Newsletter No.1
〈English version〉March 2025

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

We will establish a research field of the generative design that enables conversion and creation of protein molecular functions. Protein molecules, which are responsible for biological functions, have been continuously changing in the evolution to adapt to the environment, resulting in diverse and highly capable molecular functions.

However, the protein molecules that have emerged in this evolution are only a small fraction of the theoretically possible set of protein molecules, and design studies of artificial proteins that do not exist in nature suggest that there are an enormous number of protein molecules that have not yet appeared in the evolution. Protein molecules therefore have immense potential for modification of their molecular functions and generation of new ones.

We therefore aim at establishing guiding principles to design protein molecules with new functions in a “generative” manner. We will develop a methodology for designing functional protein molecules based on the requirement definitions for the novel function, rather than a heuristic search. For that purpose, we will integrate theoretical methods based on computational chemistry and data science, spatiotemporal measurements, and protein molecule development technologies.

When a protein performs a remarkable molecular function, it often generates a “functional state” that appears instantaneously and activates the function. Recently, it has become possible to accurately predict and analyze the functional states using advanced computational methods. In addition, time-resolved x-ray crystallography has made it possible to directly observe experimentally the movement of molecules in three-dimensional space in functional states. By making full use of the state-of-art computational and experimental techniques, we identify functional features that determine protein molecular functions, and theoretically design protein mutants of modified functions by directly analyzing the functional features. Then, by forming a feedback loop with experimental studies on the development of novel functional proteins that contribute to medicine, drug discovery, and creation of new substances, we will establish the theory of the generative design of protein functions.

Area Director, Shigehiko Hayashi (Kyoto University)

# Introduction of Research Groups

## Group A: Theory

### A01 Generative design of protein molecular functions by molecular simulations

Principal Investigator:
Shigehiko Hayashi (Professor, Kyoto University)

We aim at developing a methodology for the theoretical design of protein functions. Through identification of molecular features in functionally activated states with an advanced hybrid molecular simulation, amino acid mutations that alter protein functions are predicted theoretically.

Co-Investigator:
Akio Kitao (Professor, Institute of Science Tokyo)

We investigate activation/inactivation mechanisms of protein/ligand complexes by cutting edge molecular simulation and free energy analysis such as parallel cascade selection molecular dynamics (PaCS-MD) and Markov state model (MSM).

Research Collaborator:
Duy Phuoc Tran (Assistant Professor, Institute of Science Tokyo)

### A02 Dynamic Structure Analysis of Proteins via Integration of Data and Simulation

Principal Investigator:
Florence Tama
(Professor, Nagoya University and RIKEN Center for Computational Science)

We investigate the structure, function, and dynamics of biomolecules through the development and application of integrative modeling computational tools that combine experimental data from various sources, including X-ray crystallography, cryo-EM, SAXS, and AFM, with molecular dynamics simulations.

Co-Investigator:
Osamu Miyashita (Senior Scientist, RIKEN Center for Computational Science)

My research focuses on developing computational tools for experimental data analysis and structural modeling. This project specifically targets the analysis of time-resolved experimental data from X-ray free electron lasers and the interpretation of conformational ensembles derived from SAXS and cryo-EM data.

### A03 Protein design based on physical chemistry and data science

Principal Investigator:
Nobuyasu Koga (Professor, Osaka University)

The protein sequence space is enormously vast. Naturally occurring proteins represent only a tiny fraction ofthis immense sequence space. Using both computational and experimental approaches, We explore fundamental principles and methodologies for designing protein molecules. Current focus is on developing methods to provide functions to de novo designed protein structures.

Co-Investigator:
Rie Tatsumi (Assistant Professor, Osaka University)

Protein design rigorously tests our understanding of protein structure and function relationship. By exploring the sequence and structure space beyond evolution, we aim to design novel functional proteins. My current research focuses on design of functional proteins using de novo designed proteins as building blocks.

## Group B: Measurement

### B01 Time-resolved structural analysis using quantum beams for generative protein design

Principal Investigator:
Eriko Nango (Professor, Tohoku University)

I am working on the development of technology for time-resolved serial crystallography of proteins using an X-ray free electron laser. I will perform time-resolved measurements at SACLA to apply precise dynamic structures to protein design.

Co-Investigator:
Masahiro Fukuda (Assistant Professor, University of Tokyo)

My research interest is to visualize the molecular dynamics of various types of membrane proteins in atomic resolution. I believe that time-resolved (TR) cryo-EM analysis will open the door for the next-generation molecular movie field in combination with TR-SFX method.

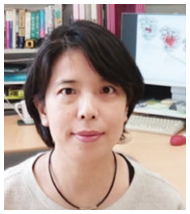
Research Collaborators:
Akira Takasu (Shinoda) (Assistant Professor, High Energy Accelerator Research Organization (KEK))
Kensuke Tono (Chief Scientist, Japan Synchrotron Radiation Research Institute)
Takaaki Fujiwara (Assistant Professor, Tohoku University)
Masahiko Taguchi (Assistant Professor, Tohoku University)
Yasumasa Joti (Chief Scientist, Japan Synchrotron Radiation Research Institute)
Shigeki Owada (Senior Scientist, Japan Synchrotron Radiation Research Institute)
Jungmin Kang (Research Scientist, RIKEN SPring-8 Center)
Takanori Nakane (Specially Appointed Associate Professor, Osaka University)
Kazuya Hasegawa (Senior Scientist, Japan Synchrotron Radiation Research Institute)
Yusuke Yamada (Associate professor, Tohoku University)

### B02 Time-resolved spectroscopic measurements of new functions generated by artificially designed proteins

Principal Investigator:
Minoru Kubo (Professor, University of Hyogo)

My area of expertise is spectroscopy. We employ infrared (IR), Raman, circular dichroism (CD), and small-angle X-ray scattering (SAXS) techniques to analyze a broad range of protein motions, from microscopic dynamics in active sites to conformational ensemble changes in intrinsically disordered proteins.

Co-Investigator:
Misao Mizuno (Associate Professor, Kyoto University)

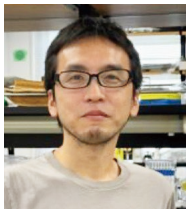


We observe protein structural dynamics using time-resolved resonance Raman spectroscopy under physiological conditions. Comprehensive understanding of our experimental observations for functionally important sites with results obtained by other experimental measurements as well as simulations will help to elucidate protein mechanisms, especially for photoreactive proteins.

Research Collaborators:
Satoshi Nagao (Tenure-track researcher, Japan Synchrotron Radiation Research Institute)

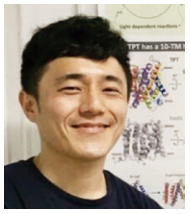
### B03 Elucidation of protein structures and dynamics by cryo-EM

Principal Investigator:
Tomohiro Nishizawa (Professor, Yokohama City University)



Our research focuses on membrane transporters. We aim to elucidate dynamic process of transporters using cryo-EM, and to control their functions based on the structural information by chemical compounds or designed proteins.

Co-Investigator:
Yongchan Lee (Assistant Professor, Yokohama City University)



My research focuses on studying the structural dynamics of membrane transporters using cryo-EM. Many of them are known to form functional complexes inside the cells – these will be the target for rational design and modification in this research area.

## Group C: Biochemistry and creation

### C01 Design and development of designer cell-surface receptors and ligand pairs using structural information

Principal Investigator:
Shigeki Kiyonaka (Professor, Nagoya University)

My research focus is the development of artificial receptors that can be selectively activated by designer ligands, which is known as chemogentics. Our goal is to generatively design the artificial receptors in collaboration with the other group members mainly focusing on structural and theoretical studies.

Co-Investigator:
Hidetsugu Asada (Associate Professor, Kyoto University)

My research interests lie in the structural analysis of membrane proteins, with a particular focus on G-protein coupled receptors (GPCRs). The objective is to identify and support the development of suitable compounds for drug discovery. The goal of this research area is to generatively design the effective GPCRs based on their structure.

Research Collaborator:
Tomohiro Doura (Assistant Professor, Nagoya University)

## C02 Elucidation of structural polymorphism of clock protein CRY and its regulation by molecular design for circadian drug discovery

Principal Investigator:

Tsuyoshi Hirota (Designated Associate Professor, Nagoya University)

We discovered unique compounds against the circadian clock protein CRY that is related to various diseases. By combining these compounds with dynamic structural analysis, molecular simulation, and generative design, we will elucidate the structural polymorphism of CRY and enable its control for circadian drug discovery.

Co-Investigator:

Kazuma Amaike (Research Scientist, RIKEN)

We focus on the design and synthesis of bioactive molecules. In this project, we will investigate circadian clock modulators with high bioactivity and also design and synthesize molecules to elucidate their molecular mechanisms.

## C03 Development of near-infrared fluorescent biosensors through generative design and directed evolution

Principal Investigator:

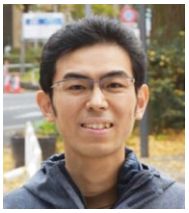
Robert E. Campbell (Professor, University of Tokyo)

My research involves the use of protein engineering, directed evolution, and chemical biology for the development of genetically encoded tools for fluorescence imaging and illumination-dependent control of cells and tissues. Our current focus is on developing far-red and near-infrared fluorescent biosensors for imaging of neural activity and metabolism.

Co-Investigator:

Takuya Terai (Associate Professor, University of Tokyo)

My research interest is in the development of functional molecular tools using the synergistic power of organic chemistry and molecular biology. In particular, we aim to develop near-infrared chemigenetic fluorescent sensors based on protein design.



## C04 Comprehensive understanding of functional expression mechanism and functional improvement of rhodopsins

Principal Investigator:

Keiichi Inoue (Associate Professor, University of Tokyo)

Microbial rhodopsins are photoreceptive membrane proteins and key optogenetic tools that transport a variety of ions using light energy. Our research aims to create novel functional rhodopsins through a synergy of experimental and computational approaches.

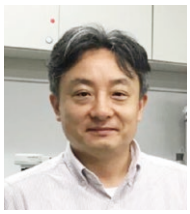
Co-Investigator:
Kazuhiro J. Fujimoto (Associate Professor, Nagoya University)

My research interest lies in the excited states of molecules in chemical and biological systems. At our laboratory, we are trying to theoretically and computationally elucidate the molecular mechanisms underlying electron transfer, excitation energy transfer, and intersystem crossing.

Research Collaborators:
Takashi Nagata (Assistant Professor, University of Tokyo)

## C05 Control, molecular design, and molecular movie analysis of dynamic catalytic reactions of useful enzymes

Principal Investigator:
Shinya Fushinobu (Professor, University of Tokyo)



We have been studying various carbohydrate-active enzymes (CAZymes) and related enzymes, with a focus on those beneficial to industry and human health. Utilizing molecular movies and other advanced methodologies, we aim to unravel the complex reaction mechanisms of these valuable enzymes.

Co-Investigator:
Akihiro Ishiwata (Senior Research Scientist, RIKEN)

My research focuses on the structure- and mechanism-based molecular design and synthesis of probesfor molecular movie analysis of dynamic catalysis of useful enzymes. Synthetic probes could be prepared especially for carbohydrate-acting enzymes, corresponding to each unique mechanism.

# Event Information

**June 13th, 2024**

The workshop "Molecular Design for Expanding Protein Functions – Challenges and Prospects -" was co-organized at the 24th Annual Meeting of the Protein Science Society of Japan.
*Organizers: Shigehiko Hayashi and Eriko Nango*

**December 13, 2024**

The IPR (Institute for Protein Research, Osaka University) seminar "Hacking Ribosomes & Translation Systems: Seminar Series on the Redesign of Naturally Occurring Biomolecules" was organized with our support.
*Organizers: Nobuyasu Koga et al.*

## Upcoming workshops

**March 26th, 2025**

The workshop "Frontier of new paradigm of molecular systems chemistry – fundamental mechanism for concerted molecular functions" will be held at the 105th CSJ (Chemical Society of Japan) Annul Meeting.
*Organizers: Shigehiko Hayashi et al.*

**April 29-30th, 2025**

The workshop "Protein Science and Engineering in the New Era" will be held at Institute of Biological Chemistry, Academia Sinica, Taiwan.
*Organizers: Shigehiko Hayashi et al.*

**June 18th, 2025**

The workshop "Understanding and expanding protein functions to enable their generative design" will be held at 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" will be held at 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" will be held in the 98th Annual Meeting of the Japanese Biochemical Society.
*Organizers: Tomohiro Nishizawa and Nobuyasu Koga*

**December 15-20th, 2025**

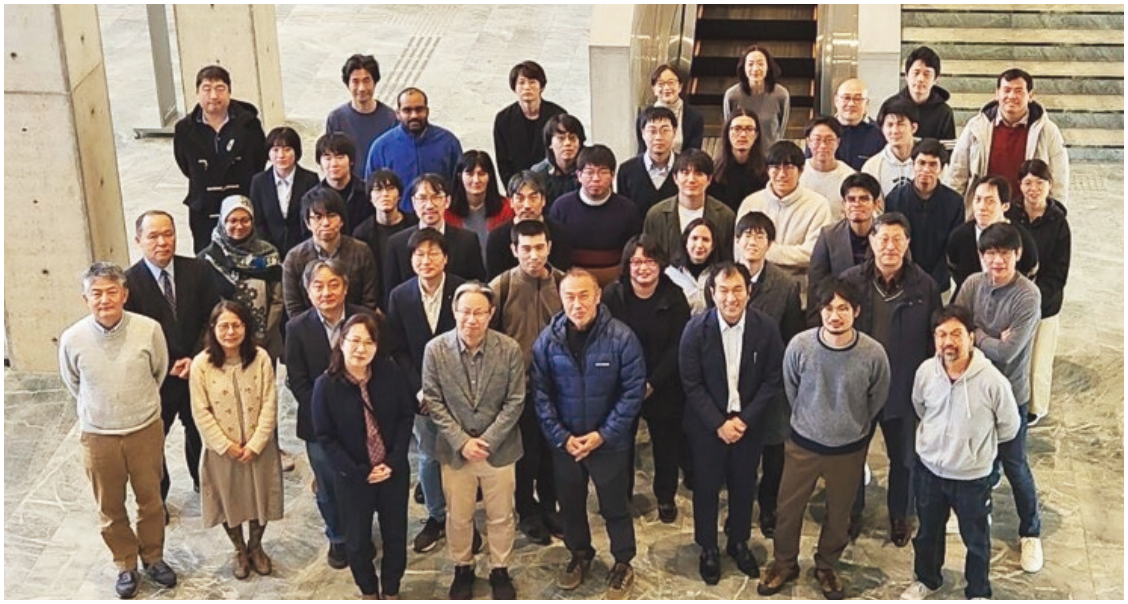
The workshop "Atomistic Understanding and Design of Enzyme Catalysis Through the Lens of Protein Dynamics" will be held in Pacifichem 2025.
*Organizers: Shigehiko Hayashi, Eriko Nango et al.*

# Activities of the Research Area

## On-site Area meeting

**May 29th, 2024** The kick-off meeting was held at Campus Plaza Kyoto, Kyoto.

**December 16-17th, 2024** The 2nd area meeting was held at Hyogo Prefectural Awaji Yumebutai International Conference Center, Hyogo.



### Upcoming area meeting

**June 17th, 2025**
The 3rd area meeting will be held in Arcrea Himeji.

## Online seminar

**January 22nd, 2025** Takuya Terai “Chemigenetic fluorescent biosensors -toward generative design-”
Osamu Miyashita “Integrative Modeling of Dynamic Biological Structures Using MD Simulations and Experimental Data”

# Announcements from the Administrative Group

### Senior Scientific Research Specialist（学術調査官）

Masanori Shigeno (Associate Professor, Tohoku University)
Yukino Baba (Associate Professor, University of Tokyo)

### Advisors（領域評価者）

Yoshie Harada (Professor, Osaka University)
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**

Theoretical Chemistry Group, Department of Chemistry, Graduate School of Science, Kyoto University
Oiwake-cho, Kitashirakawa, Sakyo-Ku, Kyoto 606-8502, Japan
E-mail: p-func_admin@theoc.kuchem.kyoto-u.ac.jp