IPR

Prospectus 2020

Institute for Protein Research
Osaka University

CONTENTS

Message from the Director

Message FROM THE DIRECTOR

From the very beginning of its foundation in 1958, researchers in the fields of chemistry, biology, physics, and medicine gathered at IPR to pursue interdisciplinary studies of proteins. Since that time, the IPR has been at the cutting edge of new developments in protein science aimed at achieving an understanding of the mechanism of living systems – from the molecular to the organism level.

The IPR started with only three divisions (laboratories): Organic Chemistry, Physical Chemistry, and Protein Metabolism. In 2020, we established a new division, Protein Network Biology, and a new research center, Research Center for Next-Generation Protein Sciences.

After more than sixty years of steady development, the IPR now consists of five divisions (19 laboratories) with an attached center (6 laboratories).

In April 2010, the IPR was designated as one of the Joint Usage/Research Centers in Japan by the Ministry of Education, Culture, Sports, Science and Technology (MEXT). In regard to its role as a Joint Usage/Research Center, the IPR offers three collaborative opportunities to help advance joint research activities in the protein science field. The IPR also manages access to a range of high performance equipment, such as the“Synchrotron Beamline at SPring-8”, the “High Magnetic Field Nuclear Magnetic Resonance (NMR) spectrometers” and the “Advanced Cryo-Electron Microscopy Suite”, all of which are open to protein science researchers both in Japan and overseas. In addition, as one of four members of the wwPDB (worldwide Protein Data Bank), the IPR operates the PDBj (Protein Data Bank Japan). The PDBj-BMRB also constructs and manages an NMR experimental database, in collaboration with the BMRB (BioMagResBank) in the USA. The IPR has also extensively organized a large number of diverse international collaborative research projects with overseas protein scientists and institutions under the auspices of the “International Collaboration Research Program”. In addition to these activities, IPR seminars (Tanpakuken Seminar) provide a place to build a research community and exchange scientific information.

A total of more than 60 academic staff at the IPR work very hard for their own scientific research, as well as for their educational activities directed towards teaching Osaka University students at the undergraduate level (placed within the Faculties of Science, Medicine, and Engineering) and Master’s course and Doctor’s course students (placed within the Graduate Schools of Science, Medicine, Frontier Biosciences, and Engineering). From those faculties and graduate schools, about 120 students study in the laboratories at IPR. In addition to this student cohort, about 30 postdoctoral fellows make their own original investigations in support of various national and international funded research project. We regard these young scientists, who come to the IPR from countries all over the world, as important human resources of the IPR and they are expected to lead new protein science fields in the future.

Recently, the life science field has made dramatic developments. We are now on the cusp of developing a new protein science field that is capable of revealing the secrets of higher-order living systems through investigation of the interactions occurring between networks of biological molecules. These functional studies are facilitated by knowledge of the precise atomic structures of biological molecules. To actualize this future potential, we aim to develop and combine a large array of different techniques. With this IPR research cohort, we are in position to strive towards the development of this new type of protein science – one that represents a future approach to the life sciences based on the eradication of borders between laboratories, scientific fields, facilities and indeed countries, and most importantly, one that is capable of developing both the scientific breakthroughs and the important human resources of the next generation.

I hope that the IPR Prospectus 2020 well demonstrates our research and activities, as well as our continued efforts for development .

Atsushi Nakagawa, D.Sci., Director

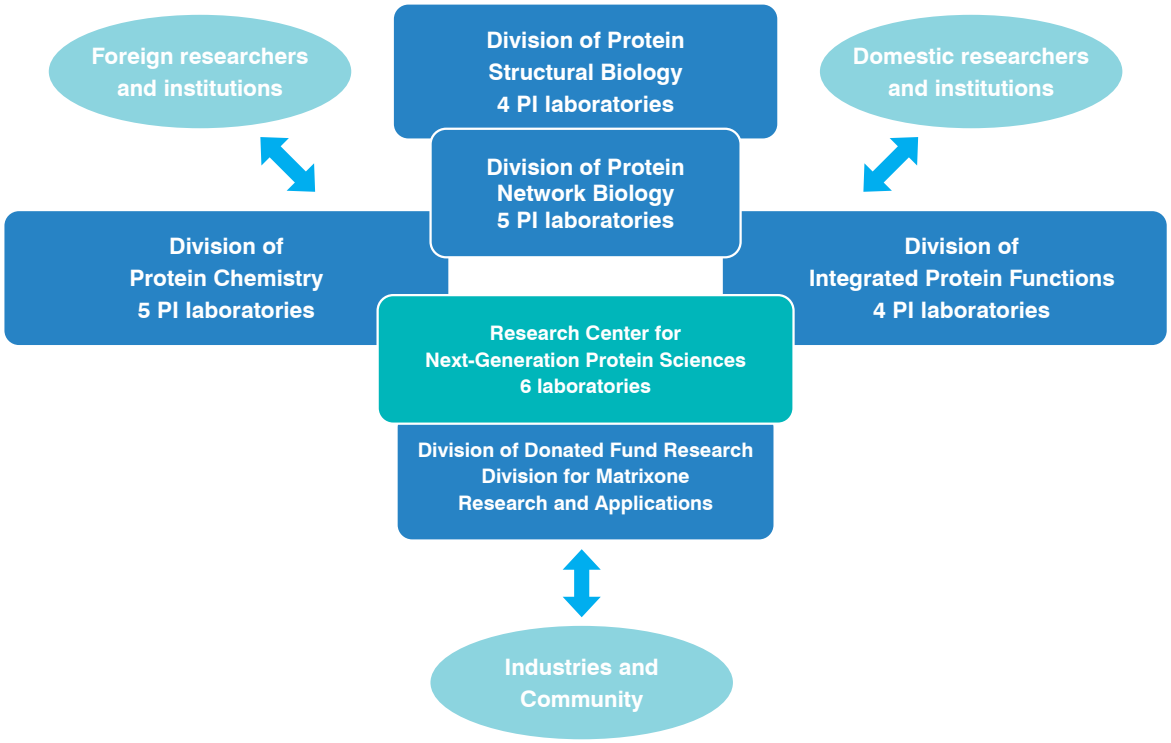
Concept and Future

The processes of life, mediated by a large variety of proteins, are very complex. Elucidation of these processes through molecular studies of proteins can be achieved by efficient cooperation of researchers in various fields of natural sciences. Another requisite is the establishment of suitable infrastructure that allows close collaborations among scientists across the country. The Institute for Protein Research (IPR) was founded as a joint-use research organization attached to Osaka University to fulfill these needs, and thus to play a central role in protein science in Japan. Now, IPR works as one of the Joint Usage/Research Centers authorized by Japanese Government.

Thus, the mission of IPR has not changed during its long history, and it is summarized as the following three concepts:

1) Basic studies are performed on the structure and function of proteins and their biological significance at the molecular level, as well as at the cellular level with tight collaborations by researchers in IPR having various backgrounds such as chemistry, physics, biology and medicine,.
2) IPR provides the resources for research, databases such as PDB, and scientific communication for domestic and international collaborative studies as the Joint Usage/Research Center, thereby promoting protein science for the scientific community and society.
3) IPR will educate undergraduate/graduate students, Ph. D. students, and young researchers not only in Osaka University but also other universities including overseas using the framework of the joint-use/research center.

Based on the above mission, IPR has so far initiated various projects on structural biology, neurobiology, proteomics, and molecular and cellular biology, and produced many excellent results. Although IPR started as a domestic center, it is now widely recognized as an international center of excellence for protein research. For instance, IPR operates the Worldwide Protein Data Bank (wwPDB) as one of four worldwide centers, mainly covering the Asia region. Because of its high standard of scientific activity, it has attracted many researchers from abroad and will continue to do so in the future. In April 2016, a new program started to aim for the international core for the multiscale integrative structural life science. IPR also invites researchers in the new research fields such as systems biology and single-molecule analysis. In addition, two new divisions of donated fund research also started their activities with the associated industries in 2016. The IPR will continue to make essential contributions to revealing the structure and function of proteins and their networks for the elucidation of life.



Organization

Director

- Faculty Meeting
- Administrative Council
- Panel on Joint Usage/Research
- Research Division
  - **Division of Protein Chemistry**
    - Laboratory for Protein Organic Chemistry
    - Laboratory for Nanobiology
    - Laboratory for Protein Synthesis and Expression
    - Laboratory for Protein Profiling and Functional Proteomics
    - Laboratory for Membrane Protein Chemistry
  - **Division of Protein Structural Biology**
    - Laboratory for Molecular Biophysics
    - Laboratory for Protein Crystallography
    - Laboratory for CryoEM Structural Biology
    - Laboratory for Supramolecular Crystallography
  - **Division of Integrated Protein Functions**
    - Laboratory for Molecular and Developmental Biology
    - Laboratory for Genome and Chromosome Functions
    - Laboratory for Advanced Brain Functions
    - Laboratory for Organelle Biology
  - **Division of Protein Network Biology**
    - Laboratory for Cell Systems
    - Laboratory for Computational Biology
    - Laboratory for Nuclear Dynamics
    - Laboratory for Infection Systems
    - Laboratory of Visiting Scientists
- Special Research Facilities
  - **Research Center for Next-Generation Protein Sciences**
    - Protein Data Bank Japan
    - Laboratory for Ultra-High Magnetic Field NMR Spectroscopy
    - Laboratory for Synchrotron Radiation Research
    - Laboratory for High Resolution Cryo-EM
    - Laboratory for Biomolecular Analysis
    - Open Space Laboratory for Advanced Protein Science
- Division of Donated Fund Research
  - **Division for Matrixome Research and Application**
- Technology Division
- Administration
  - General Affairs Section
  - Accounting Section
  - Project Team of Joint Usage / Research Center
  - Research Support Section
- Public Relations Office
- Library
- IPR Alumni Office

Renewal

IPR WEBSITE

http://www.protein.osaka-u.ac.jp/en/

Division of Protein Chemistry

- Laboratory for Protein Organic Chemistry
- Laboratory for Nanobiology
- Laboratory for Protein Synthesis and Expression
- Laboratory for Protein Profiling and Functional Proteomics
- Laboratory for Membrane Protein Chemistry

Laboratory for Protein Organic Chemistry

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Assistant Professor Yuya Asahina, Ph.D.

Study on the role of post-translational modifications through chemical synthesis

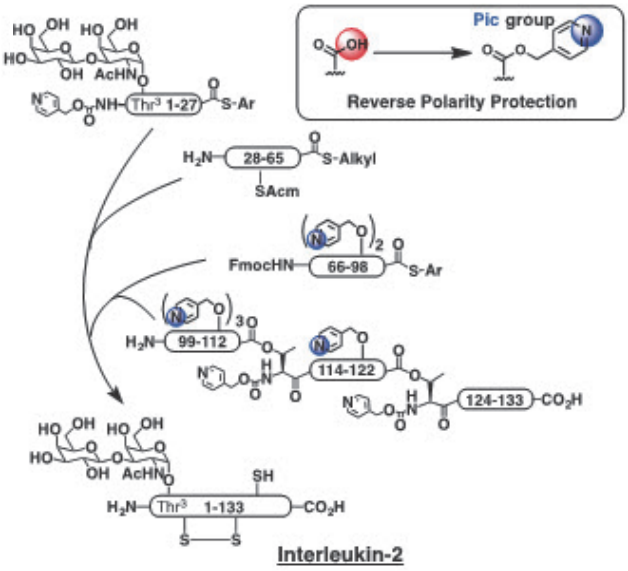
Chemical synthesis enables the preparation of proteins, which are difficult to prepare by the recombinant method, such as site-specifically labeled, glycosylated and phosphorylated proteins. The Laboratory for Protein Organic Chemistry is aiming to promote new protein researches using these synthetic proteins. Thus, our laboratory is developing facile methods for protein synthesis based on ligation chemistries. At present, we are aiming to overcome a solubility problem, which we often face during the ligation reaction. The problem is particularly serious for the synthesis of membrane proteins. To overcome this problem, we are synthesizing membrane proteins and their partial sequences to elucidate the signal transduction mechanism by solid state NMR and IR. Modified histones and their partial sequences, glycosylated proteins are also being synthesized for functional analyses.

Research Programs

1) General studies on a chemical protein synthesis
2) Synthetic studies of modified histones, and elucidation of the role of modification
3) Synthesis, structural and functional studies of membrane proteins
4) Synthesis and functional study of glycoproteins

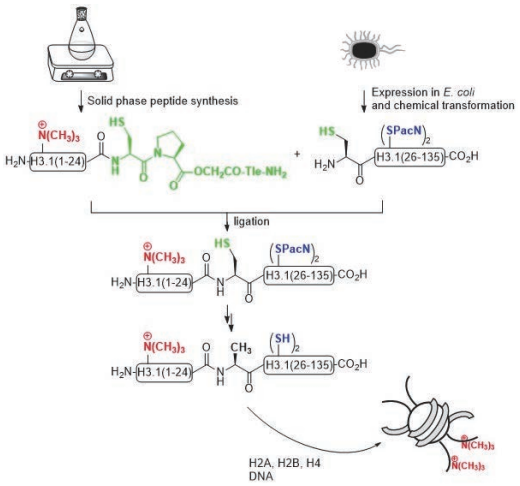
Research Topics

♦Ligation chemistries have been widely used for the synthesis of (glyco)proteins for their functional and structural studies. However, we often face a serious solubility problem during synthesis by ligation methods, even in the case of globular proteins. The same problem also occurs during the synthesis of highly hydrophobic proteins, such as membrane proteins, which are important drug targets. To overcome this problem, we examined the modification of the structure of polypeptides during the synthesis. In the case of human interleukin-2, for example, the introduction of basic picolyl ester was introduced to the side chain carboxy group of Glu residue, which reverses the polarity from negative to positive. This reverse-polarity protection efficiently increases the isoelectric point of the segment and increases its solubility, which leads to the successful synthesis of fully active interleukin-2. This method can be generally used for the synthesis of hydrophobic (glyco) proteins and further studies will be made along this line.

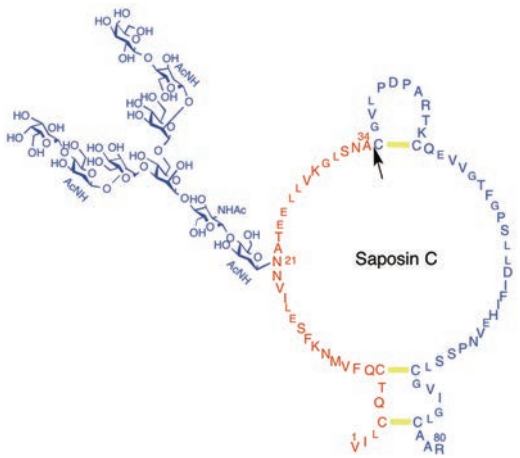


Solubilization of peptide segment by the reverse polarity protection and its application to the synthesis of human interleukin-2.

♦The post-translational modification of histones plays an important role in the epigenetic regulation of gene expression. A specifically modified histone library is extremely useful for functional studies of these molecules. Therefore, we developed methods for the synthesis of modified histones. In the figure, for an example, a method is shown for synthesizing such modified histones by ligating chemically prepared N-terminal peptides, containing specific modifications, and C-terminal recombinant peptide building blocks, in which a peptide containing a Cys-Pro ester (CPE) sequence is used as a thioester precursor. The resulting proteins could then be used to prepare a nucleosome library containing post-translational modifications.



♦Glycosylation is one of the major post-translational modifications of proteins. Recently, it has become known that glycan chains on proteins are engaged in various biological processes, such as cell differentiation, cell attachment and viral infection. However, the details of the processes are not fully understood. The major problem is that the structure of the glycan chain is highly heterogeneous, which makes the relationship between structure and function vague. To overcome this problem, we have been engaged in establishing a facile method for glycoprotein synthesis, which enables to prepare glycoproteins having homogeneous carbohydrate structures. The method has been applied to the synthesis of glycoproteins, such as saposin C, immunoglobulin domain of TIM-3 and human interleukin-2, and functional studies are being conducted.



Publications

1. Glycopeptide Synthesis Based on a TFA-Labile Protection Strategy and One-Pot Four-Segment Ligation for the Synthesis of O-Glycosylated Histone H2A, Asahina Y, Kawakami T, Hojo H (2019) *Eur. J. Org. Chem.*, **2019**, 1915-1920.
2. One-Pot Four-Segment Ligation using Seleno- and Thioesters: Synthesis of Superoxide Dismutase, Takei T, Andoh T, Takao T, Hojo H (2017) *Ang. Chem. Int. Ed.*, **56**, 15708 –15711.
3. Preparation of Selenoinsulin as a Long-Lasting Insulin Analogue. Arai K, Takei T, Okumura M, Watanabe S, Amagai Y, Asahina Y, Moroder L, Hojo H, Inaba K, Iwaoka M (2017) *Ang. Chem. Int. Ed.*, **56**, 5522-5526.
4. Structure of the Dnmt1 Reader Module Complexed with a Unique Two-Mono-Ubiquitin Mark on Histone H3 Reveals the Basis for DNA Methylation Maintenance, Ishiyama S, Nishiyama A, Saeki Y, Moritsugu K, Morimoto D, Yamaguchi L, Arai N, Matsumura R, Kawakami T, Mishima Y, Hojo H, Shimamura S, Ishikawa F, Tajima S, Tanaka K, Ariyoshi M, Shirakawa M, Ikeguchi M, Kidera A, Suetake I, Arita K, Nakanishi M (2017) *Molecular Cell*, **68**, 350–360.
5. Chemical Synthesis of O-Glycosylated Human Interleukin-2 by the Reverse Polarity Protection Strategy. Asahina Y, Komiya S, Ohagi A, Fujimoto R, Tamagaki H, Nakagawa K, Sato T, Akira S, Takao T, Ishii A, Nakahara Y, Hojo H (2015) *Ang. Chem. Int. Ed.*, **54**, 8226-8230.
6. Chemoenzymatic Synthesis of Immunoglobulin Domain of Tim-3 Carrying the Complex Type N-Glycan Using the One-pot Ligation Method. Asahina Y, Kamitori S, Takao T, Nishi N, Hojo H (2013) *Ang. Chem. Int. Ed.*, **52,** 9733-9737.

Laboratory for Nanobiology

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Associate Professor (Lecturer) Madoka Suzuki, Ph.D.
Assistant Professor Shingo Sotoma, Ph.D.

Single-molecule and single-cell physiologies using optical microscopy

Biomolecules are constantly perturbed by the environment surrounded. In contrast to the artificial large machines, biomolecules are considered to harness thermal fluctuations to increase energy efficiency. For example, without using chemical energy, or ATP, RNA polymerases are shown to diffuse one-dimensionally on DNA to find promoter sequences. One of our main goals is to understand the sophisticated mechanism of biomolecules. To this end, we have developed novel technologies to image and manipulate individual molecules under the optical microscope. We study motions and structural changes of biomolecules at the single molecule level. Furthermore, the activities of biomolecules can result in various types of cellular functions. Among those, we focus on heat release events by developing novel tools to measure temperature changes in individual cells.

Research Projects

1) Developing novel single-molecule imaging techniques using fluorescent nano-diamond
2) Developing techniques for local temperature measurement in *in vitro* and *in vivo*

Research Topics

1) Nano-diamonds

Conventional fluorescent probes have intrinsic problems such as bleaching and blinking properties, overlapping of emission spectrum with auto-fluorescence of biological samples, and difficulties in determining angular information of the molecule labelled. To overcome these issues, we have focused on the unique properties of fluorescent nano-diamonds (FNDs). Upon excitation with 560 nm light, Nitrogen-Vacancy Centers (NVCs) in the diamond crystals emit fluorescence light. The fluorescence from NVC show no photo-bleaching or -blinking, so prolonged observation is possible in biological samples. Furthermore, using an optically detected magnetic resonance (ODMR) technique, we have successfully developed a method to separate the fluorescence signal of FNDs from the auto-fluorescence in the *C. elegans*. We are also developing methods to detect rotational motions with high sensitivity using FNDs.

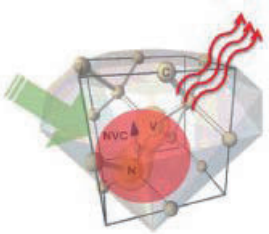
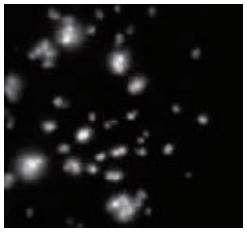


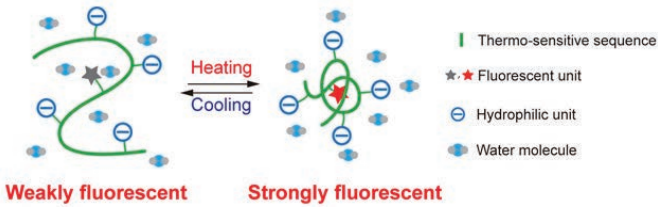
Illustration of fluorescent nano-diamond



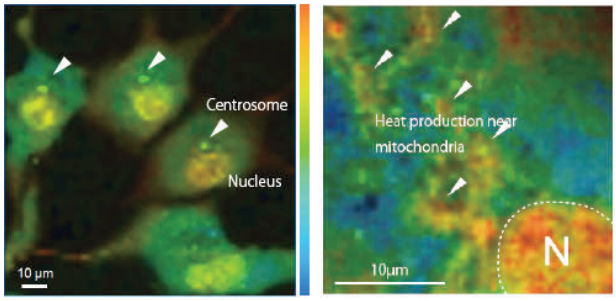
Fluorescence image of FNDs

2) Establishing ”Thermal-biology”

The temperature is a fundamental parameter in biology. Temperature affects metabolism and biorhythm. However, temperature fluctuations in space and time in individual cells has been largely unknown. We have developed methods to quantitatively image the temperature using the fluorescence microscopy and fluorescent ”nanothermometers” that are based on fluorescent polymers, FNDs, fluorescent nanoparticles and fluorescent molecules. In this research topic, we are studying the biological relevance of the thermal fluctuation that have been found in previous studies.



Linear polymeric thermometer

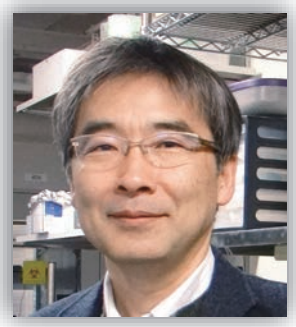


Imaging of intracellular temperature

Publications

1. Multifunctional temozolomide-loaded lipid superparamagnetic nanovectors: Dual targeting and disintegration of glioblastoma spheroids by synergic chemotherapy and hyperthermia treatment. Marino, A. et al. *Nanoscale* **11**, 21227-21248 (2019).
2. Polydopamine coating as a scaffold for ring-opening chemistry to functionalize gold nanoparticles. Sotoma, S. and Harada, Y. *Langmuir* **35**, 8357-8362 (2019).
3. HCV IRES captures an actively translating 80S ribosome. Yokoyama, T. et al. *Mol. Cell.* **74**(6), 1205-1214 (2019).
4. Bright dots and smart optical microscopy to probe intracellular events in single cells. Fujita, H. et al. *Front. Bioeng. Biotechnol.* **6**, 204 (2019).
5. 褐色脂肪細胞の温度変化をイメージングしたいのはなぜ？　鈴木団、原田慶恵 *生物物理* **58**(2), 97-99 (2018).
6. Fluorescent nanodiamonds as a robust temperature sensor inside a single cell. Sekiguchi, T. et al. *Biphys. Physicobiol.* **15**, 229-234 (2018).
7. Construction of integrated gene logic-chip. Masubuchi, T. et al. *Nature Nanotech.* **13**(10), 933-340 (2018).
8. Enrichment of ODMR-active nitrogen-vacancy centres in five-nanometre-sized detonation-synthesized nanodiamonds: Nanoprobes for temperature, angle and position. Sotoma, S. et al. *Sci. Rep.* **8** (1), 5463-3802 (2018).
9. Ca^{2+}-associated triphasic pH changes in mitochondria during brown adipocyte activation. Hou, Y. et al. *Mol. Metabolism* **6**(8), 797-808 (2017).
10. Gold Nanoshell-Mediated Remote Myotube Activation. Marino, A. et al. *ACS Nano* **11**(3), 2494-2508 (2017).
11. A Beetle Flight Muscle Displays Leg Muscle Microstructure. Shimomura, T. et al. *Biophysical journal* **111**(6), 1295-1303 (2016).
12. Single-Molecule Analysis of the Target Cleavage Reaction by the Drosophila RNAi Enzyme Complex. Yao, C. et al. *Mol. Cell* **59**, 125-132 (2015).
13. Synergistic effect of ATP for RuvA-RuvB-Holliday junction DNA complex formation. Iwasa, T. et al. *Sci Rep.* **5**, 18177 (2015).
14. Real-time background-free selective imaging of fluorescent nanodiamonds in vivo. Igarashi, R. et al. *Nano Lett.* **12,** 5726-5732 (2012).
15. Intracellular temperature mapping with a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy. Okabe, K. et al. *Nat. Commun.* **3**, 705 (2012).

Laboratory for Protein Synthesis and Expression



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Assistant Professor Yu Kitago, Ph.D.
Spec. Appoint. Assist. Prof. Takao Arimori, Ph.D.

Elucidation of the structural mechanism of transmembrane signaling and development of protein therapeutics

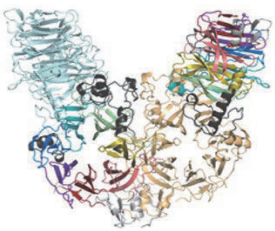
”How do things work?” - This is the question most, if not all, scientists are eager to answer. Our passion is to unravel the mechanism of function of proteins in a living organism where they work with remarkable precision as small ”molecular machines”. This lab can be described as a ”structural biology lab”, but our goal is not the determination of the three-dimensional structure of proteins. Rather, we FIRST aim to solve the structure, THEN perform biochemical, biophysical, and cell biological experiments to draw novel pictures about the molecular mechanism of proteins, taking advantage of the structural information that is exclusive to us. We are mostly focused on the molecular interactions between cell surface receptors and their extracellular ligands implicated in signal transduction in a wide variety of biological contexts, ranging from development, neurobiology, and immunity. Our study focuses on questions such as; how receptors recognize their specific ligands, how this recognition leads to structural change in the receptor complex, and how the information crosses the plasma membrane without transporting a chemical entity.
Our approach is multi-faceted. As the methodology for structural analysis, we utilize X-ray crystallography, which determines 3D structure of proteins at atomic resolution, and transmission electron microscopy (TEM), which can derive the structure of protein complexes too large for XRD or visualize the shape of proteins in their true biological environment (e.g., within cells). The latter expertise includes cutting-edge technologies such as cryoelectron microscopy and electron tomography. In order to back these structural efforts, we have also developed an array of in-house technologies critical for the production of high-quality recombinant proteins using mammalian cell expression system.

Research Programs

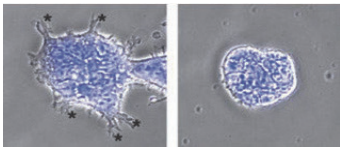
1) Structure and function of extracellular ligands and their receptors implicated in cell adhesion and neural guidance/morphogenesis
2) Structure-guided molecular design of novel proteins
3) Development of high-quality recombinant protein production system
4) Development of novel design principle for non-antibody protein biologics

Research Topic

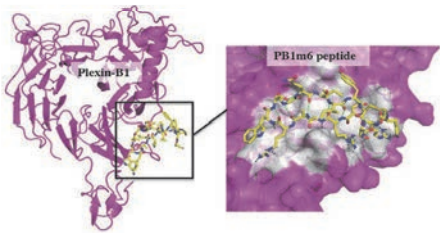
♦ Semaphorins are a class of axon guidance molecules and bind to cell surface receptor plexins. Semaphorin-plexin signaling is implicated not only in neuronal cells but also in immune cells, bone cells, and cancer cells, making it an intensive target of drug development. In 2010, we reported the first crystal structure of the semaphorin-plexin signaling complex (ref 11). Since then, we have been actively engaged in researches aimed at unraveling the mechanism of semaphorin-plexin signaling, using both crystallography and electron microscopy. In addition to the structural analyses, we try to clarify the structure-function relationship about this important signaling pathway using cell-based assays, enabling us to discover novel class of inhibitor that can potentially become a drug lead (ref 7).



Crystal structure of semaphorin- plexin complex

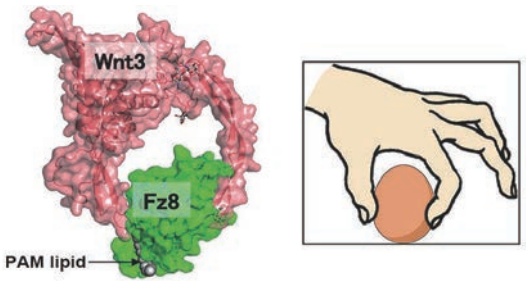


Cell morphology before (left) and after (right) stimulation by semaphorin

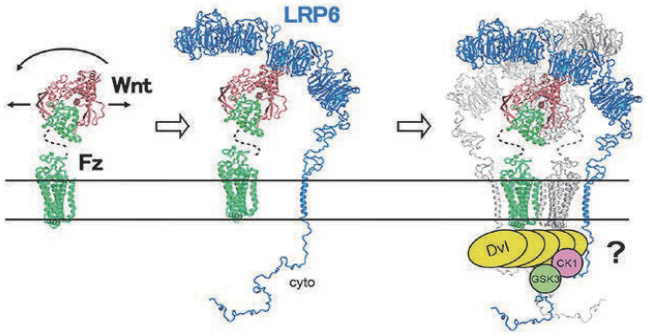


Crystal structure of plexinB1 complexed with an allosteric inhibitor peptide

♦ Wnt family includes 19 related proteins and represents one of the most extensively studied class of extracellular signaling molecules in the field of developmental biology. Wnts are also fundamentally involved in the pathogenesis of various diseases and are obligatory growth factors for virtually all kind of stem cells. Structural and biochemical characterization of Wnt proteins however has lagged behind other soluble growth factors because of their very low stability in aqueous solution due to the covalent modification by a water-insoluble lipid moiety. We applied numerous biochemical tricks to “tame” this difficult protein, including the discovery of a novel Wnt carrier protein afamin (ref 8) and succeeded in crystallizing human Wnt3 in the form of complex with its receptor Frizzled8 to yield a 2.8Å resolution structure (ref 2). Together with the structural information for a Wnt coreceptor LRP6 using EM (ref 5), we built the first realistic model of the Wnt-Frizzled-LRP6 ternary complex, which represents the molecular machinery central to the initiation of the canonical Wnt signaling at cell surface.

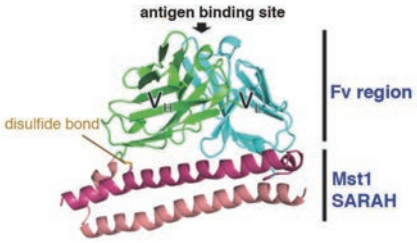


Structure of human Wnt3 in complex with Fz8



Wnt:Fz:LRP ternary complex and hypothetical signaling model

♦ Antibody fragments have been used to aid macromolecular crystallization for more than 20 years, yielding structures of numerous biologically important membrane as well as soluble proteins. Since not all high affinity antibodies exhibit ideal ”chaperoning activity”, however, large number of co-crystallization trials and errors using multiple antibodies are generally required. We developed “Fv-clasp”, a two-chain chimeric molecule composed of antibody Fv fragment and a homodimeric SARAH domain taken from human Mst1 kinase (ref 6). Fv-clasp exhibited surprisingly high heat stability and crystallizability, and lead to a determination of multiple high resolution crystal structures of biologically and medically important human proteins.



Structure of a novel small antibody format “Fv-clasp”

Publications

1. Macrocyclic peptide-based inhibition and imaging of hepatocyte growth factor. Sakai et al. (2019) *Nature Chem. Biol.,* **15**:598-606.
2. Crystal structure of mammalian Wnt–frizzled complex. Hirai et al. (2019) *Nature Struct. Mol. Biol.* **26**,372-379.
3. Structure and mechanism of cancer-associated N-acetylglucosaminyltransferase-V. Nagae et al. (2018) *Nature Commun.* **9**, 3380.
4. Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. Kang et al. (2018) *Nature Immunology*, **19**, 561-570.
5. Conformational freedom of the LRP6 ectodomain is regulated by N-glycosylation and the binding of the Wnt antagonist Dkk1. Matoba et al. (2017) *Cell Reports,* **18**:32-40.
6. Fv-clasp: An artificially designed small antibody fragment with improved production compatibility, stability, and crystallizability. Arimori et al. (2017) *Structure*, **25**:1611-1622.
7. Allosteric inhibition of a semaphorin 4D receptor plexinB1 by a high-affinity macrocyclic peptide. Matsunaga et al. (2016) *Cell Chem. Biol.* **23**:1341-1350.
8. Active and water-soluble form of lipidated Wnt protein is maintained by a serum glycoprotein afamin/ α -albumin. Mihara et al. (2016) *eLife*; **5**:e11621.
9. Structural basis for amyloidogenic peptide recognition by sorLA. Kitago et al. (2015) *Nature Struct. Mol. Biol.* **22**, 199-206.
10. Crystal structure of α 5 β 1 integrin ectodomain: Atomic details of the fibronectin receptor. Nagae et al. (2012) *J. Cell Biol.* **197,** 131-140.
11. Structural basis for semaphorin signaling through the plexin receptor. Nogi et al. (2010) *Nature* **467**, 1123-1127.

Laboratory for Protein Profiling and Functional Proteomics

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Assistant professor Toshiki Takei, Ph.D.
Spec. Appoint. Assist. Prof. Wang Qiuyi, Ph.D.

Exploring the protein world by mass spectrometry

Mass spectrometry (MS) is a well-accepted technique for the analysis of chemical structures of biological compounds. For the last four decades, we have been working to develop methods for determining primary structures and post-translational modifications of proteins using MS. In conjunction with accumulating protein and gene sequence databases, we are using state-of-the-art MS for large-scale protein identification, which is indispensable for understanding biological events. We apply the following developed methods to the structural analysis of micro quantities of peptides, proteins, and their related substances. We have found several novel post-translational modifications such as *S*-farnesyl moiety at the *C*-terminal Cys, heterogeneous fatty acids at the *N*-terminal Gly, ε-(γ-glutamyl) lysine in core histones, phosphatidylethanolamine linked to the *C*-terminal Gly, *O*-palmitoleoyl moiety at Ser, etc.

Research Programs

1) Development of chemical/analytical methods and software for analysis of protein primary structure by mass spectrometry
2) Mass spectrometric analysis of post-translational modifications
3) Development of chemical and analytical methods for proteomics
4) Study on fragmentation of peptides and carbohydrates in mass spectrometry
5) Hardware development for high-sensitivity and high-accuracy mass spectrometry

Research Topics

♦ The Wnt family of secreted signal proteins plays a key role in numerous aspects of embryogenesis, as well as in carcinogenesis. In collaboration with Prof. S. Takada (Okazaki Institute for Integrative Biosciences), we found that murine Wnt-3a is acylated at a conserved serine residue (Ser209) with palmitoleic acid (Δ9-C16:1), which was essential for Wnt secretion. The lipid was not as saturated as originally thought. Furthermore, in collaboration with Prof. A. Kikuchi (Graduate School of Medicine, Osaka University), we determined multiple *N*-glycosylation types for several Wnt-family proteins, which could regulate the direction of Wnt secretion in polarized epithelial cells.

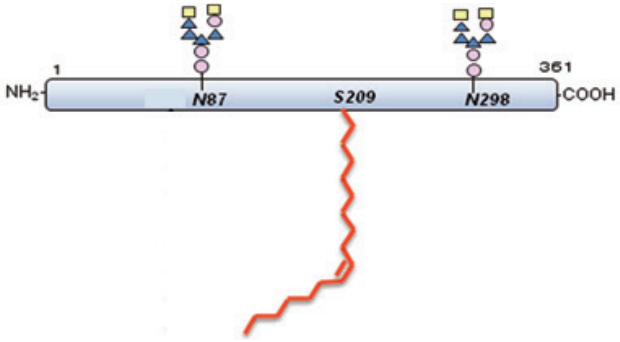

Fig.1. Novel fatty acid modification (palmitoleoyl Modification at Ser209) of Wnt protein essential for Wnt secretion (Dev. Cell 2006).

♦ Proteins often suffer from various chemical modifications *in vivo*. β2-microglobulin (β2m), a subunit of MHC class I, circulates as a free form in blood. Since β2m has Asn-Gly sequences at two sites in the molecule which are amenable to α ↔ β rearrangement, we investigated their stability in solution. As a result, deamidation/isomerization at Asn17 and Asn42 with half-lives of 33 and 347 days occurred, respectively. The rearrangement at these sites caused some structural change of β2m. In fact, the modified β2m showed higher affinity to Cu^{2+} ion, which was probed by ESI-MS.

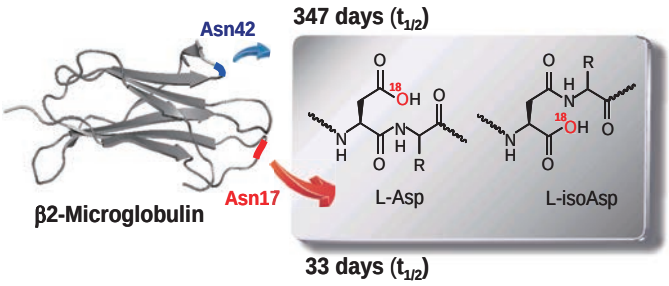

Fig. 2 Quantitative Analysis of Deamidation and Isomerization in β2-Microglobulin by ^{18}O Labeling (Anal. Chem. 2012).

♦ In collaboration with Prof. Y. Ohsumi (National Institute for Basic Biology; currently, Honorary Professor of Tokyo Institute of Technology), we found a novel lipid modification at the *C* terminus of Apg8 with a phospholipid, phosphatidylethanolamine (PE). This novel lipidation was essential for the autophagosome formation in autophagy.

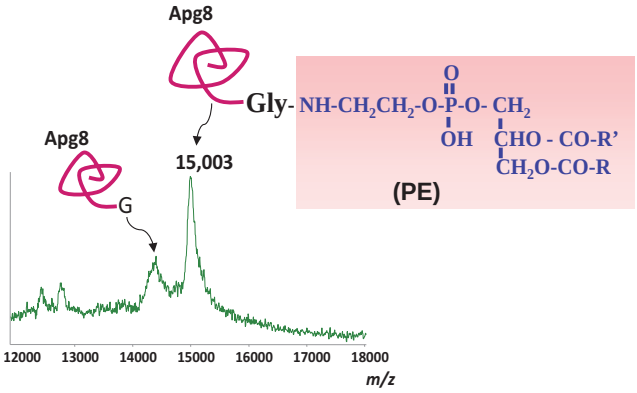

Fig. 3 Ubiquitination-like system mediates novel protein lipidation. (Nature 2000).

Publications

1. Metastable Decomposition at the Peptide *C*-Terminus. -Possible Use in Protein Identification-. Wang Y, Nakajima E, Okamura Y, Wang D, Okumura N, Takao T. (2020) *Rapid Commun. Mass Spectrom.* **34**(9):e8734.
2. Lithium ion adduction enables UPLC-MS/MS based analysis of multi-class, 3-hydroxyl group containing keto-steroids2. Wang Q, Shimizu K, Maehata K, Pan Y, Sakurai K, Hikida T, Fukada Y, Takao T. (2020) *Journal of Lipid Research,* **61**(4):570-579.
3. Free Thiol of Transthyretin in Human Plasma Most Accessible to Modification/Oxidation. Pereira CD, Minamino N, Takao T. (2015) *Anal Chem.* **87**(21), 10785-91.
4. Functional Link between Atg32-mediated Mitophagy and Phospholipid Methylation. Sakakibara K, Eiyama A, Suzuki SW, Sakoh-Nakatogawa M, Okumura N, Tani M, Hashimoto A, Kondo-Okamoto N, Kondo-Kakuta C, Asai E, Kirisako H, Nakatogawa H, Kuge O, Takao T, Ohsumi Y, Okamoto K (2015) *EMBO J*, **34**(21), 2703-19.
5. Quantitative Analysis of Deamidation and Isomerization in β2-Microglobulin by ^{18}O Labeling. Fukuda M, Takao T (2012) *Anal. Chem.* **84**, 10388-10394.
6. Affinity-Trap Polyacrylamide Gel Electrophoresis: A Novel Method of Capturing Specific Proteins by Electro-Transfer. Awada, C., Sato, T., Takao, T. (2010) *Anal. Chem.* **82**, 755–761.
7. Mono-unsaturated Fatty Acid Modification of Wnt Protein: Its Role in Wnt Secretion. Takada R, Satomi Y, Kurata T, Ueno N, Norioka S, Kondoh H, Takao T, and Takada S. (2006) *Dev. Cell*, **11**, 791-801.
8. Ubiquitination-like system mediates novel protein-lipidation. Ichimura, Y., Kirisako, T., Takao T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Ishii, T., Ohsumi, M., Noda, T., and Ohsumi, Y. (2000) *Nature* **408**, 488-492.

Laboratory for Membrane Protein Chemistry

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Reconstituting the molecular machinery of intracellular membrane trafficking

The Laboratory for Membrane Protein Chemistry is working on *in vitro* reconstituted proteoliposomal studies to understand the molecular machineries of membrane tethering, docking, and fusion processes in the endomembrane organelle systems of eukaryotic cells (from yeast to humans). Intracellular membrane tethering, docking, and fusion events are fundamental and conserved biological reactions, which are vital for vesicle trafficking between subcellular organelle membrane compartments and plasma membranes, organelle morphology, hormone secretion, and also synaptic neurotransmission. Earlier seminal studies have revealed that membrane tethering, docking, and fusion are temporally and spatially regulated in cells by the diverse sets of key protein components, which include SNARE-family proteins, SNARE-interacting chaperone proteins such as Sec1/Munc18-family proteins, Rab-family small GTPases, Rab-interacting effector proteins, and tethering multisubunit complexes. However, it has still remained enigmatic how those essential protein factors cooperate to specifically and efficiently mediate membrane tethering, docking, and fusion events. In our group, we have been and continue to explore these vital tethering/docking/fusion machineries by *in vitro* reconstitution with purified recombinant proteins (SNAREs, Rabs, and their effectors) and synthetic lipid bilayers with defined lipid compositions. Our reconstitution studies will uncover the mechanistic details of how eukaryotic cells confer the spatiotemporal specificity of the endomembrane trafficking systems.

Research Programs

1) Understanding the molecular machinery to catalyze membrane tethering, docking, and fusion events in eukaryotic endomembrane systems
2) Developing novel reconstitution systems and assays to study the mechanisms by which SNAREs, Rab GTPases, and their effectors, control the directionality of membrane trafficking

Publications

1. Homotypic and heterotypic trans-assembly of human Rab-family small GTPases in reconstituted membrane tethering. Segawa K, Tamura N, Mima J (2019) *J Biol Chem* **294.** 7722-7739.
2. Reconstituted Proteoliposome Fusion Mediated by Yeast SNARE-Family Proteins. Mima J (2019) *Methods Mol Biol* **1860.** 303-322.
3. Reconstitution of membrane tethering mediated by Rab-family small GTPases. Mima J (2018) *Biophys Rev* **10.** 543-549.
4. Human Rab small GTPase- and class V myosin-mediated membrane tethering in a chemically defined reconstitution system. Inoshita M, Mima J (2017) *J Biol Chem* **292.** 18500-18517.
5. Physiological lipid composition is vital for homotypic ER membrane fusion mediated by the dynamin-related GTPase Sey1p. Sugiura S, Mima J (2016) *Sci Rep* **6,** 20407.
6. Membrane-anchored human Rab GTPases directly mediate membrane tethering in vitro. Tamura N, Mima J (2014) *Biol Open* **3,** 1108-1115.
7. Multiple and distinct strategies of yeast SNAREs to confer the specificity of membrane fusion. Furukawa N, Mima J (2014) *Sci Rep* **4,** 4277.
8. Distinct contributions of vacuolar Qabc- and R-SNARE proteins to membrane fusion specificity. Izawa R, Onoue T, Furukawa N, Mima J (2012) *J Biol Chem* **287,** 3445-3453.

Division of Protein Structural Biology

- Laboratory for Molecular Biophysics
- Laboratory for Protein Crystallography
- Laboratory for CryoEM Structural Biology
- Laboratory for Supramolecular Crystallography

Laboratory for Molecular Biophysics

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Assistant Professor	Masatomo So, D.Sci
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Spec. Appoint. Assist. Prof.	Kenichi Harada, Ph.D.
Spec. Appoint. Assist. Prof.	Jun Fukazawa, Ph.D.

Structure and function of interacting proteins as revealed by NMR

The Laboratory for Molecular Biophysics is engaged in studying the biological macromolecular structure and its intermolecular interactions mainly by using nuclear magnetic resonance (NMR). NMR provides information on the protein structure at work with atomic resolution even in cells. This advantage allows us to understand the biological activities for signal transduction and energy conversion from structures. Structures of Fo c-ring, light-harvesting Bchl c complex, β_2-microglobulin amyloid, florigen, interacting ubiquitin, and membrane-bound mastoparan-X have been elucidated. Since supramolecular systems, such as membrane protein complexes, play important roles in biological systems, we are also developing new methodologies in NMR to analyze those challenging structures. One of our programs for solid-state NMR features high-field dynamic nuclear polarization (DNP) for a 1000-fold signal enhancement by using high-intensity terahertz light source, gyrotron. These developments aim to contribute to not only academic but also industrial NMR applications such as drug discovery.

Research Programs

1) NMR analysis of proteins and their interactions
2) Sensitivity enhancement of high-resolution NMR by hyperpolarization
3) New methodologies in biological NMR including isotope-labeled sample preparation and data analysis

Research Topics

High-resolution NMR analysis of structural and functional of proteins in solutions and biomembranes requires development of methods for sample preparation, NMR experiments and data analysis.

1) NMR spectroscopy can provide the detailed information even on transient protein-ligand and protein-protein interactions. To perform such analysis for challenging targets with large molecular masses and low yields, we introduced methodology for sample preparations such as posttranslational protein modification with high-sensitivity molecular probes, efficient amino acid selective isotope labeling, and 19F addition to ligand molecules as a sensitive spin.

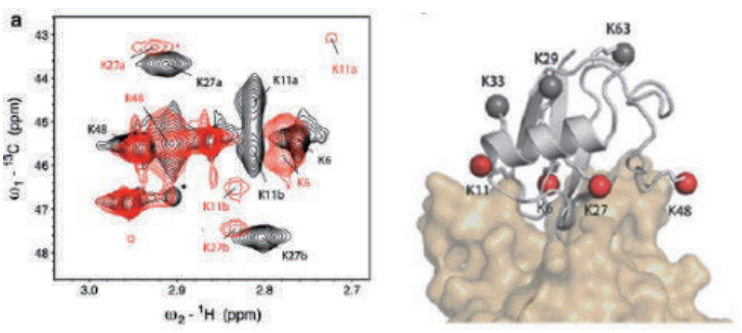

Fig. 1. ^{1}H–^{13}C HSQC spectra of methylated ubiquitin and methylated ubiquitin interacting with protein YUH1 shown on the left. Ubiquitin and YUH1 are shown in the complex (1CMX) by the ribbon and surface representation, respectively. Larger and smaller chemical shift changes are colored red and gray, respectively. This simple post-methylation method gives strong CH_3NMR signals for detecting the protein interactions. (Ref.5)

2) Themagnetic interaction of electron spins is about 1000 times stronger than that of nuclear spins. We are developing high-field DNP-NMR spectrometers for 10000-fold NMR sensitivity enhancement using electron spins at cryogenic temperatures in collaboration with scientists and engineers in academia and industries. This ultrahigh-sensitivity spectrometers are available through shared use programs at IPR.

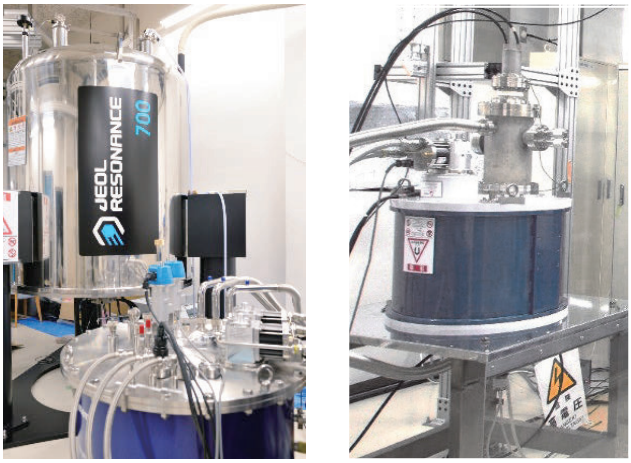

Fig. 2. NMR magnet for 700-MHz solid-state NMR with a closed-cycle cold helium magic-angle spinning system on the left and 460-GHz gyrotron for high-intensity light source of terahertz-wave on the right. Hyperpolarization generated with these instruments increases the NMR sensitivity of proteins. This DNP-NMR spectrometer was developed in the Institute for Protein Research. (Ref. 4)

3) We can now find relationships between NMR parameters and protein structures based on a huge number of protein structures in database PDB and using computational chemistry, quantum mechanical and molecular dynamics calculations. By making use of such relationships we efficiently conduct structural and functional analysis of protein systems.

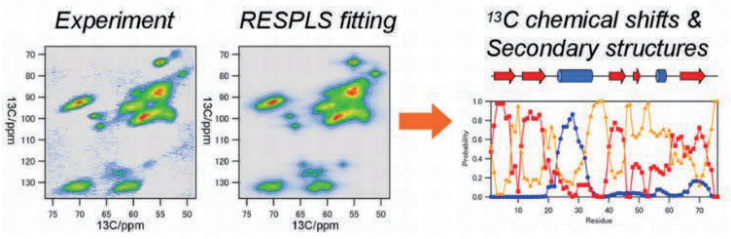

Fig. 3. Automatic solid-state NMR structural analysis of protein. ^{13}C-NMR spectra of proteins in solids often show unresolved signals. Our spectral fitting software RESPLS enables chemical shift assignments and secondary structure prediction based on the databases PDBj and BMRB. This method simplifies the structural analysis by providing reliable information even for lyophilized states. (Ref. 6)

Publications

1. The route from the folded to the amyloid state: exploring the potential energy surface of a drug-like miniprotein, Taricska, N., Horváth, D., Menyhárd, D. K., Ákontz-Kiss, H., Noji, M., So, M., Goto, Y., Fujiwara, T. and Perczel, A. (2020) Chemistry a European Journal, **26**, 1968-1978.
2. Veratridine binding to a transmembrane helix of sodium channel Nav1.4 determined by solid-state NMR, Niitsu, A., Egawa, A., Ikeda, K., Tachibana, K., and Fujiwara, T. (2018) Bioorganic & Medicinal Chemistry, **26**, 5644-5653.
3. Mechanistic and structural basis of bioengineered bovine Cathelicidin-5 with optimized therapeutic activity, Sahoo, B., Maruyama, K., Edula, J., Tougan, T., Lin, Y., Lee, Y-H., Horii, T., and Fujiwara, T., (2017) *Sci. Rep.*, **7**, 44781, 1-16.
4. Closed-cycle cold helium magic-angle spinning for sensitivity-enhanced multi-dimensional solid-state NMR, Matsuki, Y., Nakamura, S., Fukui, S., Suematsu, H., and Fujiwara, T. (2015) *J. Magn. Reson.*, **259**, 76-81.
5. Utilization of lysine ^{13}C -methylation NMR for protein-protein interaction studies. Hattori, Y., Furuita, K., Ohki, I., Ikegami, T., Fukada, H., Shirakawa, M., Fujiwara, T., Kojima, C. (2013) *J. Biomol. NMR*, **55**, 19-31.
6. Secondary structural analysis of proteins based on ^{13}C chemical shift assignments in unresolved solid-state NMR spectra enhanced by fragmented structure database, Ikeda, K., Egawa, A., Fujiwara, T. (2013) *J. Biomol. NMR*, **55**, 189-200.

Laboratory for Protein Crystallography

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Protein structure at high resolution by crystallography, and Supramolecular structure by Cryo-EM

Biological reactions in the cell are connected each other, to form a balanced network. Protein crystallography or Single particle Cryo-EM is the best method to determine the atomic structure of protein molecules in order to elucidate the molecular mechanism of highly organized biological systems. Three-dimensional protein structures, especially in photosynthesis, respiration and cytoskeletal molecular motors, bring us a deeper insight into the networking highly organized biological processes in the cell. The main aim of our research group is structure determination of biological macromolecular assemblies including membrane protein complexes at the atomic level. Current Research Projects are “Structural studies of a photosynthetic membrane protein complex, such as Cytochrome b_6f and Photosystem I complexes, and related redox enzymes”, “Crystal structure analyses of dynein motors with crystallographic technique as well as CD and NMR spectroscopic methods” and “High resolution and radiation damage-free structure analysis of metalloproteins, using X-ray free electron laser or neutron as a probe”.

Research Programs

1) Structural studies of photosynthetic energy-transducing membrane protein complex and related redox enzymes
2) Crystal structure analyses of dynein motors
3) High resolution and radiation damage-free structural analysis of metalloproteins

Research Topics

♦ Dynein is a microtubule-based motor protein, consisting of identical heavy-chains with assorted light-, light intermediate- and intermediate chains. Motor activity is located in the heavy chain; the molecular mass of which is more than 500kDa. High-resolution molecular structures including our X-ray structure (PDB ID: 3VKH) indicated that the microtubule-binding domain of dynein heavy chain is separated from the AAA core of the motor which contains the ATP hydrolysis sites, by an elongated stalk domain consisting of an anti-parallel coiled-coil structure (Fig.1). It was hypothesized that the dynein utilized small amounts of helix sliding between the AAA core and the microtubule-binding domain. Based on the currently available X-ray structures of stalk coiled-coil regions and related biophysical characterization such as molecular dynamics simulation, we proposed the “Open-zipper Model” of helix sliding. However, the structural basis of how to slide the two long coiled-coil helices in the opposite directions and couple the microtubule binding is still unknown.

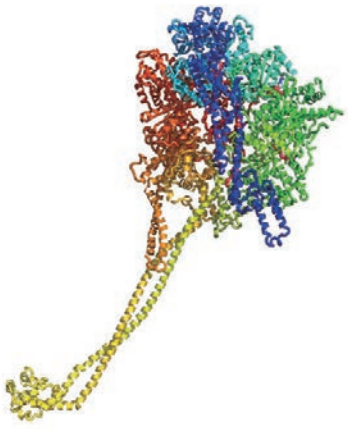

Fig. 1. Overall structure of the dynein motor domain at ADP-bound form.
A ribbon model showing linker (blue), six AAA modules constituting ring (from blue to red), stalk–strut coiled-coils (yellow and orange) and C-sequence (pink) at the outside of the ring.

♦ Life on Earth depends ultimately on sunlight through photosynthesis. Photosynthetic electron transfer consists of three membrane protein complexes: Photosytem II, Cytochrome b_6f complex and Photosystem I (PSI), which are electrically connected by the soluble electron carrier proteins, Plastocyanin or Ferredoxin (Fd). However, it remains a major challenge to predict the productive electron transfer complex formation between the membrane protein and the soluble electron carrier protein, and to understand how the reducing equivalents are delivered from the thylakoid membrane to the stromal space *via* Fd. Our group described the X-ray structure of the trimeric PSI-Fd complex and its complementary analysis in solution by NMR and flash absorption spectroscopy. We discovered that the Fd-bound PSI trimer is asymmetric and the piston-like shift of the transmembrane subunit PsaF, which imply the molecular signaling across the membrane to accomplish efficient electron transfer (Fig.2).

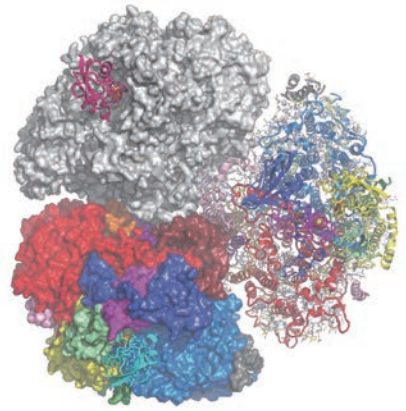

Fig. 2. Overall structure of the cyanobacterial PSI-Fd complex from *T. elongatus*.
A cytosolic view of the PSI-Fd complex. Three Fds, colored in sky blue, yellow, and magenta, are bound to respective promoters of PSI trimer.

Publications

1. X-ray structure of an asymmetrical trimeric ferredoxin-photosystem I complex. Kubota-kawai, H., Mutoh, R., Shinmura, K., Sétif, P., Nowaczyk, M.M., Rögner, M, Ikegami, T., Tanaka, H and Kurisu, G. (2018) *Nature Plants*, **4**, 218-224.
2. Calredoxin represents a novel type of calcium-dependent sensor-responder connected to redox regulation in the chloroplast. Hochmal, A. K., Zinzius, K., Charoenwattanasatien, R., Gäbelein, P., Mutoh, R., Tanaka, H., Schulze, S., Liu, G., Scholz, M., Nordhues, A., Offenborn, J.N., Petroutsos, D., Finazzi, G., Fufezan, C., Huang, K., Kurisu, G. and Hippler, M. (2016) *Nature Commun.*, **7**, 11847.
3. A structural view of synthetic cofactor integration into [FeFe]-hydrogenases. Esselborn, J., Muraki, N., Klein, K., Engelbrecht, V., Metzler-Nolte, N., Apfel, U.-P, Hofmann, E., Kurisu G. and Happe, T. (2016) *Chem. Sci.*, **7**, 959-968.
4. The 2.8 Å crystal structure of the dynein motor domain. Kon, T., Oyama, T., Shimo-Kon, R., Imamula, K., Shima, T., Sutoh, K. and Kurisu, G. (2012) *Nature,* **484**, 345-350.
5. X-ray crystal structure of the light-independent protochlorophyllide reductase. Muraki, N., Nomata, J., Ebata, E., Mizoguchi, T., Shiba, T., Tamiaki, H., Kurisu, G. and Fujita, Y. (2010) *Nature*, **465**, 110-116.
6. Structure of the Cytochrome b_6f complex of Oxygenic Photosynthesis: Tuning the cavity. Kurisu, G., Zhang, H., Smith, J. L. and Cramer, W. A. (2003) *Science* **302**, 1009-1014.
7. Structure of the electron transfer complex between ferredoxin and ferredoxin-NADP(+) reductase. Kurisu, G., Kusunoki, M., Katoh, E., Yamazaki, T., Teshima, K., Onda, Y., Kimata-Ariga, Y. and Hase, T. (2001) *Nature Struct. Biol.,* **8**, 117-121.

♦ National Affiliated Centre of CCDC in Japan

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The Cambridge Structural Database (CSD) is a database for small molecule crystal structures constructed by CCDC (The Cambridge Crystallographic Data Centre). From 1970, we served as a National Affiliated Centre of CCDC in Japan and support academic researchers in Japan. We provide a CSD portal site in Japanese to the Academic users (http://www.protein.osaka-u.ac.jp/CSD), and carry out a licensing activity collaborating with the Protein Research Foundation.

Laboratory for CryoEM Structural Biology

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Structual and functinal analysis of the motor proteins by Cryo-EM

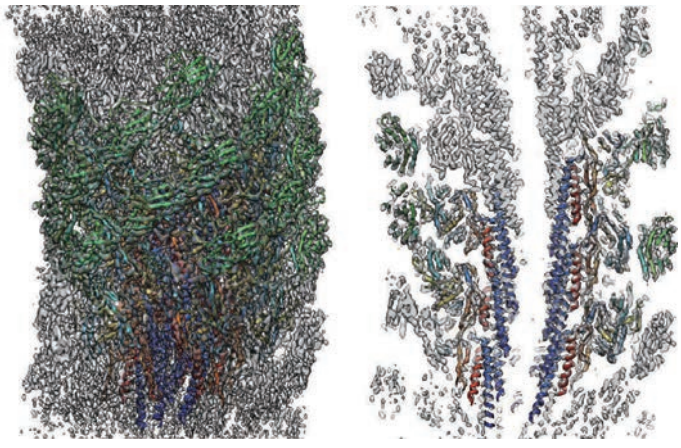
The function of proteins is closely related to the three-dimensional structure, and if the three-dimensional structure is broken, the function is lost. In addition, proteins are changing their conformation constantly and are designed to perform the necessary functions within the fluctuations. This fact indicates that knowing the protein structure and its conformational change is the best way to understanding the functional mechanisms.
The electron cryomicroscopy (cryoEM) is one of the technique to analyze the structure of proteins. Recently, cryoEM single particle image analysis became the most powerful tool for the structural analysis of large protein complexes in their native form at near-atomic resolution. Since the cryoEM can be analyzed the functional structure, we can obtain more useful information to clarify the mechanisms of disease and to design the drug.
In our laboratory, we have analyzed the structural of proteins by cryoEM to understand the mechanism of the function, developed the techniques that enable high-resolution structural analysis, and establishing methods for analyzing conformational changing of proteins.

Research Programs

1) Structual analysis for the proteins to understanding the mechanisums
2) Development methodologies high-resolution strucutal analysis by cryoEM
3) Development methodologies for thromo dynamics of proteins

Research Topics

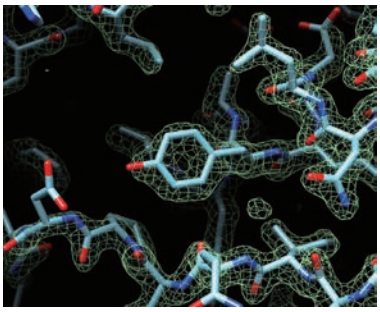
♦ The single particle analysis method using an electron cryomicroscope has a less limit on the molecular weight of a protein, and it possible to analyze the structure in a functional state. Therefore, we normally use it to solve the structure of large molecular complexes. The motor protein such as flagella motor and ATPase, achieves a high-performance motor function with quite high energy conversion efficiency and small. We analyze the structure of the several proteins by cryoEM.



Structure of supuercpiled flagella hook

♦ Recent cryoEM is capable of high-resolution structural analysis, but not all samples can be applied. Some proteins can not be analyzed the structure by the denaturing and preferred orientation on the grid. Or even if they can be analyzed, they cannot always be solved at the expected resolution. We are trying to develop the techniques that can constantly analyze the structure at high-resolution, even in membrane protein and small molecules, which are difficult samples by cryoEM.

CryoEM for high-resolution analysis



The structure of apo-ferritin at 1.9Å resolution

CryoEM for screening

♦ In cryoEM, the molecular images in quick-frozen sample are recorded, then the structure is analyzed by image analysis. The molecular images contain proteins with various conformation. By correctly classifying molecular images with various conformations, it is possible to visualize the inherent thermal fluctuations of proteins. We will clarify the nature of the protein by establishing a method to analyze the thermal fluctuation of the proteins.

Publications

1. Refined mechanism of Mycoplasma mobile gliding based on structure, ATPase activity, and sialic acid binding of machinery. Nishikawa, M.S., Nakane, D., Toyonaga, T., Kawamoto, A., Kato, T., Namba, K. & Miyata, M. (2019) mBio, **10**,e02846-19.
2. Structure of the native supercoiled flagellar hook as a universal joint. Kato, T., Makino, F., Miyata, T., Horváth, P. & Namba, K. (2019) Nature Communications, **10**, 5295.
3. Structure of Salmonella flagellar hook reveals intermolecular domain interactions for the universal joint function. Horváth, P., Kato, T., Miyata, T. & Namba, K. (2019) Biomolecues, **9**, 462.
4. CryoTEM with a cold emission gun that moves structural biology into a new stage. Kato, T., Makino, F., Nakane, T., Terahara, N., Kaneko, T., Shimizu, Y., Motoki, S., Ishikawa, I., Yonekura, K. & Namba, K. (2019) Microsc. Microanal, **21**,Suppl 2, 998.
5. Novel insights into conformational rearrangements of the bacterial flagellar switch complex. Sakai, T., Miyata, T., Terahara, N., Mori, K., Inoue, Y., Morimoto, Y.V., Kato, T., Namba, K. & Minamino, T. (2019) mBio. **10**, e00079-19.
6. Architecture of a flagellar apparatus in the fast-swimming magnetotactic bacterium MO-1, Ruan, J., Kato, T., Santini, C.-L., Miyata, T., Kawamoto, A., Zhang, W.-J., Bernadac, A., Wu, L.-F. and Namba, K. (2012) Proceedings of the National Academy of Sciences USA, **109**, 20643.
7. High-resolution structural analysis of a DNA nanostructure by cryoEM. Kato, T., Goodman, R.P., Erben, C.M., Turberfield, A.J. & Namba, K. (2009) Nano lett, **9**(7), 2747.

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Structure determination of biological macromolecues using X-ray diffraction and cryoEM

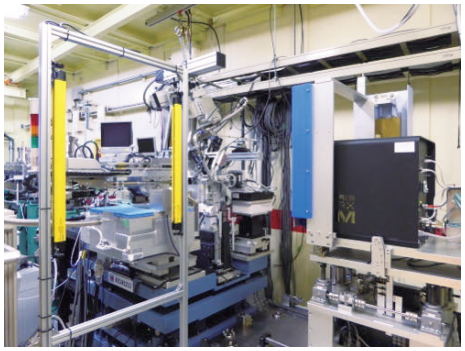
There are various biological macromolecular assemblies consisting of proteins, nucleic acids, sugars, lipids, and other substances in living cells. These macromolecular assemblies play key roles in all living systems. Our laboratory works on structure determination of biological macromolecular assemblies, as well as proteins, which play important roles in biological systems, using X-ray crystallography. Development of tools for X-ray crystal structure determination of biological macromolecular assemblies, including synchrotron radiation beamtime at SPring-8, is also one of our main works.

Research Programs

1) X-ray structure determination of macromolecular assemblies and proteins
2) Development methodologies for X-ray structure determination of biological macromolecular assemblies using synchrotron radiation and X-ray free electron laser
3) Development of data processing algorithm of diffraction data from micro-crystals

Research Topics

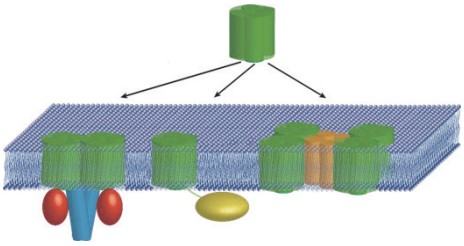
♦ Protein crystallography has made great progress in recent years, and many complex protein structures have been determined during this time. However, there are still many barriers to be overcome in the process of structure determination. High quality diffraction data collection is one of the most important steps for success of structure determination. For this purpose, we are managing the synchrotron radiation beamline at SPring-8 for high quality diffraction data collection from biological macromolecular assembly crystals. The beamline, BL44XU, is named the beamline for biological macromolecular assemblies or IPR beamline (Tanpakuken beamline). This beamline aims to collect high quality diffraction data from small size and large unit cell crystals such as membrane protein complexes, protein complexes, protein and nucleic acid complexes, and viruses. Data collection from ordinary protein crystals also can be achieved.



BL44XU at SPring-8

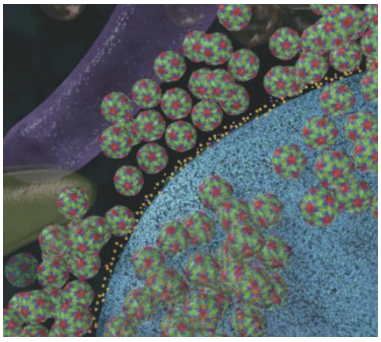
This beamline is available to any scientist in the world as one of the programs of the Joint Usage and Research for Proteins. International collaboration between the National Synchrotron Radiation Research Center (NSRRC) in Taiwan is stimulating the activities of the beamline.

♦ Electric signals are important in many living processes, such as nerve signal transmission and heart beat. Recently, new voltage-sensing proteins have been identified, and these molecules transduce electrical signals into intracellular signals in a different way from the classical voltage-gated ion channels. Our project aims to reveal the dynamic mechanism of signal transduction by the new voltage-sensing proteins based on their atomic structure. These results will open a new paradigm of life science. Our results will give valuable information for strategic development of new tools for visualization of electrical signal and will be extended to studies for medical application.



Voltage-sensing protein family

♦ We are working on structural studies of biological macromolecular assemblies, such as plan reoviruses and multi-drug efflux pump complexes. One of our main targets is a *Rice dwarf virus* (RDV), of which atomic structures of capsid has been determined. We are working to determine the complete structure of the virus including the transcriptional complex, genome and the protein component that is required for infection. In addition, non-structure proteins, which are essential for assembly, multiplication and infection to the next cell, are also targeted. We aim to understand the life cycle of RDV from the atomic structures of proteins of RDV.



Model of multiplication process of *Rice dwarf virus in a cell*

Publications

1. An assembly intermediate structure of *Rice Dwarf Virus* reveals a hierarchical outer capsid shell assembly mechanism, Nakamichi Y., Miyazaki N., Tsutsumi K., Higashiura A., Narita H., Murata K., and Nakagawa A. (2019) *Structure*, **27**, 439-448.
2. Hierarchical structure assembly model of rice dwarf virus particle formation, Nakagawa, A., Miyazaki, N., Higashiura A. (2018) *Biophysical Reviews*, **10**, 659-665.
3. Structural basis for the assembly of the Ragulator-Rag GTPase complex, Yonehara, R., Nada, S., Nakai, T., Nakai, M., Kitamura, A., Ogawa, A., Nakatsumi, H., Nakayama, K. I., Li, S., Standley, D. M., Yamashita, E., Nakagawa, A., and Okada, M. (2017) *Nature Communications*, **8**, 1625.
4. X-ray crystal structure of voltage-gated proton channel, Takeshita, K., Sakata, S., Yamashita, E., Fujiwara, Y., Kawanabe, A., Kurokawa, T., Okochi, Y., Matsuda, M., Narita, H., Okamura, Y., and Nakagawa, A. (2014) *Nature Structural & Molecular Biology*, **21**, 352-357.
5. A new protein complex promoting the assembly of Rad51 filaments. Sasanuma, H., Tawaramoto, M.S., Lao, J, Hosaka, H., Sanda, E., Suzuki, M. Yamashita, E., Shinohara, M. Hunter, N., Nakagawa A., and Shinohara, A. (2013) *Nature Communications*. **4**, 1676-1688.
6. Structural insight into maintenance methylation by mouse DNA methyltransferase 1 (Dnmt1), Takeshita, K., Suetake, I., Yamashita, E., Suga, M., Narita, H., Nakagawa, A., and Tajima, S. (2011) *Proceedings of the National Academy of Sciences USA*, **108**, 9055-9059.
7. Crystal Structure of the Membrane Fusion Protein, MexA, of the Multidrug Transporter in *Pseudomonas aeruginosa*, Akama, H., Matsuura, T., Kashiwagi, S., Yoneyama, H., Narita, S., Tsukihara, T., Nakagawa, A., and Nakae, T. (2004) *Journal of Biological Chemistry*, **279**, 25939-25942.

Division of Integrated Protein Functions

- Laboratory for Molecular and Developmental Biology
- Laboratory for Genome and Chromosome Functions
- Laboratory for Advanced Brain Functions
- Laboratory for Organelle Biology

Laboratory for Molecular and Developmental Biology

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Associate Professor Taro Chaya, Ph.D.
Spec. Appoint. Assist. Prof. Yuko Sugita, Ph.D.

Understanding of central nervous system development from gene to neuronal function and human disease

Our laboratory studies molecular mechanisms underlying the development and function of the vertebrate central nervous system (CNS) using various research methods of molecular biology, mouse genetics, biochemistry, cell biology and neural physiology. Our brain consists of more than 100 billion neurons. To function as a brain, numerous numbers of neurons must be generated at the right place and they must be interconnected each other. We use the retina as a model system to understand how DNA encodes programs to generate various neurons and glial cells, form precise neuronal circuits, and enable complicated neuronal function. We also focus on how abnormality of biological processes in development and maturation leads to human diseases. We are eager to contribute to the development of diagnosis and cure of human diseases. Together, our lab aims to elucidate the mechanisms and principles underlying CNS development from DNA programs to physiological function and human disease.

Research Programs

1) Analysis of molecular mechanisms underlying neuronal differentiation
2) Molecular analysis of neural circuit formation in the retina
3) Analysis of neuronal degeneration in the retina and development of the method to prevent neuronal degenerative diseases
4) Elucidation of functional roles of microRNAs (miRNAs) in CNS development
5) Functional analysis of cilium, an antenna of a cell, in the CNS
6) Analysis of visual function using mouse models

Research Topics

♦Exquisitely precise synapse formation is crucial for correct functioning of the mammalian central nervous system. Retinal photoreceptors transfer information to bipolar and horizontal cells at a specialized synapse, the ribbon synapse. We identified pikachurin, a novel extracellular matrix-like retinal protein, and observed its localization to the synaptic cleft in the photoreceptor ribbon synapse. *Pikachurin* null mutant mice display improper apposition of the bipolar terminus to the ribbon synapse, resulting in alterations in synaptic signal transmission and visual function as detected by electroretinogram and optokinetic responses. We observed co-localization of pikachurin with dystrophin-glycoprotein complex (DGC) molecules and direct binding of pikachurin with alpha-dystroglycan, an extracellular component of DGC. Together, our results identified a novel dystroglycan ligand protein, pikachurin, and demonstrate its essential role in the precise interactions between the photoreceptor ribbon synapse and the bipolar terminus. This may also advance our understanding of the molecular mechanisms underlying the retinal electrophysiological abnormalities observed in muscular dystrophy patients (Ref. 9).

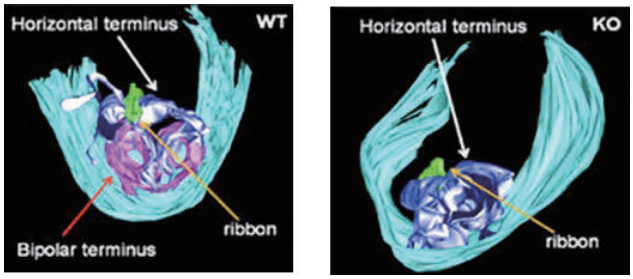

Fig. Electron tomography of rod photoreceptor synapse terminals using ultrahigh-voltage electron microscopy.
In the WT mouse retina, photoreceptor axonal terminus forms invagination to appose dendritic terminals of horizontal cells and bipolar cells to form the ribbon synapse. In contrast, Pikachurin KO mice showed improper apposition of the bipolar cell dendritic tips to the photoreceptor ribbon synapses.

♦ Micro RNA is about 21 bp small non-coding RNA which is involved in many biological processes and human diseases through translational repression. MicroRNA-124a (miR-124a) is the most abundant miRNA expressed in the vertebrate central nervous system (CNS). We explored the *in vivo* function of miR-124a by targeted disruption of *Rncr3 (retinal non-coding RNA 3)*, which is the dominant source of miR-124a. *Rncr3*$^{-/-}$ mutant mice exhibit abnormalities in the CNS, including small brain size, axonal mis-sprouting of DG granule cells, and retinal cone cell death. We found that *Lhx2* is an *in vivo* target mRNA of miR-124a. We also observed that LHX2 down-regulation by miR-124a is required for prevention of apoptosis in the developing retina and proper axonal development of hippocampal neurons. Our results showed that miR-124a plays an essential role in maturation and survival of DG neurons and retinal cones by repressing *Lhx2* translation (Ref. 7).

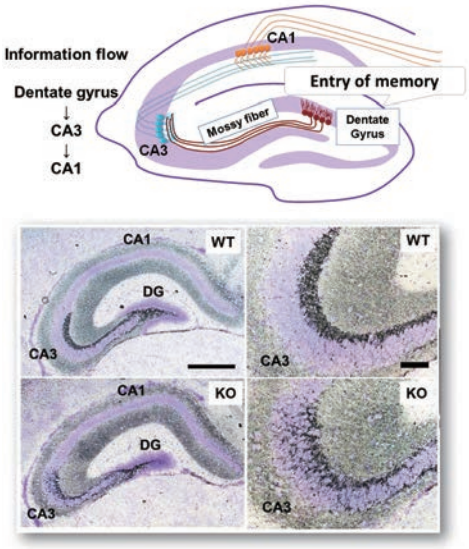

Fig. Aberrant sprouting of mossy fibers in themiR-124a KO mouse. The mossy giver terminals were visualized by Timm staining with Nissl counterstaining at postnatal day 10. Scale bars represent 500 um.

♦Cilia are microtubule-based organelles that extend from the surface of various types of cells in vertebrates. In humans, dysfunction of cilia causes a broad range of overlapping clinical phenotypes termed “ciliopathies,” which include retinal degeneration, polycystic kidney disease, polydactyly and obesity. We found that a ciliary kinase, Mak, is essential for ciliary length regulation and survival of photoreceptor cells (Ref. 8). We clarified that a Mak paralog, ICK, regulates turnaround machinery of intraflagellar transport (IFT) at ciliary tips using knockout mice and zebrafish morphants (Ref. 5).

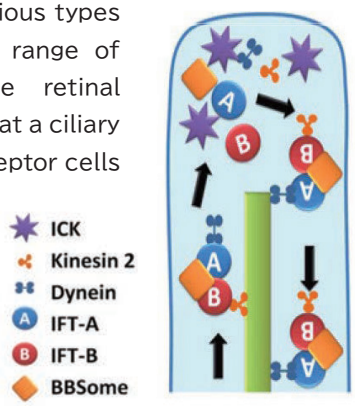

Fig. A hypothetical model for ICK function in the regulation of IFPT turnaround at ciliary tips.
ICK regulates the disassembly between IFT-A and IFT-B subcomplexes at ciliary tips through phosphorylation of IFT.

Publications

1. Cul3-Klhl18 ubiquitin ligase modulates rod transducing translocation during light-dark adaptation. Chaya T. et al. (2019) *EMBO J.* **38**, e101409.
2. Lrit1, a Retinal Transmembrane Protein, Regulates Selective Synapse Formation in Cone Photoreceptor Cells and Visual Acuity. Ueno A. et al. (2018) *Cell Rep.* **22**, 3548–3561.
3. Samd7 is a cell type-specific PRC1 component essential for establishing retinal rod photoreceptor identity. Omori Y. et al. (2017) *Proc Natl Acad Sci USA.* **114**, E8264 - E8273.
4. Protein-4.1G-Mediated Membrane Trafficking Is Essential for Correct Rod Synaptic Location in the Retina and for Normal Visual Function. Sanuki R et al. (2015) *Cell Rep.* **10**, 796–808.
5. ICK is essential for cell type-specific ciliogenesis and the regulation of ciliary transport. Chaya T, Omori Y, Kuwahara R, Furukawa T (2014) *EMBO. J.* **33**, 1227-1242.
6. Presynaptic Dystroglycan-Pikachurin Complex Regulates the Proper Synaptic Connection between Retinal Photoreceptor and Bipolar Cells. Omori et al. (2012) *J. Neurosci.* **2**, 6126-6137.
7. miR-124a is required for hippocampal axogenesis and retinal cone survival through Lhx2 suppression. Sanuki R et al. (2011) *Nature Neurosci.* **14**, 1125-1134.
8. Negative regulation of ciliary length by ciliary male germ cell-associated kinase (Mak) is required for retinal photoreceptor survival. Omori Y et al. (2010) *PNAS* **107**, 22671-22676.
9. Pikachurin, a dystroglycan ligand, is essential for photoreceptor ribbon synapse formation. Sato S et al. (2008) *Nature Neurosci.* **11,** 923-931.

Laboratory for Genome and Chromosome Functions

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Assistant Professor	Masaru Ito, Ph.D.
Assistant Professor	Yurika Fujita, Ph.D.

Studing the mechanisms of genome stability & instability in eukrayotes

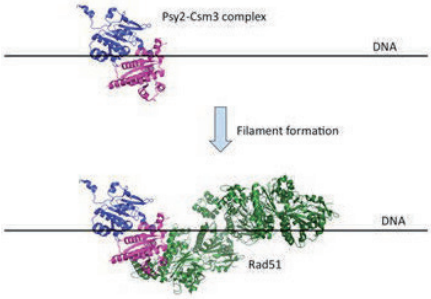
We are studying mechanisms to stabilize genome and molecular defects induced by genomic instability by visualizing molecular pathways at a molecular level, as well as deciphering the events in cells or in organisms. Among pathways to stabilize the genome, we are focusing on not only DNA biochemistry of DNA exchange, homologous recombination, and DNA replication, but also control mechanisms of their recombination events in mitotic and meiotic cell cycles by chromosome morphogenesis and movements. We employed inter-disciplinary approaches combined with molecular biology, biochemistry, structural biology and genome-wide approaches. Recently, we have been extending our analysis by targeting recombination reactions in human cells or mice. Dysfunction of recombination in mitotic cells induces genome instability which leads to tumorigenesis while defective recombination during gamete formation induces miscarriage and aneuploidy disease such as Down syndrome. We are also trying to understand molecular defects in these diseases.

Research Programs

1) Study of functions and structure of protein and protein complexes involved in homologous recombination
2) Research on interplay between recombination, and chromosome structure, motion and nuclear envelope remodeling
3) Control mechanisms of recombination by epigenetics and telomere functions
4) Biochemical and structural studies on human proteins involved in recombination and DNA replication
5) Study on recombination and DNA repair in mice and human cells

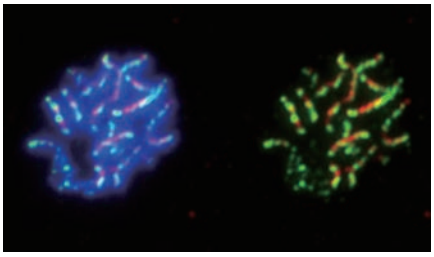
Research Topics

♦In recombination, a step called homolog search between DNAs is a key step involving dynamic assembly and disassembly of proteins on single-stranded (ss) DNA. A RecA homolog, Rad51 forms a right-handed helical filament on ssDNA, assembly and disassembly of which is tightly regulated by both positive and negative regulators. Rad51 paralogs as well as BRCA2, whose mutation causes familial breast cancer, to promote the formation of Rad51 filament. However, how these proteins work in Rad51 assembly remains unknown. We have been studying Rad51-medated reaction since we identified Rad51 as a RecA homolog. Recently we identified a new protein complex called (PCSS), which facilitates Rad51assembly and determines a crystal structure of core complex of Psy3-Csm2. These studies shed light on how this complex promotes Rad51 filament by binding to the end of filament for recruitment as well as stabilization (Ref. 7).



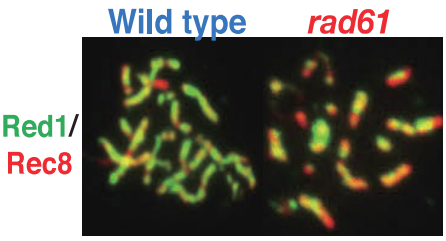
A model of Rad51-assembly by PCSS

♦During meiosis, specialized chromosome morphogenesis such as the formation of the Synanptonemal complex (SC) and dynamics with telomere clustering are believed to control DNA biochemistry on meiotic chromosomes such as recombination. It remains unsolved how the SC is formed telomere clustering is facilitated in terms of molecular machinery. Our group has been identifying proteins involved in these processes. Recently, we have been focusing on the cohesin complexes involved in sister chromatid cohesion in meiotic events and found cleavage-independent pathway of removal of a meiosis-specific cohesin complex during late meiosis I. This depends on the phosphorylation of cohesin regulators (Ref. 3, 6).



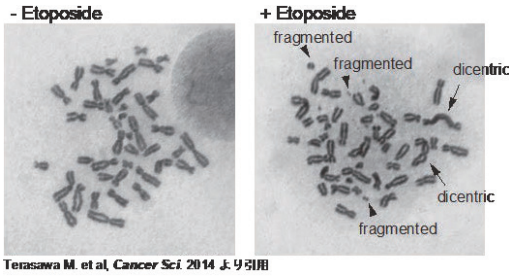
Immuno-staining image of Red1(green) and Rec8 cohesin (red)

♦Meiotic recombination plays an essential role in chromosome segregation at meiosis I. Different from mitotic recombination, meiotic recombination is highly differentiated and regulated so that recombination occurs preferentially between homologous chromosomes over a sister chromatid; crossover is more frequent than non-crossovers; the number and distribution of crossovers along chromosomes are strictly controlled. Each unique property of meiotic recombination is catalyzed by different protein complexes. Our group has isolated different proteins involved in these reactions. Recent studies revealed that DNA damage response (DDR) proteins such as DNA damage clamp promotes crossover formation by helping the assembly of so-called pro-crossover complex, suggesting interplay between DDR and meiotic recombination (Ref. 4,7, 10).



Immunostaining image of SC; Zip1 (red), Rec8 (green)

♦It is known that one of the reasons why cells become cancerous is that genomic DNA, the source of our genetic information, is physically destroyed. Thus, DNA damage often causes genomic instability such as mutation, translocation, amplification or deletion. Ionizing radiation, some chemicals, sunlight and oxygen are essential for our life; they also induce genomic instability. Therefore, we have multiple mechanisms to repair DNA damage. We have shown that human FIGNL1 protein, which interacts with a human RAD51 paralog, SWSAP1, can disrupt RAD51 filaments both in vivo and in vitro and SWSAP1 suppresses the FIGNL1 activity, suggesting a novel mechanism of RAD51 assembly by collaborative actions of RAD51 mediators and anti-recombinases (Ref.2). Single-molecule observation on human RAD51-MRE11-NBS1 (MRN) complex by atomic-force microscopy revealed that structural similarity of the MRN to the cohesin (Ref.1). These findings provide novel information to know the mechanism of homologous recombination in human.



DNA damage induced genomic instability. Mitotic chromosome from human cell without DNA damage (left), or after DNA damage induction (right).

Publications

1. Rad50 zinc hook functions as a constitutive dimerization module interchangeable with SMC hinge. Tatebe, H., Lim, C.T., Konno, H., Shiozaki, K., Shinohara, A., Uchihashi, T. and Furukohri, A. (2020) *Nature Commun.* **11,** 370.
2. Human RAD51 paralogue, SWSAP1, fosters RAD51 filament by regulating the anti-recombinase, FIGNL1 AAA+ ATPase. Matsuzaki, K., Kondo, S., Ishikawa, T., and A. Shinohara. (2019) *Nature Commun.* **10,** 1407.
3. Meiosis-specific prophase-like pathway controls cleavage-independent release of cohesin by Wapl phosphorylation. Challa, K., Fajish G.V., Shinohara, M., Klein, F., Susan M. Gasser, S.M., and A. Shinohara. (2019) *PLoS Genetics*, **15,** e1007851.
4. DNA damage response clamp loader Rad24(Rad17) and Mec1(ATR) kinase distinctly control meiotic crossover formation. Shinohara M., Bishop, D.K., and A. Shinohara. (2019) *Genetics*, **113.** 1255-1269.
5. Srs2 helicase prevents the formation of aberrant DNA damage during late prophase I of yeast meiosis. Sasanuma, H., Sabhan, H.M.S., Furihata, Y., Challa, K., Palmer, L. Gasser, S.M., Shinohara, M., and A. Shinohara. (2019) *Chromosoma*, **128**, 453-471.
6. Rad61/Wpl1 (Wapl), a cohesin regulator, controls chromosome compaction during meiosis. Challa, KK., Lee, MS., Shinohara, M., Kim, K.M and A. Shinohara. (2016) *Nucleic Acids Research*, **44**, 3190-3203.
7. DNA damage response clamp contributes to chromosomal assembly of ZMM-SIC pro-crossover factors during meiosis. Shinohara, M, Hayashihara, K., Grubb, J.T., Bishop, D.K., and Shinohara, A. (2015) *J. Cell. Sci.* **128**, 1494-1506.
8. A new protein complex promoting the assembly of Rad51 filaments. Sasanuma, H., Tawaramoto, M.S., Lao, J, Hosaka, H., Sanda, E., Suzuki, M. Yamashita, E., Shinohara, M. Hunter, N., Nakagawa A. and Shinohara, A. (2013) *Nature Commun.* **4**, 1676-1688.
9. *Saccharomyces cerevisiae* Srs2 helicase disassembles Rad51 from meiotic chromosomes. Sasanuma, H., Furihata Y., Shinohara, M. and Shinohara, A. (2013) *Genetics*, **194**, 859-872.
10. Crossover assurance and crossover interference are distinctly regulated by the ZMM proteins during yeast meiosis. Shinohara, M., Oh, S., Hunter, N., and Shinohara, A. (2008) *Nature Genet.* **40,** 299-309.
11. A protein complex containing Mei5 and Sae3 promotes the assembly of the meiosis-specific RecA homolog Dmc1. Hayase, A., Takagi, M., Miyazaki, T., Oshiumi, H., Shinohara, M. and Shinohara, A. (2004) *Cell* **119**, 927-940.

Laboratory for Advanced Brain Functions

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Assistant Professor Tom Macpherson, Ph.D.

Study on molecular and circuit mechanisms in brain functions and mental disorders using model mice

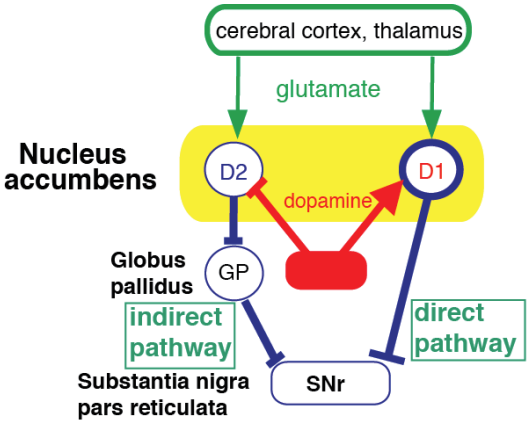
Our laboratory studies neural circuit mechanisms underlying various advanced brain functions, such as cognitive learning and decision making behaviors, using molecular techniques for neural circuit-specific manipulation. We use several mouse models to reveal molecular pathologies of neuropsychiatric diseases. In particular, we focus on molecular mechanisms of gene-environment interaction in the pathogenesis of mental disorders. We also promote translational research for targeting mental disorders in collaboration with clinical departments and pharmaceutical companies.

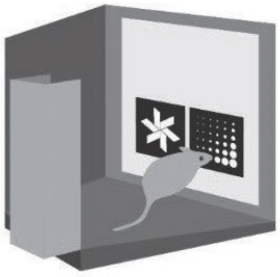
Research Programs

1) Analysis of neural circuit mechanisms in advanced brain functions
2) Molecular analysis of neuropsychiatric pathologies
3) Translational research of mental disorders

Research Topics

♦ Basal ganglia are the neural nuclei located in the center of the brain, they are covered and targeted by the cerebral cortex. The cortico-basal ganglia circuit is essential for motor control as well as advanced brain function, such as learning, cognitive, social, and mental functions. There are two distinct pathways in the basal ganglia circuit; i.e., the direct and indirect pathways from the striatum/nucleus accumbens to the substantia nigra pars reticulata. However, their roles in advanced brain functions remain unclear. We have developed pathway-specific reversible neurotransmission blocking by specifically and reversibly expressing tetanus toxin within neurons in the direct and indirect pathways (Hikida et al., Neuron 2010). We have found that the direct pathway is important for reward learning and drug addiction, while the indirect pathway is important for aversive learning (Hikida et al., Neuron 2010; Macpherson & Hikida, Front Neurosci 2018), and that dopaminergic input and pathway-specific PKA/cAMP signaling are critical for learning (Hikida et al., PNAS 2013; Yamaguchi et al., PNAS 2015). We also found that dopamine D2L receptor signaling in the indirect pathway is important for behavioral flexibility in the reward learning tasks in the IntelliCage (Macpherson et al., Learn Mem 2016), and that dopamine D2L receptor is required for visual discrimination and reversal learning in touchscreen cognitive learning tasks (Morita et al., Mol. Neuropsychiatry 2016).



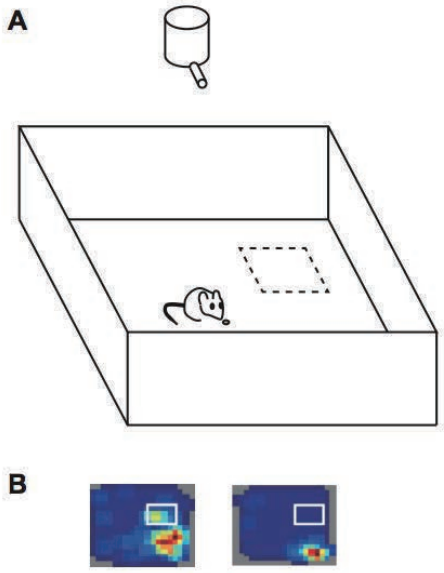


Up: Basal ganglia circuit

Left: touchscreen cognitive learning tasks for mice

♦ Animal models are required for translational research. We have developed a mouse model for mental disorders, transgenic mice overexpressing patient-type mutant DISC1 in the forebrain (Hikida et al., PNAS 2007; Niwa et al., Science 2013). These genetic model mice show behavioral abnormalities and changes in cortical dopamine signals after adolescent social isolation, indicating genetic-environmental interactions in the pathogenesis of mental disorders.

We have examined cognitive functions of this mouse model using the place preference task (Fig. A). We have found that the mutant DISC1 transgenic mice are impaired in place learning after adolescent social isolation. As the place cell in the hippocampal CA1 is known to be important for place learning, we next examined place cell activity by *in vivo* electrophysiological recording. There was no significant change in the place cell property of the mutant DISC1 transgenic mice. However, reward-related activity in the goal zone disappeared in the place cells of the mutant DISC1 transgenic mice (Fig. B) (Hayashi et al., Neurosci. Res. 2016), indicating hippocampal circuit abnormality (Kitanishi et al., JPS 2017).



A. The testing box for the place preference task. Animals were required to stay at least 1 second in the unmarked goal zone (dashed square), after which time a food pellet was dropped from the overhead dispenser.
B. Example place field map with putative goal-zone activity (*Left*) and without goal-zone activity (*Right*). Refs. 2 & 5.

Publications

1. Nucleus accumbens dopamine D1-receptor-expressing neurons control the acquisition of sign-tracking to conditioned cues in mice. Macpherson & Hikida (2018) *Front. Neurosci.* **12,** 418.
2. Network mechanisms of hippocampal laterality, place coding and goal-directed navigation. Kitanishi et al. (2017) *J. Physiol. Sci.* **67**, 247-258.
3. Dopamine D2L receptor is required for visual discrimination and reversal learning. Morita et al. (2016) *Mol. Neuropsychiatry* **2**, 124-132.
4. Nucleus accumbens dopamine D2-receptor expressing neurons control behavioral flexibility in a place discrimination task in the IntelliCage. Macpherson et al. (2016) *Learn. Mem.* **23**, 359-364.
5. Impaired hippocampal activity at the goal zone on the place preference task in a DISC1 mouse model. Hayashi et al. (2016) *Neurosci. Res.* **106**, 70-73.
6. Role of PKA signaling in D2 receptor-expressing neurons in the core of the nucleus accumbens in aversive learning. Yamaguchi et al. (2015) *Proc. Natl. Acad. Sci. U.S.A.* **112**, 11383-11388.
7. Adolescent stress-induced epigenetic control of dopaminergic neurons via glucocorticoids. Niwa et al. (2013) *Science* **339**, 335-339.
8. Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors. Hikida et al. (2013) *Proc. Natl. Acad. Sci. U.S.A.* **110**, 342-347.
9. Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. Hikida et al. (2010) *Neuron* **66**, 896-907.
10. Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans. Hikida et al. (2007) *Proc. Natl. Acad. Sci. U.S.A.* **104**, 14501-14506.

Laboratory for Organelle Biology

Independent Associate Professor **Masato Nakai, D.Sci.**
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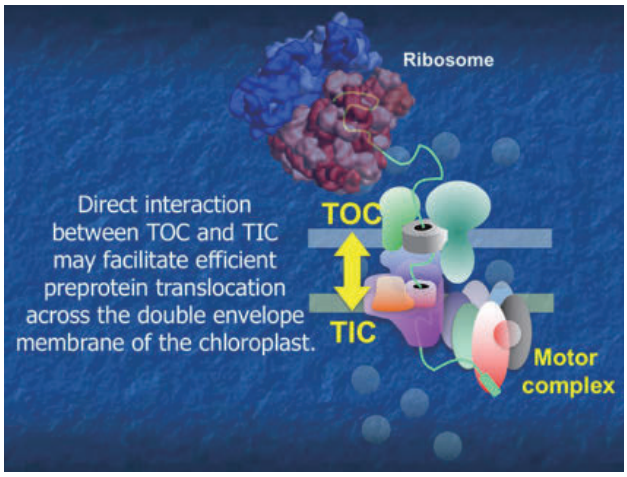
Elucidation of molecular mechanisms of chloroplast biogenesis in plants and algae.

Higher plants must import ~ 3000 different cytosolically-synthesized nuclear-encoded chloroplast proteins, across the double envelope membranes surrounding the organelle, to fulfill their complex physiological roles including photosynthetic functions. Two successive protein translocons at the outer and inner envelope membranes, termed TOC and TIC, respectively, are responsible for protein import into chloroplasts. Our recent discoveries of the genuine TIC translocon, published in ***Science*** in 2013 (1), and the completely novel TIC-associated import motor complex, published in ***Plant Cell*** in 2018 (2), provide an entirely revised view on the molecular mechanisms of protein translocation across the inner envelope membrane of chloroplast (3-5).



Research Programs

1) Elucidation of molecular mechanisms of protein import into the chloroplasts
2) Molecular evolutionary studies on the chloroplast protein import system

Publications

1. Unconvering the Protein Translocon at the Chloroplast Inner Envelope Membrane. Kikuchi S, Bédard J, Hirano M, Hirabayashi Y, Oishi M, Imai M, Takase M, Ide T, Nakai M. (2013) *Science* **339**, 571-574.
2. A Ycf2-FtsHi Heteromeric AAA-ATPase Complex Is Required for Chloroplast Protein Import. Kikuchi S, Asakura Y, Imai M, Nakahira Y, Kotani Y, Hashiguchi Y, Nakai Y, Takafuji K, Bédard J, Hirabayashi-Ishioka Y, Mori H, Shiina T, Nakai M. (2018) *Plant Cell* **30**, 2677-2703.
3. New perspectives on Chloroplast Protein Import. Nakai M. (2018) *Plant Cell Physiol.* **59**, 1111-1119.
4. The TIC complex uncovered: The alternative view on the molecular mechanism of protein translocation across the inner envelope membrane of chloroplasts. Nakai M. (2015) *Biochim Biophys Acta* **1847**, 957-967.
5. YCF1: A Green TIC. Nakai M. (2015) *Plant Cell* **27**, 1834-1838.

Division of Protein Network Biology

- Laboratory for Cell Systems
- Laboratory for Computational Biology
- Laboratory for Nuclear Dynamics
- Laboratory for Infection Systems
- Laboratory of Visiting Scientists

Laboratory for Cell Systems

Professor Mariko Okada, Ph.D.

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Spec. Appoint. Assoc. Prof. (Lecturer)	Sho Tabata, Ph.D.
Assistant Professor	Keita Iida, Ph.D.
Assistant Professor	Ayaka Ichikawa, Ph.D.
Spec. Appoint. Assist. Prof.	Ulrike Münzner, Ph.D.

Understanding a cell, the smallest unit of life, as a dynamic system of molecules

The aim of our laboratory is to define general regulatory rules in signal transduction-transcriptional networks in the cell determination processes and apply this knowledge of regulatory principles to manipulate cell fate and elucidate the mechanism of human diseases. Wet lab experiments, mathematical modeling, and informatics analysis are used to perform quantitative measurements and analysis of biological systems of interest. Using these systems biology approaches, we unravel unique properties of network topologies in signal-transcription networks in cell fate control in cancer, immune responses, development and brain systems.

Research Programs

1) Dynamics of transcription factors and gene regulation
2) Mathematical modeling of signaling networks for cell regulation
3) Multi-Omics analysis of human disease models

Research Topics

♦Intracellular signaling pathways are important to characterize the activation of transcription factors, and their dynamics are often associated with the determination of particular cellular phenotypes. Our earlier study indicated that duration of ERK activity determines switch-like all-or-none activation of the c-Fos transcription factor, thereby inducing qualitative changes in the cell fate (Nakakuki et al. *Cell* 2010). Our study on BCR-mediated NF-κB signaling pathway showed the characteristic dynamics of NF-κB; switch-like activation and oscillatory behaviors are shaped by feedback regulations. In particular, for a mechanism of switch-like activation, we found that IKK activity is regulated by positive feedback from IKK to TAK1, mediated by scaffolding protein CARMA1 at Ser-578, and this feedback regulation could induce the switch-like activation of NF-κB in single cells (Shinohara et al. *Science*, 2014), and in the later step, this NF-κB activity is involved in a transcriptional machinery to fine-tune gene expression levels (Michida et al. *Cell Rep.* 2020).

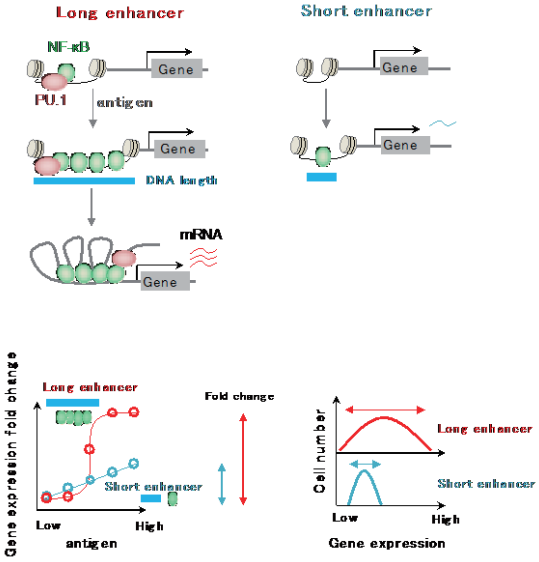


Fig 1. Quantitative control of NF-κB target gene expression by DNA length

♦ Dysregulated dynamics of signaling responses are closely related to incidence of several types of human cancer, therefore the quantitative understanding of cancer network is important for the better understanding of disease mechanisms. Our laboratory developed a mathematical model of various signaling pathways based on highly quantitative experimental data. Based on this knowledge, we are now developing a whole cell signaling network of human cancer. The integration of clinical cancer data into mathematical models allows for more mechanistic classification of cancer subtypes.

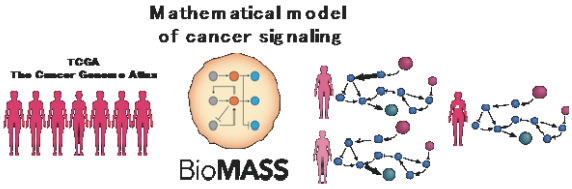


Fig 2. Mathematical modeling of cell signaling network and disease application

♦Signaling networks are essential in cellular survival and development, and regulate transcriptome, epigenetics, and metabolome landscape in mammalian systems. This system-wide data analysis will provide a better understanding of the underlying principles of how signal pathways link to other biochemical networks to determine the fate of cells and individuals. We use disease models and public data for the analysis and try to understand higher-order regulation of the biological network conserved in various levels of mammalian development.

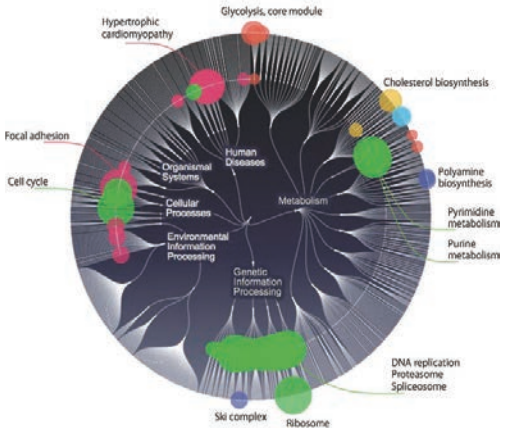


Fig 3. Signal protein knockout induces drastic changes in transcriptome landscape.

Publications

1. The number of transcription factors at an enhancer determines switch-like gene expression. Michida H, et al. (2020) *Cell Rep.* **31**(9):107724. doi: 10.1016/j.celrep.2020.107724.
2. Essential role of the Crk family-dosage in DiGeorge-like anomaly and metabolic homeostasis. Imamoto A, et al. (2020) *Life Sci Alliance*. **3**(2):e201900635. doi: 10.26508/lsa.201900635.
3. Inferring the transcriptional regulatory mechanism of signal-dependent gene expression via an integrative computational approach. Chiang S, et al. (2020) *FEBS Lett.* **594**(10):1477-1496. doi: 10.1002/1873-3468.13757.
4. Signal-dependent regulation of early-response genes and cell cycle: a quantitative view. Imoto H & Okada M. (2019) Current *Opinion in Systems Biology* **15**, 100-108 doi: 0.1016/j.coisb.2019.04.003.
5. Hunt for the tipping point during endocrine resistance process in breast cancer by dynamic network biomarkers. Liu R, et al. (2019) *J Mol Cell Biol.* **11**(8):649-664. doi: 10.1093/jmcb/mjy059.
6. Positive feedback within a kinase signaling complex functions as a switch mechanism for NF-κB activation. Shinohara H, et al. (2014) *Science* **344**, 760-764. doi: 10.1126/science.1250020.
7. Ligand-specific c-Fos expression emerges from the spatiotemporal control of ErbB network dynamics. Nakakuki T, et al. (2010) *Cell* **141**, 884-896. doi: 10.1016/j.cell.2010.03.054.
8. Ligand-dependent responses of the ErbB signaling network: experimental and modeling analysis. Birtwistle MR, et al. (2007) *Mol. Syst. Biol.* **3**, 144. doi: http://10.1038/msb4100188.
9. Quantitative transcriptional control of ErbB receptor signaling undergoes graded to biphasic response for cell differentiation. Nagashima T, et al. (2007) *J. Biol. Chem.* **282**, 4045-4056. doi: 10.1074/jbc.M608653200.

Laboratory for Computational Biology

Professor Kenji Mizuguchi, Ph.D.

kenji@protein.osaka-u.ac.jp

Associate Professor Kosuke Hashimoto, Ph.D.
Assistant Professor Chioko Nagao, Ph.D.

From the elucidation of biological mechanisms using computational approaches towards drug discovery and other applications

We aim to increase our understanding of biological systems and diseases using computational approaches, with applications to drug discovery and other research. Recognizing that the availability of a large amount of data in a computer-friendly format is key to the successful development of artificial intelligence models, our research is focused on integrating a wide array of data, including genes, proteins, chemical compounds and diseases, and developing integrated databases. We also develop methods for predicting protein structure, function and interaction, and apply them to real-life data analysis.

Research Programs

1) Data integration for linking events that occur at the molecular level to higher-order biological systems
2) Understanding and predicting molecular interactions involving proteins, and modelling biological responses

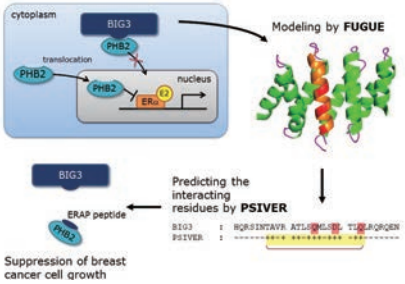
Research Topics

♦ A large volume of data, relevant to protein science and drug discovery research, are available from public databases. However, many issues exist before utilizing such “big data”. For example, the lack of details for the experimental conditions makes it difficult to search and select appropriate data entries, and terminologies and units are often unstandardized. Similar issues concerning data standardization span a wide range of domains including molecular biology and clinical informatics.

We specifically aim to bridge events occruing at the molecular level and in higher-order biological systems, and develop integrated databases and related methodologies. We have performed extensive manual curation on a dataset of chemical structures and associated pharmacokinetic parameters. We also developed TargetMine (https://targetmine.mizuguchilab.org), an integrated data warehouse for assiting early-stage drug discovery. In TargetMine, we compile relationships such as those between genes and disesase/phenotypes from multiple data sources, and we aim to introduce unified vocabularies and ontologies, as well as developing relevant anlaysis tools.

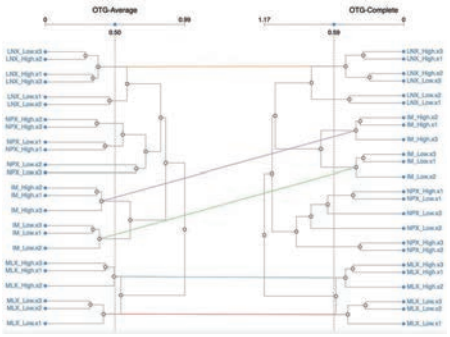
Data sources integrated in TargetMine

♦ The increasing amounts of experimentally-determined protein structures and interations have made it possible to predict protein structure, function and interaction from amino acid sequence information alone. We develop novel comutaiontal methods using machine learning and other techniques, but also focus on the analysis of specific biological systems, aiming to present hypotheses testable by direct experimentation. For example, we predicted the structure and binding-sites of BIG3, a novel gene exclusively overexpressed in breast cancer cells. A peptide designed based on this knowledge has been shown to block the interactions between BIG3 and its partner protein, and suppress the growth of breast cancer cells both *in vitro* and *in vivo*. Protein-protein intractions are attractive targets both in drug discovery and basic research, and remain one of our major research themes.



Predicting protein interactions for a new therapeutic strategy

♦ When the available data are insufficient, alternative approaches, notably, mathematical or rule-based modelling, are required. We combine molecular dynamics simuilations (physics-based) and informatic analysis for understanding molecular mechanisms of protiens. We also combine mathematical models for intra-cellular signaling and chemoinformatic anlysis for predicting biological responses to chemical perturbations. Other research projcts include the development of visualization tools, for better interpretations and assisting analysis in general.



Visualiztion tool for comparing clustering results.

Publications

1. The TargetMine data warehouse: enhancement and updates. Chen Y-A, Tripathi LP, Fujiwara T, Kameyama T, Itoh MN, Mizuguchi K. (2019) *Front Genet.* **10**, 934.
2. Curation can Improve the Prediction Accuracy of Metabolic Intrinsic Clearance. Esaki T, Watanabe R, Kawashima H, Ohashi R, Natsume - Kitatani Y, Nagao C, Mizuguchi K. (2019) *Mol Inform.* **38**, 1800086.
3. Integrating sequence and gene expression information predicts genome-wide DNA-binding proteins and suggests a cooperative mechanism. Ahmad S, Prathipati P, Tripathi LP, Chen YA, Arya A, Murakami Y, Mizuguchi K. (2018) *Nucleic Acids Res.* **46**, 54-70.
4. A-kinase anchoring protein BIG3 coordinates oestrogen signalling in breast cancer cells. Yoshimaru T, Ono M, Bando Y, Chen YA, Mizuguchi K, Shima H, Komatsu M, Imoto I, Izumi K, Honda J, Miyoshi Y, Sasa M, Katagiri T. (2017) *Nat Commun.* **8**, 15427.
5. Network analysis and in silico prediction of protein-protein interactions with applications in drug discovery. Murakami Y, Tripathi LP, Prathipati P, Mizuguchi K. (2017) *Curr Opin Struct Biol.* **44**, 134-142.
6. Ligand-induced Ordering of the C-terminal Tail Primes STING for Phosphorylation by TBK1. Tsuchiya Y, Jounai N, Takeshita F, Ishii KJ, Mizuguchi K. (2016) *EBioMedicine* **9**, 87-96.
7. Applying the Naive Bayes classifier with kernel density estimation to the prediction of protein-protein interaction sites. Murakami Y, Mizuguchi K. (2010) *Bioinformatics* **26**, 1841-8.
8. FUGUE: sequence-structure homology recognition using environment- specific substitution tables and structure-dependent gap penalties. Shi J, Blundell TL, Mizuguchi K. (2001) *J Mol Biol.* **310**, 243-57.

Laboratory for Nuclear Dynamics

Spec. Appoint. Prof. **Takashi Nagano, M.D., Ph.D.**
takashi.nagano @protein.osaka-u.ac.jp

Professor (Concurrent) Takahisa Furukawa, M.D., Ph.D.

Exploring a wonder of cell nucleus how 2-meter-long DNA is sophisticatedly packed to organize life

Nearly all somatic cells within an individual human body carry identical genome. Therefore, epigenetic information (including chemical modification of DNA or chromatin proteins and higher-order (three-dimensional) chromatin structure) that modifies genome function in cell type-specific manner is essential for appropriate cell differentiation. Among such epigenetic information, higher-order chromatin structure is still enigmatic, and our research goal is to elucidate its regulation and dynamics.

To analyze higher-order chromatin structure, typical approaches are either microscopic techniques like FISH or molecular biological methods such as Hi-C. Microscopic techniques have revealed cell-to-cell variability of the structure but not on a genome-wide scale, while molecular biological methods are comprehensive throughout the genome but ignore cell-to-cell variability.

To bridge these two approaches, we pioneered to develop single-cell Hi-C in 2013 and have further improved the technique to acquire thousands of single-cell Hi-C data with reasonable genomic coverage. As a result, we have found dynamic and continuous reorganization in higher-order chromatin structure along with cell cycle progression in interphase cells.

These unprecedented dynamics open up number of further questions regarding higher-order chromatin structure, such as the relationship to DNA replication, other epigenetic information and cell differentiation. By developing state-of-the-art technologies, we aim to pursue such questions to understand how higher-order chromatin structure is regulated and involved in essential life events.

Research Programs

1) Developing state-of-the-art technologies to analyze the dynamic aspects of higher-order chromatin structure
2) Analyses on the dynamic aspects of higher-order chromatin structure using single-cell technologies

Publications

1. Collombet S, Ranisavljevic N, Nagano T, et al. Parental-to-embryo switch of chromosome organization in early embryogenesis. (2020) *Nature*. **580**: 142-146.
2. Nagano T, Lubling Y, Várnai C, et al. Cell-cycle dynamics of chromosomal organization at single-cell resolution. (2017) *Nature*. **547**: 61-67.
3. Nagano T, Lubling Y, Yaffe E, et al. Single-cell Hi-C for genome-wide detection of chromatin interactions that occur simultaneously in a single cell. (2015) *Nat Protoc.* **10**: 1986-2003.
4. Nagano T, Várnai C, Schoenfelder S, et al. Comparison of Hi-C results using in-solution versus in-nucleus ligation. (2015) *Genome Biol.* **16**: 175.
5. Nagano T, Lubling Y, Stevens TJ, et al. Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. (2013) *Nature*. **502**: 59-64.

Laboratory for Infection Systems

Spec. Appoint. Prof. **Yumiko Imai, M.D., Ph.D.**
y-imai@protein.osaka-u.ac.jp

Professor (Concurrent) Mariko Okada, Ph.D.

Study on the response of the nuclear system to viral infection and the mechanisms involved in the severe infectious diseases

Our laboratory aims to understand host responses to virus infection and pathogenesis formation as a system. In particular, we are focusing on the responses of the host nuclear system to viral infection, and the mechanism involved in the formation of lethal disease states. With the progress of recent genome analysis technology, mass spectrometry technology, bioinformatics analysis technology, etc., it has become possible to acquire huge quantitative life science data of multiple levels even in the research field of infection and immunity. While collaborating with domestic and overseas research groups, we comprehensively analyze the quantitative life science data such as host epigenome profiling, mRNA translation profiles, metabolome analysis, single cell transcriptome analysis, human microbiome analysis, and elucidate the networks involved in the molecular pathology and severity of infectious diseases. By applying the genome synthesis and genome editing technology in mouse models, we conduct research aimed at establishing drug discovery, diagnostic methods and preemptive medical cares based on it.

Research Programs

1) Dynamics of higher-order chromatin structural changes to viral infection and their roles in disease pathogenesis
2) Elucidation of host and virus mRNA translation mechanism to viral infection
3) Neuro-immune cross talk in the pathology of virus infection
4) Prediction of dynamic network responsible for the formation of severe pathology of virus infection and its application to preemptive therapy

Publications

1. Minato T, Nirasawa S, Sato T, et al. B38-CAP is a bacteria-derived ACE2-like enzyme that suppresses hypertension and cardiac dysfunction. (2020) *Nature Communications.* **11**(1):1058.
2. Momota M, Lelliott P, Kubo A, et al. ZBP1 governs the inflammasome-independent IL-1α and neutrophil inflammation that play a dual role in anti-influenza virus immunity. (2020) *Int Immunol.* **32**(3):203-212.
3. Fujiwara S, Hoshizaki M, Ichida Y, et al. Pulmonary phagocyte-derived NPY controls the pathology of severe influenza virus infection. (2019) *Nature Microbiology.* **4**(2):258-268.
4. Yamaguchi T, Suzuki T, Sato T, et al. The CCR4-NOT deadenylase complex controls Atg7-dependent cell death and heart function. (2018) *Sci Signal.* **6**;11(516).
5. Blank T, Detje CN, Spieß A, et al. Prinz M. Brain Endothelial- and Epithelial-Specific Interferon Receptor Chain 1 Drives Virus-Induced Sickness Behavior and Cognitive Impairment. (2016) *Immunity.* **44**(4):901-12.
6. Katahira J, Dimitrova L, Imai Y, et al. NTF2-like domain of Tap plays a critical role in cargo mRNA recognition and export. (2015) *Nucleic Acids Res.* **43**(3):1894-904.
7. Morita M, Kuba K, Ichikawa A, et al. The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. (2013) *Cell.* **153**(1):112-25.
8. Ichikawa A, Kuba K, Morita M, et al. CXCL10-CXCR3 enhances the development of neutrophil-mediated fulminant lung injury of viral and nonviral origin. (2013) *Am J Respir Crit Care Med.* **187**(1):65-77.
9. Neely G, Kuba K, Cammarato A, et al. A global in vivo Drosophila RNAi screen identifies NOT3 as a conserved regulator of heart function. (2010) *Cell.* **141**(1):142-153.
10. Imai Y, Kuba K, Neely GG, et al. Identification of oxidative stress and toll like receptor 4 signaling as a key pathway of acute lung injury. (2008) *Cell.* **133**(2):235-49.
11. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. (2005) *Nature.* **436**(7047):112-6.
12. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. (2005) *Nat Med.* **11**(8):875-9.

Laboratory of Visiting Scientists

Visiting Professor **Toshio Yamazaki, D.Sci.**
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Nuclear magnetic resonance (NMR) was able to exceed the wall of hydrogen nuclear resonance frequency 1GHz by using high-temperature superconducting materials. High-temperature superconducting materials have the potential to create magnetic fields that are several times stronger than the magnetic field upper limit in conventional materials because the critical magnetic field is very high. By the frequency increases, further sensitivity and resolution can be expected. In addition to the development of NMR magnets, it is necessary to develop NMR devices and NMR measurement technologies that make use of ultra-high magnetic field magnets. We are developing equipment together with magnet development. Strengthening the uniformity and stabilization of the magnetic field, it is necessary to sophistication of the detector. In addition to sensitivity resolution, research that makes use of the characteristics of high magnetic fields is interesting. The line shape of the quadrupole nucleus is magnetic field dependent, the relaxation by molecular motion comes out strongly, and development such as easy to take out structural information by using molecular orientation can be expected. By combining molecular structure control methods, including light, we aim to diversify NMR measurements.

Research Programs

1) Development and utilization of ultra-high magnetic field NMR
2) Study on structural changes in proteins by optically synchronized NMR
3) NMR research in various states

Publications

1. Nagashima, Toshio; Ueda, Keisuke; Nishimura, Chiaki; Yamazaki, Toshio, Anal. (2015) *Chem.* **87**, 11544-52.
2. Kenjiro Hashi, Kenzo Deguchi, Toshio Yamazaki, ..., and Tadashi Shimizu, (2016) *Chemistry Letters* **45**, 209-210.
3. Guzmán-Afonso C, Hong YL, Colaux H, Iijima H, Saitow A, Fukumura T, Aoyama Y, Motoki S, Oikawa T, Yamazaki T, Yonekura K, Nishiyama Y., (2019) *Nat Commun.* **10**, 3537.

Visiting Academic Staff **Tetsuya Takeda, Ph.D.**
ttakeda@okayama-u.ac.jp

To elucidate the mechanism by which various cell shapes in organisms are produced, we are conducting research focusing on membrane remodeling molecules that control membrane deformation and fission. Furthermore, we also try to elucidate pathomechanisms of intractable diseases or other diseases such as cancer caused by defective membrane remodeling using multiple approaches including cell biology, biophysics, structural biology and model organisms.

Research Programs

1) Elucidation of membrane remodeling mechanisms by dynamin and BAR domain proteins
2) Elucidating pathogenesis of intractable diseases caused by defective dynamin and BAR domain proteins
3) Elucidation of the function of memrbane remdeling machinery in cancer invasion

Publications

1. Takeda T, Kozai T, Yang H, Ishikuro D ⋯, and Takei K. (2018) *eLIFE* **7**, e30246.
2. Zhang Y, Nolan M, ⋯, and Takeda T. Biochem. Biophis. Res. (2016) *Commun.* **480**, 409-414.
3. Takeda T, Robinson I.M., Savoian, M.M. ⋯, and Glover D.M. (2013) *Open Biol.* **3**(8), 130081.
4. Takeda T., Kawate T. and Chang F. Nat. (2004) *Cell Biol.* **6**(11), 1142-1144.

Visiting Academic Staff **Yuta Suzuki, Ph.D.**
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The ability of chemists to act as ”designers” to modify protein structure and functions using a combination of rationale design and directed evolution has the potential to usher in the new era allowing the development of “smart bionanorobots programmed to perform multiple tasks for biotechnology applications”. Such ”protein nanorobots” could play active roles in the field of medicine and biotechnology, and development of this technology may not be far away. However, existing protein designs require advanced techniques in order to control the diversity and complexity of protein structures. Therefore, the probability of such success is still low, and many trials-and-errors are required. So far, only primitive construction of protein assembly has been achieved. For this reason, I propose to conduct research to establish “new protein design and engineering methodology” that maximizes the actual and natural characteristics of protein targets toward creating “smart bionanorobots”, rather than forcefully controlling such potentials and characteristics.

Research Programs

1)Protein Design and Engineering toward Creation of Functional Biomaterials

Publications

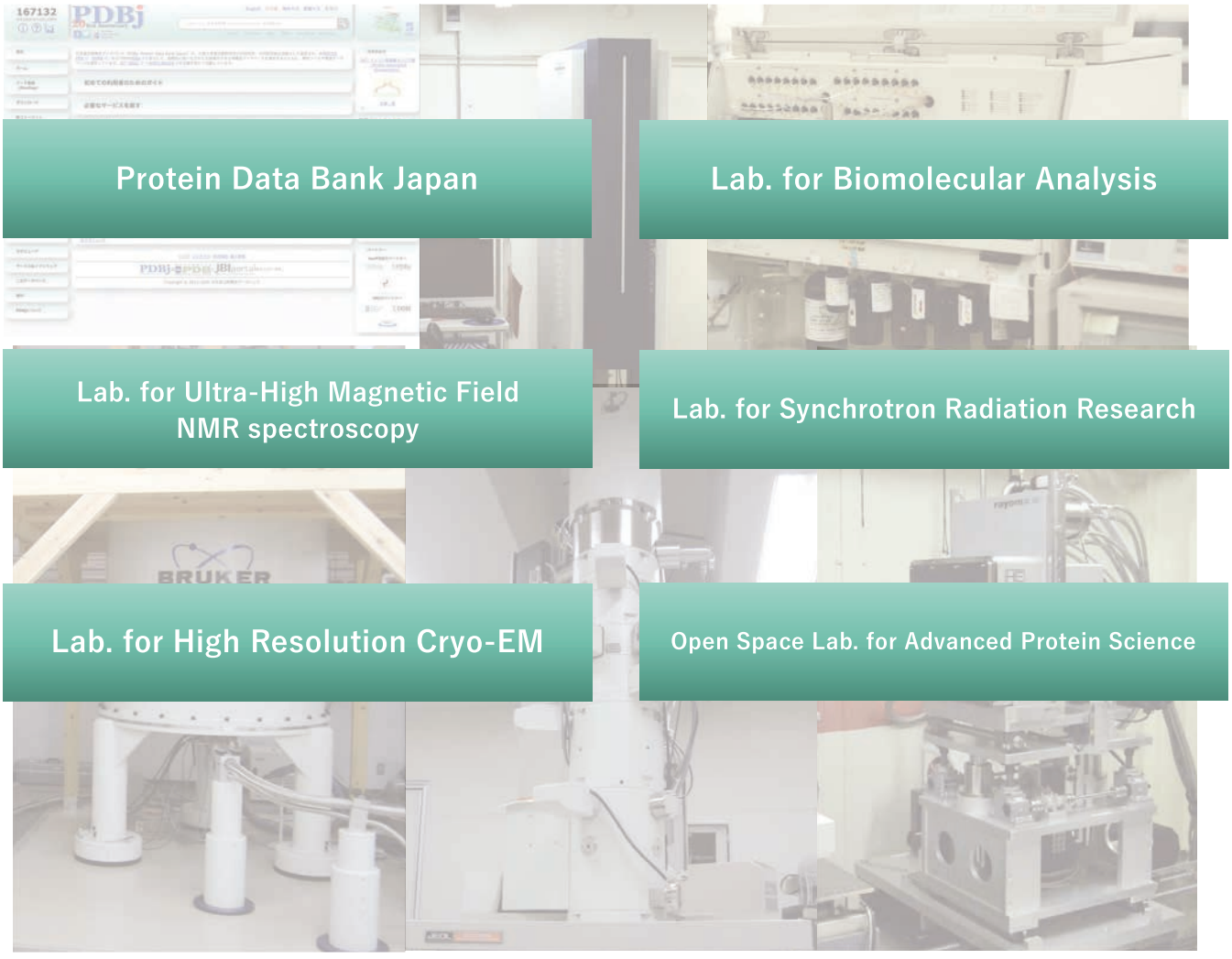
1. Alberstein, R.; Suzuki, Y.; Paesani, F.; Tezcan, F. A. (2018) *Nature Chemistry* **10**, 732 – 739.
Highlighted in Materials Today, Space Daily, and etc.
2. Suzuki, Y.; Cardone, G.; Restrepo, D.; Zavattieri, P. D.; Baker, T. S.; Tezcan, F. A. (2016) *Nature* **533**, 369 – 373.
Highlighted in C&EN, Chemistry world, ScienceDaily, Daily Mail, and Materials Today, and etc.

Research Center for Next-Generation Protein Sciences

- Protein Data Bank Japan
- Laboratory for Ultra-High Magnetic Field NMR Spectroscopy
- Laboratory for Synchrotron Radiation Research
- Laboratory for High Resolution Cryo-EM
- Laboratory for Biomolecular Analysis
- Open Space Laboratory for Advanced Protein Science

Research Center for Next-Generation Protein Sciences

The Research Center for Next-Generation Protein Sciences was founded in October 2020 as a Joint Usage/Research Center that aims to strengthen international and industry-academia collaboration.
In the field of Structural Biology, the hardware/software of Cryo-electron microscopy, X-ray crystallography, and NMR spectroscopy have improved remarkably in recent years, enabling us to analyze detailed atomic structures and molecular interactions in a short amount of time even if the targets are large protein complexes. With these advances in Structural Biology, the Protein Data Bank archive has been drastically enriched in terms of both quality and quantity, and its importance as a Biodata Resource has come to be strongly recognized. Moreover, since not only the static and detailed precise structure of each protein, but dynamic structural information such as in situ imaging has also become important, it is essential that we do not just use traditional techniques, but also move forward to the Integrated/Hybrid Method which obtains useful structural information using a combination of techniques.
The Research Center includes faculty members from the main divisions of the institute and aims to strengthen next-generation structural biology research through the integration of several sophisticated techniques, including implementation of the Integrated/Hybrid method, advancement of various structural analysis methods, construction and operation of a global database, and utilization of an open space lab. We therefore expect this research center to serve as a hub for the protein research community both domestic and internationally.

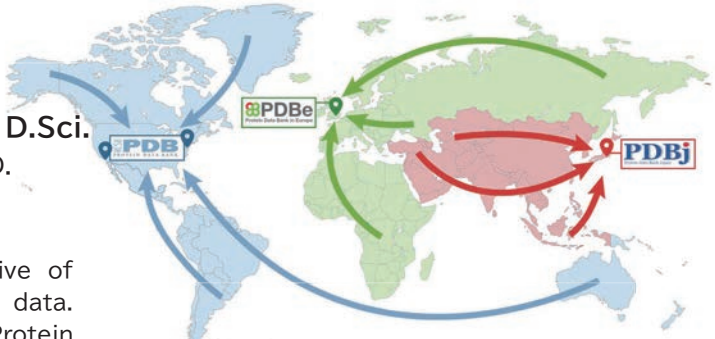


Protein Data Bank Japan

Managing the single global database (PDB) collaborating with other data centers in USA and Europe

♦ PDBj (Protein Data Bank Japan)

Professor (Concurrent)	**Genji Kurisu, Ph.D.**
Professor (Concurrent)	**Toshimichi Fujiwara, D.Sci.**
Spec. Appoint. Assoc. Prof.	Takeshi Kawabata, Ph.D.
Spec. Appoint. Assist. Prof.	Gert-Jan Bekker, Ph.D.



Protein Data Bank (PDB) is the single global archive of experimentally determined macromolecular structure data. The PDB archive is managed jointly by the worldwide Protein Data Bank (wwPDB: https://wwpdb.org/) consortium which has four partners – the Protein Data Bank Japan (PDBj), the RCSB Protein Data Bank (RCSB PDB), the Protein Data Bank in Europe (PDBe), and BioMagResBank (BMRB). The wwPDB also manages related experimental data/metadata from X-ray, NMR (Biological Magnetic Resonance Data Bank: BMRB) and cryoEM (Electron Microscopy Data Bank: EMDB).

We, the PDBj (Protein Data Bank Japan; https://pdbj.org), are the founding member of the wwPDB, and curate, annotate and process the all PDB, BMRB and EMDB data from Asia and middle-East region. Data/metadata processed locally at each PDB site are all shared by the wwPDB members (PDBj in Asia, RCSB PDB and BMRB in US, and PDBe in Europe), and then weekly updated all PDB data are released freely on each web site. In addition, we provide the new tools in the field of biological NMR spectroscopy, and various services for researchers and students who are interested in structural biology: molecular graphics viewer, Molmil; molecular surface database for functional sites, eF-site; and EM Navigator, image viewer for the structures by electron microscopy.

PDBj also serves as the broker site for EMPIAR (https://empiar.pdbj.org) database which is an archive of micrograph raw images, and started the new archive for biological structural models named "BSM-Arc" (https://bsma.pdbj.org). The RCSB PDB Molecule of the Month by David S. Goodsell (RCSB PDB) presents short accounts on selected molecules from the PDB. PDBj translates the Molecule of the Month into Japanese. Each installment includes an introduction to the structure and function of the protein molecules.

https://pdbj.org

https://bmrbdep.pdbj.org

Publications

1. New tools and functions in data-out activities at Protein Data Bank Japan (PDBj). Kinjo AR, Bekker G-J, Wako H, Endo S, Tsuchiya Y, Sato H, Nishi H, Kinoshita K, Suzuki H, Kawabata T, Yokochi M, Iwata T, Kobayashi N, Fujiwara T, Kurisu G, Nakamura H. (2018) *Protein Sci.* **27**, 95-102.
2. Protein Data Bank Japan (PDBj): updated user interfaces, resource description framework, analysis tools for large structures. Kinjo AR, Bekker G-J, Suzuki H, Tsuchiya Y, Kawabata T, Ikegawa Y, Nakamura H, (2017) *Nucl. Acids Res.* **45**, D282-D288.
3. Publication of nuclear magnetic resonance experimental data with semantic web technology and the application thereof to biomedical research of proteins. Yokochi M, Kobayashi N, Ulrich EL, Kinjo AR, Iwata T, Ioannidis YE, Livny M, Markley JL, Nakamura H, Kojima C, Fujiwara T, (2016) *J. Biomed. Semantics* **7**, 16.
4. An automated system designed for large scale NMR data deposition and annotation: Application to over 600 assigned chemical shift data entries to the BioMagResBank from the RIKEN Structural Genomics/Proteomics Initiative internal database. Kobayashi N, Harano Y, Tochio N, Nakatani E, Kigawa T, Yokoyama S, Mading S, Ulrich EL, Markley JL, Akutsu H, Fujiwara T. (2012) *J. Biomol. NMR* **53**, 311-320.

Laboratory for Ultra-High Magnetic Field NMR Spectroscopy

Associate Professor Yohei Miyanoiri, Ph.D.
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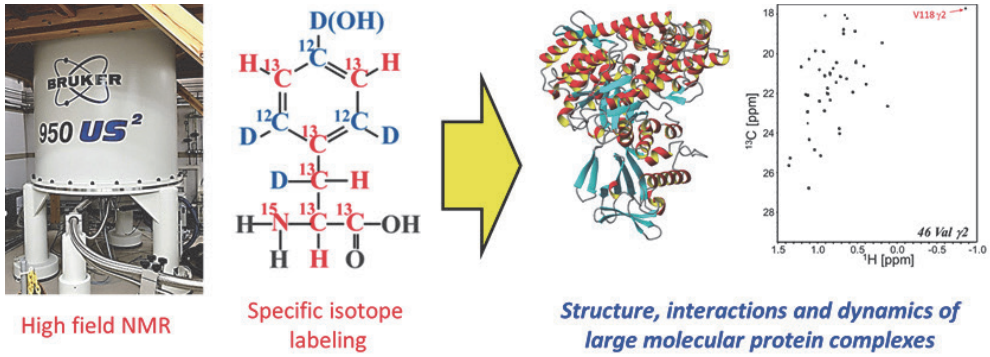
Spec. Appoint. Assist. Prof. (Concurrent) Toshihiko Sugiki, Ph.D

Elucidating correlation between structure, dynamics and biological functions of macromolecular proteins by advanced NMR

Our research is concerned with structural and dynamical aspects of protein function. We are interested in elucidating correlation between structure, dynamics and biological functions of macromolecular proteins. To pursue our goals, we use nuclear magnetic resonance spectroscopy (NMR).
At present, the solution NMR study of larger proteins (>50 kDa) relies exclusively upon the information obtained from the backbone ^{15}NH and methyl ^{13}CH$_3$ signals, which are not sufficient for precise dynamics and structural analysis of proteins. In order to solve this problem, we have been developing the Stereo-Array Isotope Labeling (SAIL) NMR methods. The new SAIL NMR methods allow us to observe the several ^{1}H-^{13}C signals in 80 – 1000 kDa large molecular proteins. This information is very useful for understanding the precise structure and dynamic property of various macromolecular proteins such as membrane proteins and protein-protein complexes.



High field NMR | Specific isotope labeling | *Structure, interactions and dynamics of large molecular protein complexes*

Research Programs

1) Development of new NMR methods for structure and dynamics study of larger proteins
2) Further optimization of stable isotope labeling of SAIL amino acids
3) Quantitative analysis of structural rearrangement for proteins upon interacting with other molecules

Publications

1. Recent Developments in Isotope-aided NMR Methods for Supramolecular Protein Complexes -SAIL Aromatic TROSY. Miyanoiri Y, Takeda M, Terauchi T, Kainosho M (2019) *BBA Gen Subj,* **1864**(2), 129439.
2. Aromatic Ring Dynamics, Thermal Activation, and Transient Conformations of a 468 kDa Enzyme by Specific 1H-13C Labeling and Fast Magic-Angle Spinning NMR. Gauto DF, Macek P, Barducci A, Fraga H, Hessel A, Terauchi T, Gajan D, Miyanoiri Y, Boisbouvier J, Lichtenecker R, Kainosho M, Schanda P. (2019) *J Am Chem Soc.* **141**(28), 11183-11195.
3. Evolution and diversification of the plant gibberellin receptor GID1. Yoshida H, Tanimoto E, Hirai T, Miyanoiri Y, Mitani R, Kawamura M, Takeda M, Takehara S, Hirano K, Kainosho M, Akagi T, Matsuoka M, Ueguchi-Tanaka M (2018) *Proc. Natl. Acad. Sci. USA,* **115**(33), E7844-E7853.
4. Perspective: next generation isotope-aided methods for protein NMR spectroscopy. Kainosho M, Miyanoiri Y, Terauchi T, Takeda M (2018) *J. Biomol. NMR,* **71**(3), 119-127.
5. Structural and functional analysis of the C-terminal region of FliG, an essential motor component of Vibrio Na^+-driven flagella. Miyanoiri Y, Hijikata A, Nishino Y, Gohara M, Onoue Y, Kojima S, Kojima C, Shirai T, Kainosho M, Homma M (2017) *Structure,* **25**, 1540-48.
6. Highly efficient residue-selective labeling with isotope-labeled Ile, Leu, and Val using a new auxotrophic *E. coli* strain. Miyanoiri Y, Ishida Y, Takeda M, Terauchi T, Inouye M, Kainosho M (2016) *J. Biomol. NMR,* **65**, 109-19.
7. Myosin VI undergoes cargo-mediated dimerization. Yu C, Feng W, Wei Z, Miyanoiri Y, Wen W, Zhao Y, Zhang M (2009) *Cell,* **138**, 537-48.

Laboratory for Synchrotron Radiation Research

Associate Professor **Eiki Yamashita, Ph.D.**
eiki@protein.osaka-u.ac.jp

Professor (Concurrent) Atsushi Nakagawa, D. Sci.

http://www.protein.osaka-u.ac.jp/rcsfp/supracryst/research/beamline/

Development of synchrotron radiation structural biology

We are working on development of new methodologies for X-ray protein crystallography using the synchrotron radiation beamline for biological macromolecular assemblies at SPring-8 (BL44XU). This beamline utilizes high-brilliant undulator radiation of SPring-8 to collect high quality diffraction data from biological macromolecular assembly crystals.

Research Programs

1) Development of high-precision diffraction data collection system using Synchrotron Radiation
2) Structural studies of biological macromolecular assemblies

Publications

1. SPring-8 BL44XU, a synchrotron radiation beamline for biological macromolecular assemblies, operated by the Institute for Protein Research, Osaka University. Yamashita E, Nakagawa A (2019) *Biophysical Reviews*, **11**, 521-523.
2. Structures of the wild-type MexAB–OprM tripartite pump reveal its complex formation and drug efflux mechanism. Tsutsumi K, Yonehara R, Ishizaka-Ikeda E, Miyazaki N, Maeda S, Iwasaki K, Nakagawa A, Yamashita E (2019) *Nature Communications*, **10**, 1520.
3. SPring-8 BL44XU, beamline designed for structure analysis of large biological macromolecular assemblies. Higashiura A, Yamashita E, Yoshimura M, Hasegawa K, Furukawa Y, Kumasaka T, Ueno G, Yamamoto M, Tsukihara T (2016) *AIP Conference Proceedings*, **1741**, 030028.
4. A high-resolution structure of pre-microRNA nuclear export machinery. Okada C, Yamashita E, Lee SJ, Shibata S, Katahira J, Nakagawa A, Yoneda Y, Tsukihara T (2009) *Science*, **326**, 1275-1279.

Laboratory for High Resolution Cryo-EM

Professor(Concurrent) Takayuki Kato, Ph.D.
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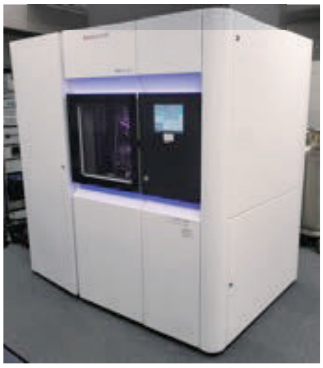
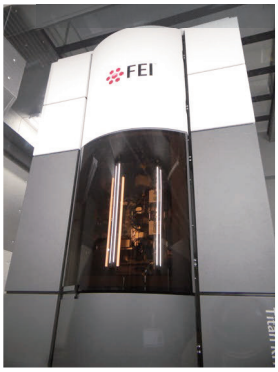
Support for the structural analysis of the biological samples by Cryo-EM

Our mission is to develop single-particle electron cryo-microscopy (cryo-EM) and provide high-quality data using cutting edge cryo-EM instruments. Our cryo-EM facility has three electron cryo-microscopes and one conventional transmission electron microscope. The best of them is Titan Krios equipped with recent advanced devices which are direct electron cameras, K3 Summit with an energy filter as well as Falcon3 Cs-corrector. In addition, both Volta phase plate (VPP) and spherical aberration corrector (Cs-corrector) are installed here. VPP is a powerful tool to visualize small biological molecules. This Cs-corrector is the only one installed within the biology field in Japan. Since the 6,000 images/day can be collected by automatic image acquisition system, it will also possible to analyze the structural variation of target molecules.

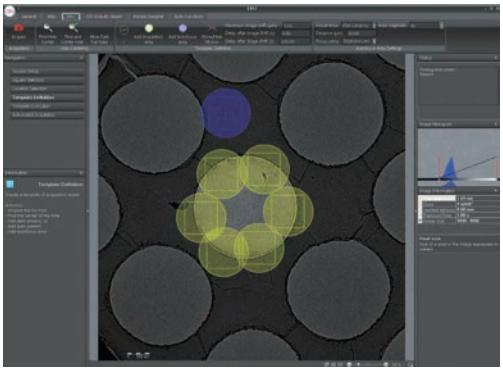
Talos Arctica



Titan Krios



Automatic Data Acquisition



Research Programs

1) Development of single-particle cryo-EM methods for drug discovery and development
2) Development of the methods for structural determination of a small protein using cryo-EM
3) Analysis of structural polymorphism of target molecules

Publications

1. Structual and Functional Comparison of Salmonella Flagellar Filaments Composed of FljB and FliC. T. Yamaguchi, S. Toma, N. Terahara, T. Miyata, M. Ashihara, T. Minamino, K. Namba, T. Kato. (2020) *Biomolecules*, **10**(2),246.
2. Strucutre of native supercoiled flagellar hook as a universal joint. T. Kato, F. Makino, T. Miyata, P. Horvath, K. Namba. (2019) *Nat. Comm.*, **10**(1), 5295.
3. Strucutre of Salmonella flagellar hook reveals intermolecular domain interactions for the universal joint function. P. Horvath, T. Kato, T. Miyata, K. Namba, (2019) *Biomoleucles*, **9**(9),462.
4. CryoTEM with a cold emission gun that moves structural biology into a new stage. T. Kato, F. Makino, T. Nakane, N. Terahara, T. Kaneko, Y. Shimizu, S. Motoki, I. Ishikawa, K. Yonekura, K. Namba. (2019) *Microsc. Microanal.*, **25**, 998-999.
5. 生命を解き明かすクライオ電子顕微鏡法の新時代　最新クライオ電子顕微鏡 CRYOARM の開発、加藤貴之、難波啓一（2018）*顕微鏡*、**53**(1), 13-17.
6. クライオ電子顕微鏡法の技術開発と生命科学への貢献、難波啓一、加藤貴之（2018）*日本電子 news*、**50**(1), 2-7.
7. Technoical Development of Electron Cryomicroscopy and Contributions to Life Sciences. (2018) *JEOL NEWS*, **50**(1), 2-7.
8. 2017 年ノーベル科学賞　クライオ電子顕微鏡の開発、加藤貴之、難波啓一（2017）*現代科学*、**12**, 40-44.

Laboratory for Biomolecular Analysis

Associate Professor **Nobuaki Okumura, Ph.D.**
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Professor (Concurrent) Toshifumi Takao, D.Sci.
Junichi Takagi, Ph.D.
Atsushi Nakagawa, D.Sci.

Support for protein analysis by N-terminal sequencing, HPLC etc.

Our laboratory works on protein analysis based on primary protein structures by N-terminal sequencing and mass spectrometry. Our laboratory supports researches that require protein identification, protein characterization and quality control of recombinant proteins, etc. We accept requests from inside and outside our institute for N-terminal protein sequencing by protein sequencers. In addition, we maintain several instruments for general protein analysis such as HPLC in addition to mass spectrometers as shared equipments. We also aim to develop advanced techniques that improve the sensitivity and efficiency for protein characterization.

Research Programs

1) Support for protein researches by N-terminal protein sequencing
2) Development and application of primary structure analysis by N-terminal protein sequencers and mass spectrometers

Publications

1. The zinc form of carnosine dipeptidase 2 (CN2) has dipeptidase activity but its substrate specificity is different from that of the manganese form. Okumura N. and Takao T. (2017) *BBRC* **494,** 484-490.
2. Evidence for an essential role of intradimer interaction in catalytic function of carnosine dipeptidase II using electrospray-ionization mass spectrometry. Okumura N, Tamura J, Takao T. (2016), *Protein Science* **25**, 511-522.
3. Carnosine dipeptidase II. Okumura N. (2013) in *Handbook of proteolytic enzymes, 3rd ed.,* pp. 1596-1600, Elsevier.
4. Diversity in protein profiles of individual calcium oxalate kidney stones. Okumura N, et al. (2013) *PLos One* **8**, e68624.
5. Identification of cargo proteins specific for importin-beta with importin-alpha applying a stable isotope labeling by amino acids in cell culture (SILAC)-based in vitro transport system. Kimura M, et al. (2013) *J. Biol. Chem.* **288**, 24540-24549.

Open Space Laboratory for Advanced Protein Science

Visiting Professor **Kazuya Matsumoto, Ph.D.**
Kazuya.Matsumoto@mitsuichemicals.com

Acceralate industrial development and international collaboration through Joint Usage/Research

Various biomarkers are being developed for personalized medicine, however few can clearly explain the relationship with diseases in molecular level. In this study, we aim to search for new biomarkers then elucidate the mechanism of biomarker production *in vivo* through detailed analysis of their molecular structures.

I have been working for industrial application of various proteins in the chemical industry. In biopharmaceutical development, we had analyzed complex interactions between hormones and membrane receptors. In the development of membrane protein sensor, discovered membrane protein stabilization and orientation control technologies. In the enzymatic process development, we have established the technology that allows enzyme works stably through tons-scale chemical reactions.

Even solved many technical issues, large number of projects have been quitted due to changes in the business situation or cost related problems. Fortunately, I was able to realize the commercialization of item from test tubes to market.

For industrial application of basic research, it is important not only to establish individual technologies but also to devise solutions by holistic approach on various issues including society, environment, cost and time. Therefore, cross-sectoral collaboration is required.

I think that the key for successful collaboration is how interesting in science. Furthermore, the project that can convey the interestingness of science to society and customers correctly will overcome various barriers such as cost or market competition etc. Intellectual curiosity moves people in all fields.

This time, I had a great opportunity to pursue science at this institute, which has a long history in cross-disciplinary collaboration in protein research. With the help of advanced technology, I would like to not only discover new biomarkers but also try to understand their roll in diseases.

Research Programs

1) Discovery of new biomarkers by mass spectrometry
2) Development of high-speed quantitative technique by immunochemical analysis
3) Structure determination of biomarkers and elucidation of their roll *in vivo*

Publications

1. Self-assembled photosystem-I biophotovoltaics on nanostructured TiO2 and ZnO. Mershin, A., Matsumoto, K, Kaiser, L., Yu, D., Vaughn, M., Nazeeruddin, MD. K., Bruce, B. D., Graetzel, M., Zhang, S. (2012) *Scientific Reports* 2: 234: 1-7.
2. Enhanced Electron Transfer Activity of Photosystem I by Polycations in Aqueous Solution. Matsumoto, K., Zhang, S., Koutsopoulos, S. (2010) *Biomacromolecules* 11: 3152-3157.
3. Designer peptides surfactants stabilize functional Photosystem-I membrane complex in aqueous solution. Matsumoto, K., Vaughn, M.; Bruce, B. D., Koutsopoulos, S., Zhang, S. (2009) *J. Phys. Chem. B* 113: 75-83.
4. Cellular activities of 20K- and 22K-hGH do not necessarily correlate with their binding affinities for rat GH receptor. Ikeda, M. Matsumoto, K., Uchida, H., Naito, N., Tsunekawa, B., Wada, M., Honjo, M. (2000) *Hormone Research*, 54; 136-142.

Division of Donated Fund Research

Division for Matrixome Research and Application

Division for Matrixome Research and Application

Professor Kiyotoshi Sekiguchi, Ph.D.
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Assistant Professor Yukimasa Taniguchi, Ph.D.

Uncovering the mechanisms governing homeostasis and dynamics of multicellularity through cell-extracellular matrix interactions

Our long-term goal is to understand the molecular mechanisms defining morphogenetic interactions of cells with their surrounding microenvironment, i.e., extracellular matrices (Fig. 1). We are particularly interested in the roles of the basement membrane in histogenesis/organogenesis, with an emphasis on the molecular interactions of basement membrane proteins with their receptors on the cell surface as well as the resulting signaling events that regulate proliferation, differentiation, apoptosis, and motility of cells. Emphasis is also given to the fabrication of culture substrates customized for individual cell types including pluripotent and tissue stem cells to promote stem cell research and regenerative medicine.

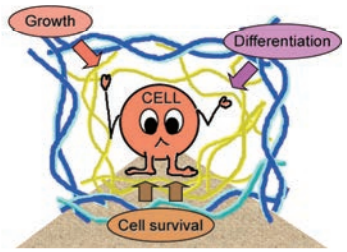

Fig. 1. Regulation of cell growth, differentiation, and survival by extracellular matrix.

Research Programs

1) Comprehensive immunohistochemical profiling of basement membrane proteins
2) Regulatory mechanisms of cellular functions by extracellular matrices
3) Molecular basis of extracellular matrix recognition by integrins
4) Physiological functions of polydom in vascular development
5) Development of culture substrates for embryonic and tissue stem cells

Publications

1. Laminin is the ECM niche for trophoblast stem cells. Kiyozumi D, Nakano I, Sato-Nishiuchi R, Tanaka S, Sekiguchi K (2020) *Life Sci. Alliance* **3**, e201900515.
2. Bipartite mechanism for laminin-integrin interactions: Identification of the integrin-binding site in LG domains of the laminin α chain. Taniguchi Y, Takizawa M, Li S, Sekiguchi K (2020) *Matrix Biol.* **87**, 66-76.
3. Molecular profiling of the basement membrane of pluripotent epiblast cells in post-implantation stage mouse embryos. Futaki S, Nakano I, Kawasaki M, Sanzen N, Sekiguchi K (2019) *Regen. Ther.* **12**, 55-65.
4. Ventricular-subventricular zone fractones are speckled basement membranes that function as a neural stem cell niche. Sato Y, Kiyozumi D, Futaki S, Nakano I, Shimono C, Kaneko N, Ikawa M, Okabe M, Sawamoto K, Sekiguchi K (2019) *Mol. Biol. Cell* **30**, 56-68.
5. Recombinant laminin fragments endowed with collagen-binding activity: a tool for conferring laminin-like cell-adhesive activity to collagen matrices. Sato-Nishiuchi R, Li S, Ebisu F, Sekiguchi, K (2018) *Matrix Biol.* **65**, 75-90.
6. Mechanistic basis for the recognition of laminin-511 by α6β1 integrin. Takizawa M, Arimori T, Taniguchi Y, Kitago Y, Yamashita E, Takagi J, Sekiguchi K (2017) *Sci. Adv.* **3,** e1701497.
7. Polydom is an extracellular matrix protein involved in lymphatic vessel remodeling. Morooka N, Futaki S, Sato-Nishiuchi R, Nishino M, Totani Y, Shimono C, Nakano I, Nakajima H, Mochizuki N, Sekiguchi K (2017) *Circ. Res.* **120**, 1276-1288.

Technology Division

Technology Division

http://www.protein.osaka-u.ac.jp/gijutsu/index.html

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Limited Term Re-emplyed Technical Staff

NORIOKA Naoko	(KURISU Laboratory	Ext. 8605)	naoko@protein.osaka-u.ac.jp

Activity Report

- Animal Facility (Tsujii)
  - Management and maintenance in an animal facility (SPF mice, rats)
  - Technical guidance for experimental animal handling methods
  - Production of genetically modified mice
  - Production of mouse frozen embryos, restoration of mouse from frozen embryos
- Radioisotope Facilities (Abe)
  - Management and maintenance of the RI Facilities
  - Operation and maintenance of the instruments in the RI facilities
  - Management of radiation workers (100 people in 2019)
- General Purpose Computer System and High-performance X-ray Diffractometers (Kosada)
  - Users of CPU servers in 2019: 13 from IPR, 2 from other universities, 1 from other organization
- Protein Data Bank Japan [PDBj] (Yamashita)
  - Management and maintenance of PDB/EMDB Archive (synchronization with wwPDB members)
  - Check the release data/service every Wednesday
  - Management and construction of weekly update system
  - XML validation / Check the XML schema
  - Gather statistics (access / download)
- Laboratory for Biomolecular Analysis (Norioka, Kawakami)
  - Operation and maintenance of the instruments in the molecular analysis laboratory (Room 509)
  - Analysis service for protein sequencing in 2019
    - ABI Procise 49cLC: Osaka University (IPR, Engineering, RIMD; 7 samples in total)
      Others (Shizuoka Univ., Kansai Univ., Toyama Univ., Ehime Univ., Nihon Univ., Niigata Univ., Nagoya Univ.; 20 samples in total)
    - Shimadzu PPSQgradient: 58 samples in total at IPR
- Work at each laboratory

About IPR

- History of IPR
- Directors
- Professors Emeriti
- Joint - Usage ／ Research Center
- Research Activities
- Educational Activities
- IPR Staff
- Administrative Council
- Staff and Accounting Data
- Floor Plan

History of IPR

The Institute for Protein Research (IPR) of Osaka University was established on April 1, 1958, as a joint research based upon the recommendation of the Science Council of Japan and the concurrence between the Ministry of Education. Professor Shiro Akabori was appointed as its director. Since its foundation IPR has been growing as shown in the chronological tables, and we are working as a research center for protein sciences in Japan.

1950s

1956 Set up of a new laboratory in Faculty of Science, Osaka University, for organic chemical studies of proteins and amino acids (the predecessor of IPR) supervised by Prof. Shiro Akabori.

1958 Establishment of Institute for Protein Research as a Joint-use Research Organization.
Advisory Committee on Administration was also founded.

1960s

1961 Main building (4,130㎡) was completed on the former Nakanoshima Campus.

1962 Set up of the Peptide Center.

1965 Set up of a Branch Division (569㎡) in Torii Memorial Hall.

1970s

1971 Main building (7,873 ㎡) of Institute for Protein Research was completed on Suita Campus.

1972 The crystal structure of bonito heart ferrocytochrome c was determined by Prof. Kakudo of IPR for the first time in Japan.

1978 Establishment of Crystallographic Research Center.

1979 Buildings for the Crystallographic Research Center (1,505 ㎡) and NMR Research Laboratory (267㎡) were completed.

1980s

1988 The Research Center for Protein Engineering was established.

1990s

1998 The Center for Structural Biology was established.

2000s

2000 The Protein Data Bank Japan (PDBj) started its operation.

2002 The Research Center for Structural and Functional Proteomics was established.

2004 Transformed into the Research Institute of Japanese National Universities under the National University Corporation Law.

2008 Building of Collaborative Research Facility (1,149㎡) was completed.

2009 Main building reinforcement for earthquake-resistance was completed.

2010s

2010 Certified as a Joint Usage/Research Center by MEXT.

2012 The Research Center for State-of-the-Art Functional Protein Analysis was established.

2016 Certified continuous activities as a Joint Usage/Research Center by MEXT.

2020s

2020 Atomic models of Cytochrome c and Taka-amylase A are registered as Chemical heritage authorized by the Chemical Society of Japan.
The next-generation protein structural analysis center was established.

Directors

1st	AKABORI Shiro	Apr. 1, 1958	-	Nov. 30, 1961
2nd	ISEMURA Toshizo	Dec. 1, 1961	-	Nov. 30, 1965
3rd	SUZUKI Tomoji	Dec. 1, 1965	-	Aug. 14, 1969
4th	NARITA Kouzo	Aug. 15, 1969	-	Aug. 14, 1971
5th	KAKUDO Masao	Aug. 15, 1971	-	Apr. 1, 1982
6th	IZUMI Yoshiharu	Apr. 2, 1982	-	Mar. 31, 1985
7th	SATO Ryo	Apr. 1, 1985	-	Mar. 31, 1987
8th	HORIO Takekazu	Apr. 1, 1987	-	Mar. 31, 1989
9th	KATSUBE Yukiteru	Apr. 1, 1989	-	Mar. 31, 1993
10th	NAKAGAWA Hachiro	Apr. 1, 1993	-	Mar. 31, 1995
11th	SAKIYAMA Fumio	Apr. 1, 1995	-	Mar. 31, 1997
12th	KYOGOKU Yoshimasa	Apr. 1, 1997	-	Mar. 31, 1999
13th	SHIMONISHI Yasutsugu	Apr. 1, 1999	-	Mar. 31, 2000
14th	NAGAI Katsuya	Apr. 1, 2000	-	Mar. 31, 2004
15th	AKUTSU Hideo	Apr. 1, 2004	-	Mar. 31, 2006
16th	TSUKIHARA Tomitake	Apr. 1, 2006	-	Mar. 31, 2008
17th	AIMOTO Saburo	Apr. 1, 2008	-	Mar. 31, 2010
18th	HASE Toshiharu	Apr. 1, 2010	-	Mar. 31, 2014
19th	NAKAMURA Haruki	Apr. 1, 2014	-	Mar. 31, 2018
20th	NAKAGAWA Atsushi	Apr. 1, 2018	-	Present

Professors Emeriti

ASANO Akira	TAKAGI Toshio	NAGAI Katsuya	AKUTSU Hideo
TSUKIHARA Tomitake	AIMOTO Saburo	SEKIGUCHI Kiyotoshi	TAJIMA Shoji
HASE Toshiharu	YOSHIKAWA Kazuaki	NAKAMURA Haruki	GOTO Yuji

Joint Usage and Research Center for Proteins

As a Joint Usage and Research Center, our major activities are to promote; 1) joint usage of advanced facilities and equipment; 2) access to databases; and 3) collaborations including those leading to the development of human resources. Through these activities, we aim to activate protein and life science communities.
IPR makes an annual call for applications to the following seven collaborative projects.

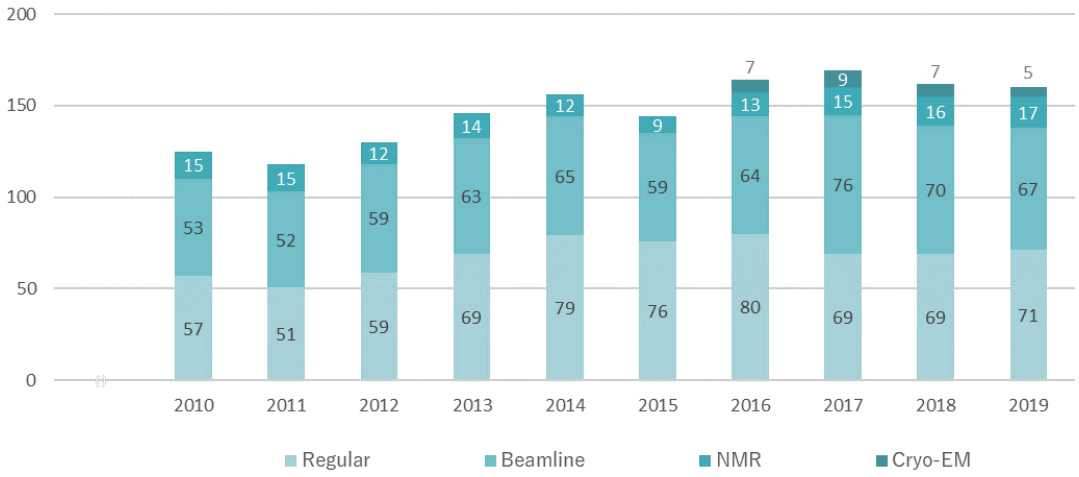
(1) Domestic Collaborators
(2) International Collaborative Research
(3) SPring-8 Collaborative Research
(4) NMR Collaborative Research
(5) Cryo-EM Collaborative Research
(6) IPR Seminars
(7) IPR Fellows

Website of the Joint Usage/Research Center
http://www.protein.osaka-u.ac.jp/en/joint/

Poster for the annual call for applications
※Regular applications are received once per year, and except for IPR Fellows, urgent applications can be accepted all year around.

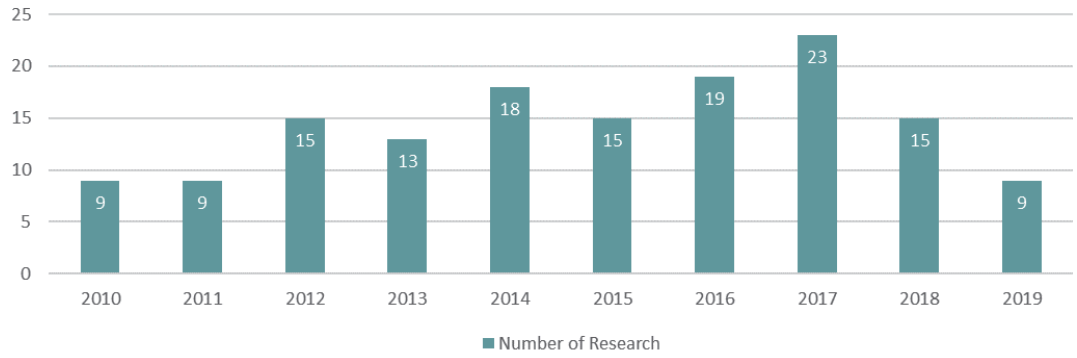
Researchers on Joint Research Program

The Joint Research Program has been established to provide visiting scientists from outside the IPR, who are engaged in studies on proteins, with an opportunity to perform coordinated research at the IPR for up to 6 months. More than 50 scientists are selected yearly from applications from various domestic institutions.
The program covers research and travel expenses, and the institute provides facilities for accommodation. Since the beginning of program in 1959, a total of 4,810 researchers have been admitted. Researchers who want to use large instruments such as X-ray analysis facilities, superconducting magnet NMR, and Cryo-Electron Microscopy (Cryo-EM) are required to apply for this program.



International Collaborative Research Program

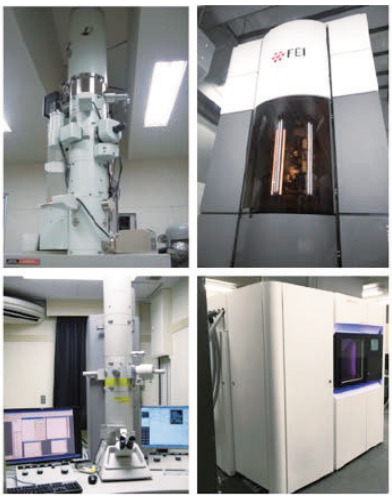
IPR established the International Collaborative Research Program in 2005, inviting researchers broadly from overseas. Under this program, research should be conducted in the form of a collaboration including at least one of the principal investigators at IPR, or it should use particular experimental facilities of IPR.
In FY2017, 34 overseas researchers from 16 countries visited IPR and conducted 9 collaborative researches with the principal investigators at IPR.



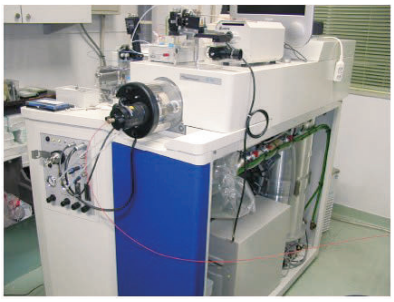
IPR Seminar

The IPR holds IPR Seminars on various topics throughout the year as one of the seven collaborative activities of Joint Usage/Research Center.

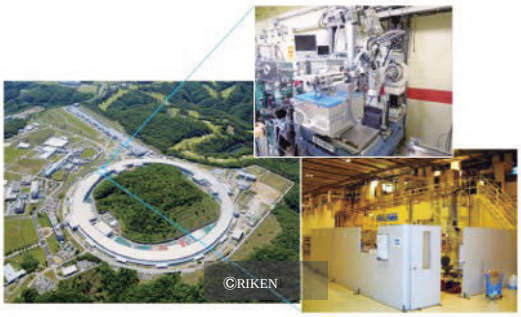
Facilities



Cryo-electron Microscopes



Analytical Apparatus for Supra-biomolecules



Beamline for Biological Macromolecular Assemblies at SPring-8

Solution NMR Spectrometer
in an Ultra-high Magnetic Field

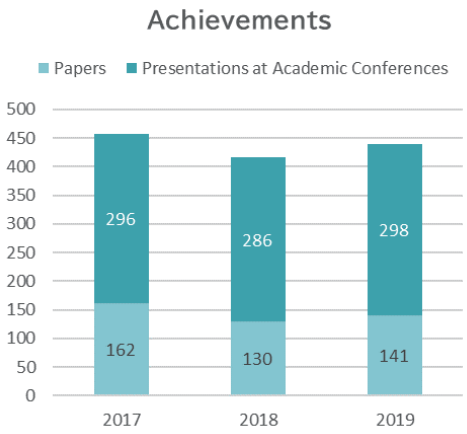
Solid-state NMR Spectrometers

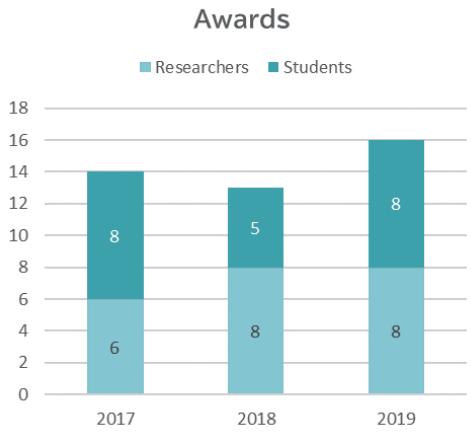
High-performance X-ray Diffractometer

Research Activities

IPR was founded by members in Faculty of Science and Medical school of Osaka University, covering different fields of sciences, such as chemistry, physics, biology and medicine. Since then, protein research in IPR has made a remarkable progress by elucidating structures and functions of proteins, and by understanding their biological roles from the molecular level to the cellular and the higher levels. Furthermore, IPR has been going forward to the integrative structural life science as the basic science, by inviting specialists in the fields of systems biology and single molecule analysis.

Recent achievements





International Exchange

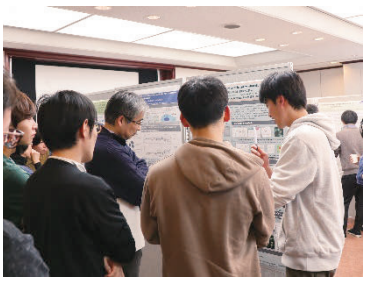
The IPR has been actively promoting international exchange through various programs. More than two hundred visiting international scientists have participated in research activities since the establishment of the IPR. In 2005, IPR started a new program, the International Collaborative Research program, for overseas researchers who perform coordinated researches at the institute and use particular experimental facilities of IPR. IPR has also been accepting international exchange students through a program called FrontierLab@OsakaU.
To promote further cooperation in research, IPR has concluded Inter-Faculty Academic Exchange Agreements with overseas institutes as listed below:

	Institution	Country	Year of agreement
1	Center for Genetic Engineering and Biotechnology	Cuba	2003
2	National Synchrotron Radiation Research Center	Taiwan	2007
3	Indian Institute of Chemical Biology	India	2009
4	Center of Protein Science, Peking University	China	2014
5	College of Pharmacy, Seoul National University	Korea	2015
6	Institute for Quantitative Biomedicine, The State University of New Jersey, Rutgers	USA	2015
7	College of Life Science, National Tsing Hua University	Taiwan	2015
8	Panjab University	India	2017
9	University College Dublin	Ireland	2017
10	Indian Institute of Science Education and Research(IISERs) Thiruvananthapuram	India	2017
11	University of Chicago	USA	2017
12	Fuculty of Biology and Biotechnology, Ruhr University Bochum(RUB)	Germany	2017
13	Fondazione Istituto Italiano di Tecnologia	Italy	2018
14	Research School of Chemistry, College of Science, The Australian National University	Australia	2020
15	Research Center for Bio-Molecule Engieering, Airlangga University	Indonesia	2020

IPR's Global Projects page on IPR website
www.protein.osaka-u.ac.jp/Global/

IPR Retreat

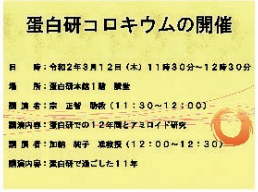
IPR holds a retreat every year with the aim to facilitate communication and interaction among researchers and students at IPR.


November 21-22, 2019　Icho Kaikan, Suita Campus, Osaka University

IPR Colloquium

As a measure for faculty development, IPR holds colloquia five times each year. Young researchers are selected to be speakers, which provides them with opportunities for presentation and discussion with other researchers. The IPR Colloquium has been a forum for active interaction and future collaboration in IPR.

Major research projects granted by government organizations

※Listed are projects in progress as of FY2020 with grants of ten million yen and over

Grants-in-Aid for Scientific Research (KAKENHI)

Organization	Program	Project	Term
JSPS	Grant-in-Aid for Scientific Research on Innovative Areas (Research in a proposed research area)	Metabolic adaptation of inflammatory diseases	2017-2021
		Neuronal circuit mechanisms of reward/goal-directed behavior	2016-2020
		Strategic design of the proton motive force based on the structural analysis	2016-2020
		research on the coordination between epigenetic information and higher-order chromatin structure during cell proliferation	2019-2023
	Grant-in-Aid for Scientific Research (A)	Molecular mechanism on the control of DNA homology search through dynamics of Rad51-Dmc1 complexes	2019-2021
		Structural elucidation of the Wnt signaling through the use of chemical biology tools	2020-2022

Other governmental research grant

Organization	Program	Project	Term
AMED	Platform Project for Supporting Drug Discovery and Life Science Research	Correlative structural analysis platform for drug discovery and life sciences	2017 – 2022
		Recombinant production of high-value target proteins and their binders via structure-based protein design	2017 – 2022
		Data science and collaborative support accelerating innovative drug discovery and life science research	2017 – 2022

Entrusted research projects

Organization	Program	Project	Term
MEXT	MEXT→RIKEN	NMR Platform	2013 – 2020
JST	Program for coordination toward integration of related databases	Enhancement of functionality and integrative management of PDB	2017 – 2022
	JST-Mirai Program	Development of cell modeling platform for drug development	2019 – 2020
	Development of advanced measurement and analysis systems	Development of ultra-high-sensitity spin-correlated high-resolution NMR spectrometer	2015 – 2020
AMED	Project Promoting Support for Drug Discovery	Development of a novel retinal protection drug by modulating epigenetic mechanisms in the retina	2018 – 2020
	Research Center Network for Realization of Regenerative Medicine	Reconstituted basement membrane-like gel for improved engraftment and maturation of transplanted iPS cell-derived cardiomyocytes	2020 – 2021
		Development of robust differentiation system for muscle stem cells using new generation extracellular matrix	2020 – 2022
	AMED→Kyoto Univ. (Project Focused on Developing Key Evaluation Technology: Development of Platform Technology for Drug Discovery through Application of Regenerative Medicine)	Development of biomimetic microdevices to recapitulate enterohepatic circulation of drugs/Supply of cryopreserved human iPS cell-derived hepatocyte-like cells	2017 – 2021
	AMED→Nagoya Univ. (Project for Supporting Drug Discovery and Life Science Research)	Platform Project for Supporting Drug Discovery and Life Science Research Cryo-EM Feedback based Multisubunit Membrane Protein Production and Innovation	2017 – 2022

JSPS: Japan Society for the Promotion of Science

MEXT: Ministry of Education, Culture, Sports, Science and Technology, Japan

JST: Japan Science and Technology Agency

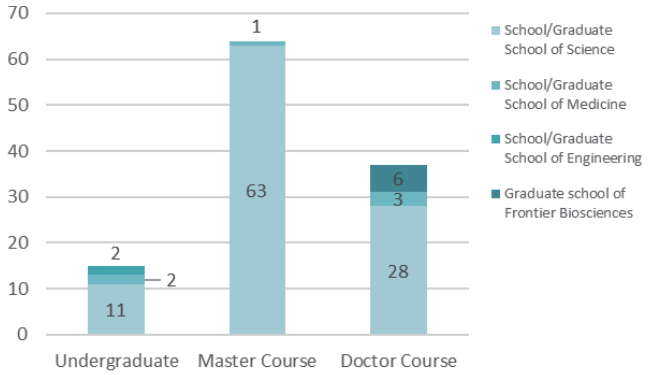
AMED: Japan Agency for Medical Research and Development

Joint research projects with private/public sectors

	Company/Institution	Term
1	JEOL Ltd.	2019-2021
2	Mandom Corporation	2016-2020
3	MATRIXOME, Inc.	2019-2020
4	FUJIFILM Corporation	2019-2020
5	ROHTO Pharmaceutical Co., Ltd.	2020
6	Morishita Jintan Co.,Ltd.	2020-2021
7	Asahi Kasei Pharma Corporation	2020
8	Mitsui Chemicals, Inc.	2019-2020

Educational Activities

Academic staff of IPR engages in graduate course education in cooperation with the Graduate Schools of Science, Medicine, Frontier Biosciences, and Engineering. In order for students to study and join the research at IPR, they are required to take courses at their enrolled department provided by IPR academic staff. Approximately 120 students, including international students, are studying at IPR every year.



Faculty members of IPR teach in Graduate School of Science (Department of Chemistry, Department of Biological Science and Department of Macromolecular Science), Graduate School of Medicine, Graduate School of Frontier Biosciences and Graduate School of Engineering.Therefore in the case that you select a professor who belongs to IPR and teach in your department as your supervisor, then you can do your education and research activities as a member of IPR.

Graduate courses by IPR

Graduate School of Science	
Chemistry	Lab. for Protein Organic Chemistry / Lab. for Protein Profiling and Functional Proteomics / Lab. for Molecular Biophysics / Lab. for Computational Biology
Biological Sciences	Lab. for Protein Organic Chemistry / Lab. for Nanobiology / Lab. for Protein Synthesis and Expression / Lab. for Protein Profiling and Functional Proteomics / Lab. for Molecular Biophysics / Lab. for Protein Crystallography / Lab. for CryoEM Structural Biology / Lab. for Supramolecular Crystallography / Lab. for Molecular and Developmental Biology / Lab. for Genome and Chromosome Functions / Lab. for Advanced Brain Functions / Lab. for Cell Systems / Lab. for Computational Biology / Lab. for Membrane Protein Chemistry / Lab. for Organelle Biology / Lab. for Biomolecular Analysis
Macromolecular Science	Lab. for Protein Crystallography / Lab. for CryoEM Structural Biology / Lab. for Supramolecular Crystallography
Graduate School of Medicine	
Medicine	Lab. for Molecular and Developmental Biology / Lab. for Advanced Brain Functions
Graduate school of Frontier Biosciences	
Frontier Biosciences	Lab. for Protein Synthesis and Expression / Lab. for Protein Profiling and Functional Proteomics / Lab. for CryoEM Structural Biology / Lab. for Supramolecular Crystallography / Lab. for Molecular and Developmental Biology / Lab. for Advanced Brain Functions / Lab. for Ultra-High Magnetic Field NMR Spectroscopy
Graduate School of Engineering	
Biotechnology	Lab. for Protein Crystallography

Graduate Program for Advanced Interdisciplinary Studies of Osaka University

A Program for State-of-the-Art Protein Structural Analysis by IPR started in 2017 as one of the Graduate Programs for Advanced Interdisciplinary Studies of Osaka University. Academic staff at IPR and invited lecturers from industry and other institutes have provided graduate students with lectures on the cutting-edge structural biology as well as sophisticated large-scale equipment and international databases that have been developed at IPR.

IPR Staff

As of October 1, 2020

Director

Professor	**NAKAGAWA Atsushi**

Vice Directors

Professor	**FURUKAWA Takahisa**
Professor	**TAKAGI Junichi**

Director of the Research Center

Professor	**KURISU Genji**

Division of Protein Chemistry

Laboratory for Protein Organic Chemistry

Professor	HOJO Hironobu
Associate Professor	KAWAKAMI Toru
Assistant Professor	ASAHINA Yuya

Laboratory for Nanobiology

Professor	HARADA Yoshie
Lecturer	SUZUKI Madoka
Assistant Professor	SOTOMA Shingo

Laboratory for Protein Synthesis and Expression

Professor	TAKAGI Junichi
Assistant Professor	KITAGO Yu
Specially-appointed Assistant Professor	ARIMORI Takao

Laboratory for Protein Profiling and Functional Proteomics

Professor	TAKAO Toshifumi
Assistant Professor	TAKEI Toshiki
Specially-appointed Assistant Professor	WANG Qiuyi

Laboratory for Membrane Protein Chemistry

Associate Professor	MIMA Joji

Division of Protein Structural Biology

Laboratory for Molecular Biophysics

Professor	FUJIWARA Toshimichi
Visiting Professor	KOJIMA Chojiro
Associate Professor	MATSUKI Yoh
Assistant Professor	EGAWA Ayako
Assistant Professor	SO Masatomo
Specially-appointed Assistant Professor	HARADA Kenichi
Specially-appointed Assistant Professor	SUGIKI Toshihiko
Specially-appointed Assistant Professor	FUKAZAWA Jun

Laboratory for Protein Crystallography

Professor	KURISU Genji
Associate Professor	TANAKA Hideaki
Specially-appointed Associate Professor	GERLE Christoph
Assistant Professor	KAWAMOTO Akihiro

Laboratory for CryoEM Structural Biology

Professor	KATO Takayuki
Assistant Professor	KISHIKAWA Junichi
Assistant Professor	TAKAZAKI Hiroko

Laboratory for Supramolecular Crystallography

Professor	NAKAGAWA Atsushi
Specially-appointed Associate Professor	YOSHIMURA Masato
Associate Professor	SUZUKI Mamoru
Associate Professor	YAMASHITA Eiki

Division of Integrated Protein Functions

Laboratory for Molecular and Developmental Biology

Professor	FURUKAWA Takahisa
Associate Professor	CHAYA Taro
Specially-appointed Assistant Professor	SUGITA Yuko

Laboratory for Genome and Chromosome Functions

Professor	SHINOHARA Akira
Associate Professor	FURUKOHRI Asako
Assistant Professor	ITO Masaru
Assistant Professor	FUJITA Yurika

Laboratory for Advanced Brain Functions

Professor	HIKIDA Takatoshi
Assistant Professor	OZAWA Takaaki
Assistant Professor	MACPHERSON Tom

Laboratory for Organelle Biology

Associate Professor	NAKAI Masato

Division of Protein Network Biology

Laboratory for Cell Systems

Professor	OKADA Mariko
Specially-appointed Lecturer	TABATA Syo
Assistant Professor	IIDA Keita
Assistant Professor	ICHIKAWA Ayaka
Specially-appointed Assistant Professor	MUENZNER Ulrike

Laboratory for Computational Biology

Professor	MIZUGUCHI Kenji
Associate Professor	HASHIMOTO Kosuke
Assistant Professor	NAGAO Chioko

Laboratory for Nuclear Dynamics

Specially-appointed Professor	NAGANO Takashi
Professor	FURUKAWA Takahisa

Laboratory for Infection Systems

Specially-appointed Professor	IMAI Yumiko
Professor	OKADA Mariko

Laboratory of Visiting Scientists

Visiting Professor	YAMASAKI Toshio
Visiting Academic Staff	SUZUKI Yuta
Visiting Academic Staff	TAKEDA Tetsuya

Research Center for Next-Generation Protein Sciences

Protein Data Bank Japan

Professor	KURISU Genji
Professor	FUJIWARA Toshimichi
Specially-appointed Associate Professor	KAWABATA Takeshi
Specially-appointed Assistant Professor	BEKKER Gert-Jan

Laboratory for Ultra-High Magnetic Field NMR Spectroscopy

Associate Professor	MIYANOIRI Yohei
Specially-appointed Assistant Professor	SUGIKI Toshihiko

Laboratory for Synchrotron Radiation Research

Professor	NAKAGAWA Atsushi
Associate Professor	YAMASHITA Eiki

Laboratory for High Resolution Cryo-EM

Professor	KATO Takayuki

Laboratory for Biomolecular Analysis

Professor	TAKAO Toshifumi
Professor	TAKAGI Junichi
Professor	NAKAGAWA Atsushi
Associate Professor	OKUMURA Nobuaki

Open Space Laboratory for Advanced Protein Science

Visiting Professor	MATSUMOTO KAZUYA

Division of Donated Fund Research

Division for Matrixome Research and Application

Professor of Donated Fund Research	SEKIGUCHI Kiyotoshi
Assistant Professor of Donated Fund Research	TANIGUCHI Yukimasa

Technology Division

Technical Staff	KAWAKAMI Keiko
Technical Staff	KOSADA Takashi
Technical Staff	YAMASHITA Reiko
Technical Staff	ABE Naoyuki
Technical Staff	TSUJII Toshinori
Technical Staff	NOROKA Naoko

Administration Office

Head	TANAKA Yoshikazu
General Affairs Section	
Chief	ICHIKI Tomoko
Deputy Chief	UMEDA Hideaki
Administrative Staff	WADA Yumi
Accounting Section	
Chief	IMADA Kaori
Deputy Chief	TAKEMOTO Isao
Administrative Staff	NAKAGAWA Yuko
Administrative Staff	KOBAYASHI Ayumi
Project Team of Joint Usage/Research Center	
Commissioned Staff	SATO Masako
Research Support Section	
Chief	IKEDA Toshihide
Deputy Chief	AKIMOTO Makiko
Deputy Chief	SAKAUE Akihiro
Specially-appointed Administrative Staff	YOSHIDA Etsuko

Public Relations Office

Specially-appointed Administrative Staff	TANAKA Yuko

Administrative Council

Chair :	NAKAGAWA Atsushi	Director, Institute for Protein Research, Osaka Univerisity
	SHIMOJO Shinji	Professor, Cybermedia Center, Osaka Univerisity
	NAGASAWA Takashi	Professor, Graduate School of Frontier Biosciences, Osaka University
	MIKI Hiroaki	Professor, Research Institute for Microbial Diseases
	SHIGA Sakiko	Professor, Graduate School of Science, Osaka University
	TSUJIKAWA Kazutake	Professor, Graduate School of Pharmaceutical Sciences, Osaka University
	ENDO Motomu	Professor, Division of Biological Science, Nara Institute of Science and Technology
	KOBAYASHI Takehiko	Professor, Institute for Quantitative Biosciences, Tokyo University
	SHIMADA Miki	Professor, Head of Division of Pharmacy, Tottori University Hospital
	NAKAYAMA Jun-ichi	Professor, National Institute for Basic Biology, National Institutes of Natural Sciences
	FUKUZAWA Hideya	Professor, Graduate School of Biostudies, Kyoto University
	KAMIMURA Midori	Distinguished research scientist, TEIJIN PHARMA LIMITED
	TSUNODA Tatsuhiko	Professor, Graduate School of Science, the University of Tokyo
	SEIMIYA Hiroyuki	Chief, Molecular Biotherapy, Cancer Chemotherapy Center of JFCR

Staff and Accounting Data

As of October 1, 2020

Staff

Full Time	Professor	13
	Associate Professor	12
	Lecturer	1
	Assistant Professor	16
	Professor of Donated Fund Research	1
	Associate Professor of Donated Fund Research	0
	Assistant Professor of Donated Fund Research	1
	Specially-appointed Professor	1
	Specially-appointed Associate Professor	2
	Specially-appointed Lecturer	1
	Specially-appointed Assistant Professor	8
	Specially-appointed Researcher	17
	Technical Staff	5
	Administrative Staff	10
	Specially-appointed Administrative Staff	4
	Commissioned Staff	1
	Subtotal	93
Part Time	Specially-appointed Professor	1
	Specially-appointed Associate Professor	1
	Specially-appointed Researcher	16
	Technical Staff	21
	Administrative Staff	19
	Commissioned Staff	2
	Subtotal	60
Total		153

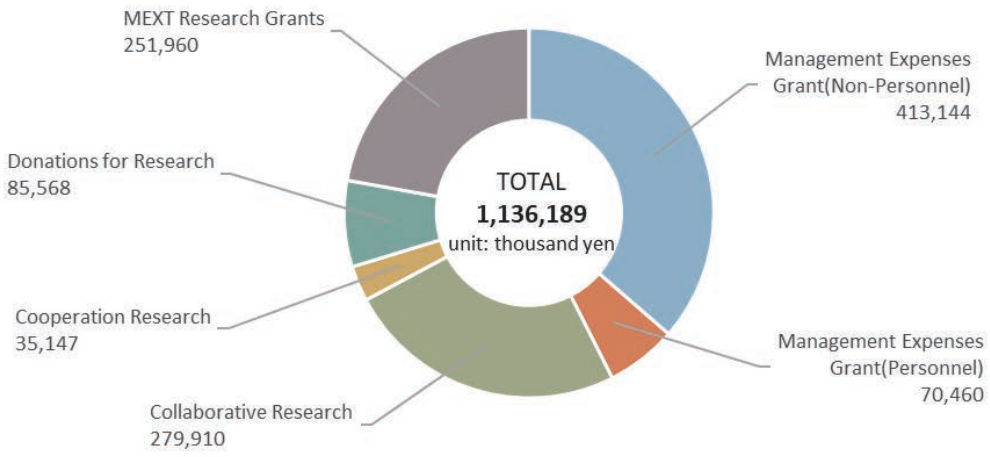
Visiting/Collaborative Researchers

Visiting Researcher	Professor	4
	Associate Professor	0
	Academic Staff	4
	Researcher	5
	Total	13
Joint Research Collaborator (total in 2017)	International Joint Research Collaborator	34
	Joint Research Collaborator	839
	(Regular	213)
	(Beamline	526)
	(NMR	73)
	(CryoEM	27)
	Total	873

Students

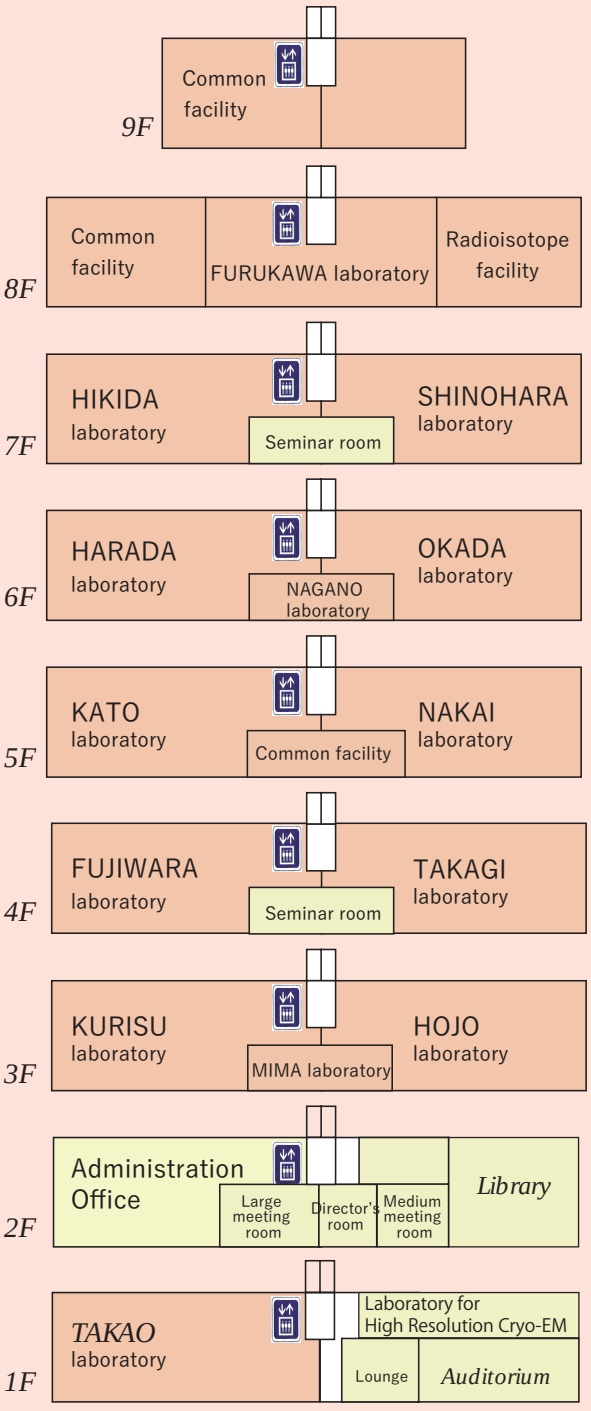
Undergraduate	15
Graduate Student (Master Course)	64
Graduate Student (Doctor Course)	37
Research Student	3
Total	119

Accounting in FY2019

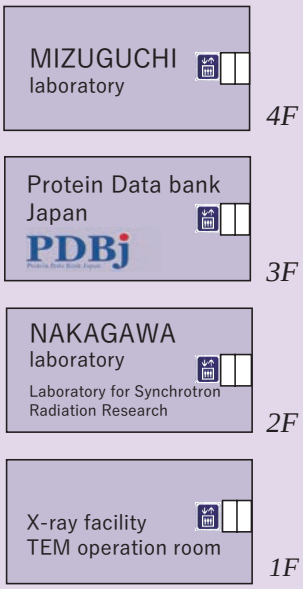


Floor Plan

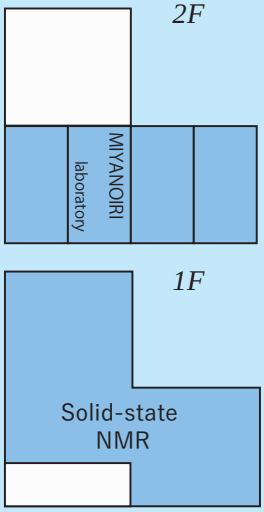
Main Building



Structural Analysis Research Building



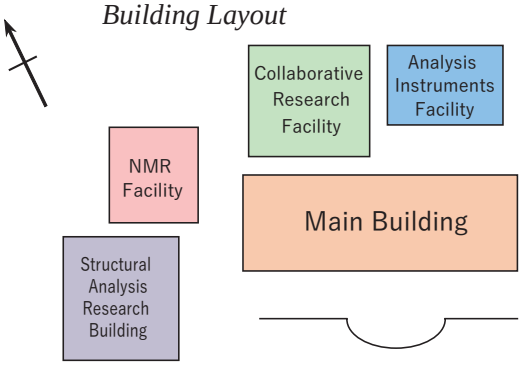
Analytical Instruments Facility



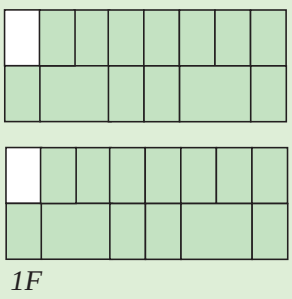
NMR Facility

Laboratory for Ultra-High Magnetic Field NMR Spectroscopy

Building Layout

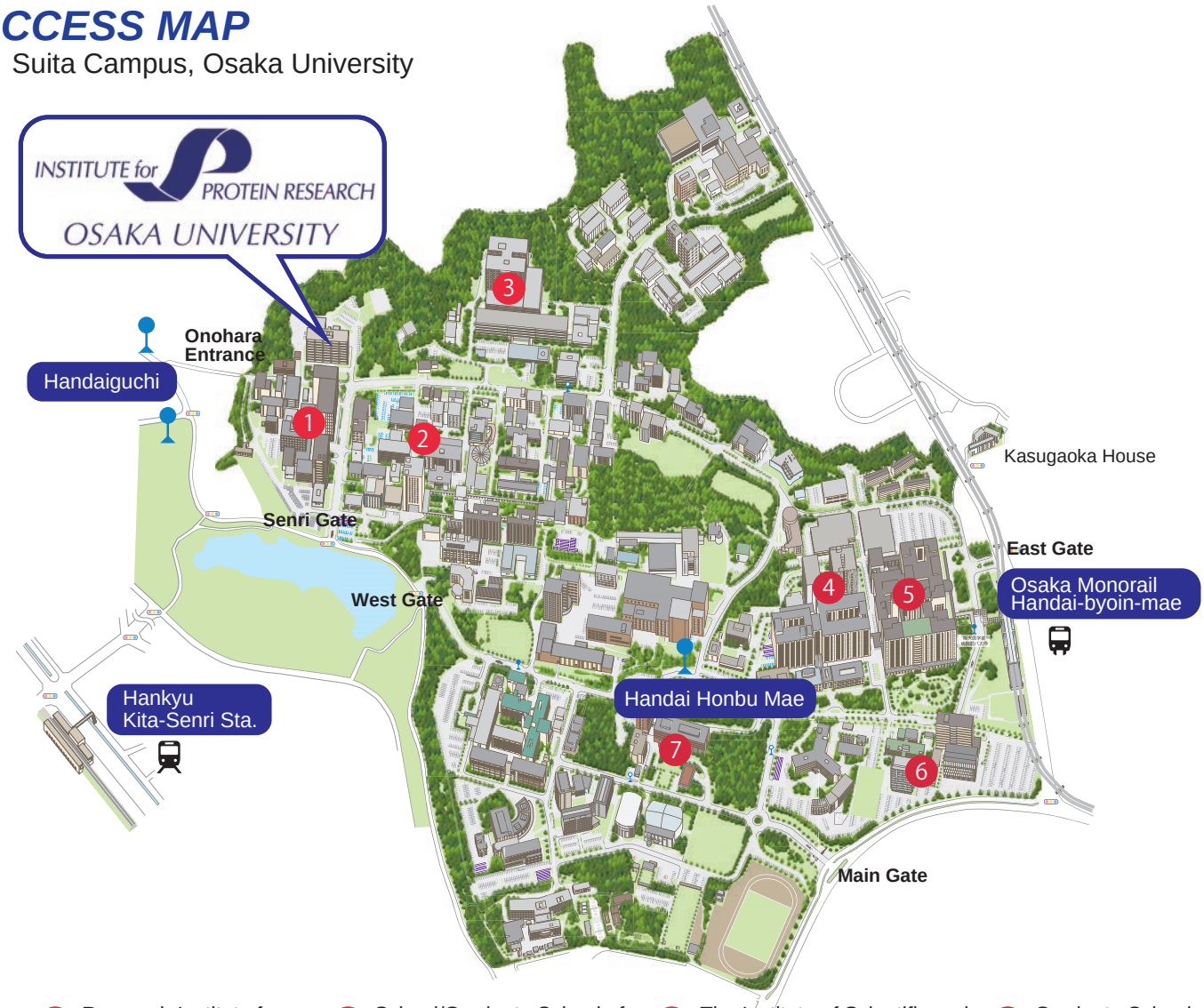


Collaborative Research Facility



ACCESS MAP

Suita Campus, Osaka University



1 Research Institute for Microbial Diseases

2 School/Graduate School of Engineering

3 The Institute of Scientific and Industrial Research

4 Graduate School of Medicine

5 Osaka University Hospital

6 Graduate School of Frontier Biosciences

7 Administration Bureau

Train

15 min. walk from Kita-Senri Station on the Hankyu Senri Line

Monorail

20 min. walk from Handai-byoin-mae Station on the Osaka Monorail

Bus

●From Senri-Chuo Station

5 min. walk from Handaiguchi bus stop on the Hankyu bus bound for Onohara Higashi or Toyokawa Eki

10 min. walk from the Handai Honbu Mae bus stop on the Hankyu bus bound for Handai Honbu Mae or Ibaraki Mihogaoka

●From Hankyu Ibaraki-shi Station（via JR Ibaraki Station）

10 min. walk from the Handai Honbu Mae bus stop on the Kintetsu bus bound for Handai Honbu Mae

Public Relations Office

Institute for Protein Research, Osaka University

3−2 Yamadaoka, Suita Osaka, 5650871, Japan

http://www.protein.osaka-u.ac.jp/en/

INSTITUTE for PROTEIN RESEARCH
OSAKA UNIVERSITY