**1P095 電子線トモグラフィーの高速化:
1F1355 繰り返し再構成に対する GPGPU の活用
Speeding up iterative 3D reconstruction techniques:
leveraging GPU technology**

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Electron tomography of biological samples through 3D reconstruction techniques (RTs) is now an important subject for life sciences including biophysics. Here we describe our strategy, implementation and results by incorporating a GPGPU (General-purpose computing on graphics processing units) technology to iterative RTs, i.e., algebraic reconstruction and simultaneous iterative reconstruction techniques (ART and SIRT). These iterative RTs are useful since object's intrinsic properties may be expressed as constraints, whereas other non-iterative methods cannot fully address such feature. However, they often require a formidable computational time because $O(n \times n \times m)$ steps are consumed in a single iteration (n : number of grids covering the object, m : number of projections). Tera flops scale throughput delivered by GPU, on the other hand, is estimated to reduce the time below several seconds. This dramatic speedup is allowed by thread parallelism with GPU-specific architecture, i.e., shared memories allowing fast read/write access in a rate comparable to the registers, and switching of threads nearly free of latency, as well as the concurrent execution mechanism of kernels introduced very recently. As a component of Eos (image processing system for macromolecular micrographs; J Struct Biol, 116(1), 155-160, 1996), our tool allows to characterize performances by using data from real samples, making comparisons with other RTs, and visualizing profiles of R-factor, information entropy, and deviations.

**1P096 非天然アミノ酸の二重導入によるタンパク質構造の精密 FRET 解析
Precise FRET analysis of protein structures by double-incorporation of nonnatural amino acids labeled with donor and acceptor**

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Fluorescence resonance energy transfer (FRET) analysis of double-labeled proteins is a powerful tool for studying protein structures and functions. We have reported position-specific and quantitative double-labeling of proteins using a nonnatural amino acid incorporation technique for FRET analysis of structures and structural changes of proteins. However, it is difficult to determine precise interresidual distances because of the presence of linkers between amino acid main chains and fluorophores.

In this research, *N*-RhodamineGreen-alanine (RhGAla) as a FRET donor and NBD-aminophenylalanine (NBDAF) as a non-fluorescent FRET acceptor were synthesized and incorporated into N-terminal and various internal tyrosine positions of maltose binding protein (MBP) in response to initiator and elongator amber codons, respectively. These nonnatural amino acids contained no linker between amino acid main chains and chromophores, and therefore, precise determination of interresidual distances was expected. Fluorescence lifetime measurements of the double-labeled MBPs and Gaussian distance distribution analysis indicated that mean values of the donor-acceptor distance distributions showed a good correlation with interresidual distances obtained from X-ray crystallographic data. These results suggest that the present method will become a novel tool to analyze protein structures and structural fluctuations.

1P097 Purification of Recombinant Protein using Cell Wall Breakage by co-Expression of VanX.

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Developing a simple and versatile purification protocol for recombinant proteins remains a major challenge in biotechnology. The first step of purification is cell wall breakage usually performed by physical/chemical methods. However, sonication or pressure can denature/aggregate the protein and soft cell wall breakage methods are expected. Here we developed a new cell breakage method that minimizes protein damage. This technique is based on our finding that VanX, which is an enzyme encoded in the vancomycin resistant VanA gene cluster, has a cell lysis activity. We assume that the lysis activity most probably results from the D-Ala-D-Ala dipeptidase activity. In our strategy, VanX is co-expressed with the target protein (in this study GFPuv). As expected, VanX triggered cell lysis during co-expression and the GFPuv leaked into the culture medium. We optimized the medium for getting high protein yields by testing various media; LB, 2xYT, SOB and ZYM-5052. The yield of the target protein released in the culture medium ZYM-5052 was the highest. Furthermore, we purified the GFPuv by collecting it from the culture medium by His affinity column and showed that its activity was identical to that of the

GFPuv purified from the soluble fraction after cell breakage by sonication. These results show that cell lysis by VanX does not affect the protein activity and that it is may replace physical/chemical cell wall breakage. We are now exploring possible application of our method to the production of recombinant protein at industrial scale.

**1P098 タンパク質進化技術「発達ライブラリー法」: 技術とコンセプト
Strategy and technology for the evolution of novel proteins:
Progressive Library Method**

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A technology which takes over the essence of the natural protein evolution has been developed, termed Progressive Library Method (PLM). PLM realizes and utilizes all of the mechanisms that have been acquired through the evolution of life (protein); module-finding, novel function-generation via module shuffling, and function-advancing by way of domain shuffling, and functional adjustment through protein mutation. Therefore, PLM consists of basic techniques such as the block shuffling (YLBS), the module-restructuring (ASAC), the module (domain) shuffling (p&p method), and others.

PLM is a highly integrative and complex technology just as its counterpart Nature is, and yet is a clear concept-led one. If we consider that the way of Nature is far more sophisticated than we can imagine, and the level of the Nature's outputs are so wonderful, the appearance of this technology must be very natural, necessary and timely. This technology seems to contribute not only to protein engineering but also to protein science due to its powerful property in finding of de novo functional structures of proteins and protein(peptide)-to-protein(peptide) interactions.

PLM has been applied to obtaining functional peptides such as cathepsin E-inhibitory peptides and others. We will discuss on the resemblance between PLM and the mechanism of the natural protein evolution based on the concrete data.

**1P099 温度制御されたマイクロ波照射下での微生物培養による蛋白質の発現
Microbial Protein Expression by Temperature Controlled
Cultivation under Microwave Irradiation**

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Microorganism cells having a number of enzymatic reactions are anticipated microwave effect because enzymatic activity is stimulated by microwave irradiation. In previous study, we confirmed proliferative effect on microwave cultivation of *Escherichia coli*. Establishment of microwave cultivation will allow for efficiency of cultivation, hyperexpression of proteins, and stimulation for viable but non-culturable (VNC) bacteria. The microwave cultivation system was composed of multi-mode microwave instrument with thermocouple, electrical transformer, microwave reflector, and air pump. The microwave instrument produced microwave irradiation at 2.45 GHz. To estimate of microwave effect on microorganism cultivation, several proteins of *Escherichia coli* in stationary phase culturing microwave heating and conventional heating was compared and analyzed by peptide mass fingerprint (PMF). As a result, difference of expressed proteins between microwave heating and conventional heating was obvious. In this study, we report the identified proteins by PMF, and discuss relativity of microwave effect.

**1P100 Towards *in vitro* autonomous evolution of self-referring in
in vitro virus displaying an RNA replicase,3**

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We have tried to construct an autonomous protein evolution system by combining the *in vitro* virus (IVV) and the one-pot isothermal RNA replication reaction. In this system, the norovirus RNA-dependent RNA polymerase (3D^{pol}) is displayed on the IVV. The 3D^{pol} IVV system is free from the disadvantage of the traditional Q β system, that is, being tetramer including three proteins of *E. coli* translation system, which may lead to complex interference behavior. Using this IVV displaying the RNA replicase and two RNA primers, we designed the one-pot isothermal replication reaction system, in which the IVV can amplify itself, and evolve autonomously. Moreover, this reaction system will evolve not only the RNA replicase but also the amplification algorithm itself, just as demonstrated in an autonomous evolution of the nucleic acids isothermal amplification system. We designed also an new isothermal RNA amplification system using the terminal transferase activity, *de novo*

initiation of polymerization and the strand displacement reaction by 3D^{pol}, which will be applied to the genomic diagnostics and the gene expression analysis. In this report, we evaluated the characteristics of the key enzyme 3D^{pol} synthesized in PURESYSYSTEM, Rabbit reticulocyte or insect cell-free protein synthesis system, regarding the effect of the post-translational modification.

1P101 FUNCTIONAL CHARACTERIZATION OF THE RECOMBINANT HUMAN TUMOR SUPPRESSOR 101F6 PROTEIN, A CYTOCHROME B561 HOMOLOGUE

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Human tumor suppressor 101F6 is a highly hydrophobic transmembrane protein and a member of the cytochrome b561 family. The 101F6 protein was expressed and purified from *Pichia pastoris* cells as a histidine-tagged protein with its molecular mass of ~26 kDa. MALDI-TOF-MS spectrometry confirmed the protein in its intact form. Heme content analysis by the pyridine hemochrome method indicated two hemes B per molecule. The midpoint potentials were found as +109 and +26 mV for the two hemes. These values were slightly lower than those reported for bovine adrenal chromaffin granule cytochrome b561 or plant *Zea mays* cytochrome b561. EPR spectra showed a highly anisotropic low-spin (HALS) signal at $g_z=3.75$ when measured at 5 K. At temperatures 15 K and 20 K, an EPR signal from a new HALS species with a different behavior in power saturation was overlapped at $g_z=3.65$. The EPR signal at $g_z=3.16$, previously seen in other cytochromes b561, was not observed. The lower homology in the amino acid sequence of 101F6 to other members of the cytochrome b561 family would account for the difference in the heme environments. Absence of the inhibition in the electron transfer activity from ascorbate upon DEPC-treatment of 101F6 protein was observed suggesting that 101F6 might accept reducing equivalent from ascorbate via a mechanism distinct from other cytochromes b561. It is very likely that these unique properties of 101F6 might be responsible for the function of this protein as a putative tumor suppressor during the development of human cancers.

1P102 T quaternary state of oxy haemoglobin in the presence of heterotropic effectors

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Oxygen affinity and its cooperative binding to hemoglobin (Hb) are related to change of the quaternary states; T (deoxy) and R (oxy) states. The binding of effectors to Hb decreased the oxygen affinity, and its cooperativity. In this study, in the presence of L35 and IHP, strong effectors, ¹H-NMR spectra of Hb were measured to clarify the correlation between the change of the oxygen affinities and the structural change of Hb by the effectors.

At pH 6.8, ¹H-NMR spectrum of deoxy-Hb in the presence of L35 and IHP showed four "T-state" signals in 10-14 ppm, originated from hydrogen bonds between Hb subunits, and hyper fine shifted (hfs) signals around 20 ppm, caused by high spin Fe(II) in the heme. With O₂ pressure increasing, the hfs signals disappeared, which means that O₂ binds to heme, but the four T-state signals were still observed under the O₂ pressure of 760 torr. In order to confirm whether O₂ is bound to the heme, visible spectrum of the NMR sample was measured, which coincides with that of oxy-Hb. Consequently, in the presence of the effectors, Hb formed T quaternary state at pH 6.8, though its heme takes 6-coordination with O₂. The pH dependence of the T (oxy) state was studied by measuring ¹H-NMR spectra between pH 6.8 and 8.0. The transition occurred at pH 7.4, and above pH 7.8, only two hydrogen bond signals remained like R (oxy). Hb in the presence of L35 and IHP takes T (oxy) state but not R (oxy) below pH 7.0, which related to the decrease of the oxygen affinity and its cooperativity.

1P103 ウシ心筋チトクロム酸化酵素の酸素還元中心への塩素イオン結合の可能性の X 線構造解析による検討

1G1255 An X-ray structural evaluation of the possibility of chloride binding to the O2 reduction site of bovine heart cytochrome c oxidase

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The resting oxidized form of bovine heart cytochrome c oxidase (CcO) does not pump protons upon initial two electron reduction, while the fully oxi-

dized CcO under turnover conditions pumps protons upon receiving electrons. The OH⁻ binding to Fe_{a3}³⁺ in the oxidized form under turnover conditions has been established by a resonance Raman analysis. The electron density between Fe_{a3} and Cu_B in the resting oxidized form is consistent either to a bridging peroxide with the O-O distance of 1.7 Å or to one equivalent of chloride ion equally (50 % each) distributed between the two sites. In fact, the chloride binding to the resting oxidized form has been proposed by EXAFS. Establishment of the structure of the resting oxidized form would provide important clues for elucidation of the proton pump mechanism. Thus, for evaluation of the possibility of the chloride binding, an anomalous dispersion analysis for the resting oxidized bovine heart CcO was performed at 50K using low energy X-rays with the wavelength of 1.7470 Å, at which the anomalous dispersion of chloride is approximately as intense as those of S, Cu and Fe atoms. The anomalous map obtained shows clear peaks at the positions of the sulfur atoms of methionine and cysteine residues, Cu and Fe sites while no peak is detectable for the electron density between Fe_{a3} and Cu_B. These results clearly exclude the possibility that chloride binds to O₂ reduction site of the resting oxidized CcO, consistent with the chloride content analyses showing 0.15 Cl/CcO.

1P104 水素原子レベルでのプロトン輸送機構解明を目的としたチトクロム酸化酵素の高分解能 X 線結晶構造解析

High resolution X-ray structural analysis of cytochrome c oxidase to clarify the proton pumping mechanism at hydrogen atom level.

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Bovine heart Cytochrome c oxidase (CcO) in the mitochondrial respiratory chain is a terminal enzyme, which catalyzes the molecular oxygen reduction coupling the proton transfer across the inner membrane. To understand proton transfer mechanism, it is necessary to determine orientations and protonation states of amino acid residues (imidazole and carboxyl groups etc.) in the proton pathways. Therefore, we have analyzed the orientations of amino acids by the temperature factor refinement of each atom in the amino acid. The result indicates that if the temperature factor is lower than 30 Å², it is possible to determine the orientation of amino acids. If we can distinguish carboxyl group bond length and observe hydrogen atom electron density, it is possible to determine the protonation states of amino acids. For the purpose, we are currently carrying out the X-ray structural analysis of bovine CcO at 1.4 Å resolution. To improve data accuracy, the diffraction redundancy was increased by merging of many image data. Besides, we select good quality reflections judging from mosaicity and linear R-factor with the program Denzo and Scalepack.

1P105 Atomic Resolution X-ray crystallographic Analyses and

1G1325 Spectroscopic Studies of a Cytochrome c' from *Alcaligenes xylosoxidans*

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Cytochrome c' (cyt c') are a class of c-type cytochrome, which have been found in several photosynthetic bacteria and denitrifying bacteria. Cyt c' has been known to be a typical four α -helices bundle structure, which attaches a heme group near the C-terminal region. The unusual magnetic properties of ferric cyt c' at neutral pH have been interpreted for the quantum mechanical admixture of an intermediate-spin (S = 3/2) and a high-spin (S = 5/2) state. The reduction potential of cyt c' from *Alcaligenes xylosoxidans* was reported to be 184 mV vs. NHE. Eady et al reported the NO and CO forms a stable 5-coordinate NO adduct and 6-coordinate CO adduct with cyt c', respectively. High quality crystal of cyt c' giving an atomic resolution data is required to add more precise knowledge to heme-protein chemistry and elucidate the small molecule recognition mechanisms of cyt c'. The optimized crystallization methods according to the crystal growth mechanism of *Alcaligenes xylosoxidans* cyt c' were reported by Kohzuma et al., and the high quality cyt c' crystals were obtained. The 0.92 - 1.11 Å resolution data were collected for the ferric, ferrous and NO-binding cyt c'. The atomic resolution structures of cyt c' from *Alcaligenes xylosoxidans* and spectroscopic properties will be presented and discussed.

1P106 Conformational Change of NOS Probed by Transpeptidase Mediated C-terminus Specific Fluorescence Labeling

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Staphylococcus aureus expresses sortase (SrtA) that catalyzes the transpepti-