- 1P062 ラマン分光法による Hen-Egg-White-Lysozyme 水溶液の低振動数領域の collective mode の帰属
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Raman spectral assignment of collective mode of hen-egg-white-lysozyme
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Vibrational spectra above 300cm⁻¹ are generally measured for analyses of the secondary structure of proteins. Local vibrational modes such as amide I and amide III modes, which are mainly associated, respectively, with C=O stretching and C-N stretching of backbone peptide, are frequently used. On the other hand, spectra in the region below 300cm⁻¹ reflects collective vibrations of characteristic chain moiety such as α -helix and β -sheet etc., and/or fluctuation of protein structure. Therefore, collective modes are useful for investigation of protein structure. Indeed, theoretical studies show that there are several collective modes¹. However, it is difficult to experimentally measure collective mode of protein by Raman scattering spectroscopy since Rayleigh scatterings from aqueous solutions are relatively strong. Thus, there have been few Raman studies focusing collective modes of proteins². The present purpose of our study is to assign the collective mode of hen-egg-white-lysozyme (HWEL) in aqueous solution. HWEL is good for the first target protein since the crystal structure of HWEL is well known. We have measured low-frequency Raman spectra of HWEL at various temperatures and various concentrations of denaturants. The present study shows that the broad peak (50cm⁻¹-120cm⁻¹) of HWEL reflects the tertiary structure.[1] K. C. Chou, Biophys. J. 48, 289 (1985), K. C. Chou, Biophys. Chem. 30, 3 (1988)[2] A. Hedeous R. Ionov J. Chem. Phys. 124 14703 (2006)

- 1P064 X線CCD検出器におけるX線溶液散乱パターンPSFデコンボリューションによる補正
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Correction of X-ray solution scattering pattern with PSF deconvolution in CCD-based detector utilizing X-ray image intensifier
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Recently, CCD-based X-ray detectors equipped with X-ray images intensifier (XRII+CCD) have become to be used in X-ray diffraction/scattering experiments, especially with synchrotron radiation. The advantages of XRII-CCD detectors are high efficiency, high spatial resolution and linearity, time resolution of submicro-second, and so on. On the other hand, the detectors have drawbacks such as the image distortion, the response non-uniformity and the contrast reduction. To solve these drawbacks, the correction methods for each are developed (K. Ito, 2005). The contrast reduction derives from a point-spread function (PSF) of the detector. The PSF of XRII+CCD detector has a high narrow peak and a very low broad tail (D. Pontoni, 2002). The observed intensity is convolution of the actual scattering intensity with the PSF. In existing correction methods, the PSF contribution is measured by covering the window with a fan-mask. But the measured PSF contribution in masked region may differ from unmasked region. We describe a new method with the PSF deconvolution from the observed image. Calculation of the PSF deconvolution is carried out with two methods, Winer inverse filtering and nonnegative constraint Jacobi interaction method. The both methods can correct effectively for the contrast reduction. By performing this method is in good agreement with the calculated high angles.

- 1P063 構造変化に伴う水和蛋白質分子のX線スペckルパターンシミュレーション
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Simulation on the X-ray speckle patterns of a hydrated protein molecule with conformational variations
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The construction of X-ray free electron laser (XFEL) in Japan will be finished in 2010. The XFEL accelerator provides ultimately intense and completely coherent X-ray of 0.06 nm with 10¹² photons per pulse. The intense X-ray pulse will make it possible to observe speckle patterns of submicron-to-nm particles. In particular, it is expected to analyze the structures of biological supramolecular complexes which is very difficult to crystallize. So far we have been developing a suite of programs to calculate the speckle scattering patterns of any objects and to reconstruct the images of objects from the scattering patterns by applying a phase retrieval algorithm in combination with the over-sampling method.

In this study, we applied the programs to examine how speckle patterns of proteins vary depending on the fluctuation of the molecules and the amount of hydration water molecules surrounding them. Here we selected glutamate dehydrogenase (GDH) as a target object for the examination. The structure of GDH of Mw of 280k was solved at a resolution of 0.018nm and its motions in solution were traced through a molecular dynamics (MD) simulation. We calculated the speckle patterns of GDH from snapshots in the MD simulation and evaluated the influences of hydration and conformational changes on the speckle patterns.

- 1P065 ヨードチロシン導入によるタンパク質の結晶構造解析
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X-ray structure analyses for iodo-tyrosine incorporated proteins
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Phasing is still one of the bottle necks for crystal structure analyses. Unless structural homologues have been known, it is required that target proteins include a key element to solve the phase problem. Structure analyses of selenomethionine substituted proteins with synchrotron radiations seem to be most popular at present time. However, the limitation of this method is access to the facilities. An in-house generator is, indeed, weaker radiation, some elements provide useful signal in longer wave-length to solve the structure. Sulfur atom could be used as one of such elements for SAD data collection. Although S-SAD is very interesting and challenging one, its anomalous signal is not very strong. Therefore, S-SAD technique is not common method yet. Here, we focus on iodine, which can be incorporated into protein as an iodo-tyrosine. Iodine shows strong anomalous signal at the wave-lengths of Cu and Cr radiations. Structure determinations have been conducted for acetyltransferase from Thermus thermophilus HB8. A series of iodo-tyrosine incorporated proteins were prepared and crystallized. The crystal structures have been successfully determined with both data sets (with Cu and Cr radiations, respectively). The calculated electron density was clear. Structure comparison suggested that the substituted iodo-tyrosine residue does not disturb native fold.