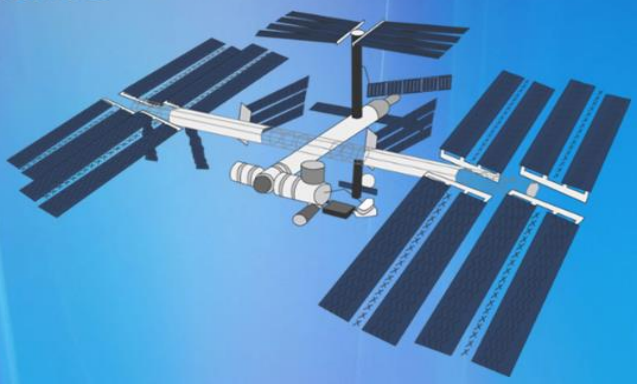
2nd International Workshop on
High Quality Protein Crystallization Technology
-Proceedings-

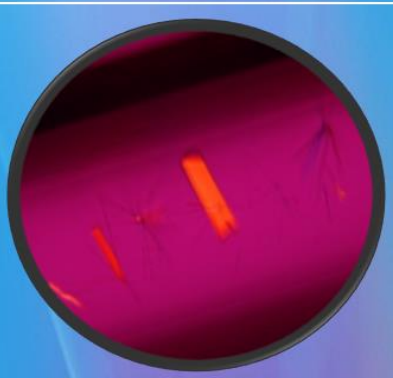
Protein Crystals

in Space



Single Crystal
Low Mosaicity, High Resolution

on Earth



Cluster Crystal
High Mosaicity, Low Resolution

Date: **October 20th (Thursday), 2016 12:00 - 17:10**
Venue: Yayoi Auditorium Annex, Angel lecture room , The University of Tokyo

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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Life Sciences, The University of Tokyo

VTT Technical Research Centre of Finland

The 2nd International Workshop

Space Science of High Quality Protein Crystallization Technology

【Date】20th October 2016（Thursday）12：00－17：10

【Venue】Yayoi Auditorium Annex, 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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Program

		Title	Presenter
	12:00 13:00		Lunch
1	13:00 13:30	High-Precision X-ray Crystallography of Proteins	Nakagawa Atsushi Professor Osaka University
2	13:30 14:00	Rational protein crystallization by controlling concentrations of main precipitant and counterion	Sachiko Takahashi Senior Engineer Confocal Science Inc.
3	14:00 14:30	How does crystal growth mechanism in space differ from that on ground?	Katsuo Tsukamoto Project Professor Graduate School of Engineering, Osaka University / Graduate School of Science ,Tohoku University
	14:30 14:50		**Coffee** break
4	14:50 15:20	Orphan drug development for Duchenne muscular	Yoshiiro Urade Professor/PI International Institute for Integrative Sleep medicine, University of Tsukuba

		dystrophy by protein crystallization in space	
5	15:20 15:50	Membrane protein crystallography using free electron laser	So Iwata Professor Department of Cell Biology, Graduate School of Medicine, Kyoto University Group Director SACLA Science Research Group Riken SPring-8 Center
6	15:50 16:20	Enzymes for the utilization of cellulosic biomass	Kiyohiko Igarashi Associate Professor Department of Biomaterial Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo
	16:20 17:00	Discussion	
	17:00 17:10	Closing	Yoshihiro Urade Professor/PI International Institute for Integrative Sleep Medicine, University of Tsukuba
	17:30		

*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

Atsushi Nakagawa

Institute for Protein Research, Osaka University

abstract

Recent development on methodologies and technologies of protein crystallography advances the structure determination of biological macromolecules, such as proteins. Number of protein structures deposited to Protein Data Bank (PDB) increasing exponentially in this couple of decades, and it is more than 120,000 in the end of August 2016. However, this number is still not enough compare to the genome data. The bottleneck of protein structure determination by X-ray crystallography is mainly caused by difficulty in crystallization. It sometimes requires tremendous effort and time to obtain good quality crystals for structure determination at atomic level. It is important to know the atomic structures of water molecules or hydrogens to understand the molecular mechanism of the protein molecules. However, only limited numbers of high-resolution structures, which give atomic information of hydrogens, 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. After the project, we joined several experiments of JAXA High-quality Protein Crystal Growth (JAXA PCG) project. I will present the technical developments of this project and further progress up to now.

Sachiko Takahashi

Senior Engineer at Confocal Science Inc

Biography

Ms. Sachiko Takahashi is a Senior Engineer at Confocal Science Inc. since 2004. Prior to that, she was a research fellow at the University of North Carolina and at Roche Research Center in Japan. She holds an MS in Agricultural Chemistry from the University of Tokyo. She has over 15 years of technical and operational experience in the JAXA protein crystallization mission, since the beginning of JAXA PCG project.

Rational protein crystallization by controlling concentrations of main precipitant and counterion

Abstract

Crystallization of protein is closely related to the neutralization of its charge with counterions to produce electroneutrality[1]. In the previous study, we showed the importance of counterions (sodium chloride) in the main precipitant (polyethylene glycol 4000)-based crystallization solution by using various concentration of counterion[2]. Protein crystals grew when the concentration of counterions and the charge density of the protein molecule were close. However, the relation between the main precipitant and the couterions was not enough demonstrated. Therefore, we crystallized protein (5-25mg/ml) in the various concentration of PEG 4000 (0-25%) and sodium chloride (0.1-1.0M) by batch method, and observed crystallization and checked crystal size and quality. Based on the results, we propose a rational protein crystallization strategy to grow high-quality and/or large crystals.

1. K. D. Collins, Methods, 34, (2004), 300.

2. M. Yamanaka, K. Inaka, N. Furubayashi, M. Matsushima, S. Takahashi, H. Tanaka, S. Sano, M. Sato, T. Kobayashi, T. Tanaka, J. Synchrotron Rad., 18, (2011), 84

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.

How does crystal growth mechanism in space differ from that on ground?

Abstract

To measure the growth rate vs supersaturation of protein solution is the key to determine the growth mechanism of the crystals. Since the growth rate of protein crystals is in the order of 10^{-2}-10^{-1} nm/s, we need precise and fast measuring methods of the growth rate if we want to measure in in space environment. We successfully utilized laser interferometry for the first time to measure the growth rate on $Ba(NO_3)_2$ in a sounding rocket in 2000 and thus we employed the same method to measure the growth rate of lysozyme crystals at ISS.

There has been an idea behind space experiments on the crystallization of protein crystals to improve the quality of the crystals. This is based on the idea that in microgravity the crystallization process is governed by diffusion of protein molecules in the fluid and thus, if there is no flow and convection, the growth rate would be much smaller that that in gravity. However this potentially accepted concept was found not to be true because growth process of lysozyme crystals is controlled by surface kinetics and not by diffusion.

We measured the growth rate *vs* supersaturation of a protein solution by laser interferometry with the same or even more accurate way at the International Space Station (ISS). It was surprising to find that the growth rate of crystals is larger at most of the supersaturated regime in space.

This rate result was analyzed based on spiral growth and 2D nucleation growth models to conclude the importance of dimer molecules as an impurity for the crystal growth and dislocation configuration. These growth mechanisms are closely related to the perfection of the protein crystals.

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 hematopoietic prostaglandin (PG) D_2 synthase (H-PGDS) was induced in the skeletal muscle with grouped necrotic muscle fibers in patients with DMD to aggravate muscular inflammation by producing a potent inflammatory mediator, PGD_2. We obtained high quality crystal of human recombine

ant H-PGDS in complexes with inhibitors, by the counter-diffusion method under a microgravity condition within the International Space Station (see http://www.nasa.gov/mission_pages/station/research/news/crystals). We determined the detailed three-dimensional structures of H-PGDS/inhibitor complexes by X-ray diffraction analysis of the space-grown crystals using an intense X-ray at SPring-8 synchrotron facility, Harima, Japan. Based on the fine structure of the inhibitor within the catalytic pocket of human H-PGDS, novel potent inhibitors TFC-007, TAS-204 and TAS-205 were developed, whose IC_{50} value was 20-80 nM. Those compounds markedly prevented the expansion of muscular necrosis and muscle atrophy without any side effects by chronic treatment of dystrophin-deficient *mdx* mice and DMD beagle dogs. Clinical trials of TAS-205 for treating DMD patients have begun sponsored by Taiho Pharmaceutical Co. Ltd. at National Center of Neurology and Psychiatry in Japan from Sept in 2014. Phase 1 study of single and multiple doses of TAS-205 in 21 patients was successfully finished to confirm the safety of this drug (see the entry in clinicaltrials.gov, NCT02246478). This is a real milestone to establish drug therapy for DMD patients. We believe that TAS-205 is able to slow down the progression of DMD boys. The fine structure of the drug-binding pocket of human H-PGDS is useful to theoretically and inexpensively develop follow-up compounds, whose chemical structures and metabolism are different from TAS-205.

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

Membrane protein crystallography using free electron laser

Abstract

At the Japanese XFEL facility, SACLA, we are currently developing a data collection system focusing on drug-target protein crystals including those from membrane proteins. X-ray free electron laser could provide a solution to the radiation damage problem, which is one of the most hampering problems in the current macromolecular crystallography. Very high dose rates delivered by the intense femtosecond pulses of XFELs reduce the amount of damage suffered by a crystal during its irradiation. Single shot diffraction patterns are collected from a series of small crystals and by combining them, we could swiftly complete the dataset without any serious radiation damage. The system is composed of a diffraction chamber with a sample injector and a fast readout multiport CCD (mpCCD) detector. The sample injector is optimized for the data collection from crystals in the lipidic cubic phase (LCP), which are common for membrane proteins. The system requires only several 100 micrograms of proteins to complete the dataset. The system can dramatically accelerate the structure determination of membrane proteins. The system is also suitable for time-resolved crystallography including visualization of ultrafast protein structural dynamics on the femtosecond to picosecond time-scale. For the time resolved studies, it is essential to obtain well diffracting crystals. In my talk, I will present recent development of membrane protein crystallization for XFEL studies 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
VTT Technical Research Centre of Finland
E-mail: aquarius@mail.ecc.u-tokyo.ac.jp

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-)

Enzymes for the utilization of cellulosic biomass

Abstract

Cellulose and chitin are major components of plant cell wall and exoskeleton in animals, respectively. They are the most abundant biomass on earth, and efficient degradation of the biomass makes it possible to produce fuels and chemicals from the resources. However, biochemical conversion of the structural polysaccharides, cellulose and chitin, by cellulase and chitinase is a bottleneck of the process, mainly because they form recalcitrant crystalline region in these biomass. The enzymes degrading the crystalline polysaccharides share a common two-domain structure, carbohydrate-binding module and catalytic domain, and these domains cooperatively function for the effective hydrolysis of the substrates. Since the reaction is carried out at the surface of insoluble substrate, it is not straightforward to analyze the reaction at a solid/liquid interface.

We have recently reported the real-time visualization of crystalline polysaccharides degradation by individual cellulase or chitinase molecules using a high-speed atomic force microscopy, having sub-second time resolution and nanometer space resolution. From the comparison among fungal (*Trichoderma reesei*[1-3] and *Phanerochaete chrysosporium*[4]) cellobiohydrolases and *Serratia marcescens* chitinases[5], we discuss possible molecular mechanisms of these processive enzymes and the natural degradation of crystalline structural polysaccharides.

We also employed a neutron diffraction analysis to investigate the catalytic mechanism of the inverting glycosdide hydrolase (GH) family 45 cellulase *Pc*Cel45A, 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 *Pc*Cel45A lacks putative general base and assisting acidic residues while it has an apparent activity towards cellulose and β-1,3-1,4-glucan[6]. To understand the catalytic mechanism of *Pc*Cel45A, we made a large crystal of 6 mm3 volume (3 mm x 2 mm x 1 mm) for the neutron protein structural study[7]. 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[8].

References

1. Igarashi, K., et al, ***J. Biol. Chem.*** 284: 36186-36190 (2009)
2. Igarashi, K., et al, ***Science*** 333: 1279-1282 (2011)
3. Nakamura, A., et al, ***J. Biol. Chem.*** 288: 13503-13510 (2013)
4. Nakamura, A., et al, ***J. Amer. Chem. Soc.*** 136: 4584–4592 (2014)
5. Igarashi, K., et al, ***Nature Commun.*** 5: 3975 (2014)
6. Igarashi, K., et al, ***Appl. Environ. Microbiol.*** 74:5628-5634 (2008)
7. Nakamura, A., et al, ***J. Sync. Rad.*** 20: 859-863 (2013)
8. Nakamura, A., et al, ***Science Adv.*** 1: e1500263 (2015)