- 1P078 コロイドプローブ原子間力顕微鏡を用いた転写タンパク質間相互作用の直接測定
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Direct measurement of interactions between the transcription proteins using colloidal probe atomic force microscopy
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Proteins play essential roles in living systems through their interactions. We study the protein interactions using colloidal probe atomic force microscopy. This approach enables us to study elementary steps of a complicated biological function. Here, in order to elucidate the known effect of heat treatment of the cell on SigB activity, the interactions between SigB, transcription factor, and RsbW, anti-SigB factor, were measured when the proteins were treated with heat at 48 degrees.

Proteins were immobilized on silica substrates employing affinity tags, glutathion-S-transferase (GST) for SigB and maltose binding protein (MBP) for RsbW. The interactions were measured in 0.1 mM Tris buffer solution containing 1.0 mM NaCl at 25±1 degrees and pH 7. Heat treatment was performed at 48 degrees for appropriate time of period, then the temperature was returned to 25±1 degrees.

The adhesion forces obtained for SigBRsbW were 2 or 3 times larger than the values for identical surfaces (SigBSigB, RsbWRsbW), indicating the measured adhesion was the specific interaction. The shape of force profiles in retraction did not change by heat treatment.

When the heat treatment time was 0.5 min and 1 min, the adhesions between SigB and RsbW were smaller than those of untreated samples. However, when the heat treatment time was further increased, the obtained adhesion was close the value of untreated samples. The changes in the interaction between SigB and RsbW could occur in rather short time.

- 1P080 フォトクロミック分子を用いたカルモジュリンの機能の光制御
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1aF14 Photo-Control of Calmodulin function using Photochromic Compound
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Calmodulin (CaM) is a well-known Ca²⁺-binding protein that participates in numerous cellular regulatory processes. CaM has a dumbbell-like shape in which two globular domains are connected by a short α -helix. Each of the globular domains has two Ca²⁺-binding site called as EF-hand. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. In this study, we have demonstrated that photo-control of CaM binding to target peptide using photochromic compound N-(4-phenylazophenyl)maleimide (PAM) which undergoes *cis-trans* isomerization by UV-Visible light irradiation reversibly. PAM was incorporated into CaM mutants that have a single reactive cysteine residue. And we prepared fluorescent fusion protein M13-YFP in order to monitor interaction between CaM and M13 peptide with HPLC using size exclusion column. The binding of PAM-CaM(N60C), PAM-CaM(D64C) and PAM-CaM(M124C) to M13-YFP were apparently photo-controlled by UV-Visible light irradiation reversibly at the appropriate Ca²⁺ concentration. Interestingly, on UV light irradiation, the binding of PAM-CaM(N60C) and PAM-CaM(D64C) increased. Contrary, the binding of PAM-CaM(M124C) was decreased. And on visible light irradiation, the binding of the PAM-CaM mutants showed opposite effect to UV light irradiation.

- 1P079 分子シミュレーションと中性子干渉性散乱で探る蛋白質の協奏的運動
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Molecular simulation study to examine the possibility of detecting collective motions in protein by coherent neutron scattering
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Collective motions play an important role in protein function. Coherent neutron scattering is potentially useful technique for studying the conformational dynamics of proteins, since it gives information on the mutual-correlation function of atoms in the specimen. However, due to limitation of neutron flux and difficulty in interpreting the experimental data, coherent neutron scattering has been rarely applied for biological systems. The flux problem will be solved by the third-generation neutron sources under construction at Tokai (J-PARC) and at Oak Ridge (SNS). Here we perform molecular simulations to examine the possibility of detecting collective motions in protein by coherent neutron scattering experiment. Molecular simulations are essential tool for studying the protein dynamics, because they simulate the detailed motion of all atoms, which cannot be observed directly by experiments. Neutron scattering data are calculated from the results of simulations. Thus, relation between atomic fluctuations and neutron scattering data is examined. Molecular dynamics simulation of bacteriophage T4 lysozyme in water is carried out here. First, we discuss the contributions of the principal component (PC) modes to the coherent scattering from the internal motions of the molecule. Then we examine the contributions of the external motions of the protein and of the solvent molecules to the total coherent scattering.

- 1P081 光分子ケージ化合物により光制御型産業用酵素の開発
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Photo-regulation of industrial enzymes using photo-responsive caged compound
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Photo-responsive caged compounds have high potency in the application concerning functional biological molecules as photo-switching device. NAD⁺-dependent formate dehydrogenase (FDH) is a pharmaceutical chemistry used enzyme, roles in the regeneration of NADH in enzymatic synthesis of optically active compounds with dehydrogenases. Whereas kinesin as a potential nanobio device is envisioned to load, transport, sort and assemble nanoscale building blocks. The photo-regulation of the catalytic activity of formate dehydrogenase and kinesin was investigated by treating these industrial enzymes with 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBB), an electrophilic caging reagent. Cysteine residue of enzymes will be selectively caged by DMNBB, pseudo irreversible inactivation results from the modification suggested by recovery with photo-release. Among the kinesin mutants which have a single cysteine at A244 and A252, around 70-80% activity inhibition have been observed after reacted with 5 times concentration of DMNBB for 14 hours. The mutant A252C recovered more than 80% of its activity by irradiating at 366nm. Wild-type FDH showed almost complete inactivation by caged modification, but no significant activity recovery has been found upon photolysis. Currently we are preparing the FDH mutant that has a single cysteine residue in the functional region in order to incorporate the caged molecules specifically. Present study successfully caged the mutant kinesin and altered its activity