Newsletter No.2
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PGFD
学術変革領域 A　蛋白質新機能生成
Protein Generative Functional Design

文部科学省 科学研究費助成事業 学術変革領域研究（A）(2024-2028)
Grant-in-Aid for Transformative Research Areas (A) (2024-2028)

タンパク質機能のポテンシャルを解放する生成的デザイン学「蛋白質新機能生成」

Generative Design to Unlock the Potential of Protein Function

Contents

Message from the Area Director

It has been almost two years since we started the research project of the Grant-in-Aid for Transformative Research Areas A, “Generative design to unlock the potential of protein function,” in 2024. The research of the Area aims at establishing guiding principles to design protein molecules with new molecular functions in a “generative” manner. This research project brings together scientists from a wide range of fields, including structural biology, spectroscopy, protein engineering, and computational sciences to elucidate the molecular basis of protein functions and to develop novel protein-based molecular functions based on the understanding of the molecular mechanism. In the second fiscal year of the research period, the publicly offered researchers joined, augmenting the research approaches and the targets in the Area.

Through the biannual Area meetings, on-line Area seminars, and co-hosted symposiums at the annual meetings of the Protein Science Society of Japan, the Biophysical Society of Japan, and the Japanese Biochemical Society, and an international joint symposium with Academia Sinica in Taiwan and PRESTO, JST, our mutual understanding of the protein molecular functions obtained through the various approaches in the Area has increased significantly. I would like to thank the members for organizing these meetings and seminars. Collaborative interactions and exchanges among the groups with different approaches in the Area have also provided fruitful insights into protein molecular functions. I am now thrilled that the novel molecular principle of the generative protein functional design is beginning to emerge from the current research in the Area. I hope that the guiding principles for designing new molecular functions will be successfully established through the research in the Area moving forward.

Area Director, Shigehiko Hayashi (Kyoto University)

Introduction of Publicly Offered Research Group

Group A: Theory

A01

Publicly offered research PI:

Kei-ichi Okazaki (Institute for Molecular Science)

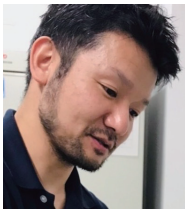
Research subject: Controlling conformational changes in biomolecular motors through the integration of AlphaFold and molecular simulation

Our research goal is to control the conformational changes of motor proteins. By combining the structure-prediction AI AlphaFold with molecular dynamics simulations, we aim to predict mutations that can alter the relative stability of conformational states, lower the energy barrier of conformational changes, and design a more effective inhibitor for motor proteins.

A02

Publicly offered research PI:

Taki Nishimura (University of Osaka)



Rational design of lipid-binding proteins guided by large-scale analysis

Natural proteins possess lipid-binding domains that can bind specifically to certain lipid species, such as phosphatidylinositol phosphates. However, the molecular mechanisms underlying their specificity for lipid species remain poorly understood. In this project, we aim to elucidate the common features of lipid-binding domains that are responsible for binding specificity by utilizing a high-throughput protein-lipid interaction analysis system, the CLiB assay, which we have recently developed. Furthermore, by analyzing lipid-binding domains from various species, we will investigate how organisms have acquired specific lipid-binding abilities through evolution.

Publicly offered research PI:

Ryuichiro Ishitani (Institute of Science Tokyo)

Development of Deep Learning-Based Molecular Docking Methods for High-Precision Protein-Ligand Complex Structure Prediction

In recent years, protein three-dimensional structure prediction has made dramatic advances, and protein-compound complex structure prediction is also progressing. However, conventional molecular docking methods have not yet been completely replaced in terms of accuracy and speed. This project aims to develop molecular docking methods using deep learning to achieve high-precision complex structure prediction. We will particularly focus on restraint-guided inference methods that apply stereochemical constraints during the diffusion process to achieve accurate reproduction of ligand chirality and bond geometries without requiring model retraining. These results will serve as the foundation for protein molecular function modification technology that can be combined with diverse compounds.

A03

Publicly offered research PI:

Masaharu Somiya (University of Osaka)

Research subject: Computational design of de novo fusogens
We have developed a computational method for designing functional membrane fusion proteins (fusogens) with enhanced activity and modularity compared to their native counterparts. This project aims to further develop this approach by designing novel fusogens with entirely new structural folds, thereby expanding both the structural and sequence space available for functional fusogens.

Publicly offered research PI:

Hideki Taguchi (Institute of Science Tokyo)

Research subject: "Seesaw" protein: Design of proteins that adopt interconvertible alternative functional conformations and its extension.
We have been working on the molecular mechanism of chaperone, protein folding, and ribosome dynamics. In this area, we will primarily study our recently developed protein, which can switch between two alternative functional conformations, termed the seesaw protein.

Publicly offered research PI:

Kiyoto Kamagata (Gifu University)

Research subject: Artificial design of phase-separating proteins
Proteins work as ensemble, utilizing the liquid-liquid phase separation. Dense protein droplets enable highly efficient chemical reactions, etc., but at the same time, there is a risk of forming insoluble aggregates that can cause diseases. We aim to (1) understand the liquid-liquid phase separation of proteins by single-molecule fluorescence microscopy, (2) control the phase separation phenomenon by peptide binder technology, and (3) design phase-separating mini-proteins.

Publicly offered research PI:

Mitsuo Shoji (University of Tsukuba)

Research subject: Reconstruction of the Mn cluster in photosystem II
My research goal is to identify or design proteins that can maintain the oxygen-evolving center of Photosystem II, whose active site consists of the Mn4CaO5 cluster. We are developing new theoretical tools to search for amino acid arrangements in proteins that can accommodate or replicate the native active site. As this study becomes increasingly challenging as it progresses, I would be very grateful for any advice you may have from various research fields.

Group B: Measurement

B01

Publicly offered research PI:

Saeko Yanaka (Institute of Science Tokyo)

Research subject: Development of Approaches to Explore Allostery Enabled by the Flexible Structures of Biomolecules
We aim to elucidate the mechanisms of allostery enabled by the flexible structures of biomolecules at the atomic level, leveraging advanced experimental measurements and computational science, using antibodies as a model system.

Publicly offered research PI:

Michi Suga (Okayama University)

Visualizing the initial charge separation reaction in photosynthesis by time-resolved structural analysis

Light energy utilization and conversion in photosynthetic Photosystem II is initiated by a photochemical reaction in which the special chlorophyll dimer P680 absorbs light and induces charge separation within femtoseconds, followed by electron transfer on the picosecond timescale. To elucidate the associated ultrafast structural dynamics, we employ pump–probe experiments using microcrystals and X-ray free-electron lasers.

Publicly offered research PI:

Kazuhiro Kobayashi (University of Tokyo)

Research subject: Systematic workflow for time-resolved cryo-EM

Proteins are composed of twenty amino acids and exert their functions upon proper folding. Beyond amino-acid composition and fold, the conformational dynamics of protein structures are crucial determinants of function. To understand and harness the diverse activities of the many macromolecular proteins that constitute living systems, it is therefore essential to visualize structural changes across time scales from femtoseconds to milliseconds. Research using X-ray free-electron lasers (XFELs) has flourished, enabling visualization of ultrafast motions from the femtosecond to microsecond range. In parallel, advances in cryo-electron microscopy (cryo-EM) have matured methods for analyzing structural changes on the second scale; however, because techniques for directly visualizing millisecond window remain underdeveloped, capturing dynamics in this regime is still challenging. Accordingly, this project aims to establish experimental methodologies for analyzing millisecond-scale conformational changes in proteins. The results will not only provide dedicated approaches for the millisecond regime but, when integrated with XFEL and cryo-EM, will broaden an analytical framework that spans virtually the entire range of time resolutions relevant to protein function.

B02

Publicly offered research PI:

Yuki Toyama (University of Tokyo)

Research subject: Functional Design of Intrinsically Disordered Proteins Guided by NMR Electrostatic Potential Measurements

My research focuses on understanding the functional dynamics of biomolecules using solution nuclear magnetic resonance (NMR) spectroscopy as the core technique. I aim to develop a theoretical and experimental framework for the functional design of intrinsically disordered regions (IDRs) in proteins, with the goal of modulating and potentially enhancing protein function.

Publicly offered research PI:

Hikaru Kuramochi (University of Osaka)

Research subject: Elucidation of primary photoreaction dynamics of photoresponsive proteins using new ultrafast multidimensional spectroscopy

We develop and apply advanced ultrafast laser spectroscopy based on state-of-the-art optical technologies to investigate chemical reaction dynamics in the condensed phase. In this area, we will establish and employ new coherent multidimensional spectroscopic techniques to provide a comprehensive understanding of the primary reaction dynamics of photoresponsive proteins.

Publicly offered research PI:

Keisuke Motone (The University of Osaka)

Research subject: Development of a next-generation measurement platform to accelerate antibody design

Antibodies are widely used in both basic and applied research. Although advances in generative AI, de novo protein design, and large-scale DNA synthesis have greatly facilitated the generation of vast antibody sequence libraries, there remain few methods capable of evaluating antibody function with both high sensitivity and high throughput. This limitation has become a major bottleneck to their effective utilization. In this study, we aim to develop a high-throughput method for monitoring single-molecule antibody kinetics and uncovering the sequence–kinetics relationship to enable the rational design of functional antibodies.

B03

Publicly offered research PI:

Daichi Morimoto (Kyoto University)

Research subject: Development of protein-based sensors for flow stress

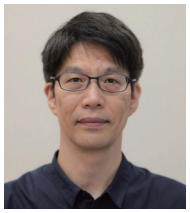
Our research aims to develop a sensor protein that can measure ‘flow stress’, a biophysical parameter that has remained elusive. Using an integrated approach combining rheological nuclear magnetic resonance (NMR) spectroscopy and molecular dynamics (MD) simulations, we develop a sensor protein that can quantify flow stress through fluorescence measurement.

Group C: Biochemistry and creation

C02

Publicly offered research PI:

Atsushi Yamagata (RIKEN IMS)



Development of engineered protein binders and fluorescent probes targeting synapses

My area of expertise is structural biology, with cryo-electron microscopy as a core technique. Our research focuses on developing binder proteins and fluorescent probes that target synapses. We will combine computational protein design with cell-free protein synthesis to efficiently produce functional proteins.

Publicly offered research PI:

Tatsuya Ikenoue (University of Osaka)

Development of liquid–liquid phase separation–controlling peptides for the dynamic regulation of reaction microenvironments

Recent studies suggest that liquid-liquid phase separation (LLPS) plays a critical role in numerous cellular processes. This research aims to develop novel peptides for target-specific LLPS control, enabling the formation of artificial organelles to induce desired reactions. The approach seeks to expand protein functionality by engineering LLPS-regulated reaction environments.

Publicly offered research PI:

Daisuke Ino (University of Osaka)

Research subject: Generative Design of Fluorescent Sensors for Appetite-Related Hormones
My research focuses on the development of genetically-encoded fluorescent sensors for the real-time visualization of extracellular signal transduction. By employing generative protein design approaches, I aim to expand the repertoire of sensors capable of detecting a wide variety of extracellular signaling molecules.

C03

Publicly offered research PI:

Yuichiro Hori (Kyushu University)

Research subject: Development of Protein-Fluorogen Hybrid Probes for Imaging Biomolecular Misfolding & Modification
Our group develops hybrid probes consisting of proteins and synthetic molecules to image biomolecular dynamics in living cells based on chemical principles. To this end, we apply our original protein labeling technique based on a protein tag named PYP-tag. In this project, we focus on various targets including DNA/RNA modifications and protein folding, image them and elucidate biological phenomena involved with these targets.

Publicly offered research PI:

Jasmina Damnjanovic (Nagoya University)

Research subject: Evolutionary engineering of oxidases using novel single-molecule display system
Our group focuses on developing an in vitro high-throughput screening platform termed SMART (Single Molecule Assay on Ribonucleic acid by Translated product) for the rapid, simple, and cost-effective evolution of enzymes, as well as other useful proteins. SMART employs mRNA display, activity-based selection resulting in labeling of active displayed enzymes, next-generation sequencing, and bioinformatics to enable the selection of desired variants in a matter of days by a single person, without specialized equipment, or necessity of cell cultivation. We established the SMART platform for oxidase evolution, which is being applied to evolve oxidases for substrate specificity and stability. We have been working on expanding the scope of SMART towards other enzymes and proteins.

C05

Publicly offered research PI:

Hiroyoshi Matsumura (Ritsumeikan University)

Research subject: Design of artificial antibodies to activate the rate-limiting enzyme in biosuccinate production
This study builds upon previous research showing that artificial antibodies can activate phosphoenolpyruvate carboxylase (PEPC), a rate-limiting enzyme in bio-succinic acid production. The aim of this research is to enhance the bio-succinic acid production capacity of Escherichia coli by constantly activating PEPC through affinity improvement and tandemization of the artificial antibodies.

Publicly offered research PI:

Takafumi Ueno (Institute of Science Tokyo)

Research subject: Generation of new functions through protein crystal design
This study develops a system to generate protein functions using protein cages. By engineering histidine clusters in crystal nanopores, we create metal-free peroxidase catalysts. In-cell crystallization enables mutant library construction, rapid screening, and XFEL-based dynamic analysis. This sustainable, cofactor-free platform positions protein crystals as programmable solid-state biocatalysts for synthetic biology and materials science.

Publicly offered research PI:

Nobutaka Fujieda (Osaka Metropolitan University)

Research subject: Controlling Plasticity of Active Sites in Metal Enzymes
We engineer non-heme metalloenzymes that catalyze stereoselective reactions. In this project, we aim to introduce fluxional behavior into the metal centers of enzymes and control their dynamic properties. By modulating metal center dynamics, we seek to enhance catalytic efficiency and facilitate both substrate binding and product release.

Event Information

March 26th, 2025

The workshop "Frontier of new paradigm of molecular systems chemistry – fundamental mechanism for concerted molecular functions" was held in the 105th CSJ (Chemical Society of Japan) Annual Meeting.
Organizers: Shigehiko Hayashi

April 29-30th, 2025

The co-hosted workshop "PROTEIN SCIENCE AND ENGINEERING IN THE NEW ERA" with PRESTO "Supra-Assembly of Biomolecule" JST and Institute of Biological Chemistry, Academia Sinica. Place: Academia Sinica (Taipei, Taiwan).
Organizers: Yusuke Nasu (Academia Sinica), Hiroyuki Noji (University of Tokyo), and Shigehiko Hayashi

June 18th, 2025

The workshop "Understanding and expanding protein functions to enable their generative design" was held in the 25th annual meeting of the Protein Science Society of Japan.
Organizers: Takuya Terai and Misao Mizuno

September 24-26th, 2025

The workshop "Integration of Quantum–Classical Mechanisms and Generative Design for the Development of Novel Functional Proteins" was held in the 63rd Annual Meeting of the Biophysical Society of Japan.
Organizers: Keiichi Inoue and Minoru Kubo

November 3-5th, 2025

The workshop "Bridging Biomolecule Structure Data to Biology" was held in the 98th Annual Meeting of the Japanese Biochemical Society.
Organizers: Tomohiro Nishizawa and Nobuyasu Koga

Upcoming workshops

December 15-20th, 2025

The workshop "Atomistic Understanding and Design of Enzyme Catalysis Through the Lens of Protein Dynamics" will be held in Pacifichem 2025.
Organizers: Shigehiko Hayashi, Eriko Nango et al.

January 22–23rd, 2026

Our research area will participate in the 4th Protein Symposium, organized by protein-related CREST programs and multiple Grant-in-Aid for Transformative Research Areas in protein science.
Organizers: Tatsuo Fukagawa

Activities of the Research Area

On-site Area meeting

June 17th, 2025 The 3rd area meeting was held at Acurie Himeji, Himeji, Hyogo.

Upcoming area meeting

December 8-9th, 2025
The 4th area meeting will be held at Hyogo Prefectural Awaji Yumebutai International Conference Center, Awaji, Hyogo.

Week of April 20th, 2026 (2 days).
The 5th area meeting will be held at Kyoto University.

November 16-17th, 2026
The 1st international symposium on "Protein Generative Functional Design" will be held at Noyoti hall, Nagoya University.

2nd online seminar

May 21st, 2025
Atsushi Yamagata (RIKEN): "Structural analyses of membrane transporters: insights into practical applications."
Masahiro Fukuda (The University of Tokyo): "Cryo-EM structural analyses of photo- and chemoreceptors."

3rd online seminar

October 14th, 2025
Daichi Morimoto (Kyoto University): "Development of protein-based sensors for flow stress"
Masaharu Somiya (University of Osaka): "Computational design and experimental characterization of functional fusogens"

Young investigator seminar

Upcoming seminar

December 8-9th, 2025
The 1st Young investigator seminar will be held at Hyogo Prefectural Awaji Yumebutai International Conference Center, Hyogo.
Duy Phuoc Tran (Institute of Science Tokyo)
Mariko Ojima (Tohoku University)
Kenji Yatsuzuka (Nagoya University)

Announcements from the Administrative Group

Senior Scientific Research Specialists（学術調査官）

Kenji Mishiro (Associate Professor, Kanazawa University)
Noriko Tsuruoka (Assistant Professor, Tohoku University)

Advisors（領域評価者）

Yoshie Harada (Professor, University of Osaka)
So Iwata (Professor, Kyoto University)
Hideaki Mizuno (Professor, KU Leuven, Belgium)
Satoshi Takahashi (Professor, Tohoku University)

Management roles of the administrative group（総括班の役割）

General affairs（総務・会計）	Shigehiko Hayashi
External relations（渉外, Co-hosting symposia）	Minoru Kubo, Shinya Fushinobu
Public affairs（広報, Newsletter）	Shigeki Kiyonaka, Keiichi Inoue
Collaboration support（共同研究支援, Online seminar）	Eriko Nango, Tomohiro Nishizawa
International activity support（国際情報発信・国際活動連携）	Florence Tama, Nobuyasu Koga, Tsuyoshi Hirota, Robert E. Campbell

Administration Office

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