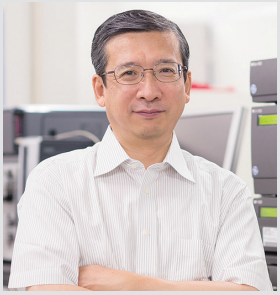
RESEARCH ACTIVITIES
Life and Coordination-Complex Molecular Science

Department of Life and Coordination-Complex Molecular Science is composed of two divisions of biomolecular science, two divisions of coordination-complex molecular science, and one adjunct division. Biomolecular science divisions cover the studies on functions, dynamic structures, and mechanisms for various biomolecules such as sensor proteins, membrane proteins, biological-clock proteins, metalloproteins, glycoconjugates, molecular chaperone, and motor proteins. Coordination-complex divisions aim to develop molecular catalysts and functional metal complexes for transformation of organic molecules, water oxidation and reduction, and molecular materials with photonic-electronic-magnetic functions. Interdisciplinary alliances in this department aim to create new basic concepts for the molecular and energy conversion through the fundamental science conducted at each divisions.

Bioinorganic Chemistry of Metalloproteins Responsible for Metal Homeostasis and Signal Sensing

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



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Keywords Bioinorganic Chemistry, Metalloproteins, Sensor Protein

Transition metal ions and metalloproteins play crucial roles in meeting the energy demands of the cell by playing roles in intermediary metabolism and in signal transduction processes. Although they are essential for biological function, metal ion bioavailability must be maintained within a certain range in cells due to the inherent toxicity of all metals above a threshold. This threshold varies for individual metal ions. Homeostasis of metal ions requires a balance between the processes of uptake, utilization, storage, and efflux and is achieved by the coordinated activities of a variety of proteins including extracytoplasmic metal carriers, ion channels/pumps/transporters, metal-regulated transcription and translation proteins, and enzymes involved in the biogenesis of metal-containing cofactors/metalloproteins. In order to understand the processes underlying this complex metal homeostasis network, the study of the molecular processes that determine the protein-metal ion recognition, as well as how this event is transduced into a functional output, is required. My research interests are focused on the elucidation of the structure and function relationships of metalloproteins responsible for the regulation of biological homeostasis.

I am also working on gas sensor proteins. Gas molecules such as O_2, NO, CO and ethylene are present in the environment and are endogenously (enzymatically) produced to act as signaling molecules in biological systems. Sensing these gas molecules is the first step in their acting as signaling molecules. Sensor proteins are usually required. Input signals generated by gas sensing have to transduce to output signals that regulate biological functions. This is achieved by biological signal-transduction systems. Recognition of the cognate gas molecules is a general mechanism of functional regulation for gas sensor proteins. This induces conformational changes in proteins that controls their activities for following signal transductions. Interaction between gas molecules and sensor proteins is essential for recognition of gas molecules. Metal-containing prosthetic groups are widely used. In my research group, our research focuses on transition metal-based gas-sensor proteins and the signaling systems working with them.

Selected Publications

- N. Muraki, C. Kitatsuji, Y. Okamoto, T. Uchida, K. Ishimori and S. Aono, "Structural Basis for Heme Transfer Reaction in Heme Uptake Machinery from Corynebacteria," *Chem. Commun.* **55**, 13864–13867 (2019).
- N. Muraki, K. Ishii, S. Uchiyama, S. G. Itoh, H. Okumura and S. Aono, "Structural Characterization of HypX Responsible for CO Biosynthesis in the Maturation of NiFe-Hydrogenase," *Commun. Biol.* **2**, 385 (12 pages) (2019).
- A. Pavlou, H. Yoshimura, S. Aono and E. Pinakoulaki, "Protein Dynamics of the Sensor Protein HemAT as Probed by Time-Resolved Step-Scan FTIR Spectroscopy," *Biophys. J.* **114**, 584–591 (2018).
- A. Pavlou, A. Loullis, H. Yoshimura, S. Aono and E. Pinakoulaki, "Probing the Role of the Heme Distal and Proximal Environment in Ligand Dynamics in the Signal Transducer Protein HemAT by Time-Resolved Step-Scan FTIR and Resonance Raman Spectroscopy," *Biochemistry* **56**, 5309–5317 (2017).
- N. Muraki, C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, "Structural Characterization of Heme Environmental Mutants of CgHmuT that Shuttles Heme Molecules to Heme Transporters," *Int. J. Mol. Sci.* **17**, 829 (2016).
- N. Muraki and S. Aono, "Structural Basis for Heme Recognition by HmuT Responsible for Heme Transport to the Heme Transporter in *Corynebacterium glutamicum*," *Chem. Lett.* **45**, 24–26 (2015).

1. Molecular Mechanisms for Biosynthesis and Maturation of Hydrogen Sensing Regulatory Hydrogenase

Regulatory hydrogenase (RH) that acts as a H_2 sensor consists of two subunits, a large subunit containing the Ni-Fe dinuclear complex and a small subunit containing iron-sulfur clusters. Though the Ni-Fe dinuclear complex in the large subunit is assumed to be the active site for H_2 sensing by RH, the molecular mechanisms of biosynthesis and maturation of the Ni-Fe dinuclear complex are not clear yet.

CO and CN^- ligands are coordinated to the Fe in the Ni-Fe dinuclear complexe in RH. These CO and CN^- are biosynthesized and assembled into the metal clusters, for which several accessory and chaperone proteins are required, as shown in Figure1. In 2019, we have determined the crystal structure of HypX, which catalyzes CO biosynthesis for the ligand of the Ni-Fe complex in RH, to find that HypX adopt coenzyme A (CoA) as a cofactor for CO biosynthesis using formyl-tetrahydrofolate as a substrate to form formyl-CoA, and that formyl-CoA is the reaction intermediate to form CO.

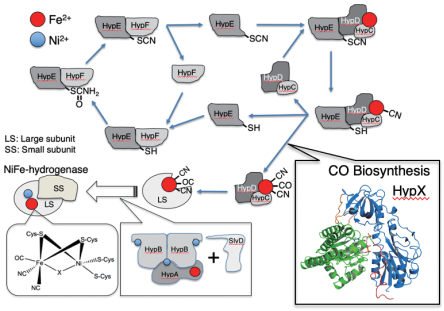


Figure 1. Reaction scheme of the biosynthesis and maturation of the Ni-Fe dinuclear complex for the active site in RH.

CO produced by HypX is used as a ligand of the iron in the $NiFe(CN)_2(CO)$ center of NiFe hydrogenases. The $Fe(CN)_2(CO)$ unit of the NiFe dinuclear center is assembled in the HypC/HypD complex as a scaffold. The binding site of the $Fe(CN)_2(CO)$ unit is proposed to be located at the bottom of a tunnel ca. 20 Å deep inside from the protein surface in the HypC/HypD complex, to which Fe is initially bound and then CN^- and CO ligands bind to the Fe. If CO produced by HypX is diffused into solvent, it will be inefficient for the assembly of the $Fe(CN)_2(CO)$ unit in the HypC/HypD. It may be a solution to utilize CO produced by HypX effectively is that HypX and HypC/HypD form a complex. The SEC analyses reveal the formation of HypC/HypD and HypC/HypD/HypX complexes as described below.

HypC (10.4 kDa in monomer) and HypD (45.0 kDa in monomer) are eluted from a Superdex75 column with an apparent mass of 21.6 kDa and 35.5 kDa, respectively, indicating that HypC and HypD exist as a homo-dimer and monomer in solution, respectively. The mixture of HypC and HypD is eluted with an apparent mass of 76.4 kDa. Though this result indicates the formation of the complex between HypC and HypD (probably $(HypC)_2HypD$ complex), its quaternary structure is not clear at present. The mixture of HypC, HypD, and HypX was eluted from a Superdex200 column with an apparent mass of 119.7 kDa, suggesting the formation of the 1:1:1 complex of HypC, HypD, and HypX. The structural characterization of this complex is now in progress.

2. Structural Basis for Heme Transfer Reaction in Heme Uptake Machinery from Corynebacteria

Corynebacteria including *Corynebacterium diphtheriae* and *Corynebacterium glutamicum*, which are classified as a high GC content Gram-positive bacteria (Actinomycetes), adopt a different heme uptake machinery (Hta/Hmu system). It consists of the membrane-bound heme binding/transport proteins (HtaA and HtaB) and the ABC-type heme transporter system (HmuTUV). HtaA and HtaB consist of two and one CR (Conserved Region) domains, respectively, which are responsible for heme binding/transport. In this study, we have determined the crystal structures of HtaA and HtaB from *C. glutamicum* to understand the structural basis of the heme-uptake in Corynebacteria.

Though these interactions and the overall structure are conserved among HtaA and HtaB, the orientation of heme is different from one another. Good fitting of the model into the electron density of heme was obtained with the single orientation of heme for HtaA. On the other hand, assuming a 1:1 mixture of two orientations of heme was needed to obtain good fitting for the electron density of heme in HtaB. Thus, heme is accommodated with a mixture of two different orientations in HtaB unlike HtaA. The difference of the heme orientation suggests that heme transfer reaction between HtaA and HtaB proceeds through the HtaA/HtaB complex formation.

We also determined the crystal structure of the apo-form of H434A-HtaA at a resolution of 2.0 Å. Though the holo-form of H434A-HtaA is a monomer, the apo-form of this variant was dimer. The N-terminal region (Ser364-Gly391) including the β1 strand and the α1 helix in each protomer are separated from the core region and each β1 strand is swapped between two protomers to form a domain-swapped dimer (Figure 2).

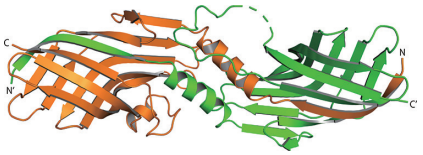
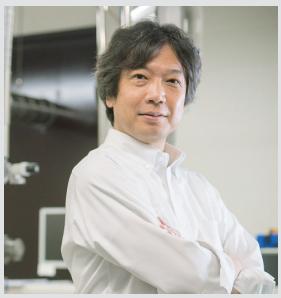


Figure 2. The structure of the apo-form of H343A-HtaA. The chains A and B are shown in orange and light green, respectively. A dotted line in the chain B is a disordered region including the α2 helix.

The structure of the domain-swapped dimer of apo-HtaA would be a model of a reaction intermediate for the heme transfer. In the domain-swapped dimer, the chain A would be a model of the holo-HtaA because it is superimposable to the structure of the holo-HtaA. The chain B in the domain-swapped dimer would be a model of the apo-HtaB in the holo-HtaA/apo-HtaB complex.

Dynamical Ordering of Biomolecular Systems for Creation of Integrated Functions

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



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Living systems are characterized as dynamic processes of assembly and disassembly of various biomolecules that are self-organized, interacting with the external environment. The omics-based approaches developed in recent decades have provided comprehensive information regarding biomolecules as parts of living organisms. However, fundamental questions still remain unsolved as to how these biomolecules are ordered autonomously to form flexible and robust systems (Figure 1). Biomolecules with complicated, flexible structures are self-organized through weak interactions giving rise to supramolecular complexes that adopt their own dynamic, asymmetric architectures. These processes are coupled with expression of integrated functions in the biomolecular systems.

Toward an integrative understanding of the principles behind the biomolecular ordering processes, we conduct multidisciplinary approaches based on detailed analyses of dynamic structures and interactions of biomolecules at atomic level, in conjunction with the methodologies of molecular and cellular biology along with synthetic and computational technique.

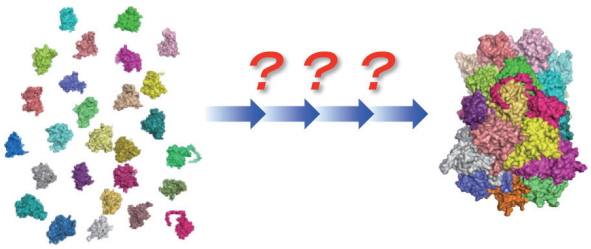

Figure 1. Formation of supramolecular machinery through dynamic assembly and disassembly of biomolecules.

Selected Publications

- S. Yanaka, R. Yogo and K. Kato, "Biophysical Characterization of Dynamic Structures of Immunoglobulin G," *Biophys. Rev.* **12**, 637–645 (2020).
- T. Satoh and K. Kato, "Structural Aspects of ER Glycoprotein Quality-Control System Mediated by Glucose Tagging," in *Glycobiophysics*, Y. Yamaguchi and K. Kato, Eds., Springer Nature; Singapore, pp. 149–169 (2018).
- K. Kato, H. Yagi and T. Yamaguchi, "NMR Characterization of the Dynamic Conformations of Oligosaccharides," in *Modern Magnetic Resonance, 2nd Edition*, G. A. Webb, Ed., Springer International Publishing, pp. 737–754 (2018).
- T. Yamaguchi and K. Kato, "Molecular Dynamics of Gangliosides," in *Gangliosides*, S. Sonnino and A. Prinetti, Eds., Methods in Molecular Biology, Humana Press; New York, **vol. 1804**, pp. 411–417 (2018).
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- K. Kato, S. Yanaka and H. Yagi, "Technical Basis for Nuclear Magnetic Resonance Approach for Glycoproteins," in *Experimental Approaches of NMR Spectroscopy*, The Nuclear Magnetic Resonance Society of Japan, Ed., Springer Nature; Singapore, pp. 415–438 (2018).

1. Conformational Dynamics of Post-Translational Protein Modifiers

A majority of proteins encoded in genomes of limited size are post-translationally diversified by covalent modifications such as glycosylation and ubiquitination. The modifiers, *i.e.*, glycans and ubiquitin (Ub) chains, carry distinct biological information in forms of "glycocode" and "Ub code," respectively, which are read out by specific interacting proteins. Because these modifiers possess considerable degrees of motional freedom, we develop methodologies for characterizing their conformational dynamics in solution by NMR spectroscopy.

Our NMR analyses enabled the quantification of populations of individual conformers of Lys48-linked Ub chains, which serve as tags for proteasomal degradation. The data indicate that the most distal Ub unit in the Ub chains is the most apt to expose its interaction surface with the Ub-recognizing proteins. We also demonstrate that a mutational modification of the distal end of the Ub chain can remotely affect the solvent exposure of the interaction surfaces of the other Ub units, suggesting that Ub chains could be unique design frameworks for the creation of allosterically controllable multidomain proteins.[1)]

We also developed an approach to improve the protein-binding affinity of an oligosaccharide by remodeling its conformational space in the precomplexed state. In this approach, based on NMR-validated molecular dynamics simulations, we created an oligosaccharide analogue with an increased population of on-pathway metastable conformers that were originally very minor but exclusively accessible to the target protein without steric hindrance (Figure 2).[2)]

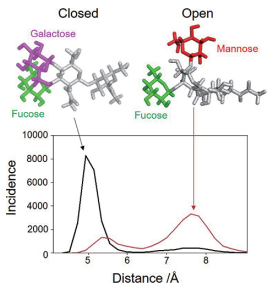

Figure 2. Remodeling of the oligosaccharide conformational space in the prebound state to improve lectin-binding affinity.

2. Integrative Biophysical Approaches to Exploring Protein Assembly Dynamics

The integrative biophysical approaches we have been developing in collaboration with several research groups in ExCELLS and our external research network could be successfully applied to a variety of biomolecular assembling systems, yielding fruitful results in the past year, as summarized below (Figure 3).

We revealed that the two functionally unannotated archaeal proteins, PbaA and PF0014, are co-assembled into a unique ancient Greek tholos-like architecture, offering a novel framework for designing functional protein cages.[3)] We successfully visualized the dynamic process by which the antibodies bound to antigens in membranes spontaneously assemble to form a hexameric ring structure, thereby recruiting complement component C1q on the membrane, which is the initial step of complement-mediated cell lysis.[4)] Assembly of amyloid β (Aβ) under microgravity conditions were explored using the International Space Station, showing that the Aβ fibrillization process significantly slowed down in the microgravity environment, giving rise to distinct morphologies of Aβ.[5)]

Furthermore, we demonstrated that the cargo receptor complex responsible for the intracellular transportation of blood coagulation factors V and VIII recognizes 10-amino acid sequence built into these glycoproteins as a "passport" in the secretory pathway.[6)] The secretion levels of recombinant glycoproteins were significantly increased simply by tagging it with the passport sequence. Our findings offer a potentially useful tool for improving the production yields of recombinant glycoproteins of biopharmaceutical interest.

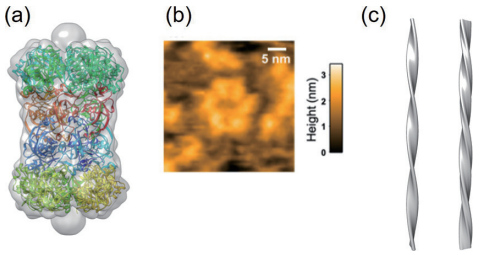

Figure 3. Integrative biophysical approaches to exploring protein assembly dynamics. The simulated model structure of the PbaA/PF0014 complex superimposed onto the cryo-EM map (a), high-speed AFM image of IgG hexamers formed on membrane (b), distinct morphologies of Aβ fibrils formed under microgravity conditions (c).

References

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2) T. Suzuki *et al. Biochemistry* **59**, 3180–3185 (2020).
3) M. Yagi-Utsumi *et al. Sci. Rep.* **10**, 1540 (2020).
4) S. Yanaka *et al. Int. J. Mol. Sci.* **21**, 147 (2020).
5) M. Yagi-Utsumi *et al. NPJ Micorgravity* **6**, 17 (2020).
6) H. Yagi *et al. Nat. Commun.* **11**, 1368 (2020).

Awards

SAITO, Taiki; Young Scientist Award, The Japanese Biochemical Society Chubu Branch (2019).
UMEZAWA, Fumiko; Young Scientist Award, the 3rd Glycolleague (2019).
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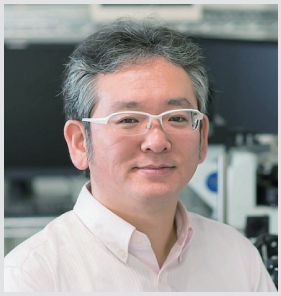
Operation and Design Principles of Biological Molecular Machines

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



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Keywords Molecular Motors, Single-Molecule Analysis, Protein Engineering

Activity of life is supported by various molecular machines made of proteins. Protein molecular machines are tiny, but show very high performance, and are superior to man-made machines in many aspects. One of the representatives of protein molecular machines is linear and rotary molecular motors (Figure 1). Molecular motors generate mechanical forces and torques that drive their unidirectional motions from the energy of chemical reaction or the electrochemical potential across the cell membrane.

We unveil operation principles of molecular motors with advanced single-molecule functional analysis. With the help of site-saturation mutagenesis and robot-based automation, we also engineer non-natural molecular motors to understand their design principles.

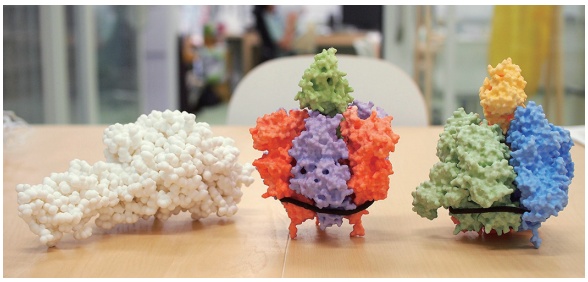

Figure 1. Protein molecular machines. (Left) A linear molecular motor chitinase A. (Center and Right) Rotary molecular motors F_1-ATPase and V_1-ATPase, respectively.

Selected Publications

- J. Ando, A. Nakamura, M. Yamamoto, C. Song, K. Murata and R. Iino, "Multicolor High-Speed Tracking of Single Biomolecules with Silver, Gold, Silver-Gold Alloy Nanoparticles," *ACS Photonics* **6**, 2870–2883 (2019).
- T. Iida, Y. Minagawa, H. Ueno, F. Kawai, T. Murata and R. Iino, "Single-Molecule Analysis Reveals Rotational Substeps and Chemo-Mechanical Coupling Scheme of *Enterococcus hirae* V_1-ATPase," *J. Biol. Chem.* **294**, 17017–17030 (2019).
- J. Ando, A. Nakamura, A. Visootsat, M. Yamamoto, C. Song, K. Murata and R. Iino, "Single-Nanoparticle Tracking with Angstrom Localization Precision and Microsecond Time Resolution," *Biophys. J.* **115**, 2413–2427 (2018).
- A. Nakamura, K. Okazaki, T. Furuta, M. Sakurai and R. Iino, "Processive Chitinase is Brownian Monorail Operated by Fast Catalysis after Peeling Rail from Crystalline Chitin," *Nat. Commun.* **9**, 3814 (2018).
- A. Nakamura, T. Tasaki, Y. Okuni, C. Song, K. Murata, T. Kozai, M. Hara, H. Sugimoto, K. Suzuki, T. Watanabe, T. Uchihashi, H. Noji and R. Iino, "Rate Constants, Processivity, and Productive Binding Ratio of Chitinase A Revealed by Single-Molecule Analysis," *Phys. Chem. Chem. Phys.* **20**, 3010–3018 (2018).
- T. Uchihashi, Y. H. Watanabe, Y. Nakazaki, Y. Yamasaki, T. Watanabe, T. Maruno, S. Uchiyama, S. Song, K. Murata, R. Iino and T. Ando, "Dynamic Structural States of ClpB Involved in Its Disaggregation Function," *Nat. Commun.* **9**, 2147 (2018).

1. Small Stepping Motion of Processive Dynein Revealed by Load-Free High-Speed Single-Particle Tracking[1)]

Cytoplasmic dynein is a dimeric motor protein which processively moves along microtubule. Its motor domain (head) hydrolyzes ATP and induces conformational changes of linker, stalk, and microtubule binding domain (MTBD) to trigger stepping motion. Here we applied scattering imaging of gold nanoparticle (AuNP) to visualize load-free stepping motion of processive dynein (Figure 2). We observed artificially-dimerized chimeric dynein, which has the head, linker, and stalk from Dictyostelium discoideum cytoplasmic dynein and the MTBD from human axonemal dynein, whose structure has been well-studied by cryo-electron microscopy. One head of a dimer was labeled with 30 nm AuNP, and stepping motions were observed with 100 μs time resolution and sub-nanometer localization precision at physiologically-relevant 1 mM ATP. We found 8 nm forward and backward steps and 5 nm side steps, consistent with on- and off-axes pitches of binding cleft between αβ-tubulin dimers on the microtubule. Probability of the forward step was 1.8 times higher than that of the backward step, and similar to those of the side steps. One-head bound states were not clearly observed, and the steps were limited by a single rate constant. Our results indicate dynein mainly moves with biased small stepping motion in which only backward steps are slightly suppressed.

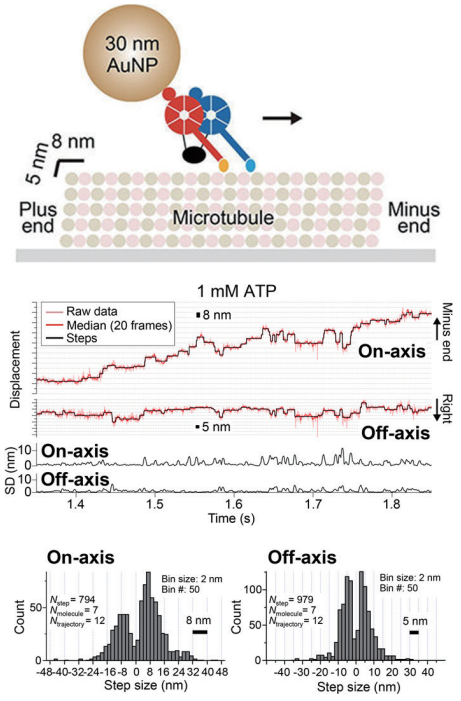

Figure 2. (Top) Schematic of single-molecule imaging of dynein motion. (Middle) Typical trajectory of motion. (Bottom) Distribution of step size in on- and off-axis.

2. Single-Molecule Imaging Analysis Reveals the Mechanism of a High-Catalytic-Activity Mutant of Chitinase A from *Serratia marcescens*[2)]

Chitin degradation is important for biomass conversion and has potential applications for agriculture, biotechnology, and the pharmaceutical industry. Chitinase A from the Gram-negative bacterium *Serratia marcescens* (*Sm*ChiA, Figure 3) is a processive enzyme that hydrolyzes crystalline chitin as it moves linearly along the substrate surface. In a previous study, the catalytic activity of *Sm*ChiA against crystalline chitin was found to increase after the tryptophan substitution of two phenylalanine residues (F232W and F396W), located at the entrance and exit of the substrate binding cleft of the catalytic domain, respectively. However, the mechanism underlying this high catalytic activity remains elusive. In this study, single-molecule fluorescence imaging and high-speed atomic force microscopy were applied to understand the mechanism of this high-catalytic-activity mutant. A reaction scheme including processive catalysis was used to reproduce the properties of *Sm*ChiA WT and F232W/F396W, in which all of the kinetic parameters were experimentally determined. High activity of F232W/F396W mutant was caused by a high processivity and a low dissociation rate constant after productive binding. The turnover numbers for both WT and F232W/F396W, determined by the biochemical analysis, were well-replicated using the kinetic parameters obtained from single-molecule imaging analysis, indicating the validity of the reaction scheme. Furthermore, alignment of amino acid sequences of 258 *Sm*ChiA-like proteins revealed that tryptophan, not phenylalanine, is the predominant amino acid at the corresponding positions (Phe-232 and Phe-396 for *Sm*ChiA). Our study will be helpful for understanding the kinetic mechanisms and further improvement of crystalline chitin hydrolytic activity of *Sm*ChiA mutants.

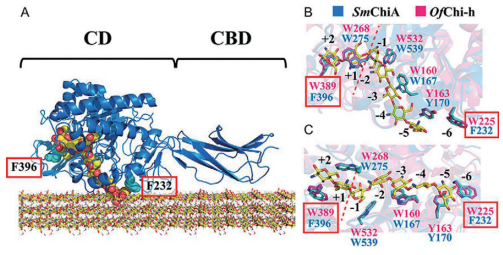

Figure 3. (A) Structural model of *Sm*ChiA bound to crystalline chitin. (B and C) Side and bottom views of aromatic amino acid residues (cyan and pink) and bound chitin (yellow) in the catalytic cleft of *Sm*ChiA and OfChi-h.

References

1) J. Ando *et al.*, *Sci. Rep.* **10**, 1080 (2020).
2) A, Visootsat *et al.*, *J. Biol. Chem.* **295**, 1915–1925 (2020).

Awards

NAKAMURA, Akihiko; Early Career Award in Biophysics, 2019 Annual Meeting of the Biophysical Society of Japan (2019).
IIDA, Tatsuya; Student Presentation Award, 2019 Annual Meeting of the Biophysical Society of Japan (2019).

* IMS International Internship Program

A Supramolecular Chemical Approach to the Construction of Artificial Cells

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Keywords Artificial Cell, Origin of Life, Droplet

The cell is the smallest unit of life, and the first simple cells evolved from simple molecular assemblies on prebiotic earth. To understand this transition from non-living to living structures, we use a supramolecular chemical approach. As shown in Figure 1, the key elements of a cell are a compartment, information, and a catalyst (*i.e.*, metabolism). We have attempted to construct a chemically based artificial cell endowed with these three elements.

In our laboratory, we constructed two types of artificial cells by using giant vesicles (GVs) as the compartment. The first, developed in collaboration with the Sugawara group (Kanagawa Univ.), is an artificial cell that can proliferate from generation to generation. We have improved this model by constructing a recursive vesicular artificial cell system with proliferation cycles. After self-reproduction, these second-generation GVs contain no PCR reagents by consuming and therefore cannot reproduce for a second time. However, the reagents can be replenished by using the vesicular transport system and changing the pH of the dispersion, resulting in the fusion of the GVs with conveyer GVs bearing the PCR reagents. After the PCR reagents are replenished, the GV can self-reproduce again. This system could lead to an evolvable artificial cellular system. The second type of artificial cell contains a catalyst-producing system. The GV system can generate catalysts and membrane molecules by transforming their respective precursors. The catalysts that are produced facilitate the proliferation of the GVs.

We are now tackling the creation of artificial cells that mimic cellular dynamics, such as cytoskeleton formation within the cell.

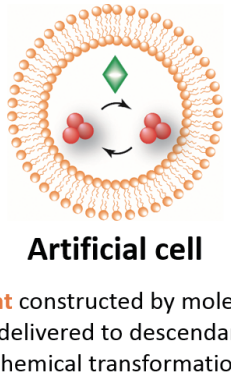

Figure 1. Artificial cell model. Materials containing heritable information are enclosed within a compartment. The reactions in the two replicating systems (compartment and information) are accelerated by appropriate catalysts The reactions in the two replicating systems are accelerated by appropriate catalysts.

Selected Publications

- Y. Natsume, E. Noguchi and K. Kurihara, "Spontaneous Localization of Particles in Giant Vesicles Owing to Depletion Force," *J. Phys. Soc. Jpn.* **88**, 033001 (2019).
- M. Matsuo *et al.*, "Environment-Sensitive Intelligent Self-Reproducing Artificial Cell with a Modification-Active Lipo-Deoxyribozyme," *Micromachines* **11**, 606 (2020). doi:10.3390/mi11060606

1. Construction of a LLPS-Droplet Based Model Protocell

In the prebiotic era, cooperative interaction between self-producing molecular aggregates and peptide polymers led to the emergence of primitive cells. Although the advanced membrane provides a field for catalytic reaction, it remains a mystery how cooperation between polymers and molecular aggregates occurred even in membraneless organisms like coacervate droplets. Since a coacervate droplet as a model of early life was created by Oparin about 100 years ago, interesting primitive cell models using coacervate droplets have been created. However, construction of the self-reproduction of coacervate droplets and the spontaneous formation of peptides, which are the constituents of the coacervate droplets has not been realized in the same environment. In the present study, we designed and synthesized a molecule that has both a peptide and a droplet formation site to enable the formation of the coacervate droplets. We attempted to construct a liquid–liquid phase-separated droplet that self-reproduces by constructing a reaction system in which a peptide is produced by spontaneous polymerization of an amino acid derivative in water.

We synthesized an amino acid derivative (monomer) with two cysteine reactive sites at the N-terminus and a thioester at the C-terminus that spontaneously polymerizes in water to form a peptide. In order to prevent undesirable oxidation, a monomer precursor was obtained by crosslinking monomers with disulfide. After the addition of a reducing agent (dithiothreitol, DTT) to the monomer precursor solution, we observed the formation of droplets. We then added more precursor and DTT and examined the changes in particle size. The mean particle size increased and decreased rapidly immediately after the addition, confirming the self-reproducibility of the formed droplets. When the precursor and DTT were continuously added every 20 hours, the particle size of the droplets fluctuated recursively, indicating autocatalytic self-reproduction of the formed liquid–liquid phase-separated droplets. A detailed analysis of the particle size distribution measurements based on dynamic light scattering revealed that the growth of the droplets can be classified into two stages: The initial autocatalytic droplet formation and the fusion of the droplets. This autocatalytic droplet formation in this system is considered to be due to a physical mechanism: When a molecular assembly is created as the dehydration condensation proceeds and it forms a hydrophobic field, the assembly functions as a site for promoting dehydration condensation, thereby allowing the autocatalytic dehydration condensation to proceed. The behavior of the interface formed by this chemical reaction replicates the autocatalytic self-reproduction that might have occurred in droplets formed by liquid–liquid phase separation on the primitive, prebiotic earth.

Furthermore, we conceived that this liquid–liquid phase separation droplet would be useful as a place to integrate biomolecules representing other origin-of-life hypotheses (*e.g.*, RNA world, lipid world, *etc.*). Therefore, we attempted to investigate whether this droplet would incorporate those biomolecules. We added 20 mer RNA fragments, DNA fragments, and phospholipids to the droplets. By fluorescence microscopy observation and Raman microspectroscopy, it was found that the droplet was consisted of the hydrophobic center region and the hydrophilic peripheral region. The highly hydrophobic lipids were concentrated in the central region of the droplets and highly hydrophilic nucleic acids concentrated in the peripheral region. This hydrophilic and hydrophobic property was clear compared to the empty droplets. We suspect that the water contained in the hydrophilic region may have been replaced by nucleic acids.

In the future, we aim to construct the Droplet World Hypothesis by inducing the emergence of the primordial cell membrane via an internal chemical reaction or by functionally expressing biologically active molecular species, such as ribozymes, inside the droplet.

Development of Heterogeneous Catalysis toward Ideal Chemical Processes

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Our research interests lie in the development of transition metal-catalyzed reaction systems toward ideal (highly efficient, selective, green, safe, simple, etc.) organic transformations. In particular, we have recently been developing the heterogeneous aquacatalytic systems, continuous flow catalytic systems, and super active catalysts working at ppm-ppb loading levels. Thus, for example, a variety of palladium catalysts were designed and prepared promoting carbon–carbon bond forming reactions at ppm-ppb loading levels (Figure 1).

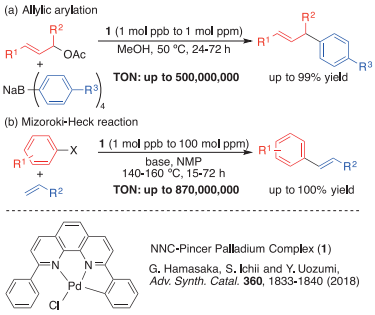

Figure 1. Typical Examples of Pd-Catalyzed Carbon–Carbon Bond Forming Reactions with ppm-ppb Loading Levels of an NNC-Pincer Pd Comples.

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- R. David and Y. Uozumi, "Recent Advances in Palladium-Catalyzed Cross-Coupling Reactions at ppm-ppb Molecular Catalyst Loadings (review)," *Adv. Synth. Catal.* **360**, 602–625 (2018).
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1. Arylation of Terminal Alkynes by Aryl Iodides Catalyzed by a Parts-per-Million Loading of Palladium Acetate[1)]

Arylation of terminal alkynes (16 varieties) by aryl iodides (28 varieties) was achieved with a mol ppm loading level of palladium catalyst, where a variety of functional groups including heteroarenes were tolerated. Thus, the arylations were carried out in the presence of palladium acetate at ppm loadings and potassium carbonate in ethanol at 80 °C to give the corresponding internal alkynes in good to excellent yields. Synthesis of 2-phenyl-3-(phenylalkynyl)benzofuran was achieved by iterative use of the alkyne arylation under mol ppm catalytic conditions. Reaction-rate analysis, transmission electron microscopic (TEM) examination of the reaction mixture, and mercury-amalgamation test were performed to gain insight into the active species of the highly active ppm catalytic species. TEM examination of the reaction mixture revealed that palladium nanoparticles were generated in situ under the reaction conditions, and their cluster size was variable during the catalytic reaction. A variation in size of palladium particles suggested that the composition-decomposition process of Pd aggregates should take place in situ via monomeric palladium(0) species and/or fine palladium(0) clusters, which might be real catalytic species in this reaction.

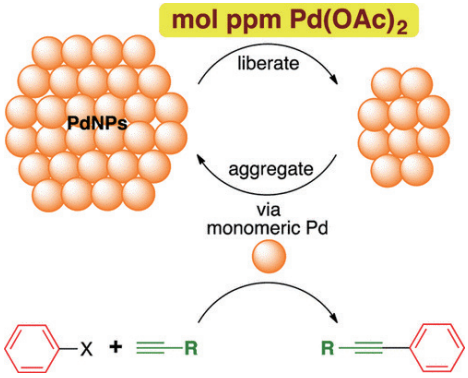

Figure 2. Arylation of Terminal Alkynes with a mol ppm Loading Level of a Palladium Catalyst.

2. Second-Generation *meta*-Phenolsulfonic Acid–Formaldehyde Resin as a Catalyst for Continuous-Flow Esterification[2)]

A second-generation *m*-phenolsulfonic acid–formaldehyde resin (PAFR II) catalyst was prepared by condensation polymerization of sodium *m*-phenolsulfonate and paraformaldehyde in an aqueous H_2SO_4 solution. This reusable, robust acid resin catalyst was improved in both catalytic activity and stability, maintaining the characteristics of the previous generation catalyst (*p*-phenolsulfonic acid–formaldehyde resin). PAFR II was applied in the batchwise and continuous-flow direct esterification without water removal and provided higher product yields in continuous-flow esterification than any other commercial ion-exchanged acid catalyst tested.

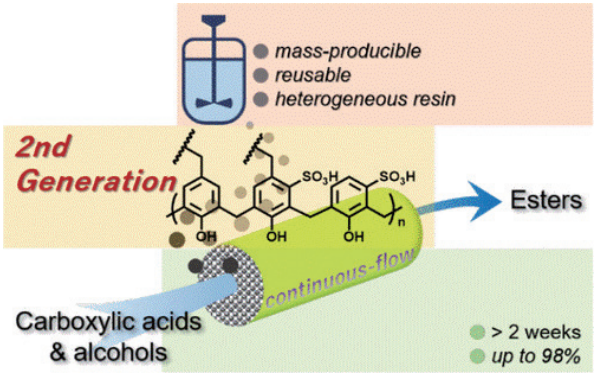

Figure 3. Continuous-Flow Esterification of Carboxylic Acid with Alcohols Using PAFR II Catalyst.

3. The Hiyama Cross-Coupling Reaction at Parts Per Million Levels of Pd: In Situ Formation of Highly Active Spirosilicates in Glycol Solvents[3)]

A palladium NNC-pincer complex at a 5 molppm loading efficiently catalyzed the Hiyama coupling reaction of aryl bromides with aryl(trialkoxy)silanes in propylene glycol to give the corresponding biaryls in excellent yields. This method was applied to the syntheses of adapalene and a biaryl-type liquid-crystalline compound, as well as to the derivatization of dextromethorphan and norfloxacin. ESI-MS and NMR analyses of the reaction mixture suggested the formation of pentacoordinate spirosilicate intermediates in situ. Preliminary theoretical studies revealed that the glycol-derived silicate intermediates formed in situ are quite reactive silicon reagents in the transmetalation step.

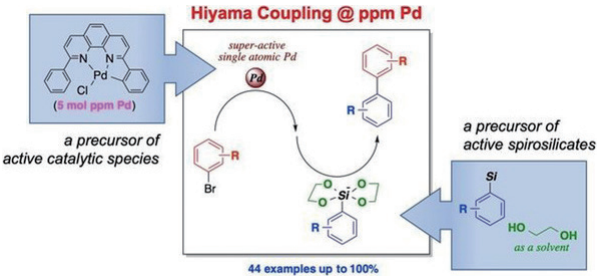

Figure 4. Hiyama Cross-Coupling Catalyzed by ppm Palladium NNC-Pincer Complex in Glycol Solvents.

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3) S. Ichii, G. Hamasaka and Y. Uozumi, *Chem. –Asian J.* **14**, 3850–3854 (2019).

Design and Synthesis of Chiral Organic Molecules for Asymmetric Synthesis

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Keywords Synthetic Chemistry, Molecular Catalyst, Non-Covalent Interaction

The field of molecular catalysis has been an attractive area of research to realize efficient and new transformations in the synthesis of functional molecules. The design of ligands and chiral molecular catalysts has been recognized as one of the most valuable strategies; therefore, a great deal of effort has been dedicated to the developments. In general, "metal" has been frequently used as the activation center, and conformationally rigid catalyst framework has been preferably components for the catalyst design. To develop new type of molecular catalysis, we have focused on the use of hydrogen and halogen atom as activation unit, and have utilized non-covalent interactions as organizing forces of catalyst framework in the molecular design of catalyst, which had not received much attention until recently. We hope that our approach will open the new frontier in chiral organic molecules from chiral molecular chemistry to chiral molecular science.

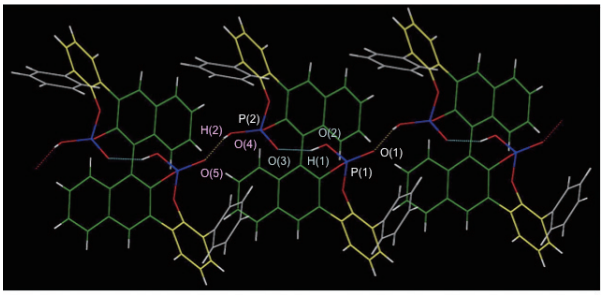

Figure 1. Hydrogen bonding network in chiral bis-phosphoric acid catalyst derived from (*R*)-3,3'-di(2-hydroxy-3 -arylphenyl)binaphthol. Hydrogen bond acts as activation unit for the substrate in asymmetric reaction space and controls atropisomeric behavior in naphthyl–phenyl axis.

Selected Publications

- T. P. Yoon and E. N. Jacobsen, *Science* **299**, 1691–1693 (2003).
- N. Momiyama and H. Yamamoto, "Brønsted Acid Catalysis of Achiral Enamine for Regio- and Enantioselective Nitroso Aldol Synthesis," *J. Am. Chem. Soc.* **127**, 1080–1081 (2005).
- N. Momiyama, H. Tabuse and M. Terada, "Chiral Phosphoric Acid-Governed Anti-Diastereoselective and Enantioselective Hetero-Diels–Alder Reaction of Glyoxylate," *J. Am. Chem. Soc.* **131**, 12882–12883 (2009).
- N. Momiyama, T. Konno, Y. Furiya, T. Iwamoto and M. Terada, "Design of Chiral Bis-Phosphoric Acid Catalyst Derived from (*R*)-3,3'-Di(2-hydroxy-3-arylphenyl)binaphthol: Catalytic Enantioselective Diels–Alder Reaction of α,β-Unsaturated Aldehydes with Amidodienes," *J. Am. Chem. Soc.* **133**, 19294–19297 (2011).
- N. Momiyama, H. Tabuse, H. Noda, M. Yamanaka, T. Fujinami, K. Yamanishi, A. Izumiseki, K. Funayama, F. Egawa, S. Okada, H. Adachi and M. Terada, "Molecular Design of a Chiral Brønsted Acid with Two Different Acidic Sites: Regio-, Diastereo-, and Enantioselective Hetero-Diels–Alder Reaction of Azopyridinecarboxylate with Amidodienes Catalyzed by Chiral Carboxylic Acid–Monophosphoric Acid," *J. Am. Chem. Soc.* **138**, 11353–11359 (2016).

1. Brønsted Acid Catalyzed Asymmetric Rearrangement: Asymmetric Synthesis of Linear Homoallylic Amines

Allylation of imines with allylic metal reagents has been one of the most valuable tools to synthesize enantioenriched homoallylic amines. Due to the inherent nature of allylic metal reagent, however, regioselectivity has been a long-standing subject in this area. To develop the synthetic reaction for enantioenriched linear homoallylic amines, we discovered chirality transferred formal 1,3-rearrangement of ene-aldimines in the presence of Brønsted acid, and developed it as synthetic method for variety of enantioenriched linear homoallylic amines.[1)] Furthermore, we studied details of reaction mechanism and succeeded catalytic asymmetric version of this rearrangement.[2)] To the best our knowledge, our discovery is the first example of asymmetric formal [1,3]-rearrangement and the new entry of the synthetic methodology for the linear enantioenriched homoallylic amines.

2. Design of Chiral Brønsted Acid Catalyst

Chiral Brønsted acid catalysis has been recognized as one of the useful tools in asymmetric synthesis. We have contributed to this area by focusing on the use of perfluoroaryls and C_1-symmetric design.

Perfluorinated aryls have emerged as an exquisite class of motifs in the design of molecular catalysts, and their electronic and steric alterations lead to notable changes in the chemical yields and the stereoselectivities. However, unfortunately, the distinctive potential of perfluorinated aryls has not been fully exploited as design tools in the development of chiral Brønsted acid catalysts. We developed the perfluoaryls-incorporated chiral mono-phosphoric acids as chiral Brønsted acid catalysts that can deriver high yields and stereoselectivities in the reactions of imines with unactivated alkenes. We have described the first example of a diastereo- and enantioselective [4+2] cycloaddition reaction of *N*-benzoyl imines, as well as the enantioselective three-component imino–ene reaction using aldehydes and $FmocNH_2$.[3)]

We have developed (*R*)-3,3'-di(2-hydroxy- 3-arylphenyl) binaphthol derived chiral bis-phosphoric acid which efficiently catalyzed enantioselective Diels–Alder reaction of acroleins with amidodienes.[4,5)] We demonstrated that two phosphoric acid groups with individually different acidities can play distinct roles in catalyst behavior through hydrogen bonding interactions. Hence, we were interested to explore whether a combination of *different acidic functional groups*, in particular an aryl phosphinic acid-phosphoric acid, would function as an efficient Brønsted acid catalyst. We developed a Brønsted acid with two different acidic sites, aryl phosphinic acid-phosphoric acid, and its catalytic performance was assessed in the hetero-Diels–Alder reaction of aldehyde hydrates with Danishefsky's diene, achieving high reaction efficiency.[6)] Furthermore, molecular design of a chiral Brønsted acid with two different acidic sites, chiral carboxylic acid–cyclic mono-phosphoric acid, was identified as a new and effective concept in asymmetric hetero-Diels–Alder reaction of 2-azopyridinoester with amidodienes.[7)]

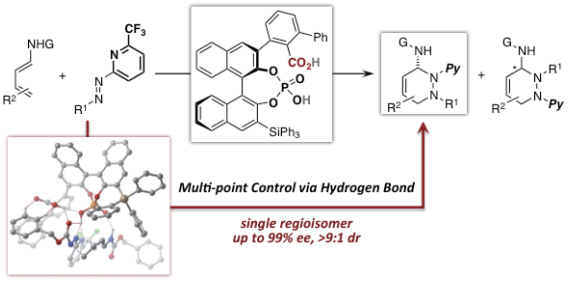

Figure 2. Chiral carboxylic acid–phosphoric acid-catalyzed azo-hetero-Diels–Alder reaction.

3. Design of Catalysis with Halogen Bond for Carbon–Carbon Bond Forming Reactions

Halogen bonds are attractive non-covalent interactions between terminal halogen atoms in compounds of the type R—X (X = Cl, Br, I) and Lewis bases LBs. It has been known that strong halogen bonds are realized when "R" is highly electronegative substituents such as perfluorinated alkyl or aryl substituents. We recently developed synthetic methodology for perfluorinated aryl compounds, and applied it for the development of chiral Brønsted acid catalysts. On the basis of our achievements, we have examined it to develop catalysis with halogen bond for carbon–carbon bond forming reactions.

We found that perfluorinated iodoaryls are able to catalyze the Mukaiyama Mannich-type reaction and allylation reaction.[8)]

References

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2) N. Momiyama *et al.*, submitted.
3) N. Momiyama, H. Okamoto, J. Kikuchi, T. Korenaga and M. Terada, *ACS Catal.* **6**, 1198–1204 (2016).
4) N. Momiyama, T. Konno, Y. Furiya, T. Iwamoto and M. Terada, *J. Am. Chem. Soc.* **133**, 19294–19297 (2011).
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6) N. Momiyama, T. Narumi and M. Terada, *Chem. Commun.* **51**, 16976–16979 (2015).
7) N. Momiyama, H. Tabuse, H. Noda, M. Yamanaka, T. Fujinami, K. Yamanishi, A. Izumiseki, K. Funayama, F. Egawa, S. Okada, H. Adachi and M. Terada, *J. Am. Chem. Soc.* **138**, 11353–11359 (2016).
8) N. Momiyama *et al.*, Two manuscripts: Submitted; four manuscripts under preparation for submission.

Creation of Novel Photonic-Electronic-Magnetic Functions Based on Molecules with Open-Shell Electronic Structures

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Keywords Radical, Open-Shell Electronic States, Photonic-Electronic-Magnetic Properties

The molecules with open-shell electronic states can exhibit unique properties, which are difficult to achieve for conventional closed-shell molecules. Our group develops new open-shell organic molecules (= radicals) and metal complexes to create novel photonic-electronic-magnetic functions.

While conventional closed-shell luminescent molecules have been extensively studied as promising components for organic light-emitting devices, the luminescent properties of radicals have been much less studied because of its rarity and low chemical (photo-)stability. We have developed a novel luminescent organic radical PyBTM, which is highly stable at ambient condition and in the photoexcited state. We have also discovered that (i) PyBTM-doped molecular crystals exhibit photoluminescence with a room-temperature emission quantum yield of 89%, which is exceptionally high in radicals, and (ii) the doped crystals show drastic changes in the emission spectra by applying a magnetic field. This is the first observation of the magnetoluminescence in organic radicals. Our studies provide novel and unique insights in molecular photonics, electronics, and spintronics, and also contribute to developing applied science for light-emitting devices.

Our group focuses on strongly-interacted spins in molecular crystals. The anisotropic assembly of open-shell molecules in crystalline states enables unique molecular materials with exotic electrical and magnetic properties, such as superconductors, ferromagnets, and quantum spin liquids.

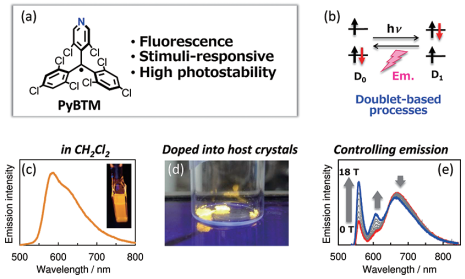

Figure 1. (a) Molecular structure of PyBTM and its characteristics. (b) Schematic photoexcitation-emission processes. (c) Emission in CH_2Cl_2. (d) Emission of PyBTM-doped molecular crystals. (e) Controlling emission by magnetic field.

Selected Publications

- S. Kimura, T. Kusamoto, S. Kimura, K. Kato, Y. Teki and H. Nishihara, "Magnetoluminescence in a Photostable, Brightly Luminescent Organic Radical in a Rigid Environment," *Angew. Chem., Int. Ed.* **57**, 12711–12715 (2018).
- Y. Hattori, T. Kusamoto and H. Nishihara, "Enhanced Luminescent Properties of an Open-Shell (3,5-Dichloro-4-pyridyl)bis(2,4,6-trichlorophenyl)methyl Radical by Coordination to Gold," *Angew. Chem., Int. Ed.* **54**, 3731–3734 (2015).
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1. Construction of Supramolecular Two-Dimensional Network Mediated via Sulfur's σ-Holes in Conducting Molecular Crystals

Metal bis(dithiolato) complexes are attractive class of multi-functional molecular systems showing magnetic, conducting, and optical properties, such as superconductivity, ferromagnetism, quantum spin liquid, and NIR absorption. These properties depend highly on the arrangement of molecules in the solid state; therefore, controlling the arrangement (*i.e.*, crystal structure) plays a crucial role for achieving novel or desired properties. We have focused on a σ-hole bond as an efficient noncovalent supramolecular interaction to control the arrangement of metal bis(dithiolato) complexes, with a goal of developing exotic magneto-conducting phenomena in molecular system. The σ-hole bond is observed between electron-deficient region on an atomic surface, so-called σ-hole, and an electron-rich moiety in a molecule. Halogen and chalcogen atoms possess one and two σ-holes capable of forming supramolecular bonds with high directionality. We have developed novel conducting molecular solids based on platinum bis(dithiolato) complex anion radicals, $(Et\text{-}4XT)_2[Pt(mnt)_2]_3$ (X = Br, I; Et-4BrT = ethyl-4-bromothiazolium, Et-4IT = ethyl-4-iodothiazolium, mnt = maleonitriledithiolato).[1,2] In their crystal structures, $X_{cation}\cdots N_{anion}$ and $S_{cation}\cdots N_{anion}$ σ-hole bonds (*i.e.*, halogen bond and chalcogen bond) were detected between the cation and the lone pairs of –CN moieties in the anion, forming a two-dimensional (2D) supramolecular network. The arrangement of the ions was similar in the two compounds (X = I and X = Br), while the orientation of the cations and the stacking manner of the anions along the direction perpendicular to the 2D supramolecular network were different (Figure 2). Such structural differences are attributed to the strength of the halogen bond, which is greater in iodine than in bromine, and resulted in distinct differences in their physical properties. For example, stronger trimerization of the anions in $(Et\text{-}4IT)_2[Pt(mnt)_2]_3$ enhanced antiferromagnetic interaction between the spins on the anions. These results suggest that sulfur-mediated chalcogen bonds can be robust enough to dominate the primary arrangement of molecules and the resulted physical properties, even in the presence of the other noncovalent intermolecular interactions or even upon the atomic replacements. Sulfur-based chalcogen bonds would be effective to realize desired structures and properties in molecular materials, while understanding the hierarchy of the noncovalent interactions operated in the crystal would be important for the precise crystal engineering.

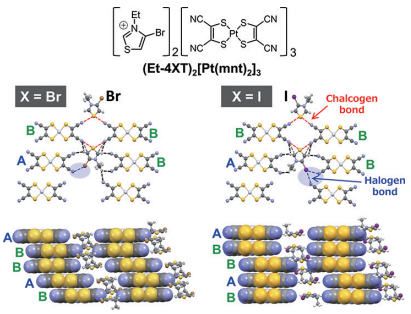

Figure 2. Chemical and crystal structures of $(Et\text{-}4XT)_2[Pt(mnt)_2]_3$ (X = Br, I).

2. Magnetoluminescence as Unique Photofunctions of Open-Shell Molecules

Controlling the spin state of open-shell molecules is a promising strategy for developing unique photochemical and photophysical properties, which are difficult to realize with conventional closed-shell molecules. We have shown that PyBTM doped into α*H*-PyBTM molecular crystals demonstrates new luminescent properties for organic radicals attributed to interplay between spin and luminescence.[3] Crystals containing 10 wt% PyBTM displayed PyBTM monomer- and PyBTM excimer-centered emissions and magnetic-field-sensitive luminescence, *i.e.*, magnetoluminescence. We have revealed that changes in spin multiplicities of aggregated radicals contribute to the magnetic-field effect. To date, magnetoluminescence of stable radicals has been observed only in a few pure organic radicals. We are developing novel PyBTM-ligated metal complexes to expand the variety of materials showing magnetoluminescence behaviour, with a goal of realizing novel spin-sensitive photofunctions. We succeeded in observing excimer-like emission and magnetoluminescence behaviour in PyBTM-ligated zinc complexes doped into host crystals (Figure 3). Detailed investigations are in progress.

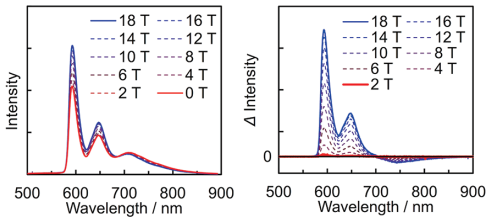

Figure 3. (left) Emission spectra of PyBTM-ligated zinc complex doped into host crystals at 4.2 K under magnetic fields. (right) Difference emission spectra (Δ intensity) under magnetic fields compared with the spectrum under 0 T.

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Award

KUSAMOTO, Tetsuro; Research Encouragement Award, Japan Society of Coordination Chemistry (2019).

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Design and Synthesis of Three-Dimensional Organic Structures

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Keywords π-Conjugated Molecules, Molecular Topology, 3D Network Polymer

Aromatic compounds are potentially useful as functional electronic materials. However, the controlled synthesis and assembly of three-dimensional complex molecules are still very difficult, especially for the crystal engineering of organic molecules. This group aims to create novel topological and reticular organic structures by using synthetic organic chemistry and geometric insights.

To achieve our purpose, this group will start electron-diffraction crystallography (MicroED) for the rapid structure determination of organic compounds. While X-ray crystallography is a general and reliable method for structure determination, it requires ~0.1 mm single crystals and making such crystal sometimes needs tremendous times and efforts. Since electron beam have much higher diffraction intensity than X-ray, structural analysis can be performed even with ultra-small crystals (1 μm or less). There are many fields such as covalent organic crystals with a three-dimensional structure and molecules with complex molecular topologies, where structural analysis has not been sufficiently developed.

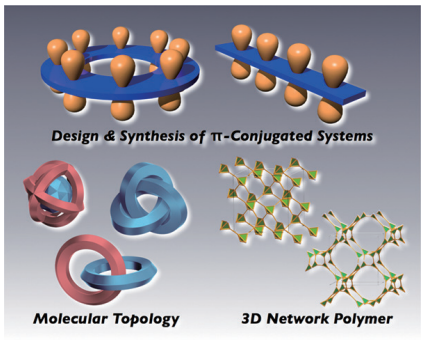

Figure 1. Design and synthesis of π-conjugated organic molecules (top); Development of novel molecular topology (bottom left); Construction of three-dimensional network polymers (bottom right).

Selected Publications

- Y. Saito, K. Yamanoue, Y. Segawa and K. Itami, "Selective Transformation of Strychnine and 1,2-Disubstituted Benzenes by C–H Borylation," *Chem* **6**, 985–993 (2020).
- Y. Segawa, D. R. Levine and K. Itami, "Topologically Unique Molecular Nanocarbons," *Acc. Chem. Res.* **52**, 2760–2767 (2019).
- Y. Segawa, M. Kuwayama, Y. Hijikata, M. Fushimi, T. Nishihara, J. Pirillo, J. Shirasaki, N. Kubota and K. Itami, "Topological Molecular Nanocarbons: All-Benzene Catenane and Trefoil Knot," *Science* **365**, 272–276 (2019).
- G. Povie, Y. Segawa, T. Nishihara, Y. Miyauchi and K. Itami, "Synthesis of a Carbon Nanobelt," *Science* **356**, 172–175 (2017).
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- Y. Segawa and D. W. Stephan, "Metal-Free Hydrogenation Catalysis of Polycyclic Aromatic Hydrocarbons," *Chem. Commun.* **48**, 11963–11965 (2012).
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1. Synthesis and Reactivity of Highly Strained Belt-Shaped Polycyclic Aromatic Hydrocarbons

We performed the synthesis of novel π-conjugated molecules with high strain energies and the investigation of its structural properties.[2–4)]

The synthesis of cycloiptycene derivatives was achieved in each one step from (6,6)carbon nanobelt (Figure 2).[3)] It was revealed that the carbon nanobelt was reacted as a diene in the Diels–Alder reaction with arynes and alkynes. Structures of all products were identified by X-ray crystallography to confirm that the Diels–Alder reactions took place at the six central benzene rings of the carbon nanobelt. DFT calculations indicated that the release of strain energy is the driving force to proceed the Diels–Alder reaction. By using this method, we have successfully synthesized cyclotetracosiptycene, the largest iptycene ever synthesized.

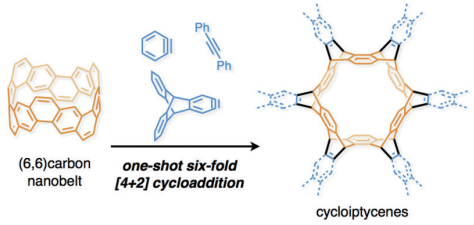

Figure 2. Diels–Alder reaction of (6,6)carbon nanobelt with alkynes and arynes for the formation of cycloiptycene derivatives.

The synthesis, structure, and properties of methylene-bridged [6]cycloparaphenylene ([6]CPP), a nonalternant aromatic belt, were investigated (Figure 3).[2)] This belt-shaped methylene-bridged [6]CPP, in which each phenylene unit is tethered to its neighbors by methylene bridges, was constructed through 6-fold intramolecular nickel-mediated aryl–aryl coupling of triflate-functionalized pillar[6]arene in 18% isolated yield. As compared to the analogous [6]CPP, the methylene bridges coplanarize neighboring paraphenylene units and enhance the degree of π-conjugation, which results in a significant decrease in energy gap. Moreover, the incorporation of small molecules in the defined pocket of methylene-bridged [6]CPP makes it an attractive supramolecular architecture. Methylene-bridged [6]CPP is characterized by high internal strain energy reaching 110.2 kcal mol^{-1}, attributed to its restricted structure. This work not only exhibits an efficient strategy to construct a new family of aromatic belt, but also showcases their properties, which combine the merits of CPPs and pillararenes.

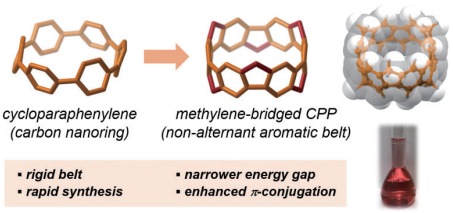

Figure 3. Synthesis of a nonalternant aromatic belt.

2. Development of a Direct Octagon-Forming Annulation Reaction by Palladium Catalyst

The discoveries of new forms of carbon have always opened doors to new science and technology. In 1991, three-dimensional (3D) periodic carbon crystals with negative Gaussian curvatures that consist of six- and eight-membered rings were proposed. To realize these 3D periodic carbon crystals, methods for creating polyaromatic structures embedding eight-membered rings must be developed. Here the two annulative coupling reactions that form an eight-membered ring through catalytic C–H functionalization are reported (Figure 4).[1)] Bay-chlorinated polyaromatics undergo either annulative dimerization or cross-coupling with biphenylene in the presence of a palladium catalyst to form various hitherto inaccessible polyaromatics embedding an eight-membered ring. The threefold annulative cross-coupling of 1,5,9-trichlorotriphenylene allowed construction of a highly curved nanocarbon. The present work not only demonstrates the potential of annulative coupling for constructing octagonal nanocarbons but also provides a conceptual pathway for the synthetic realization of 3D periodic carbon crystals.

Based on this result, our group has started the Joint Research Program in IMS with Murakami group. Our group will support the rapid structural determination of targeted π-conjugated molecules, their synthetic intermediates, as well as active species of transition metal catalysts.

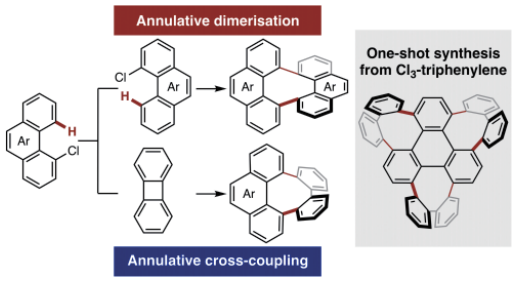

Figure 4. Palladium-catalyzed octagon-forming annulation reaction.

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Visiting Professors

Visiting Professor
FUKAZAWA, Aiko *(from Kyoto University)*

Renaissance of Nonbenzenoid π-Conjugated Systems toward Functional Materials

The work of our group has focused on exploring functional organic compounds with unusual with superb optical and/or electronic properties, based on the molecular designs of novel π-conjugated scaffolds as well as unusual functional groups. In particular, we have recently proposed a rational design of stable yet unusual π-conjugated systems based on the characteristics of nonbenzenoid hydrocarbons, *i.e.*, dehydroannulenes, non-alternant hydrocarbons, and fulvalenes, by the fusion of (hetero)arenes with small magnitude of aromaticity. In this year, we have succeeded in synthesizing several thiophene-fused antiaromatic π-systems, such as dithieno[*a*,*e*]pentalenes and their nitrogen-doped analogues. These thiophene-fused antiaromatics exhibit high thermal stability even without bearing bulky substituents while retaining pronounced antiaromatic character. These features give rise to characteristic long-wavelength absorption as well as aggregation behavior of these compounds.

Visiting Professor
WATANABE, Rikiya *(from RIKEN)*

Single Molecule Physiology

Our study aims to understand cellular functions using a bottom-up approach from the single molecule level. To achieve this, we are attempting to elucidate the mechanism by which individual biomolecules or their networks function in a precise manner, by developing novel single-molecule techniques using multidisciplinary approaches, including biophysics, bioMEMS, and chemical biology. In addition, we are developing a methodology to investigate correlations between genetic mutations, dysfunctions, and diseases with single molecule sensitivity, which would provide new insights for biological as well as pharmaceutical studies.

Visiting Associate Professor
UEDA, Akira *(from Kumamoto University)*

Development of Purely Organic Molecular Materials with Unique Structural/Electronic Properties

Design and synthesis of novel molecular materials have been a central issue for the development of molecular science. Our group has continuously focused on purely organic molecular materials with unique crystal/electronic structures and physical properties. Very recently, we have succeeded in the development of a novel neutral radical molecular conductor with a partially charge-transferred structure. Interestingly, this material forms a two-dimensional conducting layer like BEDT-TTF salts, resulting in a much higher electrical conductivity than that of the conventional neutral radical conductors. These results suggest that this material is a possible candidate of a purely organic single-component molecular metal or superconductor. The magnetic properties and phase transition behavior of this material are of interest and thus will be investigated in the near future.