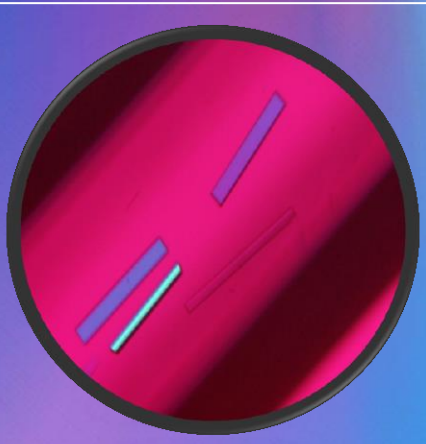
Workshop

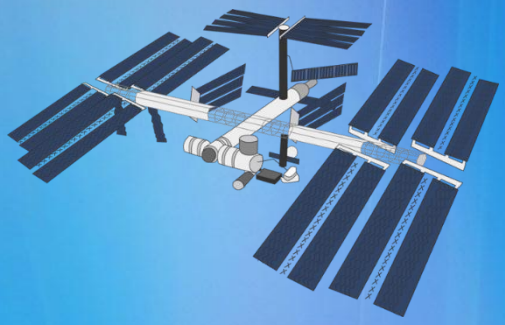
High Quality Protein Crystallization Technology

-Proceedings-

Protein Crystals

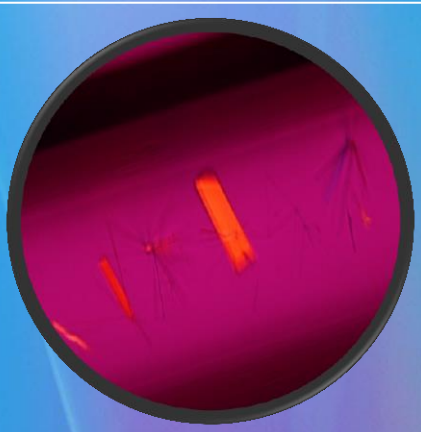
in Space





Single Crystal
Low Mosaicity, High Resolution

on Earth



Cluster Crystal
High Mosaicity, Low Resolution

Date: **October 27th, 2015 13:30 - 17:30**
Place: The University of Tokyo,
Yayoi Auditorium, Angel lecture room

http://www.spaceprotein.com

文部科学省 MINISTRY OF EDUCATION, CULTURE, SPORTS, SCIENCE AND TECHNOLOGY-JAPAN

Space Science Research Center Initiative,
High Quality Protein Crystallization Technology Program

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Space Science of High Quality Protein Crystallization Technology

Program

【Date】2015 年 10 月 27 日 (Tuesday) 13:30 - 17:30 (Open: 12:00)
18:00 - 20:00 (Party)

【Place】Yayoi Auditorium, Angel lecture room , The University of Tokyo
東京大学弥生講堂エンゼル研究棟
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【Access】http://www.a.u-tokyo.ac.jp/yayoi/map.html

【Contact】awang@mail.ecc.u-tokyo.ac.jp

1	13:30 14:00	High-Precision X-ray Crystallography of Proteins	Atsushi Nakagawa Professor Institute for Protein Research, Osaka University / JST-CREST
2	14:00 14:30	What occurs during the crystal growth?	Hiroaki Tanaka President Confocal Science Inc.
3	14:30 15:00	Exact Measurement of Growth rate of Protein Crystals in Space and Its Implication to Grow Good Crystals	Katsuo Tsukamoto Professor Graduate School of Engineering, Osaka University / Graduate School of Science ,Tohoku University
4	15:00 15:30	Orphan drug development for Duchenne muscular dystrophy by protein crystallization in space	Yoshihiro Urade Professor/PI International Institute for Integrative Sleep medicine, University of Tsukuba
5	15:30 16:00	Super high-resolution structure analysis using PHENIX	Hideaki Ogata Group Leader Max Planck Institute for Chemical Energy Conversion, Germany

6	16:00 16:30	Crystallization of membrane proteins	So Iwata Professor Department of Cell Biology, Graduate School of Medicine, Kyoto University
7	16:30 17:00	Neutron and high-resolution X-ray structural studies of cellulase	Kiyohiko Igarashi Associate Professor Department of Biomaterial Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo
	17:00 17:30	Discussion	
	17:30	Closing	Yoshihiro Urade Professor/PI International Institute for Integrative Sleep Medicine, University of Tsukuba
	18:00	Party	

*The results have been achieved by “Space Science of High Quality Protein Crystallization Technology—Research Center Initiative“, the Ministry of Education, Culture, Sports, Science and Technology (MEXT), JAPAN.

*2014 Coordination Funds for Promoting AeroSpace Utilization

Atsushi Nakagawa

Professor, Institute for Protein Research, Osaka University /
JST-CREST

Biography

2003-present	Professor, Institute for Protein Research, Osaka Univ., Japan
1999-2003	Associate Professor, Institute for Protein Research, Osaka Univ., Japan
1995-1999	Associate Professor, Graduate School of Science, Hokkaido Univ., Japan
1994-1995	Visiting Scientist, Laboratory of Molecular Biology, MRC, UK
1986-1995	Assistant Professor, Photon Factory, KEK, Japan
1999	PhD, Graduate School of Science, Osaka Univ., Japan

High-Precision X-ray Crystallography of Proteins

Abstract

Recent development on protein crystallography advances the structure determination of biological macromolecules, such as proteins. Number of protein structures deposited to the Protein Data Bank (PDB) increases exponentially in this couple of decades, and it exceeded 110,000 in October 2015. However, only very limited number of high-resolution (< 1.0Å) structures, have been reported because of limitation of resolution and quality of the crystals. Careful treatments of crystals and diffraction data are essential to obtain high resolution and high quality atomic structure of protein.

We had worked on the JAXA-GCF project ‘High-quality Protein Crystallization Project on The Protein Structure and Function Analysis for Application’ conducted by JAXA from 2004 to 2009. This project aimed to develop high precision structure determination technique for X-ray crystallography and its application to structural biology, and we are still continuing improvement of the technique after the project. I will present the technical development we have been working for high-precision protein X-ray crystallography.

Hiroaki Tanaka

President, Confocal Science Inc.

Biography

2004 Confocal Science Inc., Chief Executive Director

1994 Japan Space Utilization Promotion Center, Researcher

1988 Access Ltd., Managing Director

1981 Assistant Professor of University of Tokyo

1980 M. Pharm. Sc., Pharmaceutical Sciences, Graduate school of University of Tokyo

What occurs during the crystal growth, studied by X-ray diffraction?

Abstract

Confocal Science Inc. and Maruwa Foods and Biosciences Inc. have supported JAXA protein crystallization experiment for nearly 20 years and have compiled a lot of know how. From 2002, JAXA started PCG experiments in space with a helpful support from ESA and Prof. Garcia-Ruiz's group in Granada at the early stage. JAXA has launched protein samples 18 times till now, nearly 1.5 times a year. We have developed a lot of methods and devices, including crystallization cell, optimization methods, simulation programs, crystal harvest and cryo-protection techniques etc., to fix a standard protocol for the handling of users' proteins. From our experience of more than 500 samples, major merits of the PCG in space are the followings: (1) improvement of the maximum resolution, Rmerge and mosaicity of the X-ray diffraction, and (2) suppression of clustered form of single crystals (J. Synchrotron Rad. 2013, 20, 968-973).

The reasonable mechanism to explain these effects is the formation of protein and impurity concentration depletion zones around the growing crystal. In the terrestrial growth, those concentrations are reducing uniformly in the whole solution because of the density-driven convection. But in the convection-free environment like in space, the diffusive fields of the protein and impurity are formed around the crystal. Thus, we made two numerical models to estimate the protein super-saturation level and the impurity concentration around the crystal. It was found that major difference between the center part and the surface of the crystal is expected especially in the impurity concentration. This suggests the quality is not uniform inside the crystal (J. Synchrotron Rad. 2013, 20, 968-973).

We tried to measure local diffraction pattern of the lysozyme crystal by using micro-beam X-ray light source. We found there is an anisotropy in unit cell inflation, suggesting some anisotropy caused by the impurity attachment. This finding may give a significant suggestion that most of the crystal is not a "perfect crystal". The extent of un-uniformity is different between the space-grown crystal and the terrestrial one. There may be some best position to focus X-ray for the data collection.

Katsuo Tsukamoto

Professor,
Graduate School of Engineering, Osaka University/
Graduate School of Science, Tohoku University

Biography

Born in Osaka in 1948. Studied mineralogy and crystal growth mechanism in Tohoku University as a PhD course. PhD from Professor Sunagawa of Tohoku University. Worked with Professor Bennema in Nijmegen University, the Netherlands, in IBM Laboratory in Zurich, in Pierre and Marie Curie University in Paris and in Philips Laboratory, Eindhoven, the Netherlands. Major awards are CGCT Distinguished Explorer Award, Singapore in 2011 and Frank Prize from International Organization of Crystal Growth in 2013. Specialized in in-situ observation of crystal growth at molecular level by advanced optical methods. Experienced many invited professors.

Exact Measurement of Growth rate of Protein Crystals in Space and Its Implication to Grow Good Crystals

Abstract

Protein crystals have been grown under microgravity hoping to improve the quality of crystals, in which growth rate of the protein crystals in space has been assumed to be smaller than that in gravity because of absence of convection or flows of solutions. This condition was speculated to be better condition for the crystal quality improvement. Notwithstanding, we for the first time found by in situ laser interferometry in the International Space Station that the growth rate of lysozyme crystals under microgravity is larger by 20-30% than that in gravity in the commonly applied growth condition.

We firstly discuss on the reason why the growth rate under microgravity is larger than that in gravity based on the analysis of the growth mechanism in both environments and secondly would like to discuss whether the current strategy to grow defect-free perfect crystals are suitable or not to get good diffractivity in x-ray diffraction based on recent x-ray topographic works done by Koizumi et al.

Yoshihiro Urade

Professor/PI, International Institute for Integrative Sleep medicine (WPI-IIIS), University of Tsukuba

Biography

2013-present	Professor/PI, International Institute for Integrative Sleep Medicine, Department of Molecular Sleep Biology, University of Tsukuba
1998-2014	Director, Department of Molecular Behavioral Biology, Osaka Bioscience Institute
1993-1998	Vice Director, Department of Molecular Behavioral Biology, Osaka Bioscience Institute
1990-1993	Senior Scientist, International Research Laboratories CIBA-Geigy Japan
1988-1990	Visiting Professor, Roche Institute of Molecular Biology, USA
1987-1988	Senior Scientist, Department of Enzyme and Metabolism, Osaka Bioscience Institute
1983-1987	Senior Scientist, Hayaishi Bioinformation Transfer Project, Exploratory Research for Advanced Technology (ERATO) program, Research Development Corporation of Japan (JRDC)
1983	Ph.D. Graduate School of Medicine, Kyoto University

Orphan drug development for Duchenne muscular dystrophy by protein crystallization in space

Abstract

Duchenne muscular dystrophy (DMD) is one of the most common types of muscular dystrophy, affecting about 1 out of 3,500 boys. DMD is a severe X-linked muscle disease characterized by progressive skeletal muscle atrophy and caused by mutations in the gene of dystrophin, a cytoskeletal protein. There is still no cure for this disastrous disease. We found that grouped necrotic muscle fibers in patients with DMD expressed hematopoietic prostaglandin (PG) D_2 synthase (H-PGDS), which catalyzes the biosynthesis of PGD_2, an allergic and inflammatory lipid mediator. We obtained very high quality crystals of human recombinant H-PGDS in complexes with a variety of inhibitors, whose half maximal inhibitory concentrations (IC50s) were in the sub micro-molar range, by the counter-diffusion method onboard the ISS. We determined the detailed three-dimensional structures of H-PGDS/inhibitor complexes by X-ray diffraction analysis of the microgravity-grown crystals using an intense X-ray at SPring-8 synchrotron facility. Based on the fine structure of the inhibitor within the catalytic pocket of human H-PGDS, novel potent inhibitors TFC-007 and TAS-205 were developed, whose IC50 value was 20 nM. Both compounds prevented the expansion of muscular necrosis and muscle atrophy without any side effects by chronic treatment of genetically dystrophin-deficient mdx mice. A clinical trial study of TAS-205 for DMD patients (NCT02246478) was officially announced on Sept in 2014 and started.

Hideaki Ogata

Group Leader, Max Planck Institute for Chemical Energy Conversion, Germany

Biography

Hideaki Ogata studied physics and graduated at Kyoto University (Japan) in 1998 and obtained his Ph.D. in chemistry from Kyoto University (Japan) in 2003. He worked at Himeji Institute of Technology (Japan) as a postdoc for a half year, before joining the Max Planck Institute in Mülheim an der Ruhr in November 2003. He is a group leader at the Max Planck Institute for Chemical Energy Conversion in Mülheim an der Ruhr, Germany. His research interests are X-ray crystallographic and spectroscopic studies of metalloenzymes, in particular hydrogenases.

So Iwata

Professor, Department of Cell Biology, Graduate School of Medicine, Kyoto University
Group Director, SACLA Science Research Group, RIKEN SPring-8 Center, JAPAN

Biography

Prof. Iwata was awarded a PhD at University of Tokyo in 1991. He was then a postdoctoral research fellow at the National Laboratory for High Energy Physics, Japan (1991-2), then at the Max-Planck-Institute for Biophysics, Germany (1992-6). He accepted a position as a lecturer at Uppsala University, Sweden in 1996, where he became Professor of Biochemistry in 1999.
He joined Imperial College London in 2000 (- 2015) as the Chair of Membrane Protein Crystallography. He also served as a Diamond Fellow at Diamond Light Source, Oxford. Since 2007, he has undertaken a position of Professor at Graduate School of Medicine, Kyoto University. Since 2012, he has been serving as the group director of SACLA Science Research Group, RIKEN SPring-8 Center.
His current research includes: X-ray crystallography of membrane proteins, macromolecular assemblies, G-protein-coupled receptors (GPCR) and protein crystallography using free electron laser

Former positions

2005-2012	Senior Visiting Scientist, Riken Yokohama Institute
2005-2011	Research Director, Japan Science and Technology Agency, ERATO (Exploratory Research for Advanced Technology) IWATA Human Receptor Crystallography Project
2005-2008	Director of Centre for Structural Biology, Division of Molecular Biosciences, Imperial College London
2004-2015	Diamond Fellow, Diamond Light Source, UK
2000-2015	Division of Molecular Biosciences, Imperial College, London
1999-2000	Professor of Biochemistry at Uppsala University
1996-1999	Lecturer at Uppsala University, Department of Biochemistry
1992-1996	Postdoctoral fellow at Max-Planck-Institute for Biophysics, Frankfurt am Main, Germany
1991-1992	Research fellow with Prof. Noriyoshi Sakabe at the Photon Factory, National Laboratory for High Energy Physics, Tsukuba

Crystallization of membrane proteins

Abstract

The results of genome sequencing projects have shown that up to 30% of human proteins occur in cell membranes. Membrane proteins play crucial roles in many biological functions, including the capture of energy from sunlight by plants, the use of energy in cells, and the movement of molecules across cell membranes. They are particularly important in medicine, since ~over 50% of commercially available drugs (such as antihistamines, beta blockers, antipsychotic drugs, and morphine) target membrane proteins. We need to understand membrane protein structures to provide a basic understanding of life at the molecular level and for computer aided rational design of new drugs, which could reduce the number of animal experiments and unwanted side effects.

The G-protein coupled receptors form the largest family among these membrane proteins and play crucial roles in many biological functions. Although a large amount of work has been done in biochemistry and molecular biology, little is known about the details and the molecular mechanisms of ligand recognition and activation of the receptors. Recently, the structure determination of the receptors has been largely accelerated thanks to new technical developments including crystallization in lipidic cubic phase. To obtain large and well diffracting crystals of the receptors are, however, remains extremely difficult. In my talk, I will present recent development of membrane protein crystallization and discuss how we can take the advantages of crystallization in microgravity.

Kiyohiko Igarashi

Associate Professor, Department of Biomaterial Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo

Biography

2009-Present Associate Professor, The University of Tokyo
2002-2009 Assistant Professor, The University of Tokyo
2000-2001 Postdoctoral Fellow of Biomedical Center, Uppsala University, Uppsala, Sweden
1999 Ph.D. Biomaterial Sciences, Agriculture, The University of Tokyo
1998-2002 Research Fellow, Japan Society for Promotion of Science
1996-1998 Visiting Researcher of Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA

Awards:

1. Ichimura Academic Awards, The New Technology Development Foundation (2015)
2. Encouraging Prize, The Japanese Society of Applied Glycoscience (2013)
3. Prize of The Cellulose Society of Japan (2013)
4. Encouraging Prize of Research in Applied Enzymology, Amano Enzyme (2012)
5. Encouraging Prize, The Cellulose Society of Japan (2006)

Research Specialties:

Biochemistry and molecular biology in biodegradation of cellulose
Genomic and post-genomic analysis of wood-rotting fungi

Publications:

107 Original Papers, 31 Reviews and Book chapters, 11 Patents, 94 Invited lectures

Other activities:

Chair of Gordon Research Conference (Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes 2015)
Editorial Board Member of Journal of Wood Science (2015-)
Editorial Board Member of Applied and Environmental Microbiology (2014-)
Associate Editor of Journal of Applied Glycoscience (2013-)

Neutron and high-resolution X-ray structural studies of cellulase

Abstract

We employed a neutron diffraction analysis to investigate the catalytic mechanism of the inverting glycosdide hydrolase (GH) family 45 cellulase PcCel45A, which is an endoglucanase (EG) belonging to subfamily C of this family, isolated from the basidiomycete Phanerochaete chrysosporium. The amino acid alignment with other GH family 45 EGs indicates PcCel45A lacks putative general base and assisting acidic residues while it has an apparent activity towards cellulose and β-1,3-1,4-glucan (1). To understand the catalytic mechanism of PcCel45A, we made a large crystal of 6 mm3 volume (3 mm x 2 mm x 1 mm) for the neutron protein structural study (2). The results of a joint refinement of the neutron and high-resolution X-ray structures clarified a key role of tautomerization of asparagine 92 to imidic acid as a catalytic base in the inverting cellulase (3).

1. Igarashi, K., Ishida, T., Hori, C., and Samejima, M., Characterization of endoglucanase belonging to new subfamily of glycoside hydrolase family 45 from the basidiomycete Phanerochaete chrysosporium, Appl. Environ. Microbiol. 74:5628-5634 (2008)

2. Nakamura, A., Ishida, T., Fushinobu, S., Kusaka, K., Tanaka, I., Inaka, K., Higuchi, Y., Masaki, M., Ohta, K., Kaneko, S., Niimura, N., Igarashi K., and Samejima, M., Phase diagram-guided method for growth of a large crystal of glycoside hydrolase family 45 inverting cellulase suitable for neutron structural analysis, J. Sync. Rad. 20: 859-863 (2013)

3. Nakamura, A., Ishida, T., Kusaka, K., Yamada, T., Fushinobu, S., Tanaka, I., Kaneko, S., Ohta, K., Tanaka, H., Inaka, K., Higuchi, Y., Niimura, N., Samejima, M., and Igarashi, K., “Newton’s cradle” proton relay with amide-imidic acid tautomerization in inverting cellulase visualized by neutron crystallography, Science Adv. 1: e1500263 (2015)