see ITP3-05

- 1P-056 新規発光共役高分子による新規インスリンフィラメントの細胞毒性と内部構造解明
Cell toxicity and inner structure analysis of novel insulin filaments using novel luminescent conjugate polymers

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see ITP3-04

- 1P-057 キャピラリー内トラップによる一分子の長時間観察：蛋白質の折り畳みへの応用
Long-time observation of a single molecule trapped in a capillary cell: application for protein folding

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see IYP1-02

- 1P-058 ポリグルタミン病の新たな分子病理メカニズム—タンパク質線維の構造伝播による発症制御の可能性
Cross-seeding fibrillation of Q/N-rich proteins offers new pathomechanism of polyglutamine diseases

Yoshiaki Furukawa (1), Kumi Kaneko (1), Masaru Kurosawa (1), Gen Matsumoto (1), Nobuyuki Nukina (1) ((1) *RIKEN, Brain Science Institute*)

see IYP1-08

- 1P-059 蛋白質の体積揺らぎ動力学に対する圧力効果
Effects of Pressure on Volume-Fluctuation Dynamics of Proteins

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see ITP3-06

- 1P-060 青色光センサータンパク質フォトロピンの光反応に対するCrowding効果
The effect of macromolecular crowding on photoreaction of a blue light sensor protein; phototropin

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A characteristic of interiors of living cells is high concentrations of macromolecules. Such media are termed 'crowding condition'. Under this condition, chemical reactions could be different from those occurring in a dilute solution. Here, for elucidating the photoreaction dynamics of phototropin, which is a blue light sensor of plants to regulate the phototropism, we studied the reaction in pseudo-simulated solution by dissolving Ficoll 70 (M.W. 70000) at a high concentration (~300 g/L). Phototropin has LOV1, LOV2, and kinase domains and the reaction dynamics has been investigated previously by the time-resolved transient grating (TG) method in a dilute solution. It showed that a part of α -helix is unfolded after the photoexcitation of phot1 LOV2 and Phot1 LOV2-linker (linker is a domain between LOV2 and kinase domains) sample and these reactions should be closely related with the biological function of phototropin. It is interesting to examine the crowding effect on this reaction to elucidate the reaction in living cells. With increasing the concentration of Ficoll, the intensity of the TG signal of phot1 LOV2-linker sample decreased drastically. This change was explained by the decrease of the reaction quantum yield and the decrease of the diffusion coefficient change under the crowding condition. We

also observed that the association state of phot1 LOV2 is sensitively affected by the crowding. These crowding effects will be discussed.

- 1P-061 光センサータンパク質 TePixD の反応ダイナミクスへの圧力効果
Pressure effects on reaction dynamics of a photosensor protein TePixD

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Fluctuation during protein reaction plays an important role in biological function. However, it is very difficult to detect such fluctuation experimentally. Fluctuation is directly related to thermodynamical parameters. For example, isothermal compressibility is defined as pressure differentiation of volume change, and this parameter determines volume fluctuation. The time-resolved transient grating (TG) technique is one of powerful methods to measure thermodynamical parameters and diffusion coefficient (D) of short-lived intermediate in photosensor protein reactions. In this study, we applied the TG technique to photoreaction dynamics of a photosensor protein TePixD under various pressures up to 400 MPa. TePixD is a blue light sensor protein with a BLUF domain and forms a unique decamer in crystal. Furthermore, in solution, TePixD is in equilibrium between the decamer and pentamer. The TG signal of TePixD at 1 atm has revealed that TePixD shows volume expansion and D-change of the decamer during the photoreaction. Interestingly, the diffusion signal intensity and profile of TePixD were found to depend on the pressure dramatically. This dependence is considered to be pressure effect on the equilibrium. We will also report pressure dependence of volume change and enthalpy change, and discuss the relation between fluctuation and function of this photosensor protein.

- 1P-062 線形応答自由エネルギー法を用いた Chalcone Isomerase による触媒反応の理論的研究
Theoretical Analysis of Chalcone Isomerase Catalyzed Reaction Using Linear Response Free Energy Method

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For studying chemical reactions in solution and enzymes, we often use combined quantum mechanical/molecular mechanical (QM/MM) methods. Since a large number of degree of freedom are involved in such reaction processes, free energy surfaces instead of potential energy surfaces are necessary to be examined. Several methods based on QM/MM techniques have been developed to calculate free energy profiles of chemical reactions.

We employ QM/MM Linear Response Free Energy (LRFE) method, proposed by Higashi et al, which makes it possible to calculate free energy and free energy gradients of the systems. We have studied chalcone isomerase catalyzed reaction, using this method. Chalcone isomerase catalyzes transformation of chalcones to flavanones. Some reaction paths have been proposed in earlier studies. We optimized the geometry of chalcone and some neighbour residues, and calculated free energy profile along the reaction paths.

To analyze the catalytic effects, we defined and calculated site dipole constant based on the generalized Born formula using site electrostatic potential obtained from LRFE.

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- 1P-063 フラビン依存性オキシダーゼが示す非ミカエリス-メンテン型反応機構の理論的解析
Theoretical analysis of the reaction mechanism of non-Michaelis kinetics of flavin-dependent oxidases

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Flavin-dependent oxidases frequently exhibit non-Michaelis kinetics. For instance, monoamine oxidase A and L-aspartate oxidase show strong substrate activation. Substantial levels of substrate inhibition are more often found for various oxidases (L-amino acid oxidase is a typical one). However, why flavin-dependent oxidases are prone to show substrate activation/inhibition has not been addressed systematically. Proper analysis of the kinetic data has not been carried out either. Recently, we have found that porcine D-aspartate oxidase exhibits significant substrate activation for D-aspartate, its endogenous substrate, under physiological conditions (*J. Biochem.* (2007) 141, 363-376). To explain the observed substrate

activation, we have proposed a reaction mechanism, which is generally applicable for other flavin-dependent oxidases, and a rate equation applicable to experimentally obtained kinetic data. In the present study, we further investigated the reaction model to reveal physiological meaning of the kinetic parameters. The interaction between the oxidase and substrate/product depends on the redox state of the flavin cofactor. In the reductive half reaction, substrate is oxidized to product and the flavin is reduced concomitantly. In the oxidative half reaction, the reduced flavin is oxidized with O₂ molecule. The oxidation rate depends on whether the reduced enzyme is free or substrate- or product-bound. These redox-dependent ligand binding and ligand-dependent O₂ reactivity make flavin-dependent oxidase subject to substrate activation/inhibition. Substrate activation occurs only when the reaction rate of O₂ with substrate-bound reduced enzyme is sufficiently greater than that of free reduced enzyme.

1P-064 二重標識したマルトース結合蛋白質の基質結合と折りたたみの分子観測

Single molecule observation of the ligand binding and folding dynamics of maltose binding protein doubly labeled by a cell free system

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We investigated the single molecule fluorescence intensity for the ligand binding process of maltose binding protein (MBP), which was doubly labeled by a fluorophore and quencher in a site-specific manner. We utilized the new modification technique that enabled the introduction of aminophenylalanines linked by Alexa 532 as fluorophore and by NBD as quencher into MBP based on the cell-free translation system. The MBP derivative showed the maltose dependent changes in the fluorescence intensity at the ensemble level. We detected the fluorescence signals from the free single molecule of MBP as a function of the ligand concentration by using the hand-made observation system. The fluorescence intensity histogram from individual molecules possessed two peaks, which suggested the presence of open and close conformations of MBP. Furthermore, the histogram varied with the ligand concentration. Intriguingly, the two peaks were still present even in the histogram taken at the sufficiently low concentration of maltose. We suggest that MBP is fluctuating between the open and close conformations on the millisecond time scale in the absence of the ligand. Further experiments are underway to reveal the properties of the conformational dynamics in the ligand binding of MBP. The folding dynamics of MBP at the single molecule level will also be investigated.

1P-065 X線結晶解析における水分子位置評価および蛋白質水和構造予測プログラムの開発

Development of programs to check the hydration sites identified by X-ray crystallography and to predict protein hydration structures

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We previously calculated probability distributions of hydration water molecules around polar protein atoms from 18,007 high-resolution protein structures solved using diffraction data of better than 2.2 Å collected below 150 K in the Protein Data Bank [1]. We are developing two program suites to utilize the distributions.

One is a tool to check the 'hydration sites' deduced from X-ray crystallography. In electron density maps, water molecules are identified as spherical density peaks. However, ghost peaks resembling those of water molecules sometimes appear and water molecules may be assigned to such peaks. The tool is probably convenient for crystallographers to find readily such mis-assigned 'water molecules' by mapping hydration sites on the distributions from the database analysis.

The other is a program to predict hydration sites at around polar protein atoms by convoluting the probability distributions. The predicted hydration structure for human lysozyme is consistent well with the crystal water sites of the crystal structure analyzed by cryogenic X-ray crystallography at a resolution of 0.95 Å. The program provides the probability distribution in water-accessible narrow channels and voids inside protein molecules. Taking the results, we examined whether it predicts the hydration structure in water-accessible channels of membrane protein such as aquaporin. The predicted hydration distribution in the channel is consistent with those in the high resolution crystallography.

[1] D. Matsuoka & M. Nakasako (2009) submitted

1P-066 分子動力学法を用いた生体高分子系の緩和モード解析 Relaxation Mode Analysis of a Biopolymer System by Molecular Dynamics

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The dynamics of structural fluctuations of a biopolymer is important for the understanding of the interrelation between its structures and functions. Principal component analysis (PCA) has been widely used for analyzing static properties of structural fluctuations of biopolymers.

On the other hand, relaxation mode analysis (RMA) is a method that extracts dynamic properties of structural fluctuations. In this method, the time correlation matrices of structural fluctuations are calculated for two different times through simulations. Then, by solving a generalized eigenvalue problem for these matrices, the relaxation rates and the relaxation modes are obtained from the eigenvalues and the eigenvectors, respectively.

On the basis of RMA, we can expand the time correlation functions into exponentially relaxing terms, each of which is a contribution from each relaxation mode. Thus, we can extract slow collective motions from the relaxation of structural fluctuations as slow relaxation modes. Therefore, RMA is useful for understanding the interrelation between slow structural fluctuations and functions of biopolymers.

In our research, we perform an all atom molecular dynamics (MD) simulation of a human lysozyme in a water sphere, where Amber ff03 parameters are employed. We perform a 30 ns constant temperature simulation with the Langevin thermostat and use a MDGRAPE-3 PCI-X board for its acceleration. We analyze the simulation data by PCA and RMA methods. In this poster, we present results from these analyses.

1P-067 単純並列化によるマルチカノニカル分子動力学法のサンプリング効率の向上 Improvement of Sampling Efficiency of Multicanonical Molecular Dynamics by Trivial Parallelization

Improvement of Sampling Efficiency of Multicanonical Molecular Dynamics by Trivial Parallelization

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Multicanonical molecular dynamics (McMD) simulation is a powerful technique to enhance the conformational sampling of proteins. While a single McMD simulation has a high sampling efficiency, it is difficult to sufficiently explore the vast conformational space of a longer protein. We need more simulation time to explore it, because the degrees of freedom of the system exponentially increase with the elongation of the protein. Thus, we have developed a trivial parallelization of McMD simulations, which explores a conformational space by multiple McMD simulations started from various initial structures. In the previous study, we confirmed that physical quantities from a long McMD trajectory of a short peptide converged well with those from the sum of N short McMD trajectories with one- N th time steps of the long one. Namely, it shows that trivial parallelization of McMD with using N computing nodes can calculate N times faster than conventional McMD simulation with using a node. In the current study, we performed some trivially parallelized McMD simulations with varying N from 1 to 256 to study the dependence of N on the sampling efficiency. We used an alanine decapeptide with the all-atom model in explicit water. With increasing N , the sampling efficiency increases because of varieties of the N initial structures of the simulations. This result indicates that the trivial parallelization of McMD is not only faster, but also more accurate than the conventional McMD simulation. The details will be discussed in our poster.

1P-068 全反射赤外分光法によるV型ATPaseのNa⁺結合構造に関する研究 Sodium ion binding sites of V-ATPase studied by ATR-FTIR spectroscopy

Sodium ion binding sites of V-ATPase studied by ATR-FTIR spectroscopy

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V-ATPases couple ion movement with ATP hydrolysis, whose structure and mechanism resemble to F-ATPases. Like F-ATPases, V-ATPases have a globular catalytic domain, V₁ (equivalent to F₁) where ATP is hydrolysed. This is attached by central and peripheral stalks to an intrinsic membrane domain, V_o (equivalent to F_o) which pumps ions across the membrane. In both F- and V-ATPases, ATP hydrolysis causes rotation of the central stalk and the attached membrane ring comprised of hydrophobic subunits. One of the authors (T. M.) has identified a variant of V-ATPase in a fermentative bacterium *Enterococcus hirae*, which physiologically transports Na⁺ as well as Li⁺, and revealed the structure of the sodium ion binding site by use of X-ray crystallography in atomic detail (1).

Recently, we have succeeded in measuring the ion-binding induced difference infrared spectra of the V-ATPase by using ATR-FTIR technique for the first time. The protonation of carboxylate was observed in the negative side of the Na⁺/H⁺ and Li⁺/H⁺ difference spectra, respectively. The structural difference between Na⁺ and Li⁺ bound forms are also detected in the Na⁺/Li⁺ difference spectra. In the conference, we would like to discuss the relationship between the structure of the sodium binding sites and the pumping mechanism of the V-ATPase.