In the EM preparation, the RPs tend to dissociate from the holoenzyme and so we applied chemical fixation in density gradient centrifugation (GraFix) to the complex. For the optimal contrast of the images, we used energy-filter to estimate the thickness of the ice layer. For the easier pickup of the particles, we used the defocus pairs. By these improvements, now we can collect suitable images for the single particle analysis, which can deduce the three-dimensional architecture of macro-molecules without crystallization. We would like to discuss the structural implications by the single particle analysis, too.

1PT181 Free energy decomposition and functional sites of proteins

Mami Saito, Shoji Takada (*Grad.Sch.Sci., Kyoto Uni.*)

Prediction of functional sites of proteins has long been studied, but still has room to improve. A major way of predicting functional sites of proteins relies on sequence and/or structure alignments. When a target protein has a novel function or motif or under 30% homology, however, alignment-based methods do not work well. For overcoming this problem, someone have paid attention to free energy analysis because it should mirrors sequence and structure. In particular, it was reported that free energy of residues in functional sites tends to be higher than that in non-functional sites. Here, based on these observation, we extend free energy analysis of functional sites using some standard molecular mechanics force field. We choose MMPBSA.PY script in AMBER11, which automates post-processing of MD trajectories, to analyze energetics using continuum solvent models. It can be used to break energies into "pieces" arising from different residues. It can also calculate contribution of energy component (electrostatic energy or so). Using them, we survey free energy of residues in functional sites and non-functional sites for an ensemble of enzyme set.

1PT182 時間分解蛍光偏光法によるタンパク質周囲の水和層の局所粘性測定

Measurement of the local viscosity of the hydration shell around protein by time-resolved fluorescence anisotropy spectroscopy

Tetsuichi Wazawa, George Mogami, Nobuyuki Morimoto, Noriyoshi Ishida, Makoto Suzuki (*Dept of Material Processing, Grad Sch of Engin, Tohoku Univ*)

Hydration water is vital for the function, dynamics, and conformation of protein, and analysis of how the hydration states of protein change during the molecular processes would give better understandings of the molecular mechanisms. In this study, we have investigated time-resolved fluorescence anisotropy of a fluorophore tethered to a protein in order to apply the technique to the analysis of hydration states of protein.

Cy3, a fluorophore, was tethered to Cys-374 of actin, as a model protein, through a flexible linker so that the dye underwent rotational Brownian motion in the hydration shell. The rotational motion was anticipated to be subject to the local viscosity of the hydration shell. A time-correlated single photon counting method was used to measure the time-resolved fluorescence anisotropy of Cy3 tethered to actin.

The fluorescence anisotropy of Cy3 tethered to G-actin was investigated at room temperature. The anisotropy decay was decomposed into two components, which showed the time constants of >40 ns and ~ 200 ps with anisotropy amplitudes of 0.29 and ~ 0.04 , respectively. The slow component reflects the tumbling motion of G-actin molecules. The fast decay component would be attributed to the rotational Brownian motion of Cy3. Now, the response of the fast decay component to solvent viscosity is being investigated to establish the technique to detect changes in the hydration state of protein.

1PT183 Highly accurate statistical pickup method for single particle 3D analysis using electron microscope

Masaaki Kawata¹, Chikara Sato² (¹*ITRI, AIST*, ²*BRI, AIST*)

Single particle analysis is an information technology-based structure determination method of biological macromolecules. Among data processing in the three-dimensional (3D) reconstruction, particle pickup from micrographs is of critical importance for the final resolution. Quality of the image library also seriously affects the processing time using the following sub-algorithms, i.e., classification, alignment and 3D reconstruction. We found a new approach based on classification of local similarity (LC method), and compares its performance with other various pickup methods. We further developed an extension of the LC method using statistical particle recognition techniques to increase reliability of particle recognition. The recognition- and computation-efficiency of this method was superior to those of other methods; the modified LC method was

especially robust against noise and independent of parameter settings. We demonstrate the performance both with synthetic micrographs and cryo-micrographs. The new method is a key for fully automated cropping of high quality particle images from micrographs, and a step for a fully automated single particle reconstruction algorithm.

1PT184 中程度傾斜撮影した電子顕微鏡画像からの単粒子 3 次元再構築 Single Particle Reconstruction with Medium Tilt Images by Electron Microscopy

Yutaka Ueno, Emiko Kobayashi, Shouhei Mine, Kazunori Kawasaki (*AIST Kansai*)

In three dimensional reconstruction of single particle molecules taking tilted pairs of the same specimen in different angles greatly contributes reliability of the reconstruction of the volume data from projection images. While the random conical tilt method has been widely employed for a specimen with the same face up, a more general method for randomly oriented particles was studied. We have introduced medium tilt by plus-minus 20 degrees that comprises 40 degrees tilted pairs. The method relies on the fact: the images in the same orientation are found in both tilted images and they are grouped into unified clusters in terms of viewing directions. Once a major group of images of the particle in a certain viewing angles exist in images from different tilt angles, these tilted pair gives additional tilted view of the particle beyond the major tilt observation. They contribute to cover wider projection angles minimizing loss of projection information known as the missing cone situation. The method was first examined by simulated reconstruction with a theoretical model phantom. As with the case with traditional tilting observation data collection requires adjusting the viewing scope to locate the same as another tilted pair. Introducing recent electron microscope with CCD camera and the tilting stage controller greatly facilitated data collection. Possible automation for this tedious task will be discussed together with our preliminary results with our single particle analysis of deacetylase from Archaea.

1PT185 拘束付き分子動力学法を適用した、電子顕微鏡法のための原子モデル構築ツールの開発

Development of atomic modelling tool for Electron Microscopy, applying steered Molecular Dynamics method

Risa Yamashita, Ryota Nakao, Hiroshi Sakamoto, Takuo Yasunaga (*Kyushu Institute of Technology*)

In most of studies of proteins using electron microscopy (EM), it is difficult to discuss detail at the atomic level because three-dimensional electron microscopic maps (3D-EM maps) are mostly low or medium resolution and the typical resolution of the 3D-EM map of proteins is about 1 to 4nm. Thus we have developed an atomic modelling tool based on a 3D-EM map under an image processing package of Eos, which we called SRMD and planed atomic models constructed even from the typical resolutions of EM maps. The SRMD method is one of the extensions of steered molecular dynamics (MD) simulation techniques, in which EM maps are used as a potential for MD and constrain each atoms within a target protein. This SRMD method tool did not have GUI yet: When we use SRMD method tool, we need to use an editor to set temperatures for simulated annealing and conditions of constraints from EM-maps. Furthermore, we cannot watch atomic models during modelling in real time. To construct an atomic model precisely, there are two planning points. One is embedding of SRMD into a model viewer with GUI to monitor models and change modeling conditions easily during calculation. The other is to change molecular conditions by a pointing device like IMD. Thus, it will be possible to make atoms stacked unnaturally by 3D-EM map potentials rescued. Here, we will report our progress of the SRMD techniques and examples for a complex map of heme oxygenase and cytochrome P450 reductase, which is reconstructed from EM images.

1PT186 Comparative survey of image processing packages for electron computed tomography

Nan Shen, Mingyue Jin, Takuo Yasunaga (*Dep. Biosci. Bioinf., Inst. Tech. Kyushu*)

Electron computed tomography (ECT) is one of the most widely applicable methods for obtaining the three-dimensional (3D) reconstruction of organelles or cell components. First, tilt series of two-dimensional (2D) projection images of the specimen are recorded in electron microscope, and then 3D structure is computed from the tilt series using some softwares. A common problem