FY2021 Supporting Materials for External Evaluation

The Exploratory Research Center on Life and Living Systems (ExCELLS)

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1

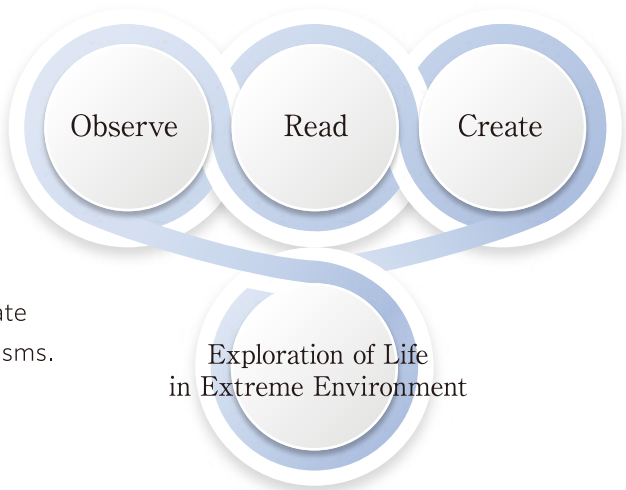
The Current Status and Future Plan of the Exploratory Research Center on Life and Living Systems (ExCELLS)

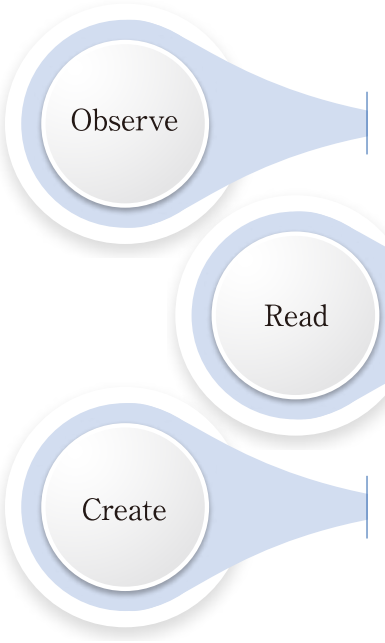
Introduction

What is life? The Exploratory Research Center on Life and Living Systems (ExCELLS) was established in April 2018 to address this fundamental question. This issue has been explored not only by life scientists, but also by researchers in many other fields. Omics-based approaches that have been developed in recent decades have provided comprehensive knowledge on biomolecules as parts of living systems. However, the fundamental question of how these biomolecules are integrated into living systems remains unanswered. ExCELLS aims to achieve a comprehensive understanding of living systems beyond reductionism by utilizing large-scale data analyses and synthetic biology approaches. For this purpose, ExCELLS develops novel approaches for observing biological entities, deciphering hidden information, and creating living systems to improve understanding of their nature. Moreover, ExCELLS promotes collaborative, interdisciplinary research involving investigators who explore organisms living in extreme environments and provides a unique platform for cross-disciplinary research in an inter-university, collaborative environment, using the "Observe, Read, and Create" approach. Furthermore, for developing a strong research and innovation base, ExCELLS enlightens young people to become the next generation scientists. To achieve our aims, we would like to expand our international collaborative network. Therefore, your cooperation and evaluation would be greatly appreciated.

Current research organization structure

ExCELLS consists of the Department of Creative Research and Section for Exploration of Life in Extreme Environments.
Department of Creative Research develops novel approaches for "Observe, Read, and Create", and aims to achieve a comprehensive understanding of living systems.
Section for Exploration of Life in Extreme Environments explores living systems in extreme environments to elucidate original modes of living and adaptation strategies of organisms.





To develop innovative methods for observing dynamic behaviors of biomolecules in situ and for visualizing changes in quantities of various physical components in complex living systems

To develop theoretical and computational approaches to decode, interpret, and predict biological patterns from varying data

To understand the design principles of dynamically ordering, and robust systems in varying environment by creating experimental and computational living systems

Organization Chart

2022.2.1

Collaboration Partnership Agreements

ExCELLS has collaboration partnership agreements with various research institutes and other organizations to promote mutually cooperative and collaborative activities.

April 2018

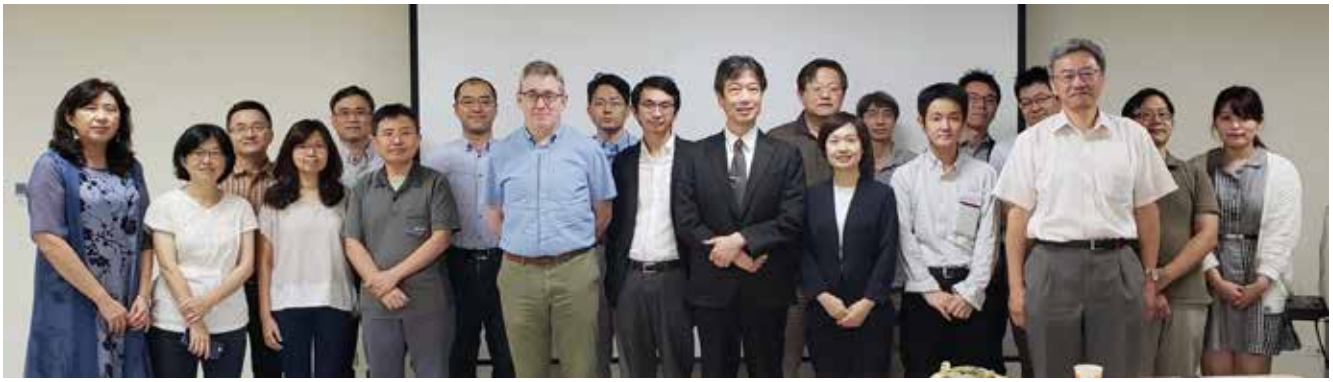
Collaboration partnership agreement with the Department of Subsurface Geobiological Analysis and Research (currently Institute for Extra-Cutting-Edge Science and Technology Avant-Garde Research) at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

April 2018

Academic exchange agreement with Nagoya City University

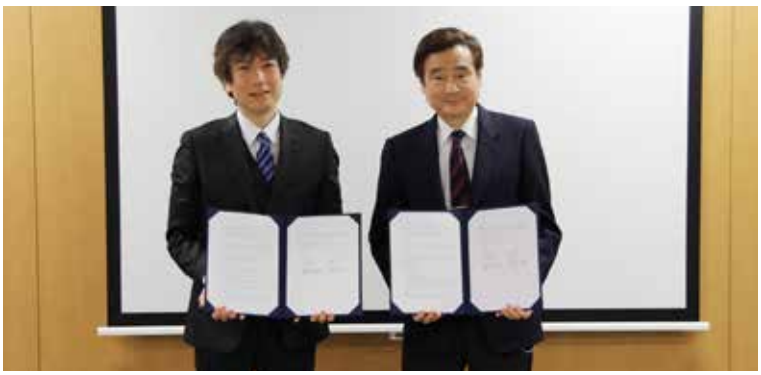
June 2019

Academic exchange agreement with the Institute of Biological Chemistry, Academia Sinica (Taiwan)



February 2020

Cooperation and collaboration pertnership agreement with the Institute for Advanced Biosciences, Keio University



December 2020

Collaboration partnership agreement on the Human Glycome Project

Joint Research Projects

General joint research

A type of collaborative research project that is conducted by researchers in universities/public research institutes in cooperation with faculty members of ExCELLS.

(FY2018) 6 projects
(FY2019) 25 projects
(FY2020) 28 projects
(FY2021) 26 projects

Research utilizing equipment

A type of collaborative research project using the following equipments available at ExCELLS by researchers in universities/public research institutes.

- Equipments
  1. Combined system of high-speed atomic force microscopy and fluorescence microscopy
  2. Q-TOF mass spectrometer for native MS
  3. High-speed live imaging system
  4. Total internal reflection fluorescence (TIRF) microscope system
  5. Super-resolution microscopy systems
  6. Biomolecular interaction analysis system
  7. 4-Dimensional tissue imaging system
  8. Cell sorting and measurement system
  9. Single-cell multi-omics analysis system
  10. Dynamic Light Scattering instrument
  11. Laser-Based quartz glass micropipette puller

(FY2018) 4 projects
(FY2019) 6 projects
(FY2020) 12 projects
(FY2021) 14 projects

ExCELLS Themed Research (Seeds Discovery-type)

A type of collaborative research related to the following research themes of the ExCELLS' goal "understanding the design principles of life". This collaborative research is organized by researchers in universities and public research institutes and two or more research groups in ExCELLS.

● Research themes

1. Research and development of basic technologies for creating artificial cells
   (1) Theoretical and computational sciences and chemical approach
   (2) Molecular and cell biology approach
2. Research on artificial creation of cellular networks
3. Research on the adaptation of life for extreme environments

(FY2018) 6 projects
(FY2019) 8 projects
(FY2020) 6 projects
(FY2021) 6 projects

ExCELLS Themed Research (General-type)

ExCELLS Themed Research (General-type) is collaborative research that conducts a specific research topic related to ExCELLS'goal by invited researchers in universities and public research institutions and two or more research groups from ExCELLS. For this purpose, the invited researcher is allowed to employ ExCELLS assistant professors.

(FY2019) 1 project

ExCELLS Collaborative Research

To pursue the goal of ExCELLS, that is, the understanding of the design principles of life, ExCELLS Collaborative Research invites external researchers in universities and institutions to create a research team and network. The project leader proposes a research theme to promote further collaboration with existing ExCELLS groups and to develop new research and measurement methods.

(Start in FY 2018) 1 project
(Start in FY 2019) 1 project

Cultivation of Young Scientists

ExCELLS Retreat for Young Scientist

To encourage and empower young scientists, ExCELLS hosts Retreat for Young Scientists annually, in which young scientists also act as the planners and organizers of the event.

1st ExCELLS Retreat for Young Scientists

Date: 1st February 2019 – 2nd February
Venue: Mikawa Onsen Kaiyutei (Nishio, Aichi)

2nd ExCELLS Retreat for Young Scientists

Date: 7th February, 2020 – 8th February
Venue: Nishiura Onsen Hotel Tatsuki
(Gamagori, Aichi)

3rd ExCELLS Retreat for Young Scientists

Date: 4th February 2021
Venue: Online Zoom meeting

Research grant for Young Scientists in ExCELLS

To promote interdisciplinary collaborative research, ExCELLS asks the young scientists in ExCELLS to make the team with two or three researchers from the different research groups and to propose collaborative research projects that are initiated and conducted by leveraging young scientists.

(FY2018) 14 projects

(FY2019) 13 projects

(FY2020) 5 projects

(FY2021) 4 projects

2

Research groups' activities

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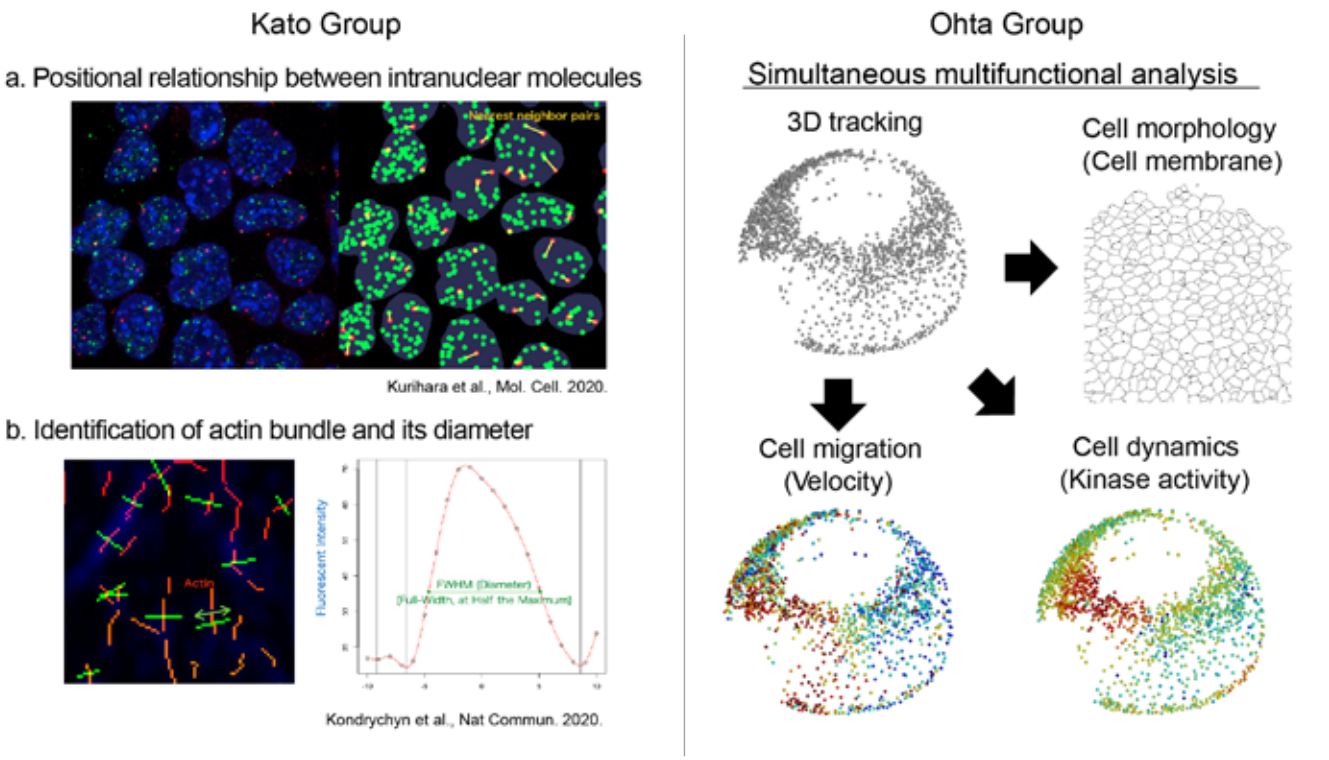
Bioimage Informatics Group

AOKI, Kazuhiro ／ KATO, Kagayaki ／ OHTA, Yusaku



Left, Kato Group: (a) The nuclei of cultured cells (left, blue) are distinguished as individual nuclei, and the closest pairs of intranuclear molecules (green and red) in each nucleus are obtained, and their positional relationships were analyzed. (b) Cortical actin bundles (left, blue) were modeled as freeline (left, red). The diameter of the actin bundle was determined by the intensity profile along with the linear segments, which are orthogonal to the model (right, green segment). Right, Ohta Group: 3D cell tracking and functional imaging by whole embryo imaging during embryogenesis of zebrafish.

【Research Result】

Modern microscopic techniques in life science have yielded vast amounts of imaging data with multiple dimensions. To address this issue, we aim to develop algorithms for extracting biological features from multi-dimensional imaging data. We developed a system to extract individual nuclear regions and analyze the mutual localization of intranuclear molecules from images obtained by multi-point microscopy on cultured cells (Kurihara et al., 2020). In addition, to elucidate the molecular basis of the actin network formation, we measured the density and diameter of the cortical actin bundle in the cultured cell system (Kondrychyn et al., 2020). We are developing an image processing pipeline to visualize and analyze the developmental process of early embryogenesis of zebrafish with a whole embryo scale at the single-cell resolution. By combining 3D cell tracking and functional imaging, we aim to extract multiple types of information such as cell morphology, cell motility, and signaling dynamics.

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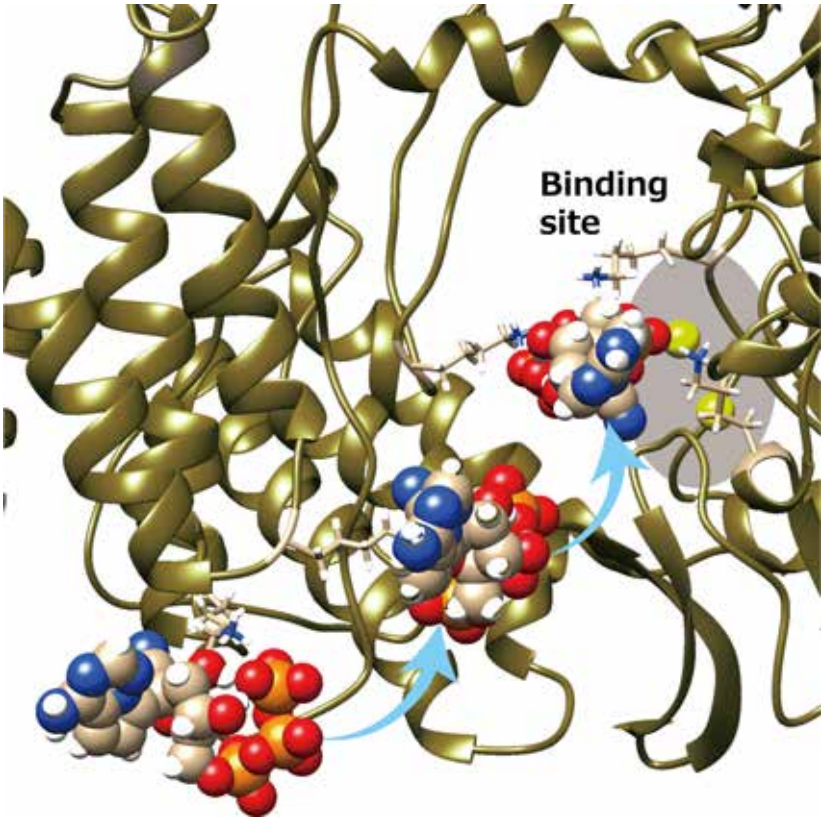
Biomolecular Dynamics Simulation Group

OKUMURA, Hisashi



Remdesivir (shown in the sphere model) is transferred to two magnesium ions (yellow-green spheres) at the binding site of the RNA polymerase (shown in the ribbon model) while being passed from one lysine residue (shown in the stick model) to another. We can see that the lysine residues transfer remdesivir like a bucket brigade.

【Research Result】

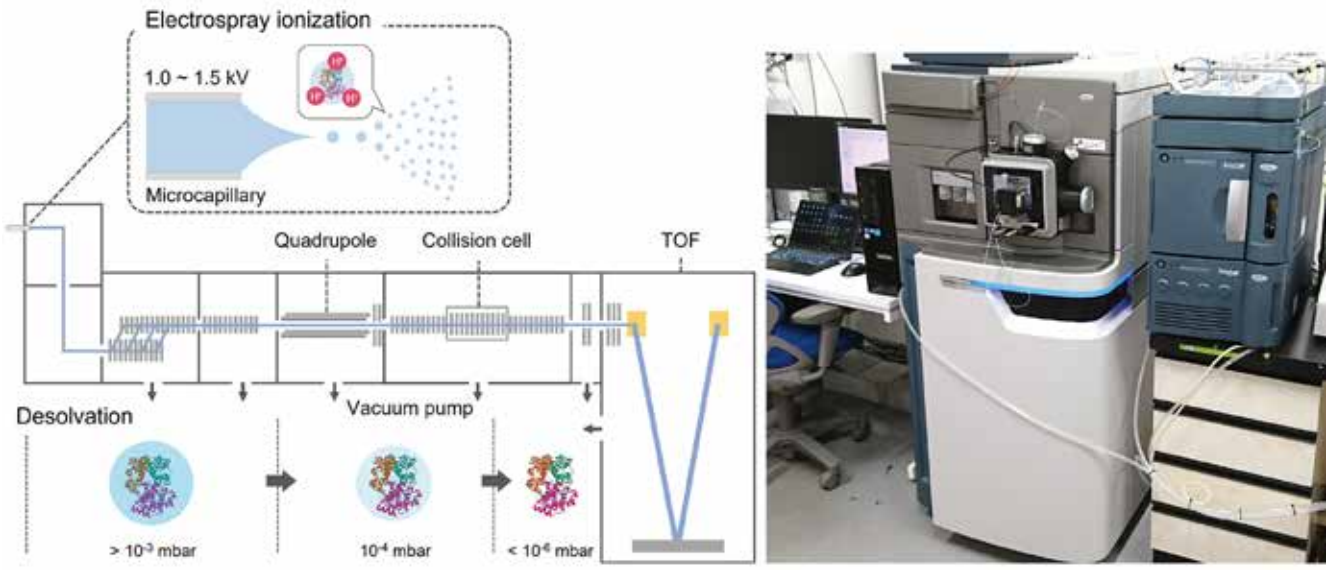
We proposed new computational methods, such as the replica-permutation method, for efficient molecular dynamics simulations of proteins and peptides. Applying these methods to amyloid-β (Aβ) peptides, which are believed to cause Alzheimer's disease, we found that the concentration of Aβ peptides is high at hydrophilic/hydrophobic interfaces such as cell-membrane surfaces, and that each Aβ peptide forms a structure that facilitates aggregation. This is the reason why the aggregation of Aβ peptides is enhanced at the hydrophilic/hydrophobic interfaces. We also elucidated the process by which drugs such as remdesivir are taken up into the RNA-dependent RNA polymerase of SARS-CoV-2. Furthermore, we clarified the dynamic properties of a protein that is thought to play an important role in tardigrade anhydrobiosis.

【References】

■ S. Tanimoto, S. G. Itoh, H. Okumura, ""Bucket brigade" using lysine residues in RNA-dependent RNA polymerase of SARS-CoV-2", Biophys. J. (2021). ■ K. Miyazawa, S. G. Itoh, H. Watanabe, T. Uchihashi, S. Yanaka, M. Yagi-Utsumi, K. Kato, K. Arakawa, H. Okumura, "Tardigrade secretory-abundant heat-soluble protein has a flexible β-barrel structure in solution and keeps this structure in dehydration", J. Phys. Chem. B (2021).■ H. Okumura, S. G. Itoh, K. Nakamura, T. Kawasaki, "Role of water molecules in the laser-induced disruption of amyloid fibrils observed by nonequilibrium molecular dynamics simulations", J. Phys. Chem. B 125, 4964-4976 (2021).■ Y. Tachi, Y. Okamoto, H. Okumura, "Conformational change of amyloid-β 40 in associated with binding to GM1-glycan cluster", Sci. Rep. 9, 6853 (11 pages) (2019).■ S. G. Itoh, M. Yagi-Utsumi, K. Kato, H. Okumura, "Effects of a hydrophilic/hydrophobic interface on amyloid-β peptides studied by molecular dynamics simulations and NMR experiments", J. Phys. Chem. B 123, 160-169 (2019).

Biomolecular Interaction Research Group

UCHIYAMA, Susumu



Schematic image of Native MS. Combination of electrospray ionization and gradual desolvation of samples dissolved in volatile buffer make it possible to keep whole structures of molecular complexes in mass spectrometry.

【 Research Result 】

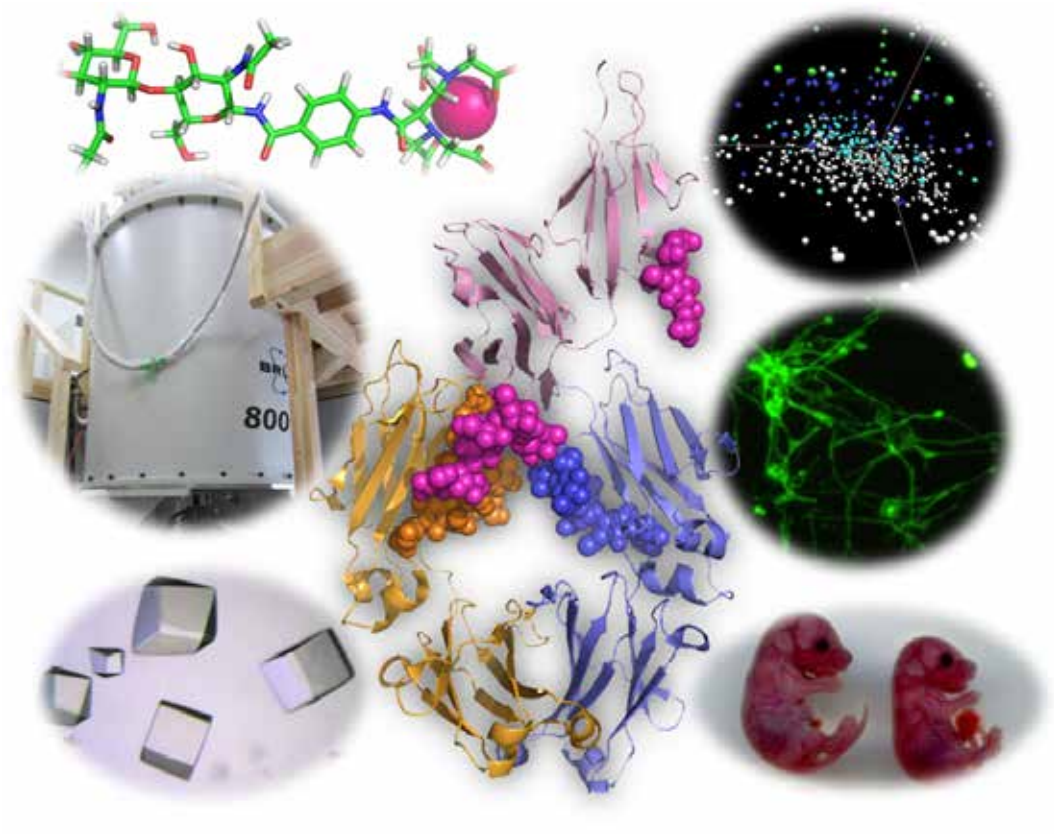
We have been studying dynamic molecular complexes using native mass spectrometry (Native MS). Native MS enables molecular complexes formed through non-covalent interactions to keep their whole structures in mass spectrometry and can approach to their stoichiometries and dissociation constants, which are strong points of Native MS. Using Native MS, we have ever successfully characterized complex formations of proteins such as proteasome and molecular chaperone, specific structural formations of artificial nucleic acids through different bond scheme from Watson-Crick base pair, and synthetic supramolecular formations such as nanocube. Through these researches, we have proved that Native MS is a strong analytical method for research of molecular complexes and well compliments structural biological methods such as NMR and crystal structure analysis.

【 References 】

■ Y. Kamiya, T. Satoh, A. Kodama, T. Suzuki, K. Murayama, H. Kashida, S. Uchiyama, K. Kato, H. Asanuma, “Intrastrand backbone-nucleobase interactions stabilize unwound right-handed helical structures of heteroduplexes of L-aTNA/RNA and SNA/RNA”, Commun. Chem. 3(156), (2020). ■ M. Yagi-Utsumi, A. Sikdar, C. Song, J. Park, R. Inoue, H. Watanabe, R.N. Burton-Smith, T. Kozai, T. Suzuki, A. Kodama, K. Ishii, H. Yagi, T. Satoh, S. Uchiyama, T. Uchihashi, K. Joo, J. Lee, M. Sugiyama, K. Murata, K. Kato, “Supramolecular tholos-like architecture constituted by archaeal proteins without functional annotation”, Sci. Rep. 10(1) 1540 (2020). ■ Y. Zhan, T. Kojima, K. Ishii, S. Takahashi, Y. Haketa, H. Maeda, S. Uchiyama, S. Hiraoka, “Temperature-controlled repeatable scrambling and induced-sorting of building blocks between cubic assemblies”, Nat. Commun. 10(1) 1440 (2019). ■ T. Sekiguchi, T. Satoh, E. Kurimoto, C. Song, T. Kozai, H. Watanabe, K. Ishii, H. Yagi, S. Yanaka, S. Uchiyama, T. Uchihashi, K. Kato, “Mutational and Combinatorial Control of Self-Assembling and Disassembling of Human Proteasome α Subunits”, Int. J. Mol. Sci. 20(9) 2308 (2019). ■ T. Uchihashi, Y. Watanabe, Y. Nakazaki, T. Yamasaki, H. Watanabe, T. Maruno, K. Ishii, S. Uchiyama, C. Song, K. Murata, R. Iino, T. Ando, “Dynamic structural states of ClpB involved in its disaggregation function”, Nat. Commun. 9(1) 2147 (2018).

Biomolecular Organization Research Group

KATO, Koichi



We explore the principles underlying biomolecular organization by multidisciplinary approaches.

【Research Result】

We have developed an approach to observe the dynamic ordering of biomolecules including proteins and carbohydrates in detail. For this purpose, we have integrated nuclear magnetic resonance, X-ray crystallography, solution scattering, native mass spectrometry, electron microscopy, high-speed atomic force microscopy, fluorescence microscopy, and computational methods through collaborative research networks within and outside ExCELLS. Using this approach, we have elucidated the dynamic organization mechanisms of circadian clock proteins and antibodies on membranes and clarified the molecular mechanisms of selective sorting and transport of glycoproteins and their quality control in cells. Furthermore, through the dynamic structural analysis of glycans, we have established a new approach for creating artificial glycans with improved functionality.

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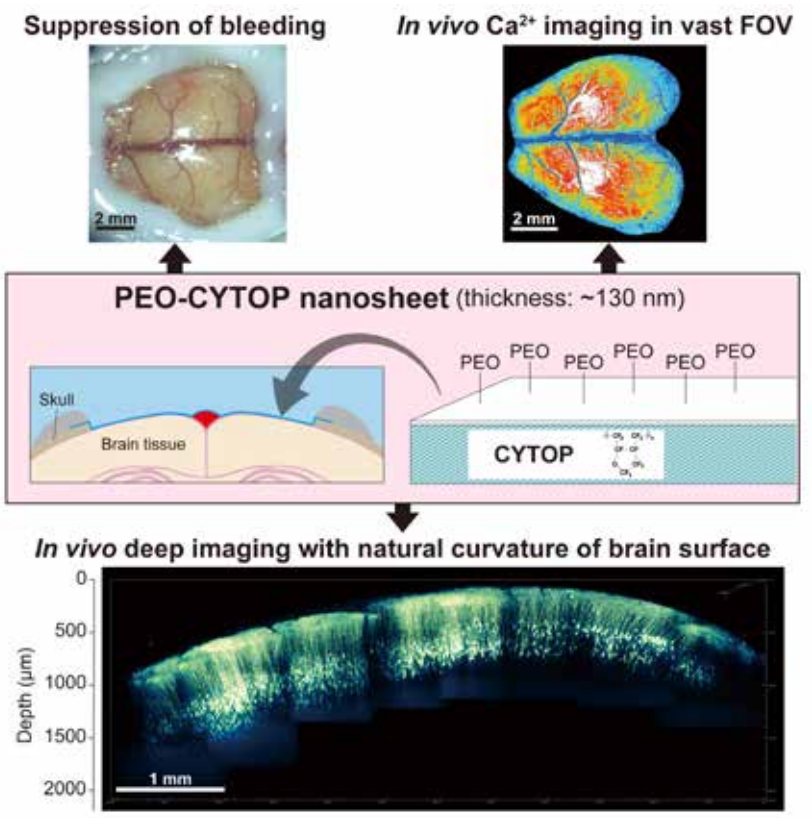
■ T. Watanabe, H. Yagi, S. Yanaka, T. Yamaguchi, K. Kato, “Comprehensive characterization of oligosaccharide conformational ensembles with conformer classification by free-energy landscape via reproductive kernel Hilbert space”, Phys. Chem. Chem. Phys. 23, 9753-9760 (2021). ■ H. Yagi, M. Yagi-Utsumi, R. Honda, Y. Ohta, T. Saito, M. Nishio, S. Ninagawa, K. Suzuki, T. Anzai, Y. Kamiya, K. Aoki, M. Nakanishi, T. Satoh, K. Kato, “Improved secretion of glycoproteins using an N-glycan-restricted passport sequence tag recognized by cargo receptor”, Nature Commun. 11, 1368 (2020). ■ T. Suzuki, S. Yanaka, T. Watanabe, G. Yan, T. Satoh, H. Yagi, T. Yamaguchi, K. Kato, “Remodeling of the oligosaccharide conformational space in the prebound state to improve lectin-binding affinity”, Biochemistry 59, 3180-3185 (2020). ■ S. Yanaka, R. Yogo, H. Watanabe, Y. Taniguchi, T. Satoh, N. Komura, H. Ando, H. Yagi, N. Yuki, T. Uchihashi, K. Kato, “Onmembrane dynamic interplay between anti-GM1 IgG antibodies and complement component C1q”, Int. J. Mol. Sci. 21, E147 (2019). ■ Y. Yunoki, K. Ishii, M. Yagi-Utsumi, R. Murakami, S. Uchiyama, H. Yagi, K. Kato, “ATP hydrolysis by KaiC promotes its KaiA binding in the cyanobacterial circadian clock system”, Life Sci. Alliance, 2, e201900368 (2019).

Biophotonics Research Group

NEMOTO, Tomomi ／ ENOKI, Ryosuke



Wide-field in vivo brain imaging using a novel nanosheet

【Research Result】

We were transferred from the Institute of Electronic Science, Hokkaido University, and inaugurated in October 2019. We are exploring the development of innovative bio-imaging methodologies and their applications to life sciences by using advanced technologies such as lasers, nonlinear optics, and nanomaterials. Since his move, he has successfully innovated imaging technologies such as developing a method for wide-field deep imaging of the mouse biological brain using novel nanosheets and the improvement of spatial resolution in deep biological brain imaging using adaptive optics. In collaboration with data science experts and quantitative biology research groups, we have also succeeded in visualizing the photo-attraction and three-dimensional transmission of ERK signaling in three-dimensional cultured specimens of cancer cells. We have also obtained essential insights into the molecular mechanisms of circadian rhythm centers.

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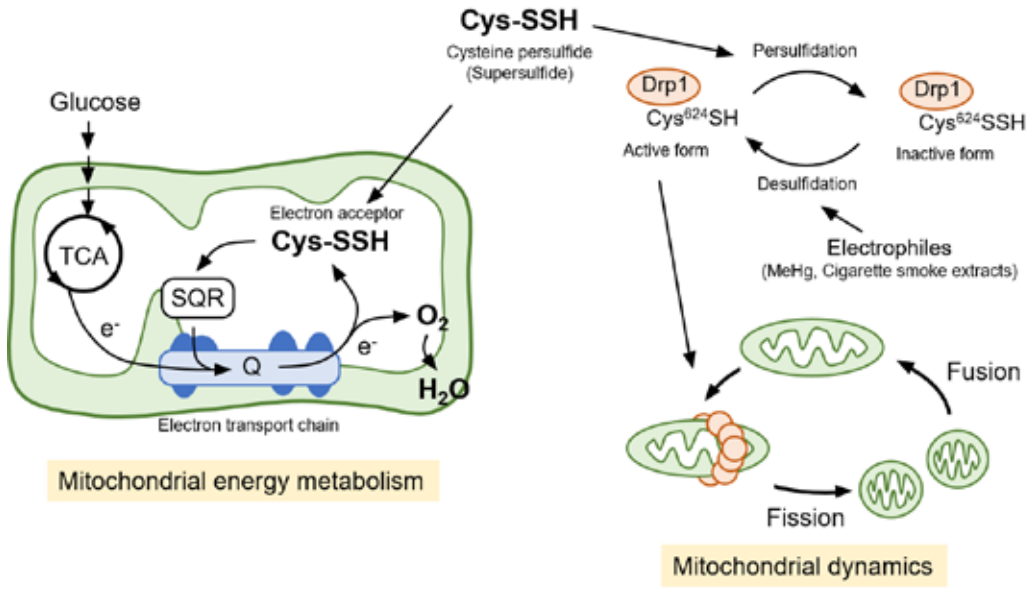
■ K. Yamaguchi, K. Otomo, Y. Kozawa, M. Tsutsumi, T. Inose, K. Hirai, S. Sato, T. Nemoto*, J. Uji-I* (*corresponding authors), “Adaptive Optical Two-photon Microscopy for Surface profiled Living Biological Specimens”, ACS Omega 6, 438-447 (2021). ■ T. Maejima, Y. Tsuno, S. Miyazaki, Y. Tsuneoka, E. Hasegawa, M. Islam, R. Enoki, T. Nakamura, M. Mieda, “GABA from vasopressin neurons regulates the time at which suprachiasmatic nucleus molecular clocks enable circadian behavior”, Proc. Natl. Acad. Sci. USA 118, e2010168118-1 - e2010168118-11 (2021). ■ S. Nakamura, S. Hagihara, K. Otomo, H. Ishida, J. Hidema, T. Nemoto, M. Izumi, “Autophagy contributes to quality control of leaf mitochondria”, Plant Cell Physiol. 62, 229–247 (2020). ■ K. Iijima, T. Oshima, R. Kawakami, T. Nemoto, “Optical clearing of living brains with MAGICAL to extend in vivo imaging”, iScience 24, 101888-1 - 101888-11 (2020). ■ T. Takahashi, H. Zhang, R. Kawakami, K. Yarinome, M. Agetsuma, J. Nabekura, K. Otomo, Y. Okamura, T. Nemoto, “PEO-CYTOP Fluoropolymer Nanosheets as a Novel Open-Skull Window for Imaging of the Living Mouse Brain”, iScience 23, 101579-1 - 101579-13 (2020).

Cardiocirculatory Dynamism Research Group

NISHIDA, Motohiro ／ NISHIMURA, Akiyuki



The role of supersulfides. Supersulfides such as cysteine persulfide (Cys-SSH) act as an electron acceptor in the mitochondrial electron transport chain. Cys-SSH that is incorporated into protein (persulfidation) also regulates mitochondrial quality control.

【Research Result】

Supersulfides, consisting of multiple sulfur atoms, were revealed to act as a molecular entity that mediates the redox reaction required for respiration/energy metabolism and stress adaptation in robust myocardium. Particularly, mitochondria-localized Cys tRNA synthase produces cysteine persulfide (Cys-SSH), which is more nucleophilic than CysSH, and Cys-SSH acts as an electron acceptor in the mitochondrial respiratory chain, and Cys-SSH is preferentially incorporated into the mitochondrial fission-accelerating protein Drp1 during translation, leading to maintenance to mitochondrial quality. Furthermore, we demonstrated that the inhibition of mitochondrial hyperfission via Drp1 desulfidation leads to prognosis improvement of mouse heart failure.

【References】

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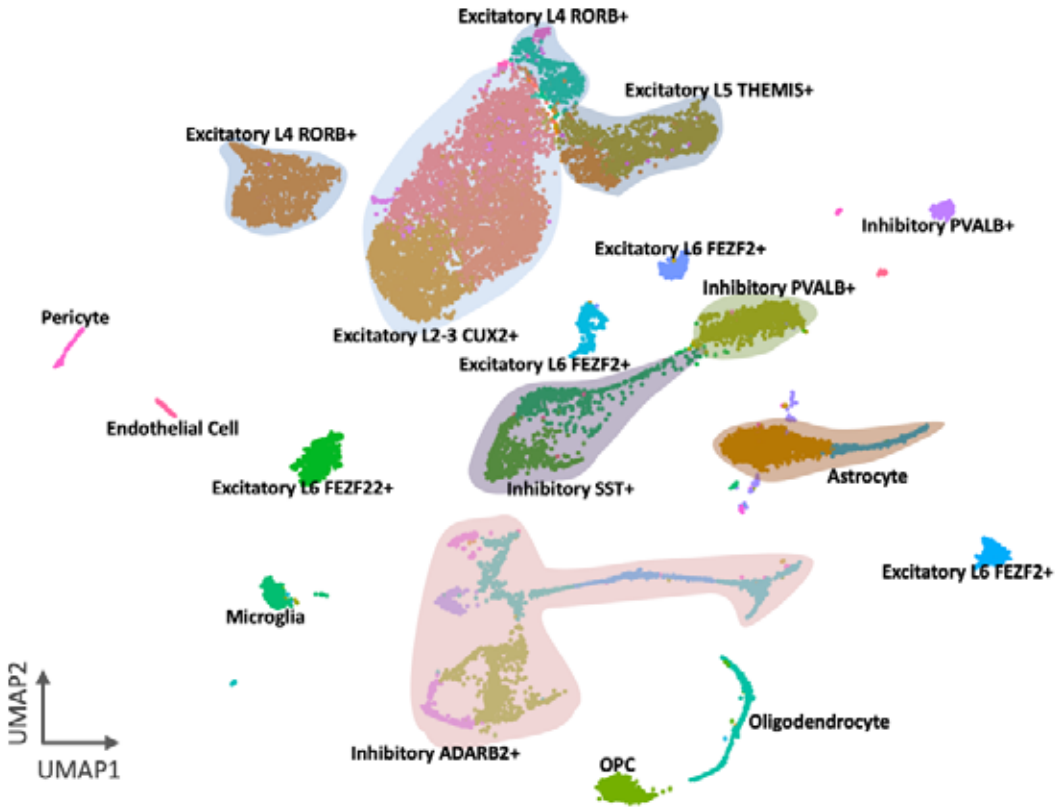
Cognitive Genomics Research Group

GO, Yasuhiro



Single-nucleus RNA-seq reveals various types of brain cells in the marmoset cortex

【Research Result】

We analyze the spatiotemporal gene expression dynamics in pharmacologically generated primate disease models to understand the molecular causality of the disease. As a result of comparing single-cell expression between the marmoset autism model and human postmortem autism brain, we identified common molecular changes in some glial cells. In addition, we are analyzing neuropsychiatric genes in over 1000 macaque monkeys and marmosets. We have identified several individuals with loss-of-function mutations in genes responsible for human neuropsychiatric disease. We are now creating new primate disease models by homologating such genes to understand and elucidate the pathogenesis.

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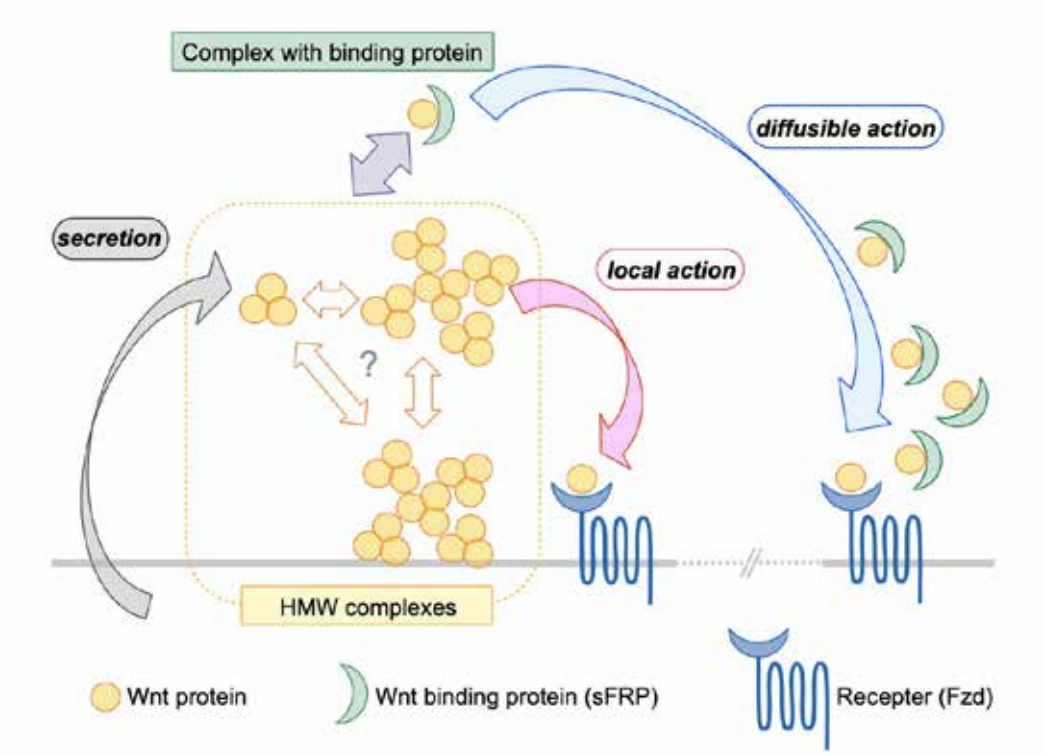
Developmental Signaling Research Group

TAKADA, Shinji



Model of Wnt protein diffusion: Wnt trimers are the smallest unit of the HMW complex. Both the trimer and the HMW complex appear to exist in the extracellular milieu although it is uncertain when the assembly to the HMW complex occurs during the process of Wnt secretion. The HMW complex is probably less mobile when interacting with the plasma membrane, resulting in the restriction of Wnt diffusion range. Some Wnt molecules can be dissociated by local interaction with Frizzled receptor (Fzd), resulting in a short-range signal (local action). In contrast, the HMW complex, probably as well as the trimer itself, can also be dissociated by interaction with soluble Wnt binding protein (partner protein), including sFRP. By this dissociation, Wnt turns to be more mobile and its diffusion range is expanded (diffusible action).

【Research Result】

We have been investigating several questions related to cell-to-dell signaling in tissue development; how Wnt, a secreted signaling protein, is actually distributed in tissues, how its distribution is regulated, and what physiological implications this distribution has, by utilizing multiscale approach from molecules to embryos. As a result, we found that secreted Wnt forms high-molecular-weight complexes with a homotrimer as the basic unit, and that its diffusivity changes as the complex composition changes. We also quantitatively analyzed the diffusion of Wnt in embryos and proposed a new model to explain the diffusion of Wnt in tissues. Furthermore, we reported that Wnt expression in the dorsal-most portion of the developing neural tube localizes to the lumenal side and that the dorsal-most portion undergoes dynamic morphological changes.

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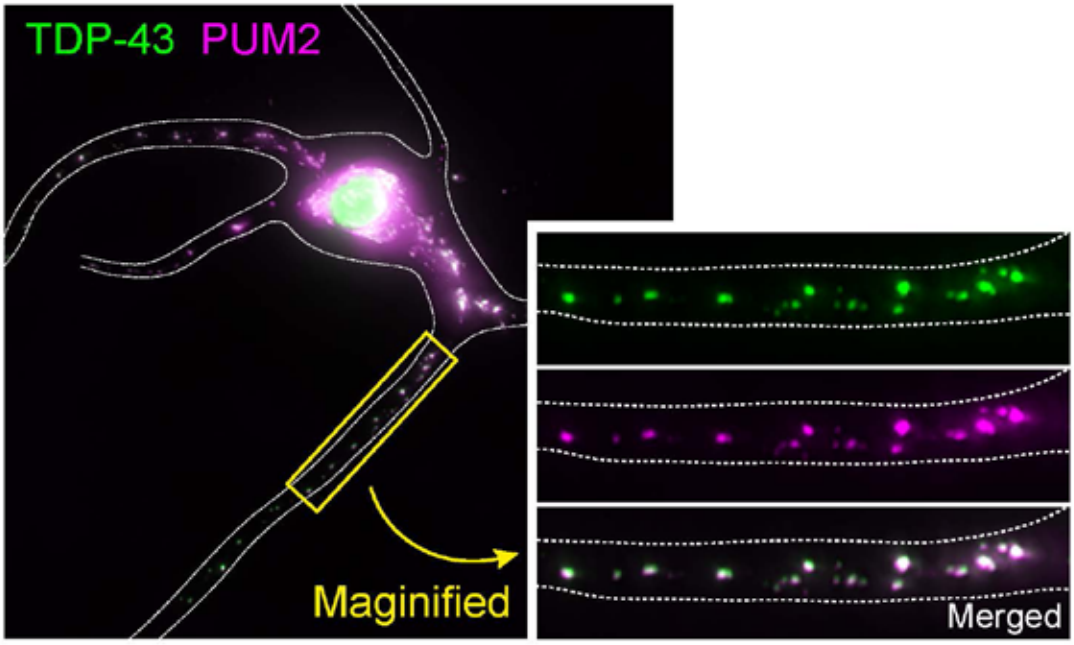
Dynamic Molecular Neurobiology Group

SHIINA, Nobuyuki



Live imaging of two factors (TDP-43, PUM2) localized in RNA granules of neurons derived from mouse cerebrum. The punctate structures in the magnified images are RNA granules transported to dendrites. By performing FRAP during such imaging, the fluidity of the two factors in RNA granules can be measured simultaneously.

【Research Result】

Long-term potentiation of neural synapses is required for the formation of long-term memory and is mediated by "local translation" that involves mRNA transport to the vicinity of synapses and subsequent protein synthesis. The local translation machinery "RNA granule" is formed by liquid-liquid phase separation, and it is thought that the fluidity of RNA granules is regulated to control mRNA transport and local translation spatiotemporally. We have developed methods for measuring and quantifying the fluidity of RNA granule-constituting proteins in cells, and found the relationship between fluidity and local translation within RNA granules. In addition, we have found the effects of proteins responsible for neurodegenerative diseases on fluidity and local translation. Furthermore, besides neurons, we have revealed that a specific RNA granule-constituting protein is involved in lens differentiation through translational regulation in the eye.

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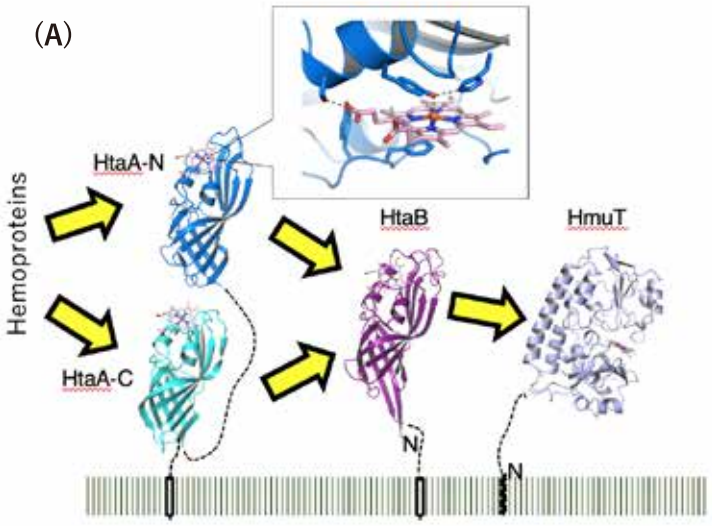
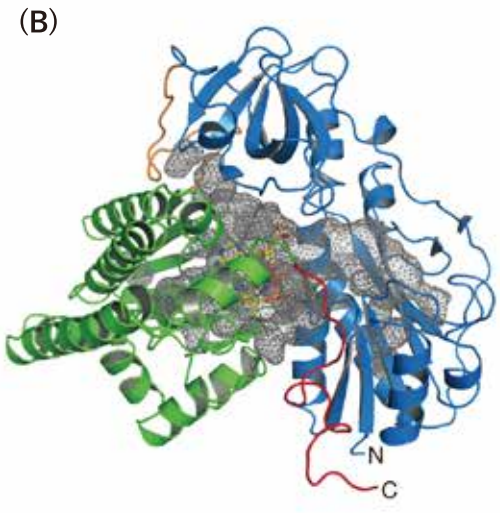
Metallobiology Group

AONO, Shigetoshi



(A) Structure of the heme uptake machinery consisting of HtaA, HtaB, and HmuT in corynebacteria. Heme molecules are transported among these proteins on the cell surface of corynebacteria in order as shown in yellow arrows.



(B) Structure of HypX responsible for biogenesis of CO, which is used as a component of the active site of Ni-Fe hydrogenase. CoA retained in the cavity (grey mesh) is formylated to form formyl-CoA, from which CO is produced by decarbonylation reaction.

【Research Result】

1. Heme uptake machinery of Corynebacteria including *Corynebacteria glutamicum* and *Corynebacterium diphtheriae* consists of heme binding proteins, HtaA and HtaB, and the ABC-type heme transporter HmuTUV. We have determined the crystal structures of the N-, and C-terminal domains of HtaA (HtaA-N and HtaA-C, respectively), HtaB, and HmuT. Based on these structures and spectroscopic analyses, we have elucidated the molecular mechanism of heme transport in this system.

2. We have determined the crystal structure of HypX, which catalyzes the biosynthesis of CO that is used as a component of the active site in Ni-Fe hydrogenase. HypX binds CoA constitutively as a prosthetic group in the continuous cavity connecting the N- and C-terminal domains. Based on these crystal structures and MD simulations, we propose the molecular mechanism of CO biosynthesis consisting of formylation of CoA and decarbonylation of formyl-CoA.

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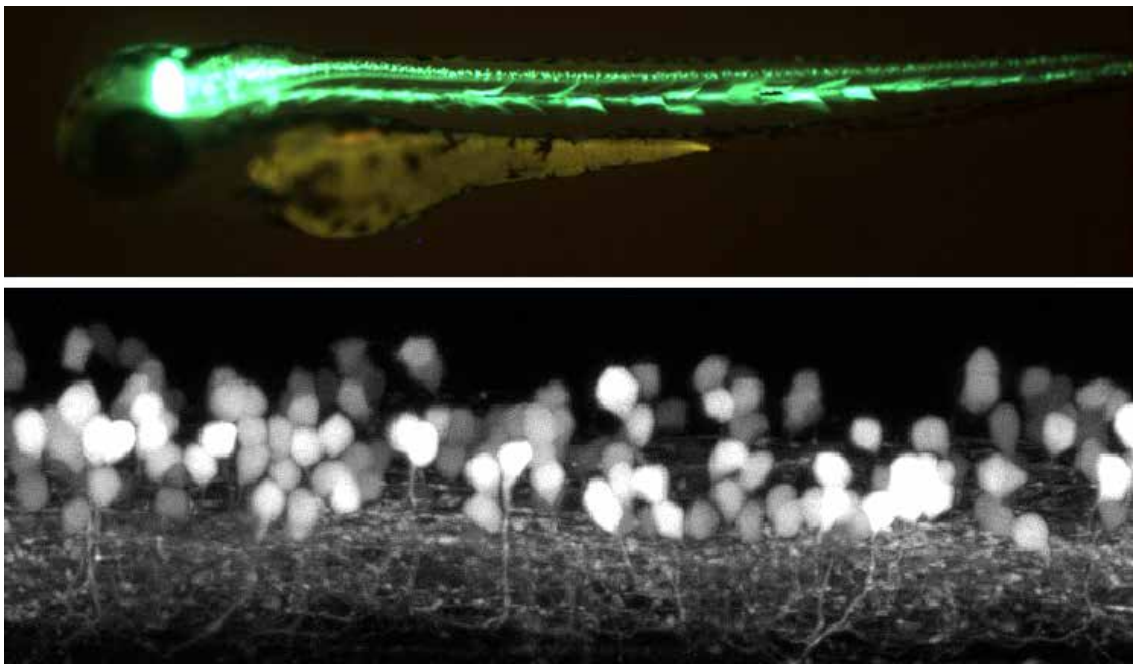
Neuronal Networks Research Group

HIGASHIJIMA, Shin-ichi



Transgenic zebrafish that express GFP in spinal V1 neurons (neurons that express transcription factor En1). The top panel shows a low magnification view of the transgenic fish, while the bottom panel shows a hagh magnification view of the spinal cord.

【Research Result】

Using larval zebrafish, we are studying neuronal circuits that control locomotion. Central to our approach is to visualize specific classes of neurons by making transgenic zebrafish that express fluorescent proteins in these cells. Using such transgenic fish, we have investigated the function of specific classes of cells by using electrophysiology, calcium imaging, optogenetics, and laser ablation. For the past 4 years, we have done the following studies. (1) We revealed behavioral role of the reciprocal inhibition between a pair of Mauthner cells during fast escapes. (2) We revealed that spinal V1 neurons regulate locomotor speed and selection of active sets of neurons during swimming. (3) We have uncovered functional diversity of glycinergic commissural inhibitory neurons that are involved in swimming. (4) We studied neuronal circuits that control rhythmic pectoral fin movements.

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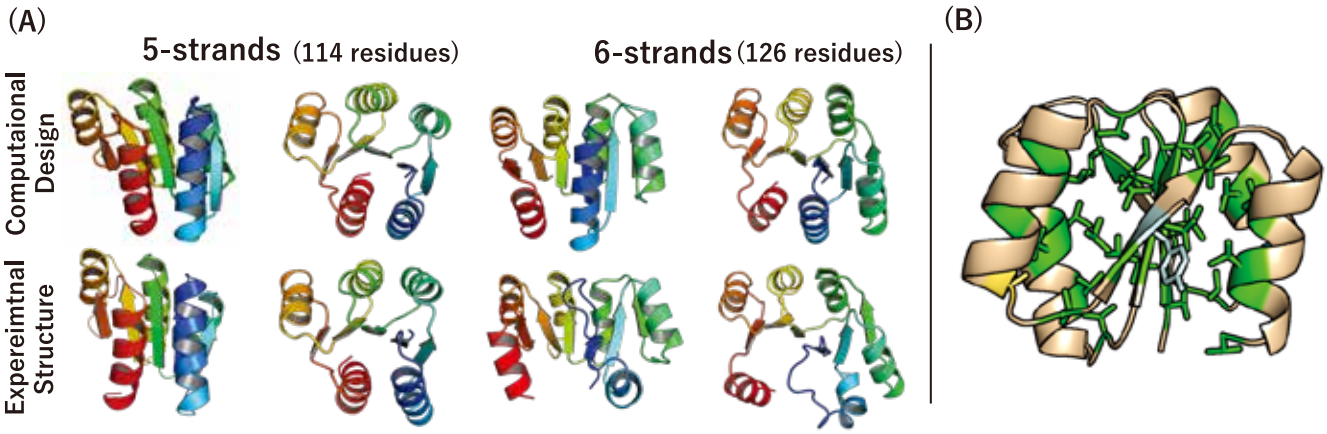
Protein Design Group

KOGA, Nobuyasu



(A) Success in designing large and complex $\alpha\beta$-protein structures. One of the long standing problems was that the designs for large and complex proteins with five or more β-strands fold into different topologies from the targeted topologies. Our newly developed protein design methodologies made it possible to design such proteins [1].

(B) A de novo designed protein with most of the core filled with valine residues. This de novo designed protein, created using our developed design methodology, shows folding ability even with most of the core filled with valine residues and high thermal stability above 100℃ [3].

【Research Result】

Protein molecules spontaneously fold into unique three-dimensional structures specified by their amino acid sequences from random coils to carry out their functions. We seek the principles for protein folding and functions by computationally designing proteins completely from scratch and develop the methods to design proteins of our interests. We succeeded in developing the methods for designing protein structures completely from scratch and thermally stabilizing protein structures. We are currently studying the relations between the evolution of life and protein folds by designing proteins with folds that do not exist on earth.

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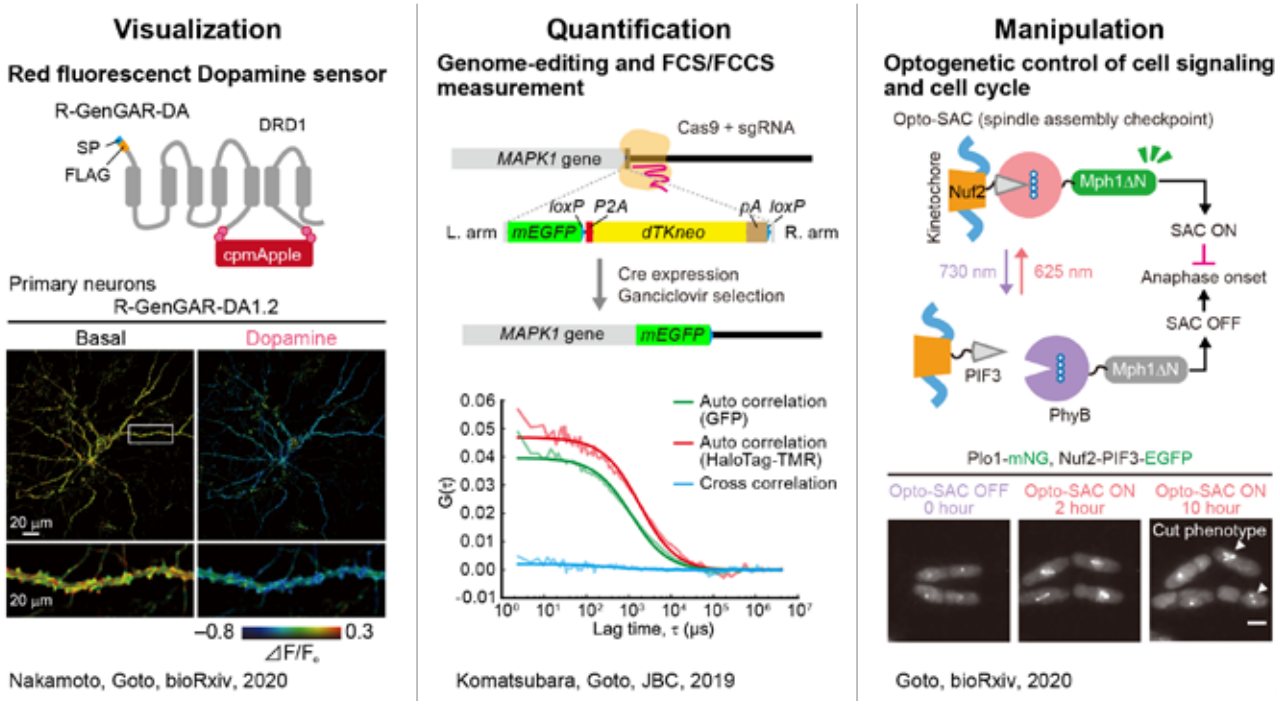
Quantitative Biology Group

AOKI, Kazuhiro



Representative results of visualization, quantification, and manipulation of the intracellular signal transduction system.

【Research Result】

A living cell senses various stimuli from the surrounding environment and processes the information inside the cell, resulting in cellular behaviors adapting to environmental changes. Our research group aims to quantitatively understand and control the molecular machinery underlying cellular input/output responses. We have so far reported visualization of the intracellular signal transduction system involved in cell death (Miura 2018), development of a new Dopamine biosensor (Nakamoto 2020), and quantification of dissociation constants in living cells (Komatsubara 2019). We recently succeeded in developing new optogenetic tools for the optical manipulation of cell cycle (Goto 2020; Yamamoto 2021).

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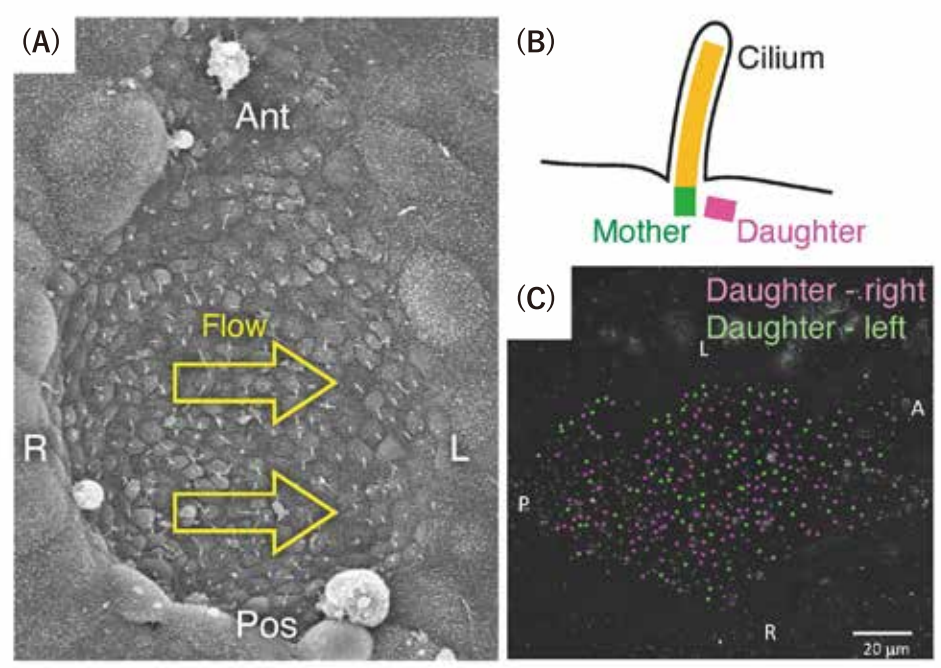
Spatiotemporal Regulations Group

NONAKA, Shigenori



(A) Scanning electron micrograph of the mouse embryonic node, ventral view. Leftward flow occurs within this area.
(B) Illustration of the bottom structure of the node cilium. The mother centriole works as the basal body of the cilium, whereas the daughter is solely located nearby.
(C) Each daughter centriole's positions relative to its mother in the node. Magenta dots indicate the daughter at the right, green dots at the left. Number of the magenta is larger than the green.

【Research Result】

We have been investigating the initial left-right (L-R) determination in mammalian development. On a small patch called 'the node' on the ventral surface of a mouse embryo, leftward fluid flow generated by vortical motion of primary cilia directs future L-R asymmetry. We have analyzed this step using ultra-fast imaging by light-sheet microscopy and super-resolution imaging by STED microscopy, and recently found a certain bias along L-R within the cytoskeletal structure at the bottom of the cilia. Whereas the role of this bias remains unclear, we are testing two hypotheses: 1) the bias is a part of conversion mechanism by which the flow direction determines L-R asymmetric gene-expression, or 2) the bias arise independently from previously known L-R determination process. In addition, we carry on a number of collaborations using light-sheet and super-resolution microscopes.

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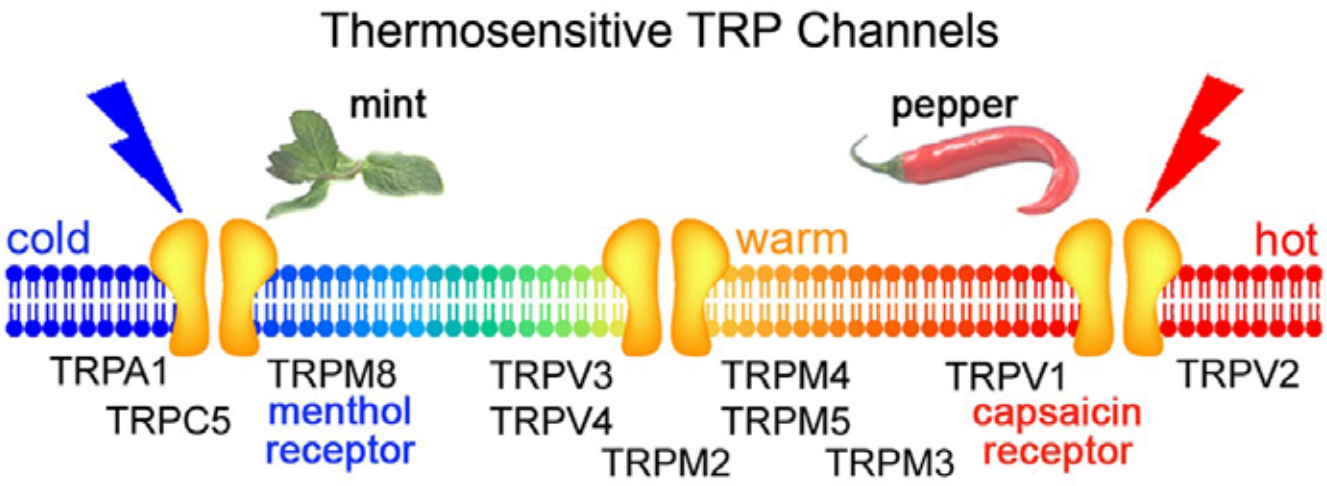
Thermal Biology Group

TOMINAGA, Makoto ／ SOKABE, Takaaki



Thermosensitive TRP channels. There are various kinds of thermosensitive TRP channels sensing cold to heat. TRPV1 activated by heat is also sensitive to an ingredient of pepper, capsaicin, and TRPM8 activated by cold stimulus is also sensitive to an ingredient of mint, menthol.

【Research Result】

We mainly investigate molecular mechanisms of thermosensation and their physiological significance by focusing on thermosensitive TRP ion channels from insects to mammals. We are also trying to clarify the nociceptive mechanisms at peripheral nerve endings by focusing on TRPV1 and TRPA1. We are doing behavioral analyses of mice lacking the thermosensitive TRP channels. Furthermore, we are cloning the thermosensitive TRP channels genes from various species, which would help us to understand the mechanisms of thermosensation in the evolution. We also utilize fruit flies as a model to investigate temperature preference and adaptation, particularly focusing on regulatory roles of lipid components.

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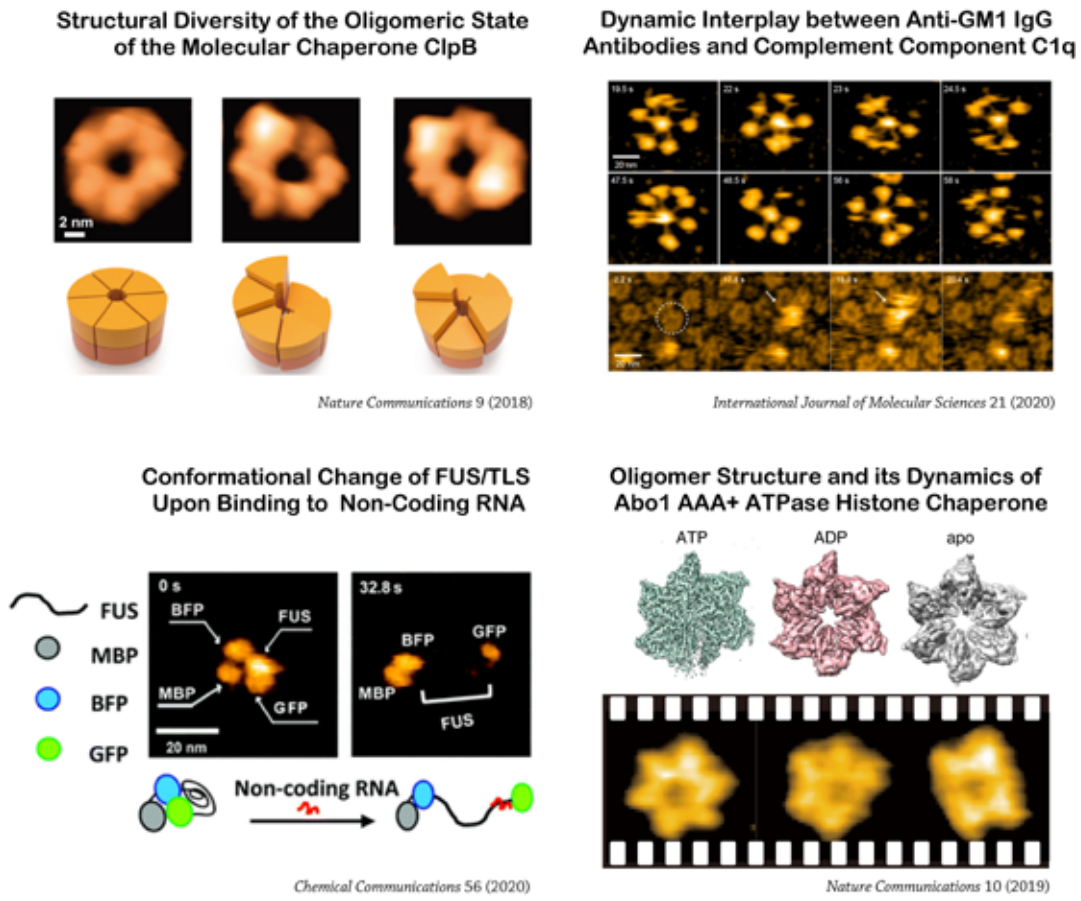
Biomolecular Dynamics Observation Group

Collaborative Research Group

UCHIHASHI, Takayuki



Representative Results of Collaborative Research on Protein Dynamics Revealed by High-Speed Atomic Force Microscopy

【Research Result】

Our collaborative research group has been conducting collaborative research on the functional dynamics of biomolecules with and outside of the ExCELLS, focusing on the development of novel techniques for multimodal and multi-scale analysis of various biological phenomena from various viewpoints. We have successfully developed a high-speed AFM/single molecule fluorescence microscopy system and a high-speed force mapping method to visualize the dynamics of biomolecular mechanical properties as well as their structures. In addition, we have actively promoted collaborative research using high-speed AFM technique especially for analyzing single-molecule dynamics, and published 5 papers in collaboration within ExCELLS and 29 papers in collaboration with external institutes, and made 7 press releases.

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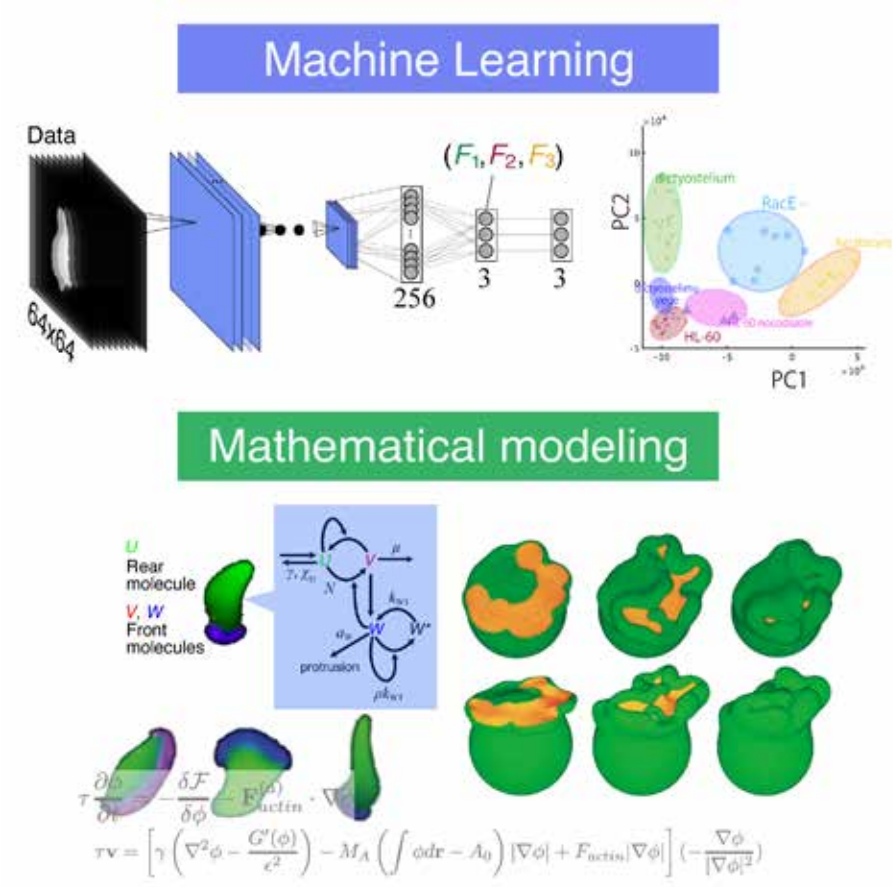
Theoretical Biology Group

HONDA, Naoki ／ SAITO, Nen

Collaborative Research Group



Cell morphology analysis by machine learning (top panel).
Deciphering mechanisms by mathematical models (bottom panel).

【Research Result】

Now is a moment we need a fusion of quantitative experiments and mathematics in biology. Recently, measurement technologies such as live imaging and next-generation sequencers have been rapidly developed, and we have entered a new era in which molecular activities and gene expression levels in living tissues can be measured at single-cell resolution in a high throughput manner. Our laboratory aims to elucidate theoretical logic of dynamic living systems from such data by combining mathematical modeling and machine learning.

In the research on gene expression data, we proposed a framework for accurate reconstruction of spatial transcriptomes by integrating single-cell RNA sequencing data and in situ hybridization data. We also conducted a quantitative comparison between mathematical models and experiments on the shape data of migrating cells. In addition, we have studied collective migration of epithelial cells, and the model analysis of microtubule transport by molecular motors.

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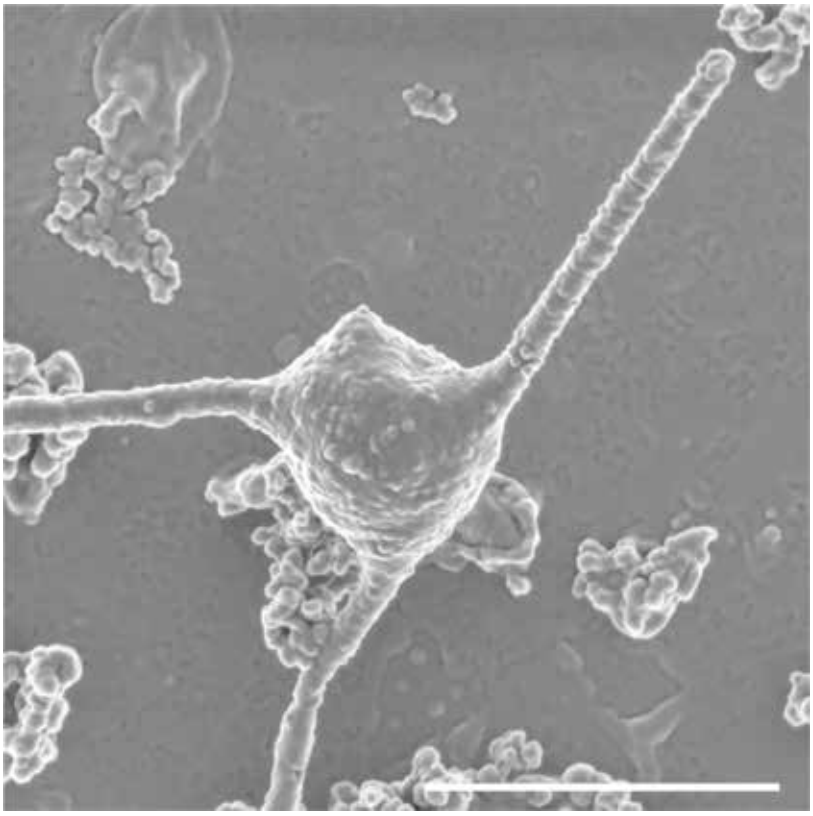
Deep-Sea and Deep Subsurface Life Research Group

TAKAI, Ken／NAKAGAWA, Satoshi



An electron micrograph of the 1st isolate of Asgaard archaea from subseafloor sediments of deep-sea. Scale bar indicates 1 μm.

【Research Result】

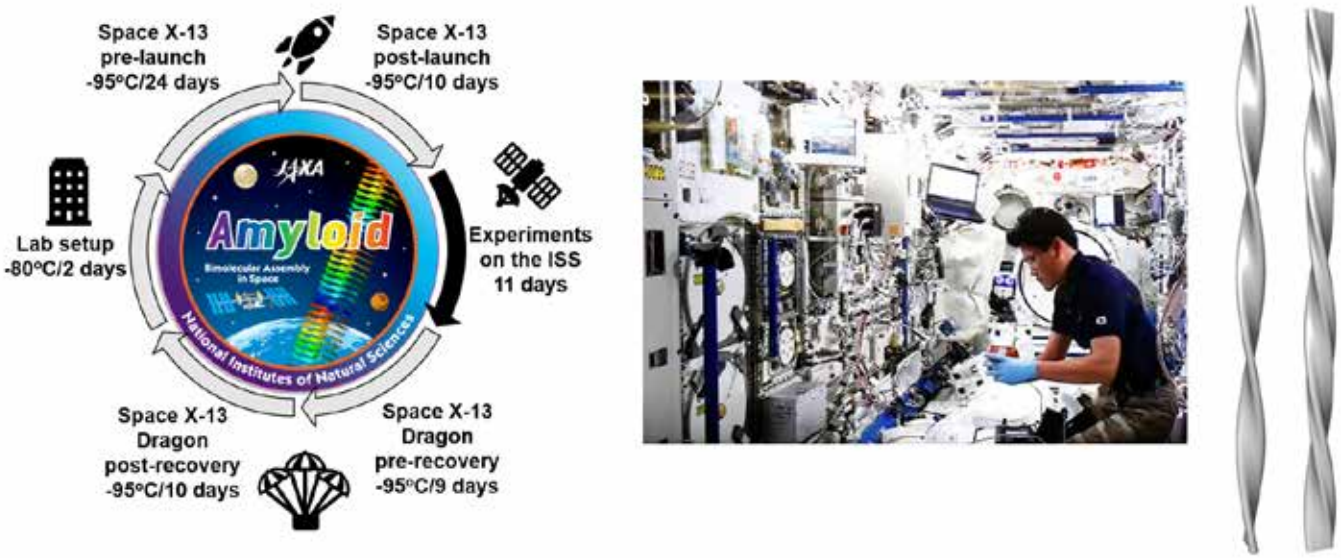
We look for real limits of life and biosphere and boundary conditions between habitable and uninhabitable in the dark world, namely deep-sea and deep subsurface environments by means of top-rated exploration platforms such as human-occupied submersible vehicle (HOV), remotely operative vehicles (ROV), research vessels including scientific drilling vessels. In 2020, we for the first time succeeded in cultivation and isolation of the Asgaard archaea that had been known as one of the most predominant "microbial dark matter" in the global subseafloor sediments and as the ancestral archaea for "Eucaryogenesis". In addition, we have investigated the diversity and structures of polysaccharides and glycome in the cellular surface off such "microbial dark matter".

【References】

H. Imachi, M. K. Nobu, N. Nakahara, Y. Morono, M. Ogawara, Y. Takaki, Y. Takano, K. Uematsu, T. Ikuta, M. Ito, Y. Matsui, M. Miyazaki, K. Murata, Y. Saito, S. Sakai, C. Song, E. Tasumi, Y. Yamanaka, T. Yamaguchi, Y. Kamagata, H. Tamaki, K. Takai, "Isolation of an archaeon at the prokaryote–eukaryote interface", Nature 575 519-525 (2020).

Extreme Environmental Biomolecular Research Group

KATO, Koichi



Timeline of the microgravity experiments (left), ISS experiments by the JAXA astronaut (middle), and the distinct morphologies of Aβ fibrils formed under microgravity conditions (right).

【Research Result】

Organisms living in extreme environments develop unique ecological adaptation mechanisms. We have identified novel glycans in deep-sea microorganisms and explored the molecular mechanism of tardigrade anhydrobiosis through biomolecular structural analysis. In addition, we have found that a macromolecular complex of unknown function from a hyperthermophilic archaeon has a shape similar to that of "tholos" in ancient Greek architecture, providing interesting insights into molecular evolution. Furthermore, we are exploring the boundary between matter and life by targeting giant viruses. On the other hand, in collaboration with the Japan Aerospace Exploration Agency (JAXA), we have conducted an experiment using the International Space Station "Kibo", showing that amyloid fibrils formed under microgravity conditions have characteristic morphologies not found on the ground.

【References】

■ M. Yagi-Utsumi, T. Tanaka, Y. Otsubo, A. Yamashita, S. Yoshimura, M. Nishida, K. Kato, “Cold atmospheric plasma modification of amyloid β”, Int. J. Mol. Sci. 22, 3116 (2021). ■ M. Yagi-Utsumi, S. Yanaka, C. Song, T. Satoh, C. Yamazaki, H. Kasahara, T. Shimazu, K. Murata, K. Kato, “Characterization of amyloid β fibril formation under microgravity conditions”, NPJ Microgravity 6, 17 (2020). ■ M. Yagi-Utsumi, A. Sikdar, C. Song, J. Park, R. Inoue, H. Watanabe, R.N. Burton-Smith, T. Kozai, T. Suzuki, A. Kodama, K. Ishii, H. Yagi, T. Satoh, S. Uchiyama, T. Uchihashi, K. Joo, J. Lee, M. Sugiyama, K. Murata, K. Kato, “Supramolecular tholos-like architecture constituted by archaeal proteins without functional annotation”, Sci. Rep. 10, 1540 (2020).

Extremotolerance Research Group

ARAKAWA, Kazuharu

Tardigrade expressing GFP designed to be localized in nucleus using the novel vector expression system.

【Research Result】

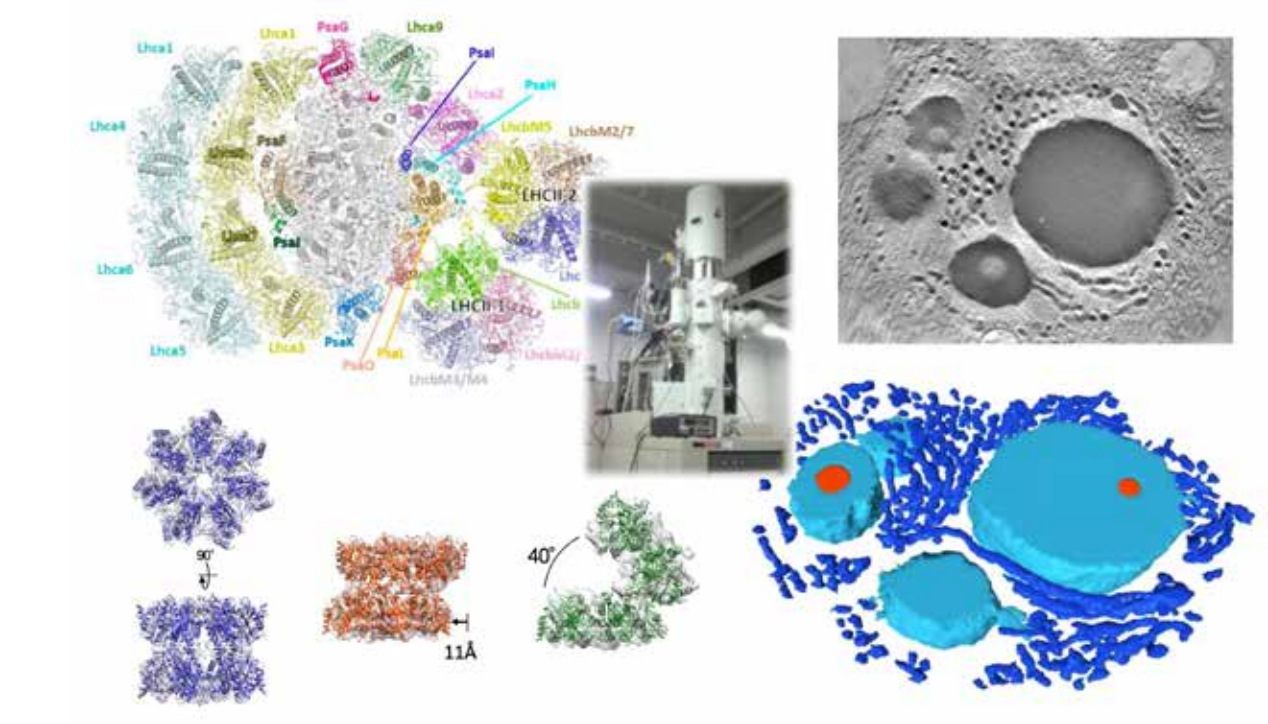
Using original ultra-low input method, we have sequenced dozens of tardigrade genomes, including species that cannot be cultured in laboratory. Through comparative analysis of these genomes, convergent evolution and two independent acquirement events of anhydrobiosis machinery even within the phylum Tardigrada are suggested. We also identified a novel tardigrade-specific peroxidase that can improve oxidative stress tolerance of human cells, based on cross-tolerance assay with UV irradiation. Moreover, in collaboration with Prof. Kato, Prof. Aoki, and Prof Uchihashi, we revealed that CAHS protein, which is significantly overexpressed upon desiccation, forms reversible fibrous matrix to protect cellular contents. Furthermore, we have constructed a vector system that can express any protein in live tardigrades, which would be a fundamental tool to “observe” and to “create” tardigrade extremotolerance machineries.

【References】

■ J. Fleming, D. Pisani, K. Arakawa, “New Tardigrade Opsins and Differential Expression Analyses shows ontogenic variation in light perception”, Genome Biology and Evolution evab164 (2021). ■ K. Arakawa, K. Numata, “Reconsidering the "glass transition" hypothesis of intrinsically unstructured CAHS proteins in desiccation tolerance of tardigrades”, Mol Cell 81 409-410 (2021). ■ J. Fleming, K. Arakawa, “Systematics of Tardigrada: A reanalysis of tardigrade taxonomy with specific reference to Guil et al (2019)”, Zoologica Scripta 50 376-382 (2021). ■ K. Arakawa, “Simultaneous metabarcoding of eukaryotes and prokaryotes to elucidate the community structures within tardigrade microhabitats”, Diversity 12 110 (2020). ■ K. Kondo, M. Mori, M. Tomita, K. Arakawa, “Pre-treatment with D942, a furancarboxylic acid derivative, increases desiccation tolerance in an anhydrobiotic tardigrade Hypsibius exemplaris”, FEBS Open Bio 10 1774-1781 (2020).

Material-Life Boundary Research Group

MURATA, Kazuyoshi



Structural analyses by Electron Microscopy. Left top: Structure of photosytem I supercomplex in a state transition. Left bottom: Dynamic structure of α7 homo-tetradecamer double ring. Right: Viral factory formed by cottonvirus.

【Research Result】

Our group is studying the structure of biomolecules in extreme environments and extreme states, which has been difficult to analyze, using a cryo-electron microscope. Although it has only been six months since its inception, several novel structures have been reported: (1) the dynamic structural change of the α7 complex ring (Song et al. 2021), which has been thought to be involved in the formation of the proteasome, (2) the structure of the state transition from Photosystem II to I (Pan et al. 2021), and (3) the characteristic ultrastructure of the virus factory (Takahashi et al. 2021) formed by the novel giant virus "cotton virus" discovered in Japan. In the future, I would like to visualize further unknown life phenomena.

【References】

■ C. Song, T. Satoh, T. Sekiguchi, K. Kato, K. Murata, "Structural fluctuations of the human proteasome α7 homo-tetradecamer double ring imply the proteasomal α-ring assembly mechanism", Int J Mol Sci 22(9), 4519 (2021). ■ X. Pan, R. Tokutsu, A. Li, K. Takizawa, C. Song, K. Murata, T. Yamasaki, Z. Liu, J. Minagawa, M. Li, "Structural basis of LhcbM5-mediated state transitions in green algae", Nat Plant 7(8), 1119-1131 (2021). ■ H. Takahashi, S. Fukaya, C. Song, K. Murata, M. Takemura, "Cotonvirus japonicus using Golgi apparatus of host cells for its virion factory phylogenetically links tailed tupanvirus and icosahedral mimivirus", J Virol 95(18), e0091921 (2021).

3

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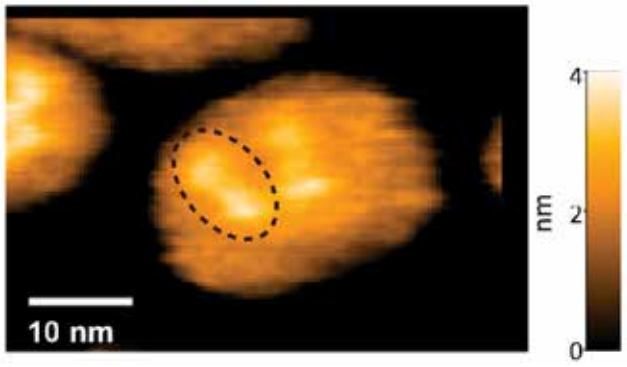
Major Achievements Resulting from Joint Research Projects

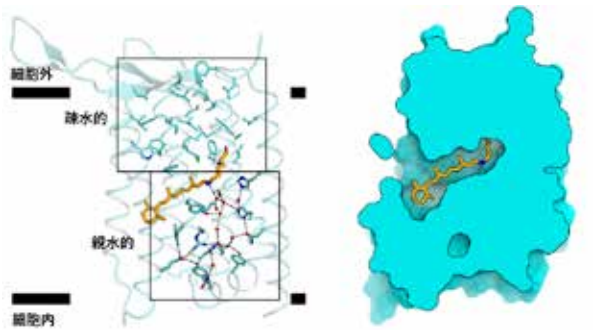
September 2019

Shihoya W, Inoue K, Singh M, Konno M, Hososhima S, Yamashita K, Ikeda K, Higuchi A, Izume T, Okazaki S, Hashimoto M, Mizutori R, Tomida S, Yamauchi Y, Abe-Yoshizumi R, Katayama K, Tsunoda SP, Shibata M, Furutani Y, Pushkarev A, Béjà O, Uchihashi T, Kandori H, Nureki O.
Crystal Structure of Heliorhodopsin, ***Nature*** 2019, 574: 132-136.

【 Outline of achievement 】

The 3-D structure and dynamics of heliorhodopsins were elucidated using X-ray crystal structure analysis and high-speed atomic force microscopy. The study has found that if heliorhodopsins are combined with different amino acids, they could possibly manifest light-induced structural changes much like bacteriorhodopsins would. The findings from this study are thought to be useful for further research on rhodopsin diversity and the exploration of novel rhodopsins.



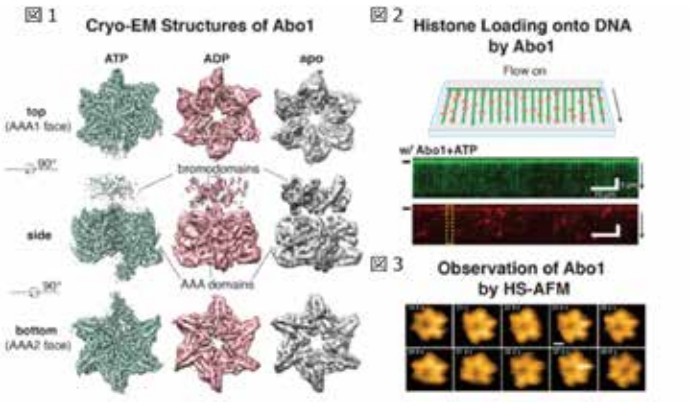


December 2019

Cho C, Jang J, Kang Y, Watanabe H, Uchihashi T, Kim SJ, Kato K, Lee JY, Song JJ.
Structural basis of nucleosome assembly by the Abo1 AAA+ ATPase histone chaperone, ***Nature Commun.*** 2019, 10: 5764.

【 Outline of achievement 】

For the first time in scientific research, the study was able to perform a detailed structural analysis of chaperone protein Abo1 that regulates chromatin assembly containing DNA which is commonly known as the blueprint of life, using cryo-electron microscopy, and observe its dynamics using high-speed atomic force microscopy. This finding serves as a basis for further research to elucidate the structure and functions of histone chaperones, and might lead to a deeper understanding of chromatin remodeling regulation.

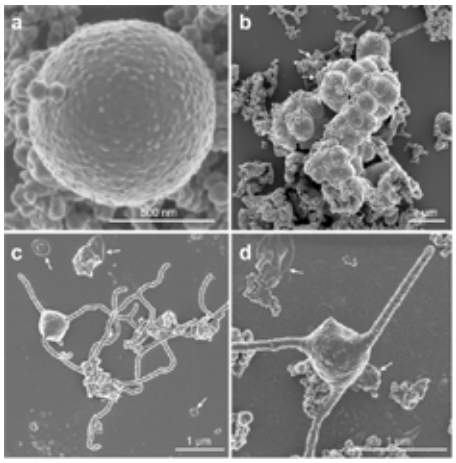


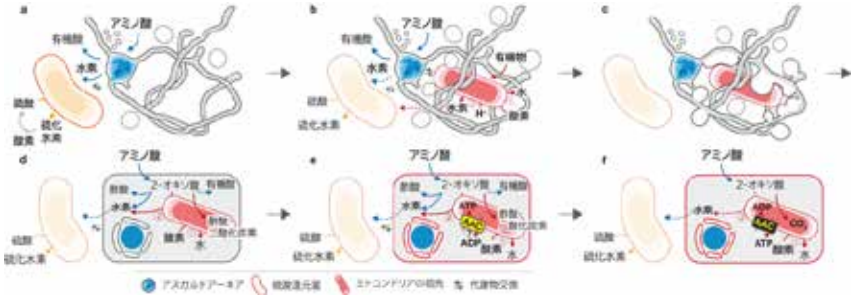
January 2020

Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, Takaki Y, Takano Y, Uematsu K, Ikuta T, Ito M, Matsui Y, Miyazaki M, Murata K, Saito Y, Sakai S, Song C, Tasumi E, Yamanaka Y, Yamaguchi T, Kamagata Y, Tamaki H, Takai K.
Isolation of an archaeon at the prokaryote–eukaryote interface, *Nature* 2020, 577: 519-525.

【 Outline of achievement 】

This interdisciplinary research, conducted jointly with JAMSTEC, succeeded in culturing the archaea that are most closely related to the ancestor of all eukaryotes for the first time in the world, from certain deep-sea sediments. This finding has elucidated that the archaea grow in a manner that is dependent on their symbiosis with other microorganisms and have a number of genes (e.g., actin-producing genes) that were previously thought to be specific to eukaryotes, and has significantly advanced science on the origin of eukaryotes, which is considered one of the biggest mysteries that remain in biology.



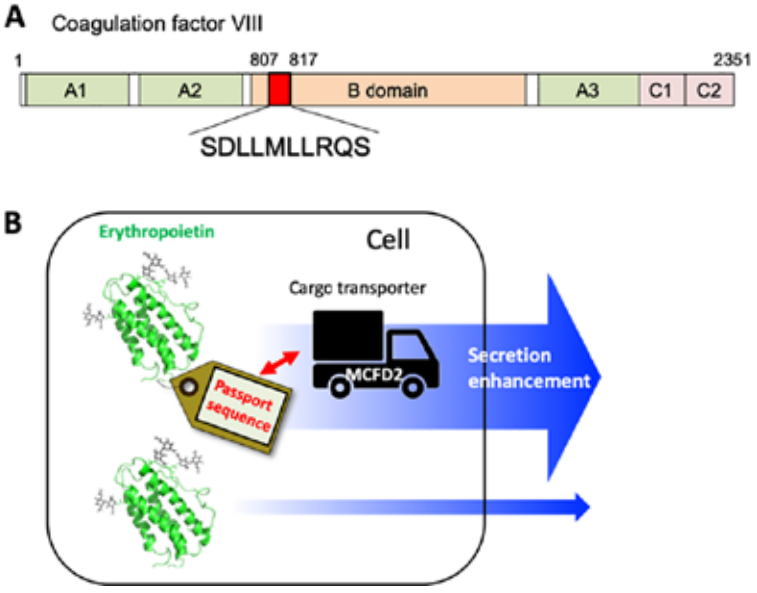


March 2020

Yagi H, Yagi-Utsumi M, Honda R, Ohta Y, Saito T, Nishio M, Ninagawa S, Suzuki K, Anzai T, Kamiya Y, Aoki K, Nakanishi M, Satoh T, Kato K.
Improved secretion of glycoproteins using an N-glycan-restricted passport sequence tag recognized by cargo receptor, ***Nature Commun.*** 2020, 11: 1368.

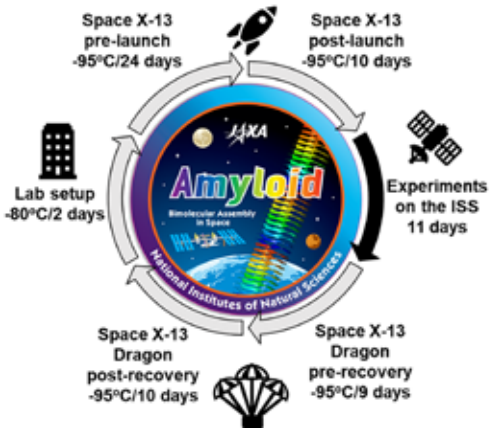
【 Outline of achievement 】

This interdisciplinary research encompassing medicine, pharmacy, and life science,was conducted on the mechanism of blood coagulation factor secretion. They found evidence indicating that their production yields could be improved significantly by giving molecular “passports” to certain glycoproteins being used in biopharmaceuticals to enable their intracellular transportation.



June 2020

Yagi-Utsumi M, Yanaka S, Song C, Satoh T, Yamazaki C, Kasahara H, Shimazu T, Murata K, Kato K.
Characterization of amyloid β fibril formation under microgravity conditions, ***NPJ Microgravity.*** 2020, 6: 17.

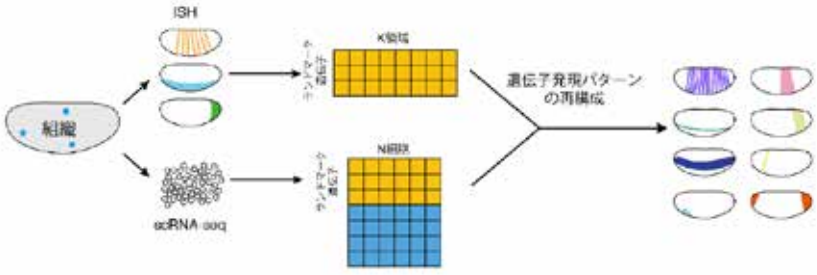


【 Outline of achievement 】

This interdisciplinary research transcending space science and life science waas jointly conducted with the Japan Aerospace Exploration Agency (JAXA). They investigated the amyloid fibrillization under microgravity conditions in the Japanese Experiment Module Kibō on the International Space Station, made the first-in-the-world discovery that uniquely-shaped amyloid fibrils would be formed under microgravity conditions.

June 2021

Okochi Y, Sakaguchi S, Nakae K, Kondo T, Naoki H.
Model-based prediction of spatial gene expression via generative linear mapping, ***Nature Commun.*** 2021, 12: 3731.

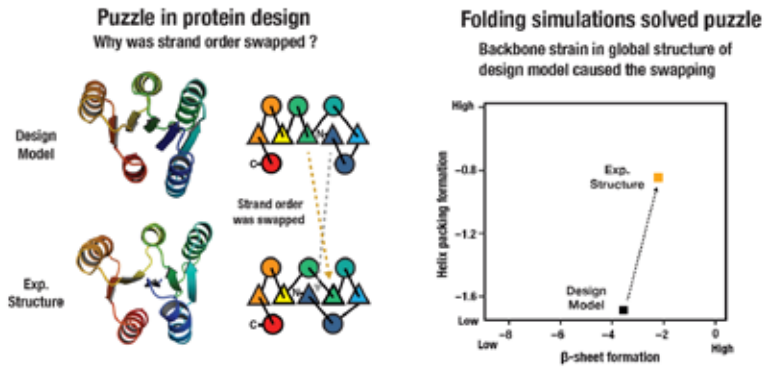


【 Outline of achievement 】

This study utilized gene expression data measured by single-cell RNA sequencing (scRNA-seq) and developed a machine learning method (Perler) that could reconstruct the spatial patterns of gene expression in a manner analogous to solving puzzles. The ability to accurately reconstruct spatial gene expression patterns achieved through this study is expected to improve scientific knowledge on how their shapes are formed during the generative process as well as the functions of related multicellular systems.

June 2021

Koga N, Koga R, Liu G, Castellanos J, Montelione GT, Baker D.
Role of backbone strain in de novo design of complex α/β protein structures, ***Nature Commun.*** 2021, 12: 3921.



【 Outline of achievement 】

This study discovered that, to design α/β protein structures consisting of over 100 residues artificially, it is necessary to consider the strain of the whole system on the foundational backbone structure. This research finding has enabled designing of artificial proteins that are larger and more complex than before and might lead to the creation of functional proteins.

Symposiums, etc.

ExCELLS Symposiums

ExCELLS regularly hosts scientific symposium for research communities encompassing a wide range of scientific research fields to communicate the recent researches.

1st ExCELLS Symposium

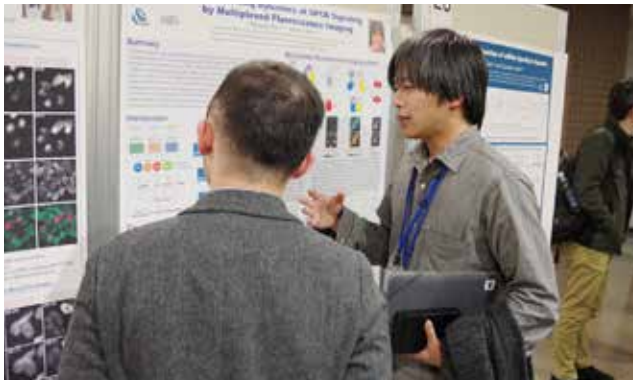
Date: 15th October 2018 – 16th October
Venue: Okazaki Conference Center

2nd ExCELLS Symposium

Date: 18th November 2019 – 19th November
Venue: Okazaki Conference Center

3rd ExCELLS Symposium

Date: 21th December 2020
Venue: Online Zoom meeting



National Institutes of Natural Sciences (NINS) Symposium

NINS annually hosts Symposium to share with the public its cutting-edge research findings from its scientific studies of space, energy, matter, life, and other fields as well as new future-forming projects. For FY2020, the symposium was held online, showcasing ExCELLS.

31st National National Institutes of Natural Sciences Symposium — What is life? —

Date: 13th March 2021
Venue: Online

Prize winners

FY2018

Biomolecular Organization Research Group: 5 prizes
Cardiocirculatory Dynamism Research Group: 6 prizes
Dynamic Molecular Neurobiology Group: 2 prizes
Protein Design Group: 1 prize

FY2019

Biomolecular Organization Research Group: 5 prizes
Biophotonics Research Group: 4 prizes
Cardiocirculatory Dynamism Research Group: 3 prizes
Protein Design Group: 1 prize
Thermal Biology Group: 1 prize
Biomolecular Dynamics Observation Group: 1 prize

FY2020

Biomolecular Organization Research Group: 5 prizes
Cardiocirculatory Dynamism Research Group: 3 prizes
Quantitative Biology Group: 1 prize
Theoretical Biology Group: 2 prizes

PR Activities

(Data as of October 1, 2021)

To disseminate information on its research activities both domestically and globally, ExCELLS actively shares its research findings through press releases.

Japanese press releases

(FY2018) 11 press releases

(FY2019) 19 press releases

(FY2020) 13 press releases

(FY2021) 9 press releases (As of October 1, 2021)

English press releases

(FY2019) 6 Press releases

- Fishing a line coupled with clockwork for daily rhythm
- High-speed AFM reveals accelerated binding of Agitoxin-2 to K+ channel by Induced-fit
- It's Fab! A hidden touch of antibody
- Antibodies gather and form a circle for defensive attack!
- Ancient Greek tholos-like architecture composed of archaeal proteins
- Passport tagging for express cargo transportation in cells

(FY2020) 6 Press releases

- Light driven proton pump in distant relative
- Dock and Harbor: A Novel Mechanism for Controlling Genes
- Amyloid formation in the International Space Station
- New role of arginine metabolism in plant morphogenesis identified
- The helix of life: New study shows how 'our' RNA stably binds to artificial nucleic acids
- Researchers find why 'lab-made' proteins have unusually high temperature stability

(FY2021) 2 Press releases
(As of October 1, 2021)

- Researchers solve a puzzle to design larger proteins
- “Bucket brigade” using lysine residues in RNA-dependent RNA polymerase of SARS-CoV-2

Outreach activity

ExCELLS periodically hosts science-themed events, etc. in cooperation with municipalities and other organizations.

(FY2018) 4 events

(FY2020) 1 event

(FY2021) 1 event (As of October 1, 2021)

4

External Evaluation Reports

Christian Griesinger, Director, Professor,
Max Planck Institute for Multidisciplinary Sciences

ExCELLS has a daunting mission, i.e. asks the question: “What is life” and follows three routes of activities: Observe, read and create. The consortium approaches these questions by international collaborations in addition to having put together a team of scientists in Japan which are experts in their respective disciplines. The added value of the consortium comes from collaborations within and beyond the consortium which have developed despite the Covid pandemic in a commendable fashion. The output publicationwise both qualitative and quantitative is impressive and number of meetings held and the outreach activites are amazing given the pandemia. Just to highlight the three symposia that already took place, only the one in 2020 was by zoom.

Highlights of the consortium in my view are the collaborative activities in which groups team up and produce scientific results that could not be achieved by the individuals. These include the determination of the dynamic structure of heliorhodopsin from X-ray crystallography combined with fast AFM from the Uchihashi group, the structural basis of the Abo1 triple A ATPase histone chaperone including Uchihashi’s and Kato’s group, the formulation of a new hypothesis on the origin of the new Archeon Candidatus Prometheoarchaeum syntrophicum that is at the prokaryote-eukaryote interface where the authors propose the so called entangle-engulf-endogenize model. Another investigations under extreme conditions is the investigation of aggregation of amyloid beta in space. The finding of differences of structures of fibrils grown under gravity and under microgravity shows the extreme dependence of the aggregation process on conditions leading to a vast number of polymorphs to which the one grown in space adds another one. Another highlight on the topic of going in the direction of creation of life is the collaboration with David Baker on de novo design of proteins containing beta sheets and alpha helices.

There is a lot of excellent science being done by the individual groups using state of the art technology such as high field nuclear magnetic resonance, cryo-electron microscopy, superresolution STED fluorescence imaging and light sheet microscopy, mass spectrometry, superfast atomic force microscopy, optogenetic tools. To highlight here collaborations between the different groups there is investigation of extremotolerance in which a newly tardigrade-specific peroxidase confers tolerance against oxidative stress. The range of topics is really large from protein design, mathematical modelling, structural biology and structural modelling, mass spectrometry, circadian clock and motor proteins, metallobiology, glycans, signal transduction, imaging of aggregating proteins in C.elegans, brain imaging, heart failure research, neuropsychatric research using gene expression, mechanisms and life under extreme conditions. These topics are rightfully very broad and are clearly fully within the scope of the ExCELLS mission. Yet, due to the diversity of topics, it is a big challenge to use all possibilities of cooperation. Thus a recommendation would be to focus on projects where collaboration is possible. For example the modelling work on remdesevir in RNA polymerase could be teamed up with cryo EM work on such complexes. Regarding novel glycans found in organisms living in extreme conditions collaboration with experts in the consortium of structural biology and function of oligosaccharides will enhance the impact of the project. For Abeta fibrils grown in microgravity a cryo-EM structure would clarify whether novel polymorphs not observed before could be found.

Finally, ExCELLS has shown that despite Covid restrictions new levels of research collaboration are possible and have lead to great results published. I recommend to discuss among the scientists to identify projects with potential for collaborations. While the fields: “observe” and “read”are well developed, the biggest challenge lies in the projects: “create”.

Yoshie Harada, Professor,
Institute for Protein Research, Osaka University

1. Research organization structure
ExCELLS consists of a “Department of Creative Research” and a “Section for Exploration of Life in Extreme Environment”. The former, Department of Creative Reseach, aims to elucidate the fundamental question "What is life?" through the development of three approaches of "Observe, Read, and Create." The organization structure and goals of the center are well thought out, and the system is in place to promote interdisciplinary research. The ExCELLS research groups, including the Okumura, Takada, Tominaga, Aoki, and Koga groups, are all doing very unique research. It is also notable that PIs of many research groups are young researchers. The establishment of two ExCELLS Collaborative Research Groups, namely Biomolecular Dynamics Observation Group and Theoretical Biology Group, has accelerated collaborative research within and outside the ExCELLS. These three collaborative research groups, including Chromosome Engineering Research Group to be established in the near future, are expected to accelerate the formation of a domestic and international network of researchers in different fields. Although I pointed out the lack of female researchers in senior PI positions, I found that the gender balance has been taken into consideration, with four of the nine members of the External Steering Committee being female. I hope that the issue of gender balance will continue to be considered in the future.

2. Joint research
The major mission of the ExCELLS to create and provide the world's most advanced collaborative research environment. About 30 General Joint Research projects are conducted each year. The number of Research Utilizing Equipment projects is increasing each year. It is expected to increase further in the future with the establishment of new ExCELLS Cooperative Research Groups. Although the number of adopted research projects is not large, the ExCELLS Themed Research (Seeds Discovery-type), in which research themes are set, is interesting, including the method for determining research themes. In addition, Mr. Honda and Mr. Kazuki's ExCELLS Collaborative Research groups were also selected from the ExCELLS Themed Researches (Seeds Discovery-type) originally adopted by ExCELLS. In this way, the purpose of “Seeds Discovery” has been successfully achieved, and this is a very good system. The website for these joint researches and equipment is well-organized, and the application procedures are easy to understand. The descriptions of available equipment with photos are helpful to understand. There are many expensive and valuable devices in the ExCELLS, and I would definitely like to use them if I have the chance.

3. Fostering young researchers
The faculty members of the ExCELLS also serve as faculty members of the Graduate University for Advanced Studies (SOKENDAI) and have opportunities to educate graduate students. Under the current situation where many students go on to master's programs but very few go on to doctoral programs, it is likely that not many students will enroll in SOKENDAI, where students are basically supposed to go on to doctoral programs. There seems to be a system to accept graduate students from other universities. I am interested in the actual enrollment of graduate students at the ExCELLS. On the other hand, for young researchers such as assistant professors and project researchers, the ExCELLS is a good environment where they can concentrate on their research. The ExCELLS supports young researchers by offering the ExCELLS Young Researcher Incentive Research program, a research grant targeting young researchers. It was concerning that in FY 2018, 14 projects were adopted, but in FY 2021, only 4 projects were adopted. Although it was not possible due to limited time for this evaluation, it would have been nice to have an opportunity to hear directly from graduate students and young researchers.

Continued from previous page.

4. Publicity activities
The website of the ExCELLS is very fascinating, with a large video and color photos on the top page. It is also designed to make it easy for visitors with various purposes to obtain the information they are looking for. The introductory video shown at the beginning of this external evaluation committee meeting is also very well done and easy to understand. In addition, while it is difficult to conduct face-to-face outreach activities under Covid-19 pandemic, the video of a lecture at the 31st National Institutes of Natural Sciences Symposium held last year will be useful in informing many people about the research activities of the ExCELLS.

5. Conclusion
The ExCELLS contributes to the advancement of life science research in Japan and abroad by developing innovative measurement methods and conducting collaborative research. The center also pursues cutting-edge research, including the Section for Exploration of Life in Extreme Environment. Four years after its establishment, the center is now well organized, and I look forward to the future development of its research.

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1. Real "Exploratory Research"
Exploring new phenomena in nature and elucidating their dynamics and mechanism are the role of natural science, and the most charming and important subject is indeed "Life and Living Systems" in microscopic level. In the past century after quantum mechanics was proposed, solving a variety subjects of physics, chemistry, and biology and contributing to the development of micro and nano technology are considered to be "Exploratory Research". Nowadays micro and nano systems are considered to be the problems which can be solved, and now life and living systems are the most important exploratory subject. Accumulated biological knowledge in molecule, tissue, cell, organ, and individual is coupled with modern scientific instrumentation, computer technology, sophisticated chemical synthesis and analysis, and so on under the atmosphere of interdisciplinary collaboration, A new stage of research has been opened, that is ExCELLS, and I highly evaluate that its setting and organization are being well performed.

2. Organization Structure
The function of ExCELLS is classified into Observe, Read, Create, and Exploration of Life in Extreme Environment, and its structure consists of Department of Creative Research and Section for Exploration of Life in Extreme Environment. In general institute structure and function has one-to-one correspondence, but here one Department structure assists more collaboration and multidisciplinary approach. Exploratory research is often far from the trend and sometime becomes selfish, which is avoided by carrying out practical subjects. From this viewpoint the study on Exploration of Life in Extreme Environment is an exceptionally useful, keeping ExCELLS realistic. I think the structure of ExCELLS is well designed.

3. Cultivation of Young Scientists
The importance of Cultivation of Young Scientists and Outreach is pointed out by many funding agencies and project leaders. Although those are preferred socially, I feel these are especially meaningful for researchers of ExCELLS. The study on Live and Living Systems needs various unconventional concepts and ideas which may be generated by interactions between professionals and amateurs.

4. Research Groups' Activity
It is well summarized and enough attractive for me, non-biological scientist. So-called high IF journals are often published, which is quite reasonable as "Life and Living Systems" is a traditionally most important subject. On the other hand, many different approaches are involved in ExCELLS and some of them are "Only One" and do not attract many interests at the moment. Multidisciplinary research does not always enable to publish high IF magazines. Steady works toward the target can be published in society journals, which, I consider, is also important contribution.

5. Scientific Discussion
It is not easy for me to follow the real content and scientific details, but I was very much impressed by many scientifically nice logics characteristic of ExCELLS. Excellent imaging and spectroscopic data, computer simulations predicting the coming concepts, and modeling which will contribute to understand life have attracted my attention. I have enjoyed listening all the presentation and reading the summary on members' activity. Here I describe some examples which show how the study on Life and Living Systems is important and have high future potential.
Prof. Koich Kato of Biomolecular Organization Group: Magnetic resonance, X-ray crystallography, solution scattering, native mass spectrometry, electron microscopy, high-speed atomic force microscopy, fluorescence microscopy, computational methods and so on are integrated, and a new approach for observing dynamic ordering of biomolecules is developed. Prof. Kato has long experience to complete this strategy, and I believe his strategy will be well summarized and shown as a general method to researchers in the relevant fields.

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Profs. Naoki Honda and Nen Saito: It is indeedtimely and important to elucidate theoretical logic of dynamic living systems, which will be made possible by combining mathematical modeling and machine learning with accumulated data in living imaging and quantitative gene expression levels. I think the approaches are not simple and quite diverse, so I hope various trials will be performed.
Prof. Kazuyoshi Murata of Material-Life Boundary Research Group: Utilization a cryo-electron microscope, shape and size of many kinds of virus, giant virus, and so on are made clear, directly triggering our thinking what is life. Also, I was charmed by the fact that some of them show hexagonal packing, which may suggest their components are sphere.

6. Conclusion
ExCELLS clearly demonstrates the very interesting start and nice progress in the past few years and one of the most valuable Research Centers and Projects in which I have recently been involved for evaluation. I expect that ExCELLS will involve more PhD students and will be recognized widely and internationally.

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