules by focusing on density field, flux and electro-static field created by them because those fields satisfies the equation of continuity thus have slow timescale and are expected to reflect slow cooperative dynamics of water molecules. We apply the above method to analyze cooperative modes of water molecules around solute molecules and how these modes and dynamics of solute molecules couple with each other by checking the mutual correlation between the two.

3P-106 蛋白質と水和水の構造変化相関：PYPの分子動力学的研究

Correlation of structural change in protein and hydration water: Molecular dynamic study of PYP

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Intensive studies on the relationship between protein and water molecule were done in the last several decades. One of the central interests in this field is the water contribution on the stability and/or dynamics of protein structure that relates strongly to folding, reaction, and expression of function of protein. Most noteworthy point is that protein needs the (translational) motion of the hydration water for the protein conformational change. Understanding such a tightly coupled dynamics among protein and water is important not only for science but for applications.

In this study, we carried out the molecular dynamic simulation for the Photoactive Yellow Protein (PYP) in the motivation to explore and analyze a protein-water coupled motion and energetics in terms of the potential landscape and/or inter-basin transitions. The global water and protein structural changes were estimated as a time-resolved 3-D distribution spectrum that derived by 5 ps accumulating atomic positions of protein and water molecule, respectively, in the Euclid-space in picosecond time region. The hierarchical cluster analysis was applied to RMSD between two distribution spectra in time for drawing dendrograms that enables us to classify the time-spacio structures (similarity) quantitatively. The events of simultaneous structural changes among protein and water were salvaged in comparison of protein- and water-dendrogram(s). We estimate protein-protein, protein-water, and water-water potential energy, solvation free energy and interpret the protein-water correlation motion with the aid of ballistic inter-basin transitions extracted by the cluster analysis for two-dimensional PC (principal component) projection map.

3P-107 アクチンフィラメントへのトロポミオシンの協同的な結合の可視化

Visualizing the cooperative binding of tropomyosin molecules along an actin filament

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Tropomyosin binds to actin filaments at ever seven monomers along the helical cleft of the filament. It would include a stochastic difficulty to reconstitute the tropomyosin-binding actin filament in vitro with maintaining this physiological stoichiometry. There should be some crucial mechanism to fulfill this restriction. In order to reveal the mechanism, we have been trying to visualize the process of binding of tropomyosin onto an actin filament under fluorescent microscope. A few amino-groups of tropomyosin were labeled with Cy3 and imposed on the actin filament under the microscope at the molar ratio of seven actin monomers to one tropomyosin, where actin filaments were attached to HMM spread on a glass

surface. We could easily observe the binding process of tropomyosin, which is dot-like for the first time transformed into line-like indicating the processes should be cooperative.

For the further investigation, observation of a single molecule of TM should be necessary. Q-dot-labeled tropomyosin was used to the same experiment. The results should be reported on the session.

3P-108 アクチン繊維内のモノマー配置の揺らぎ

Fluctuating configurations of monomers within an actin filament

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Fluctuations of local configuration for an actin filament were measured by monitoring the efficiency of inter-molecular FRET, fluorescent resonance energy transfer, between adjacent monomers. Actin monomers were selectively labeled at lys335 residue with either Cy3 or Cy5 subjected to co-polymerization and incorporated into unlabeled filament, named as FRET-spot. We have selectively picked up the spot containing 18 monomers; six Cy3-labeled, six Cy5-labeled and six unlabeled monomers. Because the fluorescent fragment within a filament figures a small spot, the number of monomers constituting the fragment was estimated from its fluorescent intensity.

The efficiency of the resonance transfer from single FRET-spot was about 50% under the conventional EPI-fluorescent microscope and was essentially the same to that obtained from spectrophotofluorometer. The series of fluorescent intensities were acquired from microscope at every 1/30 second (TV frame rate). The amplitude of fluctuation was determined to be the standard deviation of 400 sequential data of intensities, which were normalized against those in the absence of Cy5 acceptor molecules.

The fluctuation of an actin filament interacting with myosin molecules in the presence of ATP was about 4.0% in comparison with that floating in solution about 0.8%. The amplitude was not appeared to depend on ATP concentrations. However, some types of periodicity or step-like features were observed. Structural implications of these features will be discussed.

3P-109 アクトミオシンによる微小検出システム構築の試み

An attempt nano-detection system applying actomyosin motility systems

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Actin and myosin molecules could bring the protein molecules within a cell at the scale of several micrometers. The system had been reconstructed as an in vitro motility assay system under fluorescent microscope. In recent years, a few kinds of micro-devices using the motor proteins, such as dynein or kinesin, have been tried to be evolved as novel techniques. Among them, one of the most outstanding features of the system is to bring a molecule without its solvent. Here, we attempt to utilize this feature into micro-detection system, which could enable the chemical reactions without mixing the solutions. First of all, antigen-antibody reactions have been tried to realize under light microscope without mixing any solutions.

Before to the detection of antigen, avidin-biotin conjugation reaction was adopted for the calibration of experimental setup. We have separately spread two kinds of molecules over glass slide; one was the HMM molecules alone and the other was the mixture of HMM and avidin molecules. Following, the filament mixtures of both biotin-labeled and unlabeled filament were dropped to the surface. As times go on, biotin labeled filaments were found to be selectively collected to avidin-mixed area. This system might be useful to detect some kinds of antigen molecules at extremely high sensitivity with several micro liters of the specimen.

3P-110 蛍光クラスター法によるアクトミオシン単分子相互作用の可視化

Observing the interaction of single actin molecules with myosins in vitro

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Observation of single protein molecules functioning in a physiological manner should be one of the crucial goals for understanding protein functions in cells. Recent improvement for microscopic technique enabled us to detect a single fluorophore by total internal reflection microscope. Because of weak fluorescent emission and photo-bleaching, this type of observation is not always easy to use. Previously, we have developed a cluster of fluorescent dyes to occur these problems. This cluster was made from penta-peptides of lysine conjugated with Cy3 dyes (called K5Cy3). K5Cy3 was bright enough to observe single molecule under conventional EPI-fluorescent microscope. We measured the fluctuation of single actin monomers within a single actin filament by using K5Cy3. The observation revealed the fluctuations to be unusually large in amplitude and rapid in time. Additionally, the fluctuations from separated positions along a filament had a strong delayed correlation from a front to a rear end.

In spite of these new observations, detailed analysis of K5Cy3 molecule by TOF-