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-anchored proteins, biological-clock proteins, metalloproteins, glycoconjugates, and molecular chaperone. Coordination-complex divisions aim to develop molecular catalysts and functional metal complexes for transformation of organic molecules, water oxidation and reduction, and molecular materials such as molecular wires. 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 Signal Sensing

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Professor, Okazaki Institute for Integrative Bioscience (–2018)
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Keywords Bioinorganic Chemistry, Metalloproteins, Sensor Protein

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 signalling 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 heme-based gas-sensor proteins and the signalling systems working with them.

The prosthetic group heme acts as the active center of hemeproteins that show a variety of functions, including O_2 or NO storage/transport, electron transfer, redox catalysis of various substrate, and dehydration of aldoxime. In the present context, it acts as the active site for sensing of diatomic gas molecules such as NO, O_2, and CO. These gas molecules are able to bind to heme iron as an axial ligand, which is a reason why heme can be adapted as the active center for sensing gas molecules. Heme-based gas-sensor proteins constitute a major group in the gas-sensor proteins. Binding of a cognate gas molecule to heme is the initial step for gas sensing, which is followed by the signalling processes. The binding affinities of gas molecules, that measures of the sensitivities of the sensor proteins, can be controlled by heme environmental structures. Differences in the heme coordination structure of the axial ligand(s) and/or of interaction(s) between the heme-bound gas molecule and surrounding amino acid residue(s) in a heme pocket play important roles. They not only regulate the binding affinities of gas molecules but also discriminate one cognate effector gas molecule from others, allowing the sensor to respond with the proper signal transductions. We have been elucidating the relationships of structures and functions of heme-based sensor proteins by crystallographic, biochemical, biophysical, and molecular biological studies.

Selected Publications

- 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).
- C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, "Molecular Mechanism for Heme-Mediated Inhibition of 5-Aminolevulinic Acid Synthase 1," *Bull. Chem. Soc. Jpn.* **87**, 997–1004 (2014).
- Y. Okamoto, H. Sawai, M. Ogura, T. Uchida, K. Ishimori, T. Hayashi and S. Aono, "Heme-Binding Properties of HupD Functioning as a Substrate-Binding Protein in a Heme-Uptake ABC-Transporter System in *Listeria monocytogenes*," *Bull. Chem. Soc. Jpn.* **87**, 1140–1146 (2014).

1. Protein Dynamics and Signal Transduction of Heme-Based Oxygen Sensor Protein HemAT-Bs

HemAT from *Bacillus subtilis* (HemAT-*Bs*) is a heme-based O_2 sensor protein responsible for aerotaxis (chemotaxis toward molecular oxygen) control in this bacterium. It consists of the N-terminal sensor domain in which heme acts as an oxygen sensing site and C-terminal signaling domain. The binding of O_2 to the heme induces a conformational change in the sensor domain of HemAT-*Bs*, triggering intramolecular signal transductions to result in the regulation of the chemotactic signaling in *B. subtilis*. An important issue to understand the signal transduction mechanism of HemAT-*Bs* is to reveal the pathway to transmit the conformational changes induced upon ligand binding/dissociation to/from the heme. In this study, we have elucidated conformational changes upon CO ligand dissociation from the heme for full-length wild-type HemAT, and the Y70F (B-helix), L92A (E-helix), T95A (E-helix), and Y133F (G-helix) HemAT mutants by time-resolved step-scan FTIR spectroscopy.

These mutations perturb hydrogen bonding and electrostatic interactions between the heme-bound ligand and the surrounding amino acid residues. While Tyr70 and Thr95 in the distal heme pocket form hydrogen bonds to the heme-bound O_2, a reversible hydrogen bond formation/cleavage takes place between Tyr133 and His123 upon ligand binding/dissociation to/from the heme in the proximal heme pocket. Rebinding of CO to the heme is biphasic in the sensor domain and full-length HemAT as well as in the mutants, with the exception of the Y133F mutant protein. The monophasic rebinding of CO in Y133F suggests that the ligand rebinding process is significantly affected in the absence of the hydrogen bond between Tyr133 and His123 residue in the proximal heme pocket.

Time-resolved step-scan FTIR studies reveal the spectral components to discrete substructures, which originate from a helical structure that is solvated (1638 cm^{-1}) and a native helix that is protected from solvation by interhelix tertiary interactions (1654 cm^{-1}). The full-length protein is characterized by an additional amide I absorbance at 1661 cm^{-1}, which is attributed to disordered structure suggesting that further protein conformational changes occur in the presence of the signaling domain in the full-length protein. The kinetics monitored within the amide I absorbance of the polypeptide backbone in the sensor domain exhibit two distinct relaxation phases (t_1 = 24 and t_2 = 694 μs), whereas that of the full-length protein exhibits monophasic behavior for all substructures in a time range of t = 1253–2090 μs. These observations can be instrumental in monitoring helix motion and the role of specific mutants in controlling the dynamics in the communication pathway from the sensor to the signaling domain. The kinetics observed for the amide I relaxation for the full-length protein indicate that the discrete substructures within full-length HemAT, unlike those of the sensor domain, relax independently.

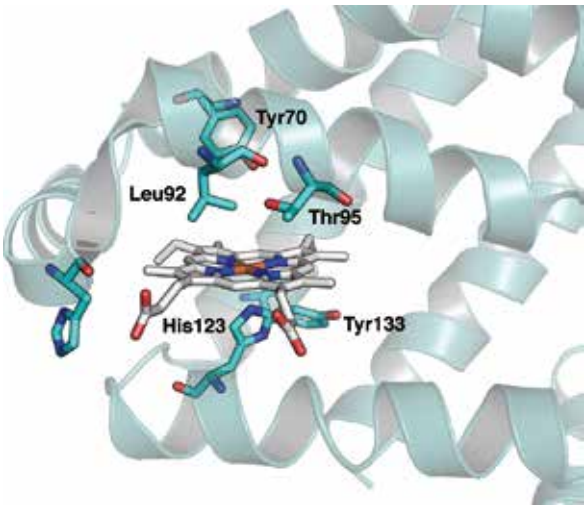

Figure 1. Heme environmental structure of HemAT-*Bs*.

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

Regulatory hydrogenase (RH), HoxJ, and HoxA proteins consist a H_2-dependent regulatory system of gene expression for proteins involved in hydrogen metabolism, in which RH acts as a molecular hydrogen sensor. RH 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 maturation of the Ni-Fe dinuclear complex and RH protein remain elusive. Several accessory proteins are involved in the formation of the Ni-Fe complex and its insertion into the large subunit to mature RH. We are now elucidating the structural and functional relationships of the accessory protein HypX responsible for the construction of the Fe(CO) unit in the Ni-Fe dinulear complex in RH. We have obtained single crystals of HypX and the crystallographic analyses of HypX are now in progress.

Dynamical Ordering of Biomolecular Systems for Creation of Integrated Functions

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Professional Employment
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Keywords Biomolecule, Dynamical Ordering, NMR

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.

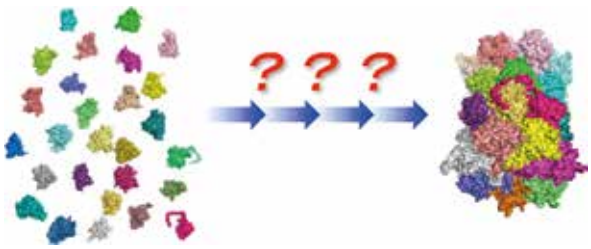

Figure 1. Formation of supramolecular machinery through dynamic assembly and disassembly of biomolecules.

Selected Publications

- 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).
- K. Kato and T. Satoh, "Structural Insights on the Dynamics of Proteasome Formation," *Biophys. Rev.* **10**, 597–604 (2018).
- 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).
- T. Ikeya, D. Ban, D. Lee, Y. Ito, K. Kato and C. Griesinger, "Solution NMR Views of Dynamical Ordering of Biomacromolecules," *Biochim. Biophys. Acta, Gen. Subj.* **1862**, 287–306 (2018).

1. Characterization of Structural Dynamics and Interactions of Immunoglobulin G Glycoprotein

Immunoglobulin G (IgG) is a major serum glycoprotein that exerts antibody functions in the immune system, coupling antigen recognition in the Fab region with a variety of effector functions promoted in the Fc region. Therefore, detailed exploration of the structural dynamics and interactions of IgG is essential to gain deeper insights into their immune functions. We characterized the interaction of IgG-Fc with its cognate receptor (FcγR) using small-angle neutron scattering and molecular dynamics simulation.[1,2] The results revealed conformational deformation of Fc upon the interactions, underscoring the significance of existence of the *N*-glycans of these glycoproteins. In particular, our studies demonstrate that core fucosyaltion of the Fc *N*-glycans disrupts optimum intermolecular interactions mediated by the complex-type *N*-glycan at specific position of FcγRIIIa.[2,3]

We also characterized the antibody interactions using stable-isotope-assisted NMR spectroscopy. We established spectral assignments of FcγRIIIb and IgG-Fc.[4,5] Moreover, we successfully observed methyl-TROSY peaks originating from IgG with a molecular mass of 150 kDa, employing tailored deuteration.[6] On this technical basis, we conducted *in-serum* NMR observation detecting semi-specific antibody interactions in physiologically relevant heterogeneous environments.[4,6]

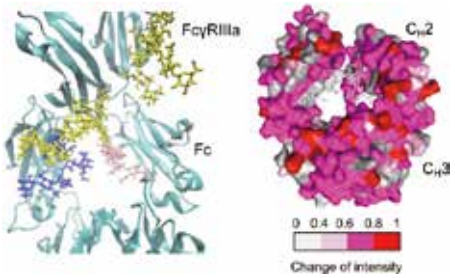

Figure 2. The dynamic view of IgG and its interactions.
Left figure: A snapshot from molecular dynamics simulation of Fc-FcγRIIIa complex. Right: Mapping of the interactions of IgG-Fc and serum components.

2. Dynamical Structures of Biomolecules toward Understanding the Mechanisms Underlying Their Functions

Our group employs multilateral biophysical and biochemical approaches for characterizing dynamical structures of various biomolecular systems that involve proteins, by integrating NMR spectroscopy, X-ray crystallography, high-speed atomic force microscopy, small-angle X-ray scattering, and cryo-electron microscopy. Our data demonstrated a two-step process for the disassembly mechanism of the eukaryotic proteasome, suggesting that scrap-and-build processes are involved in proteasome formation.[7] Based on structural information, we successfully endowed a functionally undefined archaeal protein with proteasome-activating activity by modifying its flexible C-terminal segment.[8] Furthermore, we revealed that an endoplasmic reticulum enzyme UGGT has a flexible modular structure in which the smaller catalytic domain is tethered to the larger folding-sensor region with variable spatial arrangements, offering structural insights into its working mechanism in the glycoprotein quality control system.[9] On the other hand, we created a series of synthetic neoglycolipids displaying functional glycotopes.[10] Surprisingly, the neoglycolipid micelles evoked selective apoptosis in undifferentiated neural stem cells. This serendipitous finding may offer a new strategy for controlling neural cell fates using artificial glycoclusters.

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2) Y. Sakae, T. Satoh, H. Yagi, S. Yanaka, T. Yamaguchi, Y. Isoda, S. Iida, Y. Okamoto and K. Kato, *Sci. Rep.* **7**, 13780 (2017).
3) H. Yagi, D. Takakura, L. T. Roumenina, W. H. Fridman, C. Sautès-Fridman, N. Kawasaki and K. Kato, *Sci. Rep.* **8**, 2719 (2018).
4) S. Yanaka, T. Yamazaki, R. Yogo, M. Noda, S. Uchiyama, H. Yagi and K. Kato, *Molecules* **22**, 1619 (2017).
5) R. Yogo, S. Yanaka and K. Kato, *Biomol. NMR Assignments* **12**, 201–204 (2018).
6) S. Yanaka, H. Yagi, R. Yogo, M. Yagi-Utsumi and K. Kato, *J. Biomol. NMR* **71**, 193–202 (2018).
7) T. Kozai, T. Sekiguchi, T. Satoh, H. Yagi, K. Kato and T. Uchihashi, *Sci. Rep.* **7**, 15373 (2017).
8) M. Yagi-Utsumi, A. Sikdar, T. Kozai, R. Inoue, M. Sugiyama, T. Uchihashi, H. Yagi, T. Satoh and K. Kato, *Protein Eng., Des. Sel.* **31**, 29–36 (2018).
9) T. Satoh, C. Song, T. Zhu, T. Toshimori, K. Murata, Y. Hayashi, H. Kamikubo, T. Uchihashi and K. Kato, *Sci. Rep.* **7**, 12142 (2017).
10) H. Yagi, G. Yan, T. Suzuki, S. Tsuge, T. Yamaguchi and K. Kato, *Neurochem. Res.* **43**, 212–218 (2018).

Awards

YOGO, Rina; Poster Presentation Award, The 6th International Symposium on "Dynamical Ordering and Integrated Functions" (2018).
YAGI-UTSUMI, Maho; The Pharmaceutical Society of Japan Award for Young Scientists '18, The Pharmaceutical Society of Japan (2018).
SAITO, Taiki; Poster Presentation Award, ExCELLS Young Scientists Forum 2018 (2018).

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Operation and Design Principles of Biological Molecular Machines

Department of Life and Coordination-Complex Molecular Science
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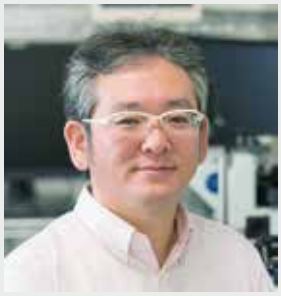

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Keywords Molecular Motors, Single-Molecule Analysis, Protein Engineering

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 will unveil operation principles of molecular motors with advanced single-molecule functional analysis and structural analysis. With the help of computer science, we will also engineer new, non-natural molecular motors to understand their design principles. Our ultimate goal is controlling living organisms with created molecular machines.

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

- 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).
- F. Kawai, A. Nakamura, A. Visootsat and R. Iino, "Plasmid-Based One-Pot Saturation Mutagenesis and Robot-Based Automated Screening for Protein Engineering,"*ACS Omega* **3**, 7715–7726 (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).
- H. Isojima, R. Iino, Y. Niitani, H. Noji and M. Tomishige, "Direct Observation of Intermediate States during the Stepping Motion of Kinesin-1," *Nat. Chem. Biol.* **12**, 290–297 (2016).
- A. Nakamura, T. Tasaki, D. Ishiwata, M. Yamamoto, Y. Okuni, A. Visootsat, M. Maximilien, H. Noji, T. Uchiyama, M. Samejima, K. Igarashi and R. Iino, "Direct Imaging of Binding, Dissociation, and Processive Movement of *Trichoderma reesei* Cel6A and Its Domains on Crystalline Cellulose," *J. Biol. Chem.* **291**, 22404–22413 (2016).
- H. Ueno, Y. Minagawa, M. Hara, S. Rahman, I. Yamato, E. Muneyuki, H. Noji, T. Murata and R. Iino, "Torque Generation of *Enterococcus hirae* V-ATPase," *J. Biol. Chem.* **289**, 31212–31223 (2014).
- T. Uchihashi, R. Iino, T. Ando and H. Noji, "High-Speed Atomic Force Microscopy Reveals Rotary Catalysis of Rotorless F_1-ATPase," *Science* **333**, 755–758 (2011).

1. Single-Nanoparticle Tracking with Angstrom Localization Precision and Microsecond Time Resolution[1)]

Gold nanoparticle (AuNP) has been used as a probe of single-molecule imaging of molecular motors. We investigated lower limit of the localization precision in dark-field imaging of AuNP. We confirmed that the localization precision is inversely proportional to square root of photon number, and the lower limit is determined by detector saturation. To overcome the limit, we developed an axicon lens-based annular illumination total internal reflection dark-field microscopy, which illuminates AuNP with high laser intensity to obtain high signal intensity and observes AuNP with small image pixel size to avoid detector saturation. As results, with 40 nm AuNP, 1.3 Å and 5.4 Å localization precisions have been achieved at 1 ms and 33 μs time resolutions, respectively (Figure 2). We then observed transition pathways from bound to unbound states of the kinesin-1 head (Figure 3). During transitions, large leftward trails were not observed along the microtubule short axis indicating that the rear head passes the microtubule-bound leading head from the right, which results in unidirectional rotation of kinesin-1.

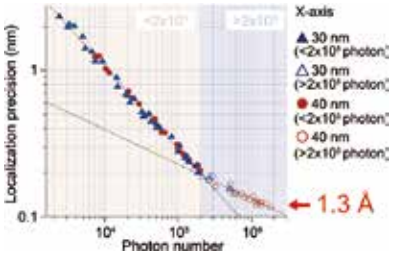

Figure 2. Photon number dependence of the localization precision for the 40 nm and 30 nm AuNPs.

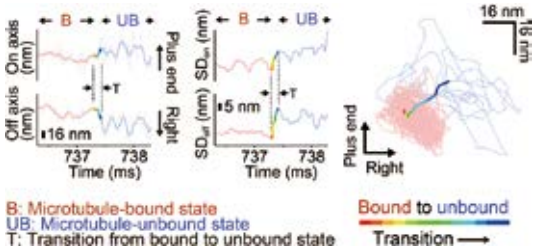

Figure 3. Transition from bound to unbound state of the kinesin-1 head labeled with 40 nm AuNP at 10 μs time resolution.

2. Processive Chitinase Is Brownian Monorail Operated by Fast Catalysis after Peeling Rail from Crystalline Chitin[2)]

Processive chitinase is a linear molecular motor which moves on the surface of crystalline chitin driven by processive hydrolysis of single chitin chain (Figure 4). Here, we analysed the mechanism underlying unidirectional movement of *Serratia marcescens* chitinase A (*Sm*ChiA) using high-precision single-molecule imaging, X-ray crystallography, and all-atom molecular dynamics simulation. *Sm*ChiA showed fast unidirectional movement of ~50 nm s^{-1} with 1-nm forward and backward steps, consistent with the length of reaction product chitobiose. Analysis of the kinetic isotope effect revealed fast substrate-assisted catalysis with time constant of ~3 ms. Decrystallisation of the single chitin chain from crystal surface is the rate-limiting step of movement with time constant of ~17 ms, achieved by binding free energy at the product binding site of *Sm*ChiA (Figure 5). Our results demonstrate that *Sm*ChiA operates as a burnt-bridge Brownian ratchet wherein the Brownian motion along the single chitin chain is rectified forward by substrate-assisted catalysis.

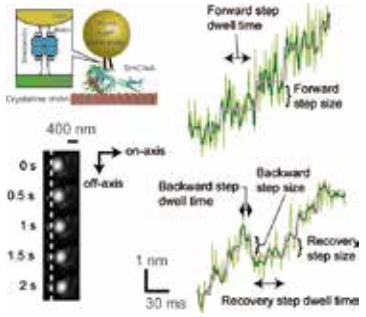

Figure 4. Examples of image and trajectory of *Sm*ChiA probed by 40 nm AuNP.

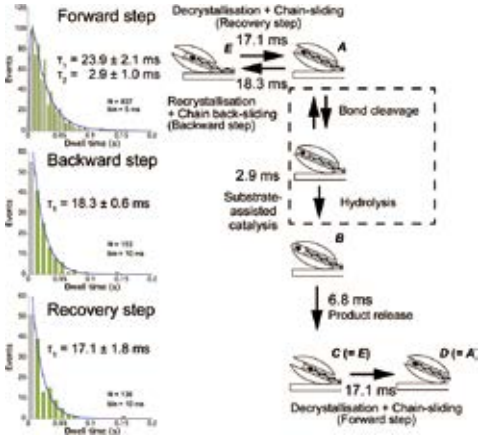

Figure 5. Distribution of dwell times and summary of time constants of elementally steps.

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2) A. Nakamura, K. Okazaki, T. Furuta, M. Sakurai and R. Iino, *Nat. Commun.* **9**, 3814 (2018).

Award

ANDO, Jun; Young Scientist Presentation Award of the Spectroscopical Society of Japan 2017 (2017).

* IMS International Internship Program

A Supramolecular Chemical Approach to the Construction of Artificial Cells

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Keywords Artificial Cell, Origin of Life, Vesicle

Exploring the boundary between living and non-living matter is one of the most challenging problems for contemporary scientists. To understand the cell, which is considered the smallest unit of life, a plausible strategy is to synthesize an artificial cell by using a supramolecular chemical approach, because simple molecular assemblies at one time evolved to create the simple cell on prebiotic earth. As shown in Figure 1, the key elements of a cell are the 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 attempted to construct two artificial cells by using giant vesicles (GVs) as the compartment. One, developed in collaboration with the Sugawara group (Kanagawa Univ.), is an artificial cell that can proliferate from generation to generation. Now, we have constructed a recursive vesicular artificial cell system with proliferation cycles. By using the vesicular transport system, the second generation GVs, which contain no PCR reagents after self-reproduction, can be replenished by fusing them with conveyer GVs bearing the PCR reagents by changing the pH of the dispersion. After the PCR reagents are replenished, the GV can self-reproduce again. This system could lead to an evolvable artificial cellular system. The other artificial cell is an artificial cell that contains a catalyst-producing system. The GV system can generate catalysts and membrane molecules by transforming their respective precursors, thereby facilitating the proliferation of the GVs with the produced catalyst.

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

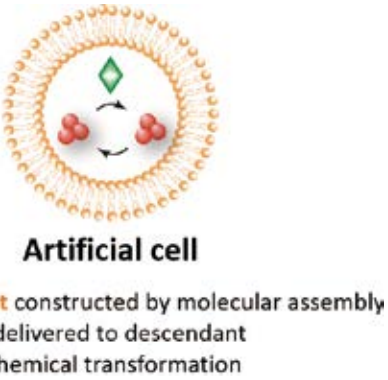

Figure 1. Artificial cell model. The replicating systems of the compartment and the information materials are combined. The reactions in the two replicating systems are accelerated by appropriate catalysts.

Selected Publications

- K. Kurihara, M. Tamura, K-I. Shohda, T. Toyota, K. Suzuki and T. Sugawara, "Self-Reproduction of Supramolecular Giant Vesicles Combined with the Amplification of Encapsulated DNA," *Nat. Chem.* **3**, 775–781 (2011).
- K. Kurihara, Y. Okura, M. Matsuo, T. Toyota, K. Suzuki and T. Sugawara, "A Recursive Vesicle-Based Model Protocell with a Primitive Cell Cycle," *Nat. Commun.* **6**, 8352 (2015).

1. An Artificial Cell Using a Self-Reproducing Oil Droplet as a Scaffold

A cell is a self-organized system that can maintain its state via metabolism. Our previously developed artificial cellular system is robust, but it can self-reproduce only a specific state in the any environments.[1–3] Research on transforming oil droplets into vesicles by use of chemical reactions and self-assembly processes is expected to facilitate our understanding of the origin and definition of life from a chemistry perspective.

The mixing of an aqueous solution of an aldehyde containing an imidazole hydrochloride group with octylaniline led to the spontaneous formation of autocatalytic oil droplets[5] (Figure 2). An aldehyde-bearing quaternary ammonium salt that does not react well with octylaniline was added to this autocatalytic droplet system. As a result, the catalytic molecules that formed within the oil droplets promoted the condensation reaction between the octylaniline and the non-catalytic aldehyde, which ultimately led to the synthesis of vesicular membrane molecules with imine functionality within the molecular aggregates; thus self-reproducible oil droplets were successfully transformed into vesicles upon the addition of the membrane precursor.

We will construct an oil droplet system that synthesizes peptides (simple proteins) and an oil droplet system that forms vesicles. The former is an oil droplet that incorporates amino acids and synthesizes peptides internally. In addition, peptide synthesis inside the vesicles is performed using a water-soluble condensing agent through our developed water-in-oil emulsion centrifugation method.[5]

2. Vesicular System Containing Peptide Synthesis

In the prebiological era, cooperative interaction of spontaneously polymerizing polymer and self-producing molecular aggregates led to the emergence of primitive cells. Although the membrane potentially provides a reactive field of catalytic reaction, it remains the mystery the cooperation between polymer and molecular aggregates occur without advanced catalyst.

Therefore, we designed and synthesized monomer precursors that spontaneously peptide-polymerized in a reductive water and generated molecular aggregates as the polymerization reaction proceeds. This monomer precursor has disulfide and thioester sites. In the first step of the reaction, the disulfide of the monomer precursor is reduced and a thiol-bearing monomer is produced. Next, benzylmercaptan, which generates the oil droplet, is eliminated by replacing the thiol of the monomer. Finally, the occurrence of S-N acyl transfer forms amide bonds (native chemical ligation).

From the reaction trace by NMR spectrum, the release of benzylmercaptan and subsequent formation of amide bond were detected. Furthermore, as turbidity increased with progress of reaction, the reaction solution formed cell-size oil droplets under microscope observation. These results mean that benzylmercaptan forms oil droplets without layer separation because the generated peptides stabilize the interface. In this system, since spontaneous polymerization of polymer and oil droplet generation are coupled, application of this system to artificial cells is expected.

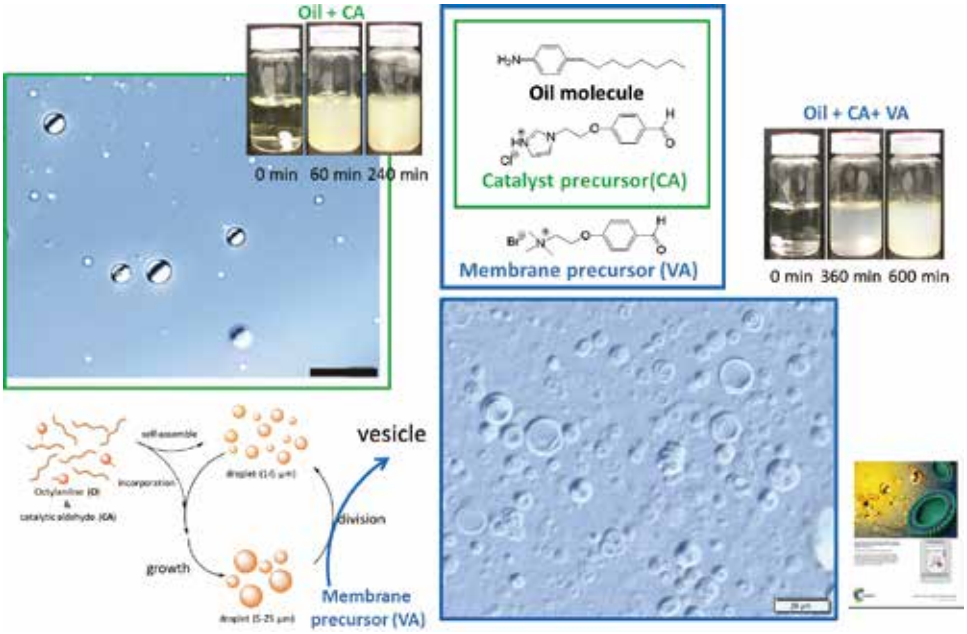

Figure 2. Scheme of the self-reproducing oil droplet (oil-in-water emulsion) and vesicular transformation system.

References

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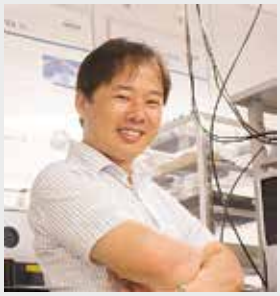
2) K. Kurihara, Y. Okura, M. Matsuo, T. Toyota, K. Suzuki and T. Sugawara, *Nat. Commun.* **6**, 8352 (2015).
3) L. Sheng and K. Kurihara, *Chem. Lett.* **45**, 598–600 (2016).
4) L. Sheng and K. Kurihara, *Chem. Commun.* **52**, 7786–7789 (2016).
5) Y. Natsume, H. Wen, T. Zhu, K. Itoh, L. Sheng and K. Kurihara, *J. Vis. Exp.* **119**, e55282 (2017).

Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

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



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Keywords Infrared Spectroscopy, Membrane Protein, Ion Channel

Membrane proteins are important for homeostasis and signaling of living cells, which work as ion channel, ion pump, various types of chemical and biophysical sensors, and so on. These proteins are considered as one of important targets for biophysical studies. Our main goal is to clarify molecular mechanisms underlying functions of the channels, transporters and receptors mainly by using stimulus-induced difference infrared spectroscopy, which is sensitive to the structural and environmental changes of bio-molecules.

We applied attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to extract ion-binding-induced signals of various kinds of membrane proteins. For example, KcsA is a potassium channel, which is highly selective for K^+ over Na^+, and the selectivity filter binds multiple dehydrated K^+ ions upon permeation. Shifts in the peak of the amide-I signals towards lower vibrational frequencies were observed as K^+ was replaced with Na^+ (Figure 1). These vibrational modes give us precise structural information of the selectivity filter. Moreover, by changing concentrations of K^+ in buffer solutions, we can estimate affinity of the selectivity filter for K^+ ions.

Recently, we have developed a rapid-buffer exchange apparatus for time-resolved ATR-FTIR spectroscopy, which can be utilized for studying dynamics of structural transition in membrane proteins.

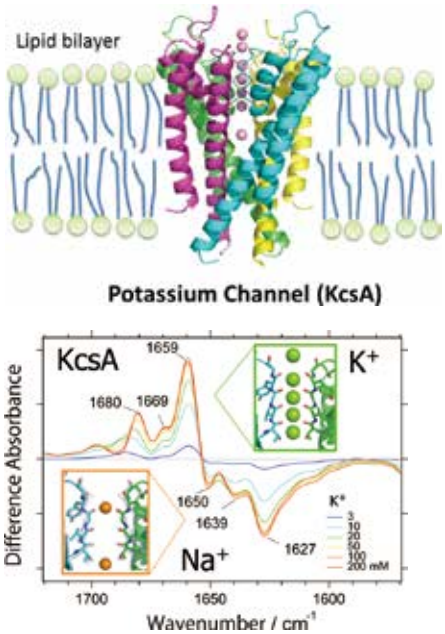

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

Selected Publications

- Y. Furutani *et al.*, "ATR-FTIR Spectroscopy Revealed the Different Vibrational Modes of the Selectivity Filter Interacting with K^+ and Na^+ in the Open and Collapsed Conformations of the KcsA Potassium Channel," *J. Phys. Chem. Lett.* **3**, 3806–3810 (2012).
- Y. Furutani *et al.*, "Development of a Rapid Buffer-Exchange System for Time-Resolved ATR-FTIR Spectroscopy with the Step-Scan Mode," *Biophysics* **9**, 123–129 (2013).

1. Ion–Protein Interactions of TWIK1 Potassium Channel with Alkali Metal Cations and Its Implication for the Ion Selectivity[1)]

Potassium channels are selectively permeable to potassium ions (K^+) in cell membrane and function for shaping neuroral signals in nerve cells or maintaining ionic compositions in various cells. The ion selectivity of potassium channels for K^+ over Na^+ is extremely high (typically 1000:1 ratio). The molecular mechanism of the selectivity has been greatly understood by determination of three-dimensional structure of a bacterial potassium channel, KcsA, which stimulates further discussion on the potassium selectivity. As we have shown in previous studies, infrared spectroscopy can detect molecular vibrations and could be a key technique for studying ion–protein interactions in membrane proteins. Indeed, ion-exchange induced difference FTIR spectroscopy successfully discriminated structures of the selectivity filter of the KcsA channel interacting with each kind of alkali metal cations.[2)]

Two-pore domain potassium channel possesses two pore domains in a monomer unit and composes the ion selectivity filter by forming a dimer instead of a conventional tetramer. Among them, TWIK1 shows the peculiar ion selectivity in which sodium ions permeate under low potassium concentration or acidic pH in the extracellular side. The pseudo four-fold rotational symmetry along the pore axis may cause the low potassium selectivity. To understand the molecular mechanism underlying the low potassium selectivity of TWIK1, we applied ion-exchange induced difference FTIR spectroscopy on this protein. Then, we compared the difference spectra of a high potassium selective variant, T118I, and a L228F variant, which is altered on the filter but known to be unaffected to the ion selectivity, with those of the wild-type TWIK-1.

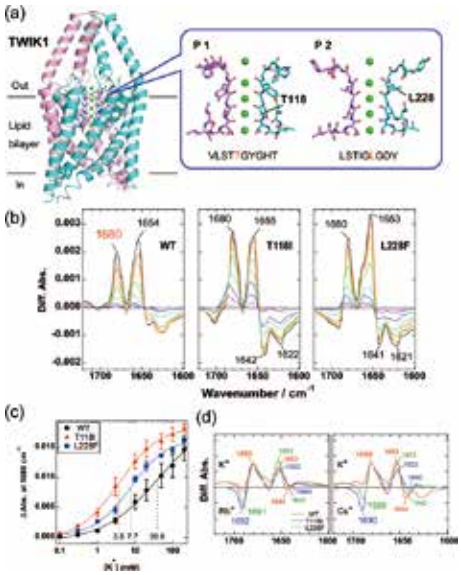

Figure 2. (a) The selectivity filter of TWIK1. (b) The difference IR spectra of WT, T118I and L228F mutants. (c) K^+-concentration dependence for the 1680-cm^{-1} band (d) The difference spectra upon replacement with Rb^+ or Cs^+.

The ion-exchange induced difference spectra in the amide-I region of WT, T118I and L228F are basically similar to the previous spectrum obtained in KcsA. Especially, we found that the 1680-cm^{-1} band would be a general marker band for the selectivity filter interacting with potassium ions. Interestingly, the band at 1680 cm^{-1} shifts to ~1690 cm^{-1} upon replacing K^+ with Rb^+ or Cs^+ in the T118I and L228F variants, but not in WT. The titration experiments provided quantitative information about affinity of the channels with potassium ions and T118I exhibited the highest affinity. Thus, we conclude that the low potassium selectivity of TWIK-1 is well correlated with the structural dynamics of the filter region and its affinity to potassium ions.

2. PDMS-Based Microfluidic Device for Infrared Spectroscopy with an Electro-Chemical Reaction[3)]

Microfluidic technique is a promising method for characterizing chemical and biological reactions with low sample consumption. Polydimethylsiloxane (PDMS) is transparent under visible light and is a soft material, which widely used for making microfluidic devices feasible for various kinds of experimental techniques. A paper applying FTIR microspectroscopy with a PDMS microfluidic device was published as a collaborative work with Dr. M. Srisa-Art in Chulalongkorn University, Thailand. After that, Mr. A. Suea-Ngam stayed in our group as an IMS-IIPA internship student. He tried to fabricate a droplet-based microfluidics coupled with amperometric detection using chip-based carbon paste electrodes (CPEs) for FTIR spectro-electrochemistry. We succeeded to demonstrate infrared spectroscopy of electrochemical reactions of ferrocyanide ($[Fe(CN)_6]^{4-}$) in the microchannel.

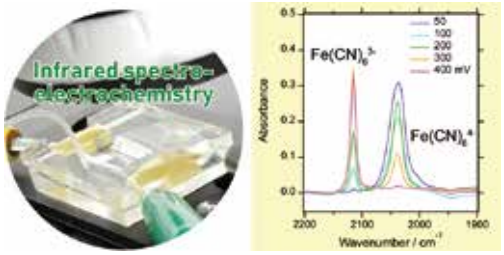

Figure 3. Infrared spectro-electrochemistry in PDMS device.

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2) Y. Furutani, *Biophys. Rev.* **10**, 235–239 (2018).

3) A. Suea-Ngam, M. Srisa-Art and Y. Furutani, *Bull. Chem. Soc. Jpn.* **91**, 728–734 (2018).

Development of Heterogeneous Catalysis toward Ideal Chemical Processes

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Professor, The Graduate University for Advanced Studies
2007 Research team leader, RIKEN
2014 Distinguished Professor, Three George University
2003 Research Project Leader, JST CREST Project (–2008)
2008 Research Project Leader, NEDO Project (–2012)
2011 Deputy Research Project Leader, JST CREST (–2016)
2014 Research Project Leader, JST ACCEL Project (–2019)

Awards
1991 Eisai Award, Synthetic Organic Chemistry
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Keywords Transition Metal Catalysis, Green Chemistry, Organic Synthesis

Our research interests lie in the development of transition metal-catalyzed reaction systems toward ideal (highly efficient, selective, green, safe, simple, *etc.*) organic transformation processes. In one active area of investigation, we are developing the heterogeneous aquacatalytic systems. Various types of catalytic organic molecular transformations, *e.g.* carbon–carbon bond forming cross-coupling, carbon–heteroatom bond forming reaction, aerobic alcohol oxidation, *etc.*, were achieved in water under heterogeneous conditions by using amphiphilic polymer-supported transition metal complexes and nanoparticles (**Figure 1**), where self-concentrating behavior of hydrophobic organic substrates inside the amphiphilic polymer matrix played a key role to realize high reaction performance in water.

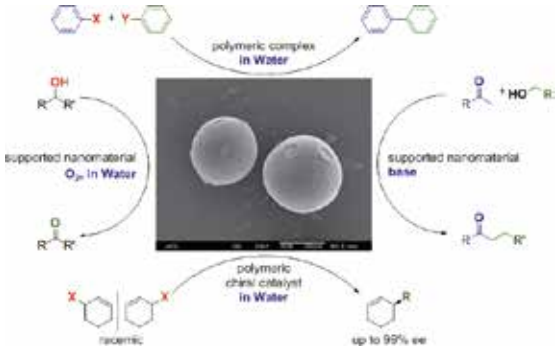

Figure 1. Typical Examples of Heterogeneous Aquacatalyses using Amphiphilic Polymer-Supported Metal Complexes and Metal Nanoparticles.

Selected Publications

- T. Osako, K. Torii, S. Hirata and Y. Uozumi, "Chemoselective Continuous-Flow Hydrogenation of Aldehydes Catalyzed by Platinum Nanoparticles Dispersed in an Amphiphilic Resin," *ACS Catal.* **7**, 7371–7377 (2017).
- Y. M. A. Yamada, S. M. Sarkar and Y. Uozumi, "Self-Assembled Poly(imidazole-palladium): Highly Active, Reusable Catalyst at Parts per Million to Parts per Billion Levels," *J. Am. Chem. Soc.* **134**, 3190–3198 (2012).
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- Y. Uozumi, Y. M. A. Yamada, T. Beppu, N. Fukuyama, M. Ueno and T. Kitamori, "Instantaneous Carbon–Carbon Bond Formation Using a Microchannel Reactor with a Catalytic Membrane," *J. Am. Chem. Soc.* **128**, 15994–15995 (2006).

1. Chemoselective Continuous-Flow Hydrogenation of Aldehydes Catalyzed by Platinum Nanoparticles Dispersed in an Amphiphilic Resin[1)]

A chemoselective continuous-flow hydrogenation of aldehydes catalyzed by a dispersion of platinum nanoparticles in an amphiphilic polymer (ARP-Pt) has been developed. Aromatic and aliphatic aldehydes bearing various reducible functional groups, such as keto, ester, or amide groups, readily underwent flow hydrogenation in aqueous solutions within 22 seconds in a continuous-flow system containing ARP-Pt to give the corresponding primary benzylic or aliphatic alcohols in up to 99% yield with excellent chemoselectivity. Moreover, the long-term continuous-flow hydrogenation of benzaldehyde for eight days was realized, and the total turnover number of the catalyst reached 997. The flow hydrogenation system provides an efficient and practical method for the chemoselective hydrogenation of aldehydes bearing reducible functional groups.

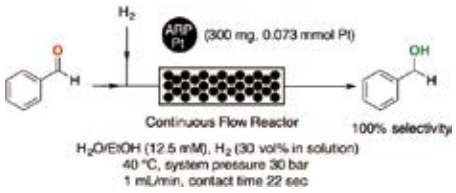

Figure 2. Aqueous continuous flow hydrogenation of benzaldehyde in water in the presence of amphiphilic resin-supported nano particles of platinum (ARP-Pt).

2. A Palladium NNC-Pincer Complex as an Extremely Efficient Catalyst Precursor for the Mizoroki–Heck Reaction[2,3)]

The Mizoroki–Heck reaction of aryl halides (iodides, bromides, or chlorides) with activated alkenes in the presence of a palladium NNC-pincer complex at ppb to ppm loadings gave the corresponding internal alkenes in excellent yields. The total turnover number and turnover frequency reached up to 8.70×10^8 and 1.21×10^7 h^{-1} (3.36×10^3 s^{-1}), respectively.

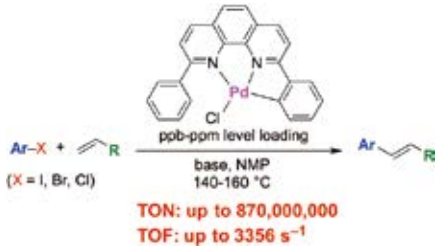

Figure 3. Heck reaction with a mol ppb loading level of an NNC-pincer palladium complex.

The catalyst was applied in a ten-gram-scale synthesis of the UV-B sunscreen agent octinoxate (2-ethylhexyl 4-methoxycinnamate). Reaction-rate analyses, transmission electron microscopic examination of the reaction mixture, and poisoning tests suggested that a monomeric palladium species is the catalytically active species in the catalytic cycle.

3. Aqueous Asymmetric 1,4-Addition of Arylboronic Acids to Enones Catalyzed by an Amphiphilic Resin-Supported Chiral Diene Rhodium Complex Under Batch and Continuous-Flow Conditions[4)]

A rhodium–chiral diene complex immobilized on amphiphilic polystyrene–poly(ethylene glycol) (PS–PEG) resin (PS–PEG–diene*–Rh) has been developed. The immobilized rhodium–chiral diene complex (PS–PEG–diene*–Rh) efficiently catalyzed the asymmetric 1,4-addition of various arylboronic acids to cyclic or linear enones in water under batch conditions to give the corresponding β-arylated carbonyl compounds in excellent yields and with excellent enantioselectivity. The catalyst was readily recovered by simple filtration and reused 10 times without loss of its catalytic activity and enantioselectivity. Moreover, a continuous-flow asymmetric 1,4-addition in a flow reactor containing PS–PEG–diene*–Rh proceeded efficiently at 50 °C with retention of high enantioselectivity. Long-term continuous-flow asymmetric 1,4-addition during 12 hours readily gave the desired product on a ten-gram scale with high enantioselectivity.

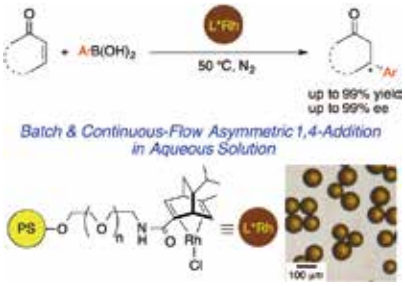

Figure 4. Asymmetric 1,4-addition of aryl boronic acids to enones in the presence of a PS–PEG resin-supported homochiral rhodium complex.

References

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2) D. Roy and Y. Uozumi, *Adv. Synth. Catal.* **360**, 602–625 (2018).
3) G. Hamasaka, S. Ichii and Y. Uozumi, *Adv. Synth. Catal.* **360**, 1833–1840 (2018).
4) G. Shen, T. Osako, M. Nagaosa and Y. Uozumi, *J. Org. Chem.* **83**, 7380–7387 (2018).

Awards

OSAKO, Takao; Thieme Chemistry Journals Award 2018 (2018).
HAMASAKA, Go; Mitsubishi Gas Chemical Award in Synthetic Organic Chemistry, Japan (2018).
HAMASAKA, Go; The Chemical Society of Japan Lecture Award for Young Chemists (2018).

Design and Synthesis of Chiral Organic Molecules for Asymmetric Synthesis

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Awards
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2005 Damon Runyon Cancer Research Foundation Post Doctoral Research Fellowship
2008 Thieme Chemistry Journals Award
2014 The 17th Morita Science Research Award
Central Glass Co., Ltd. Award in Organic Chemistry, Japan

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Keywords Organic Synthesis, 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, and C_2- or pseudo C_2 symmetry 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 conformationally flexible components 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.

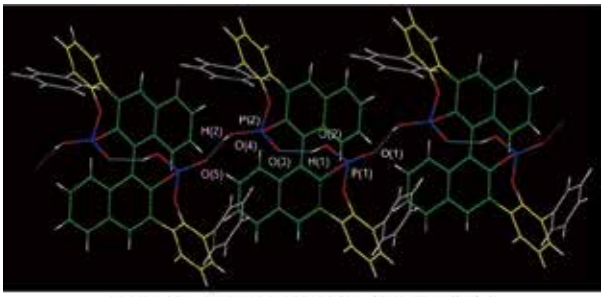

Figure 1. Hydrogen bonding network in chiral bis-phosphoric acid catalyst derived from (*R*)-3,3'-di(2-hydroxy-3 -arylphenyl)binaphthol. Hydrogen bond acts as activation unit for the substrate in asymmetric reaction space and controls atropisomeric behavior in naphthyl–phenyl axis.

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 1,3-Alkyl Migration of 1,2,2-Substituted Butenyl Amines: Asymmetric Synthesis of Linear Homoprenylamines

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 homoprenylic amines, we discovered chirality transferred 1,3-alkyl migration of 1,2,2-substituted butenyl amines in the presence of trifluoromethyl acetic acid, and developed it as synthetic method for variety of enantioenriched linear homoprenylic amines.[1)] In sharp contrast, Ollis *et al*. previously reported that chirality was significantly dropped in 1,3-alkyl migration of *N*,*N*-dimethyl-1-substituted-3-buten-1-amine.[2)] To the best our knowledge, our discovery is the first example of chirality transferred 1,3-alkyl migration 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)]

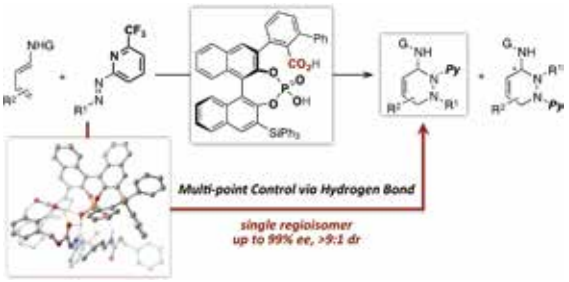

Figure 2. Chiral carboxylic acid–phosphoric acid-catalyzed azo-hetero-Diels–Alder reaction.

3. Halogen Bond Donor Catalyzed Reaction of *N*-Heteroaromatics with Allylsilatrane

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 LB. 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 halogen bond donor catalyzed allylation reaction.

We found that iodopentafluorobenzene was able to catalyze the reaction of isoquinolines, quinolines, and pyridines with allylsilatrane, crotylsilatrane, and prenyl silatrane to give the corresponding products in good yields.[8)]

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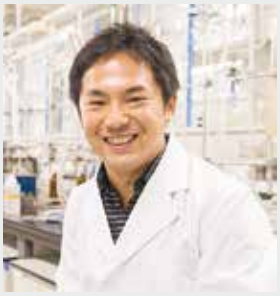
Development of Functional Metal Complexes for Artificial Photosynthesis

Department of Life and Coordination-Complex Molecular Science
Division of Functional Coordination Chemistry



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Keywords Metal Complex, Multi-Electron Transfer Reactions, Artificial Photosynthesis

Artificial photosynthesis is a solar energy conversion technology that mimics natural photosynthesis, and considered to be one of the next big breakthroughs in the research field. Our group studies the development of functional metal complexes toward the realization of artificial photosynthesis. Specific areas of research include (i) creation of cluster catalysts for multi-electron transfer reactions, (ii) frontier-orbital engineering of metal complexes for multi-electron transfer reactions, (iii) application of proton-coupled electron transfer toward multi-electron transfer reactions, (iv) electrochemical analysis of catalytic reactions, (v) development of novel photo-induced electron transfer systems, (vi) establishment of electrochemical method for the photoreactions of metal complexes in homogeneous solutions, and (vii) development of framework catalysts for small molecule conversion via the self-assembly of catalyst modules.

Figure 1. An overview of our work.

Selected Publications

- S. K. Lee, M. Kondo, G. Nakamura, M. Okamura and S. Masaoka, "Low-Overpotential CO_2 Reduction by Phosphine-Substituted Ru(II) Polypyridyl Complex," *Chem. Commun.* **54**, 6915–6918 (2018).
- T. Enomoto, M. Kondo, M. Asada, T. Nakamura and S. Masaoka, "Near-IR Light-Induced Electron Transfer via Dynamic Quenching," *J. Phys. Chem. C* **122**, 11282–11287 (2018).
- P. Chinapang, M. Okamura, T. Itoh, M. Kondo and S. Masaoka, "Development of a Framework Catalyst for Photocatalytic Hydrogen Evolution," *Chem. Commun.* **54**, 1174–1177 (2018).
- M. Okamura, M. Kondo, R. Kuga, Y. Kurashige, T. Yanai, S. Hayami, V. K. K. Praneeth, M. Yoshida, K. Yoneda, S. Kawata and S. Masaoka, "A Pentanuclear Iron Catalyst Designed for Water Oxidation," *Nature* **530**, 465–468 (2016).
- M. Yoshida, M. Kondo, S. Torii, K. Sakai and S. Masaoka, "Oxygen Evolution Catalysed by a Mononuclear Ruthenium Complex bearing Pendant -SO_3^- Groups," *Angew. Chem., Int. Ed.* **54**, 7981–7984 (2015).
- M. Yoshida, M. Kondo, T. Nakamura, K. Sakai and S. Masaoka, "Three Distinct Redox States of an Oxo-Bridged Dinuclear Ruthenium Complex," *Angew. Chem., Int. Ed.* **53**, 11519–11523 (2014).

1. Low-Overpotential CO_2 Reduction by Phosphine-Substituted Ru(II) Polypyridyl Complex[1)]

Catalytic CO_2 reduction into liquid fuels and commodity chemicals under benign condition has drawn tremendous attention, not only as a means to decrease the competition for limited fossil fuel reserves but also help to reduce the concentration of atmospheric CO_2. There are a continuously increasing number of molecular catalysts to convert CO_2 into fuels, such as HCOOH and deeply reduced products. In addition, the reduction of CO_2 to carbon monoxide (CO) is also favourable because a wide variety of fuels and commodity chemicals can be produced from CO via Fischer–Tropsch synthesis. Therefore, the development of a catalyst that can convert CO_2 to CO is an attractive research target.

In this study, we investigated electrochemical CO_2 reduction by a polypyridyl Ru complex with a mixed phosphine-pyridine and a labile ligands. Electrochemical measurements and controlled potential electrolysis revealed that the complex can promote electrocatalytic CO_2 reduction to produce CO at a lower overpotential than those of the relevant metal-complex-based catalysts. Mechanistic investigations using spectroscopic measurements clarified that the introduction of a phosphine donor at the *trans* position to the labile ligand is the key to reduce the overpotential for CO_2 reduction. In other words, a simple introduction of a phosphine moiety to the ligand largely affect the reactivity of the Ru centre, which collectively allow the complex to reduce CO_2 at a low overpotential. The results presented in this work provides a novel versatile strategy to reduce the overpotential of molecular catalysts for CO_2 reduction, which is possibly applicable to a wide variety of catalytic systems.

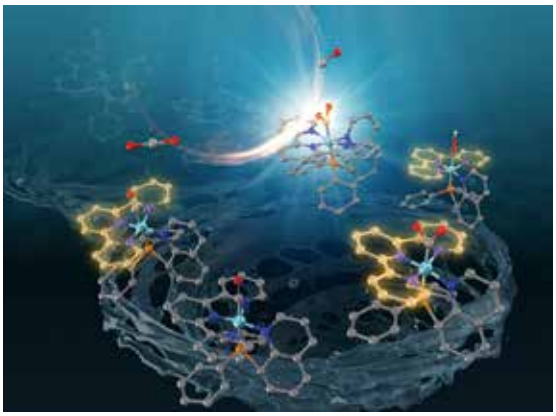

Figure 2. CO_2 reduction catalyzed by the ruthenium complex.

2. Development of a Framework Catalyst for Photocatalytic Hydrogen Evolution[2)]

The photocatalytic production of H_2 from water is a promising way to provide a sustainable and environmentally friendly chemical fuels. Thus far, considerable efforts have been devoted to the development of molecular-based homogeneous photocatalytic systems. However, homogeneous systems are considered unsuitable for future practical applications because of their moderate reusability and stability, for which heterogeneous photocatalytic systems are rather advantageous.

In this study, we propose an effective approach to construct a heterogeneous photocatalytic system based on the supramolecular assembly of molecular catalyst modules. In this system, a discrete catalyst module, which has a metal-complex-based catalytic centre (catalytic node) and intermolecular interaction sites (molecular connector), can be assembled into an ordered structure via non-covalent interactions to afford a heterogeneous framework catalyst. Therefore, our system provides two prominent features: (1) well-defined catalytic sites attributed to the molecular-based modules and (2) reusability and high durability based on the heterogeneous nature. The controlled self-assembly of a catalyst module composed of a Rh(II) paddle-wheel dimer bearing 1,8-naphthalimide-based moieties afforded a novel heterogeneous framework catalyst. The framework catalyst exhibited long-lived activity for photocatalytic hydrogen production from water and was easily reused without considerable loss of catalytic activity. The present work offer novel strategy constructing molecular-based heterogeneous catalytic systems for small molecular conversions.

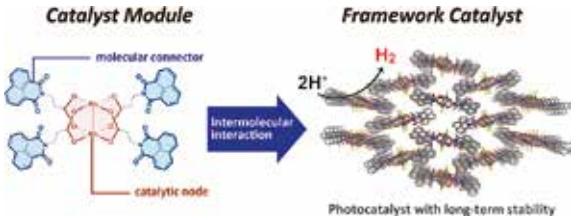

Figure 3. Construction of a framework catalyst via the self-assembly of catalyst modules.

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2) P. Chinapang, M. Okamura, T. Itoh, M. Kondo and S. Masaoka, *Chem. Commun.* **54**, 1174–1177 (2018).

Awards

KONDO, Mio; Chemical Society of Japan Award for Young Women Chemists (2018).
KONDO, Mio; The 7th Young Scientists Award of National Institutes for Natural Sciences (2018).
CHINAPANG, Pondchanok; CSJ Student Presentation Award 2017, The 98th CSJ Annual Meeting (2018).
CHINAPANG, Pondchanok; Poster Prize, the 67th JSCC Symposium (2017).
LEE, Sze Koon; Chemistry Letters Young Award, The 4th Japan-Taiwan-Singapore-Hong Kong Quadrilateral Symposium on Coordination Chemistry (2017).
IZU, Hitoshi; Oral Award, Interdisciplinary Symposium for Up-and-Coming Material Scientists 2017 (ISUMS2017) (2017).
IZU, Hitoshi; Poster Award, The 50th symposium on Chemical and Biochemical Oxidation (2017).

Control of Electron Transfer for Efficient Oxygenation Reactions

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

KURAHASHI, Takuya
Assistant Professor

Electron transfer is the most fundamental reaction to govern chemical reactions. To find an effective way to control electron transfer, electronic structures of key active species were investigated in detail with various techniques including absorption, ^{1}H and ^{2}H NMR, EPR, IR resonance Raman spectroscopy and magnetic susceptibility measurements. Correlations between electronic structures and electron transfer ability are the main focus. The insight obtained from electronic structural studies is utilized to create a new catalyst, which is applied for the reactions of gaseous methane under photoirradiation.

1. Reactions of Gaseous Methane as a Substrate

One of the problems for the reactions of gaseous hydrocarbon substrates is low solubility in organic catalyst solution. To overcome this problem, a new reaction system using fine bubbles of methane gas is constructed. In this system, an organic catalyst solution is dispersed by fine bubbles in aqueous solution. Aqueous solution serves as a coolant and also plays a role in extracting methanol product from organic catalyst solution.

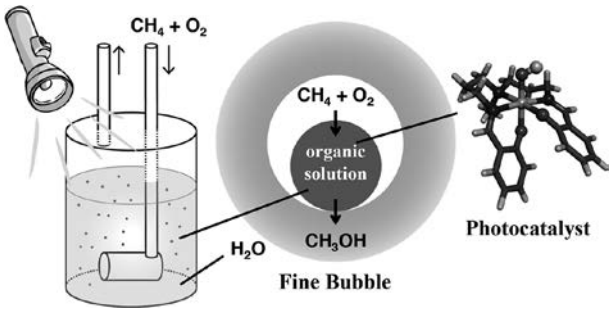

Figure 1. Photocatalytic Methane Oxygenation using Fine Bubbles under Biphasic Conditions.

Visiting Professors

Visiting Professor
SAKURAI, Hidehiro *(from Osaka University)*

Nanoscience Based on the Synthetic Organic Chemistry

Bowl-shaped π-conjugated compounds including partial structures of the fullerenes, which are called "buckybowls," are of importance not only as model compounds of fullerenes but also as their own chemical and physical properties. Very few buckybowls has been achieved for preparation mainly due to their strained structure. We develop the rational route to the various buckybowls and investigate their physical properties. We also investigate to develop novel catalytic properties of metal nanoclusters. We focus on the following projects: Preparation of size-selective gold and gold-based alloy nanoclusters supported by hydrophilic polymers and its catalytic activity: Development of designer metal nanocluster catalyst using the highly-functionalized protective polymers.

Visiting Professor
UCHIHASHI, Takayuki *(from Nagoya University)*

Dynamic Structural States of ClpB Involved in Its Disaggregation Function Revealed by High-Speed Atomic Force Microscopy

Protein disaggregation machines, ClpB in bacteria belonging to the AAA+ superfamily, refolds toxic protein aggregates into the native state in cooperation with the cognate Hsp70 partner. The ring-shaped hexamers of ClpB uses ATP to unfold and thread its protein substrate through the central pore. However, their function-related structural dynamics has remained elusive. We directly visualized the ClpB using high-speed atomic force microscopy (HS-AFM) to gain a mechanistic insight into its disaggregation function. The HS-AFM movies demonstrated massive conformational changes of the hexameric ring during the ATPase reaction, from a round ring to a spiral and even to a pair of twisted half-spirals. HS-AFM observations of Walker-motif mutants unveiled crucial roles of ATP binding and hydrolysis in the oligomer formation. Furthermore, repressed and hyperactive mutations resulted in significantly different oligomeric forms. These results lead to a comprehensive view for the ATP-driven oligomeric-state transitions that enable ClpB to disentangle protein aggregates.

Visiting Associate Professor
YAMADA, Teppei *(from Kyushu University)*

Ionic Motion in Soft Molecular Space

Dynamics of ionic species are affected by the surrounding intermolecular interaction, Madelung potential as well as the external electric field. We intended to control the intermolecular interaction of ions in the molecular scale by designing the molecular assembly. To date, the phase transition of the rotating mode of tetraethylammonium in plastic crystal phase was investigated (*J. Am. Chem. Soc.* 291–297 (2018); *Chem. Lett.* 497–499 (2018)). Ionic motion in the ionic crystal was also applied as an electrolyte of thermocell (*Chem. Lett.* 261–264 (2018)). Recently we focus on the ionic motion in chiral nanospace. A porous metal–organic framework, Labtb, was synthesized with an enantioselective method. After the collaborative work with Prof. Okamoto and Dr. Narushima in IMS, high enantiomer-excess of Labtb in particle-level was visualized by circular dichroism imaging (a paper to be submitted). The obtained enantiomeric Labtb is highly stable from heat, chemicals and has 1D pore of *ca.* 13 Å in diameter, and we are searching the wide application of it.