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 the Homeostasis Control

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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.

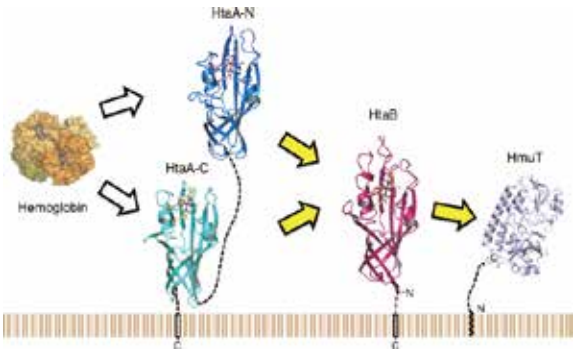

Figure 1. The protein machinery for heme uptake and transport in *Corynebacterium glutamicum*.

Selected Publications

- 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).
- S. Aono, "The Dos Family of Globin-Related Sensors Using PAS Domains to Accommodate Haem Acting as the Active Site for Sensing External Signals," *Adv. Microb. Physiol.* **63**, 273–327 (2013).
- H. Sawai, M. Yamanaka, H. Sugimoto, Y. Shiro and S. Aono, "Structural Basis for the Transcriptional Regulation of Heme Homeostasis in *Lactococcus lactis*," *J. Biol. Chem.* **287**, 30755–30768 (2012).

1. Structure and Function of Heme Uptake Machinery in *Corynebacterium glutamicum*

Most commensal or pathogenic bacteria have heme acquisition systems for utilizing host heme as an iron source. The heme acquisition systems consist of cell surface localized heme binding proteins and heme transport proteins. In several Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus anthracis*, the iron regulated surface determinant (Isd) proteins capture and transfer heme to ABC transporter-type heme transport proteins. Though a similar ABC-type heme transporter consisting of HmuT, HmuU, and HmuV proteins is used by *Corynebacteria glutamicum* and *Corynebacterium diphtheriae*, they adopt different heme binding proteins, HtaA and HtaB, instead of Isd proteins (Figure 1). The ABC-type heme transporter system is widely used for heme acquisition, which consists of an ATP-binding protein, heme permease, and heme-binding protein (substrate-binding protein).

In this work, we have studied the structural and functional relationships of heme-binding and heme-trnasport proteins, HtaA, HtaB and HmuT in *Corynebacterium glutamicum*. Where HtaA and HtaB act as heme-binding and heme-transport proteins in the heme acquisition system of HtaAB-HmuTUV for *Corynebacterium glutamicum*, HmuT is a substrate (heme) binding protein for the ABC-type heme transporter HmuUV. Our working hypothesis is that heme captured in HtaA is transferred into cytoplasm by the ABC-type HmuUV heme transporter, but the detailed molecular mechanisms of heme transport in these systems remain to be elucidated. In this work, the molecular mechanisms of heme acquisition in *Corynebacterium glutamicum* have been elucidated based on the structural analyses of HtaA, HtaB, and HmuT.

Sequence analysis identified a conserved region (CR) of approximately 150 amino acids that is duplicated in HtaA and present in a single copy in HtaB. HtaA consists of two homologous CRs in the N- and C-terminal regions. We have determined the crystal structures of the N-terminal and C-terminal CRs of HtaA (HtaA-N and HtaA-C, respectively) at the resolution of 2.0 and 1.3 Å, respectively. The crystal structure of HtaB has also been determined at the resolution of 1.7 Å. HtaA-N consists of 11 β strands and two short α helices and accommodates one heme molecules with Tyr58 located in the first α helix as an heme axial ligand. Tyr58 forms a hydrogen bond with His111 (Figure 2A). A heme propionate forms hydrogen bonds with Ser54 and Tyr201. Heme is accommodated in an open pocket formed by hydrophobic amino acid residues including Phe55, Val63, Ile118, Leu119, Phe146, Phe197, and Phe200. Phe200 forms π-π stacking with heme and heme propionate forms a hydrogen bond with Ser54. These residues including the axial ligand and residues involved in the hydrogen bonding interactions are responsible for heme recognition by HtaA-N, which are conserved among HtaA-C and HtaB, as shown in Figure 2.

We have also determined the crystal structure of HmuT at the resolution of 1.4 Å. HmuT consists of structurally similar two domains located in the N-terminal and C-terminal regions connected a long α helix. A single heme molecule is bound in the cleft between these domains. Heme iron is ligated by His141 and Tyr240, and Tyr240 forms a hydrogen bond with Arg242. There is no amino acid residue interacting heme except for His141 and Tyr240 indicating that heme is recognized by the axial ligation in HmuT. Intriguingly, HmuT binds a heme with two different orientations. As the protoheme bound to HmuT has an asymmetric structure, there are two possible orientations of heme when it is accommodated in the heme-binding site of HmuT.

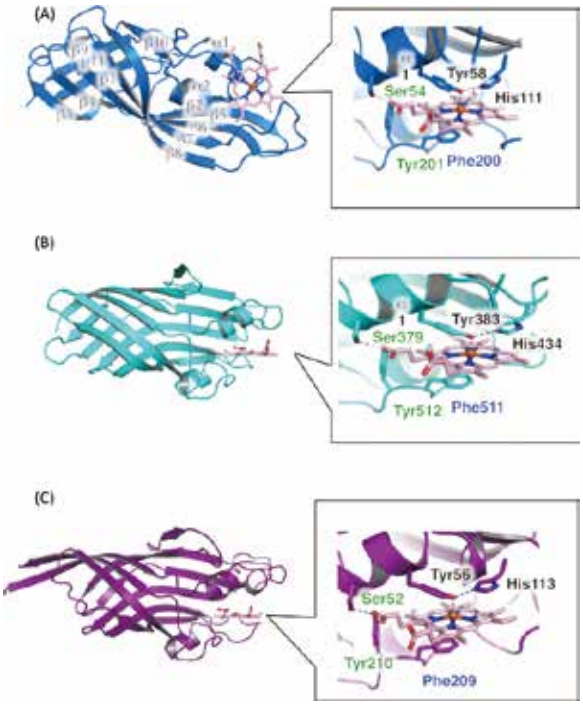

Figure 2. The crystal structures of (A) CgHtaA-N, (B) CgHtaA-C, and (C) CgHtaB with the close-up view of their heme binding sites.

2. A Novel Photosensor Protein CarH Using Vitamin B12 as a Photosensing Unit

We have studied the structure and function relationships of a novel photosensor protein CarH from *Thermus thermophilus* (*Tt*-CarH). In dark, adenosylcobalamin-bound CarH forms tetramer, which has a DNA binding ability. We have determined crystal structures of a C-terminal domain of *Tt*-CarH and full-length *Tt*-CarH at 2.5 Å and 3.0 Å resolution, respectively. *Tt*-CarH consists of an N-terminal DNA-binding domain and a C-terminal sensor domain. Adenosylcobalamin is bound in a cavity of the sensor domain with base-off form, where cobalt is coordinated by 5'-deoxyadenosine and His177 as axial ligands. The crystal structures suggest that interaction between adeonsyl group and surrounding amino acid residues plays a crucial role in photosensing by CarH.

* IMS International Internship Program

Dynamical Ordering of Biomolecular Systems for Creation of Integrated Functions

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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.

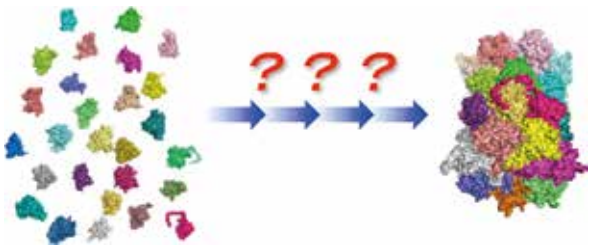

Figure 1. Formation of supramolecular machinery through dynamic assembly and disassembly of biomolecules.

Selected Publications

- M. Yagi-Utsumi, T. Yamaguchi, Y. Uekusa and K. Kato, "NMR Characterization of the Conformations, Dynamics, and Interactions of Glycosphingolipids," in *NMR in Glycoscience and Glycotechnology*, K. Kato and T. Peters, Eds., RSC Publishing; Cambridge, pp. 161–178 (2017).
- Y. Yamaguchi, H. Yagi and K. Kato, "Stable Isotope Labeling of Glycoproteins for NMR Study," in *NMR in Glycoscience and Glycotechnology*, K. Kato and T. Peters, Eds., RSC Publishing; Cambridge, pp. 194–207 (2017).
- M. Yagi-Utsumi, T. Yamaguchi, R. Kitahara and K. Kato, "NMR Explorations of Biomolecular Systems with Rapid Conformational Exchanges," in *Molecular Science of Fluctuations Toward Biological Functions*, M. Terazima, M. Kataoka, R. Ueoka and Y.Okamoto, Eds., Springer; Japan, pp. 87–103 (2016).
- M. Yagi-Utsumi and K. Kato, "Structural and Dynamic Views of GM1 Ganglioside," *Glycoconjugate J.* **32**, 105–112 (2015).
- T. Satoh, T. Yamaguchi and K. Kato, "Emerging Structural Insights into Glycoprotein Quality Control Coupled with *N*-Glycan Processing in the Endoplasmic Reticulum," *Molecules* **20**, 2475–2491 (2015).

1. Design and Creation of Neo-Glycomolecules Baced on Knowledge of Conformational Dynamics of Oligosaccharides

Exploration of the conformational spaces of flexible oligosaccharides is essential to gain deeper insights into their functional mechanisms. We characterized dynamic conformation of a high-mannose-type dodecasaccharide with a terminal glucose residue, a critical determinant recognized by molecular chaperones.[1] The dodecasaccharide was prepared by our developed chemoenzymatic technique, which uses ^{13}C labelling and lanthanide tagging to detect conformation-dependent paramagnetic effects by NMR spectroscopy. The NMR-validated molecular dynamics simulation visualized the dynamic conformational ensemble of the dodecasaccharide, thereby delineating the spatial distribution as well as the glycosidic linkage conformation of the terminal glucose determinant. Moreover, comparison of our results with previously reported crystallographic data indicates that the chaperone binding to its target oligosaccharides involves an induced-fit mechanism (Figure 2). Furthermore, our crystallographic data of glucosidase II revealed how the catalytic subunit recruits the regulatory subunit for cooperative recognition of the glucosylated high-mannose-type oligosaccharide, thereby providing structural basis of glycoprotein quality control in the endoplasmic reticulum.[2]

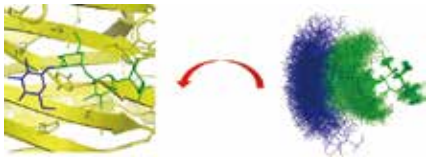

Figure 2. Comparison of our NMR-validated simulation results (right) with the previously reported crystallographic data (left) indicate that the chaperone binding to its target oligosaccharides involves an induced-fit mechanism.

We also attempted to design and develop *cyborg* supramolecular systems having unique molecular recognition properties. We successfully created a self-assembled, Lewis X-expressing glycocluster by hybridizing a spherical metal–organic complex with a synthetic oligosaccharide derivative.[3] The self-assembled glycoclusters exhibited homophilic hyper-assembly in aqueous solution in a Ca^{2+}-dependent manner through specific carbohydrate–carbohydrate interactions, offering a unique structural scaffold for functional biomimetic systems (Figure 3).

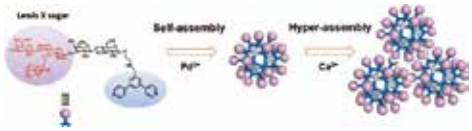

Figure 3. Ca^{2+}-dependent hyper-assembly of self-assembled glycoclusters mediated by specific carbohydrate–carbohydrate interactions.

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

Here we summarize our recent findings obtained by employing integrative biochemical and biophysical approaches for structural characterization of various biomolecular systems that involve proteins, including NMR spectroscopy, X-ray crystallography, solution scattering, and mass spectrometry (MS).

We determined a crystal structure of the human proteasome assembling chaperone PAC4 and characterized the interaction with its binding partner PAC3.[4] Furthermore, we applied native MS and small-angle neutron scattering data to structural characterization of the circadian clock protein complex.[5] Moreover, we successfully addressed the functional roles of the enzymes related to dystroglycanopathy, which is a major class of congenital muscular dystrophy caused by a deficiency of functional glycans on α-dystroglycan (αDG) with laminin-binding activity. By employing nanoLC-MS/MS analytical workflow in conjunction with a panel of mutated cells deficient in one of these enzymes, we revealed additional modifications on phosphorylated *O*-glycans of αDG, suggesting functional interplay among these enzymes through their interactions.[6]

References

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2) T. Satoh, T. Toshimori, M. Noda, S. Uchiyama and K. Kato, *Protein Sci.* **25**, 2095–2101 (2016).
3) G. Yan, T. Yamaguchi, T. Suzuki, S. Yanaka, S. Sato, M. Fujita and K. Kato, *Chem. –Asian J.* **12**, 968–972 (2017).
4) E. Kurimoto, T. Satoh, Y. Ito, E. Ishihara, K. Okamoto, M. Yagi-Utsumi, K. Tanaka and K. Kato, *Protein Sci.* **26**, 1080–1085 (2017).
5) M. Sugiyama, H. Yagi, K. Ishii, L. Porcare, A. Martele, K. Oyama, M. Noda, Y. Yunoki, R. Murakami, R. Inoue, N. Sato, Y. Oba, K. Terauchi, S. Uchiyama and K. Kato, *Sci. Rep.* **6**, 35567 (2016).
6) H. Yagi, C.-W. Kuo, T. Obayashi, S. Ninagawa, K.-H. Khoo and K. Kato, *Mol. Cell. Proteomics* **15**, 3424–3434 (2016).

Awards

YOGO, Rina; The Best Poster Award, OIIB retreat (2016).
YUNOKI, Yasuhiro; The Young Scientist Award, The 4th Joint Nagoya Meeting: Future perspectives on structural/functional analyses and molecular design of biomolecules (2016).
YOGO, Rina; Poster Award, The 81st Annual Meeting of Chubu Branch, the Japanese Biochemical Society (2017).
YUNOKI, Yasuhiro; Poster Award, The 81st Annual Meeting of Chubu Branch, the Japanese Biochemical Society (2017).

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§ carrying out graduate research on Cooperative Education Program of IMS with the University of Tokyo

Operation and Design Principles of Biological Molecular Machines

Department of Life and Coordination-Complex Molecular Science
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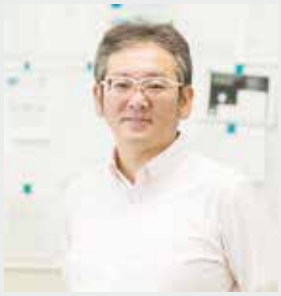

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Keywords Molecular Motors, Single-Molecule Analysis, Protein Engineering

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

- 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).
- Y. Shibafuji, A. Nakamura, T. Uchihashi, N. Sugimoto, S. Fukuda, H. Watanabe, M. Samejima, T. Ando, H. Noji, A. Koivula, K. Igarashi and R. Iino, "Single-Molecule Imaging Analysis of Elementary Reaction Steps of *Trichoderma reesei* Cellobiohydrolase I (Cel7A) Hydrolyzing Crystalline Cellulose I_α and III_I," *J. Biol. Chem.* **289**, 14056–14065 (2014).
- Y. Minagawa, H. Ueno, M. Hara, Y. Ishizuka-Katsura, N. Ohsawa, T. Terada, M. Shirouzu, S. Yokoyama, I. Yamato, E. Muneyuki, H. Noji, T. Murata and R. Iino, "Basic Properties of Rotary Dynamics of the Molecular Motor *Enterococcus hirae* V_1-ATPase," *J. Biol. Chem.* **288**, 32700–32707 (2013).
- 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. One Nanometer Steps and Rate-Limiting Step of *Serratia marcescens* Chitinase A Resolved by Gold Nanoprobe[1)]

Serratia marcescens chitinase A (*Sm*ChiA) is a monomeric linear molecular motor moving on and hydrolyzing crystalline chitin processively. We have directly resolved steps and pauses in the motion of *Sm*ChiA with high-resolution single-molecule imaging analysis with gold nanoparticle. By using total internal reflection dark-field microscopy and 40-nm gold nanoparticle as a low-load probe, movement of *Sm*ChiA was observed at 1,000–2,000 frames/s with 0.3 nm localization precision (Figure 2). The step sizes were 1.1 nm and –1.2 nm for forward and backward steps (Figure 3), respectively, consistent with the length of the product, chitobiose (~1 nm). The ratio of forward to backward steps was 5.5, corresponding to the energy difference of 1.7 k_BT. Frequent backward steps and low energy difference indicate that *Sm*ChiA operates as the Brownian ratchet. Furthermore, detailed analysis of the distribution of pause duration revealed that the rate-limiting step of chemo-mechanical coupling of *Sm*ChiA is the decrystallization of single polymer chain from the crystalline chitin, not bond cleavage and product release. These results give us important insights to engineer non-natural chitinases which show better performances than the natural ones.

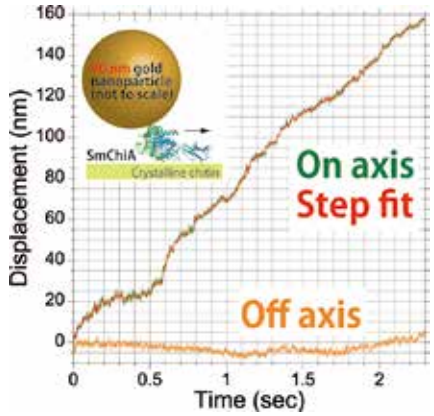

Figure 2. Example of stepping movement of *Sm*ChiA.

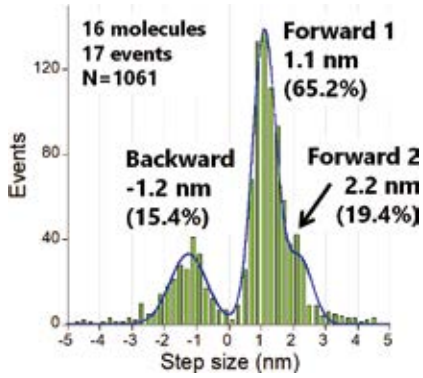

Figure 3. Distribution of step size of *Sm*ChiA.

2. Chemo-Mechanical Coupling Scheme of Rotary Molecular Motor *Enterococcus hirae* V_1-ATPase[2)]

A rotary molecular motor V-ATPase (Figure 4, left) is an ion pump driven by ATP hydrolysis. To understand the chemo-mechanical energy conversion mechanism, we conducted single-molecule analysis of V_1 moiety of *Enterococcus hirae* V-ATPase (Figure 4, right). We found that 120° steps (3 pausing positions per turn) reflecting the coordinations among three catalytic sites of V_1 were further divided into 40° and 80° substeps. At low ATP concentration ([ATP]), pause duration before 40° substep was dependent on [ATP], indicating that ATP binding triggers 40° substeps. On the other hand, at high [ATP], two time constants (both ~1 ms) independent on [ATP] were obtained. When slowly hydrolyzing ATPγS was used as a substrate, the pause before 40° step became longer (140 ms), indicating that cleavage of phosphate bond of ATP occurs during this pause. Time constant (2.5 ms) of pause duration before 80° step was also [ATP] independent. In the presence of ATPγS and high concentration of ADP, 80° backward steps were frequently observed, indicating that ADP binding triggers 80° forward step. From these results and rotation behavior of an arginine finger mutant, we proposed a model of chemo-mechanical coupling scheme of V_1 (Figure 5).

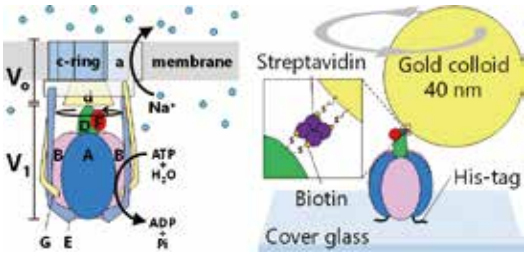

Figure 4. V-ATPase (left) and single-molecule rotation assay of V_1 (right).

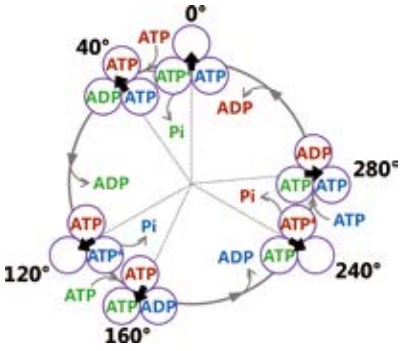

Figure 5. Chemo-mechanical coupling scheme of V_1.

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1) A. Nakamura and R. Iino, in preparation.
2) T. Iida, Y. Minagawa, H. Ueno, F. Kawai, T. Murata and R. Iino, in preparation.

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.

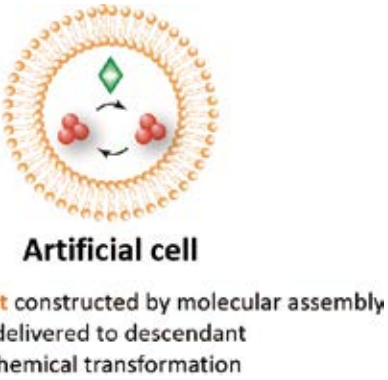

Figure 1. Artificial cell model. The replicating systems of the compartment and the information materials are combined. The reactions in the two replicating systems are accelerated by appropriate catalysts.

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 Containing a Catalyst-Producing System

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)]

Here, our goal was to create a new artificial cellular system in which the GV self-organizes its composition spontaneously according to its environment. For a GV to self-reproduce (grow and divide spontaneously) and self-maintain, it is necessary to combine the metabolism and the compartment.[4)] By introducing a cross-catalysis system (Figure 2), we constructed an artificial cell in which catalysts are produced. After addition of a membrane precursor aldehyde, the production of the catalyst and the membrane molecule was confirmed by nuclear magnetic resonance (NMR) and microscopic observation. In this system, the GV was reproduced by the catalyst, which catalyzed the production of the GV membrane lipid molecule. The GV membrane provides the field where the catalyst is synthesized.

In addition, by changing the composition of the vesicular membrane, the production of the catalyst and that of the membrane molecule fluctuated due to the components interacting each other; in effect, the artificial cell incorporated a negative feedback loop.

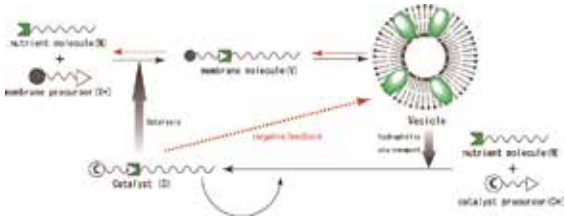

Figure 2. Scheme of our new artificial cellular system. The membrane molecules of the GV was synthesized by the catalyst produced in the GV.

We are also studying the oscillation in generation of membrane molecule and catalyst occurred by depletion of nutrient molecules.

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

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 3). 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.

In this way, we created a protocell model that can construct boundaries by using this new process that relies on the formation of robust vesicles through the use of an existing autocatalytic, self-reproducing oil drop system as a scaffold.

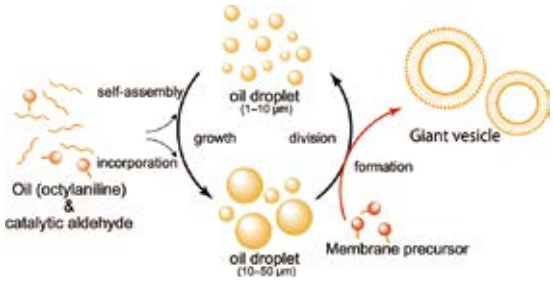

Figure 3. Scheme of the self-reproducing oil droplet (oil-in-water emulsion) and vesicular transformation system.

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)]

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Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

Department of Life and Coordination-Complex Molecular Science
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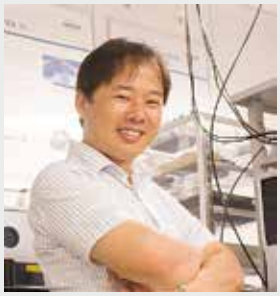

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Keywords Infrared Spectroscopy, Membrane Protein, Ion Channel

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.

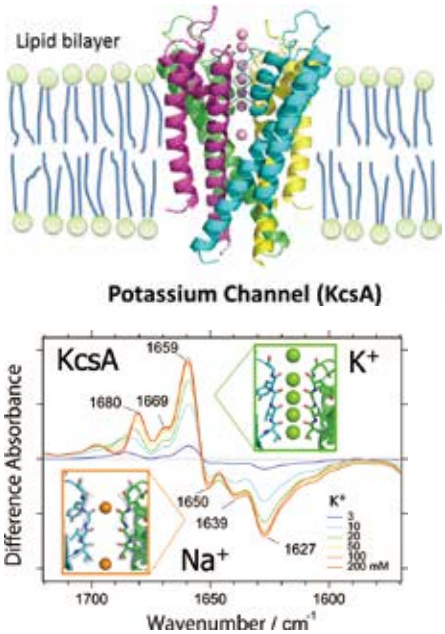

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

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. Nucleotide Base Specificity of P2X Receptors[1)]

P2X receptors are cation channels activated by adenosine-5'-triphosphate (ATP), which is a well-known biomolecule utilized for various biological activities. The ATP-gated cation channels sense extracellular ATP concentration and function for muscle contraction, taste signal transduction, nociception and so on. Three monomers form a trimeric structure and three nucleotide binding sites exist in the interfaces between two nearby monomers (Figure 2a).

X-ray crystal structure of P2X associated with cytidine-5'-triphosphate (CTP) was resolved (Figure 2a; the right bottom) and its functionality and nucleotide base specificity was examined by applying electrophysiological and spectroscopic measurements.

As shown in Figure 2b, ATP was tightly bound to WT and most of the bound ATP retained after 15–30 min wash. T189S, which loses a methyl group in the side chain, exhibited similar affinity for ATP, while T189V, which loses a hydroxyl group in the side chain, exhibited very low affinity for ATP. Therefore, the hydroxyl group in the side chain of T189 is very important for the ATP binding to P2X, which is consistent with the X-ray crystal structure (Figure 2a; the right top) showing that the hydroxyl group is located in hydrogen-bonding distance from the adenine ring of ATP. On the other hand, WT exhibited relatively higher affinity for CTP than T189S and T189V mutants, while both mutants exhibited similar affinity for CTP. Therefore, the hydroxyl group of T189 is not so important for the CTP binding to P2X, which is also supported by the X-ray crystal structure (Figure 2a; the right bottom).

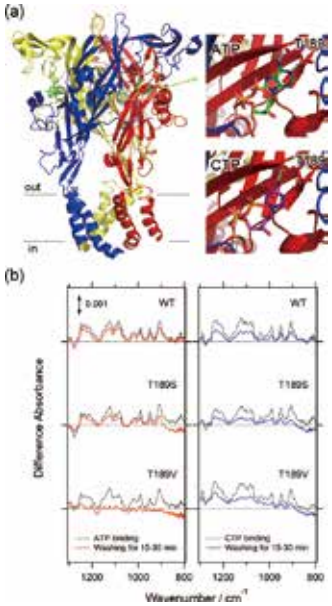

Figure 2. (a) X-ray crystal structure of P2X with ATP or CTP molecules. A nucleotide binding site is expanded in the right panel. The dotted lines indicate that the atoms of Thr189 and the nucleotides exist in hydrogen-bonding distance. (b) Ligand-binding-induced difference IR spectra of P2X measured for WT, T189S and T189V mutants with ATP or CTP.

2. UV-Sensing Protein in the Brain of a Marine Zooplankton[2)]

Most of animals show some circadian (daily) behaviors. On the earth, two large-scale daily movements of biomass are known. One is human commuting and the other is daily vertical migration of zooplanktons. Zooplanktons move downward in water during daytime and upward at night, in order to avoid predators and UV damage from sunlight. Thus, it is important to understand how zooplankton species sense ambient UV signals.

Larva of the marine ragworm (*Platynereis dumerilii*) has been studied as a zooplankton model, and the larvae possess photoreceptor cells in the brain to control circadian swimming behavior. The brain photoreceptor cells express an opsin (named as c-opsin) that is closely related to visual pigments in our eyes. We expected that *Platynereis* c-opsin is involved in UV detection, and assessed spectral and biochemical properties of the opsin.

We purified the c-opsin protein that was expressed in mammalian cultured cells. The purified opsin showed an absorption maximum at 383-nm in the UV region (Figure 3a). Also, *Xenopus* oocytes expressing the opsin showed electrophysiological responses upon UV irradiation (Figure 3b). These results clearly indicate that the c-opsin is a UV-sensitive pigment. Mutagenesis analyses identified that a single amino acid residue is responsible for UV sensing. Thus, the single residue is essential for the opsin to achieve the ability to receive UV signals. Taken together, the c-opsin would enable the brain of *Platynereis* to sense ambient UV signals.

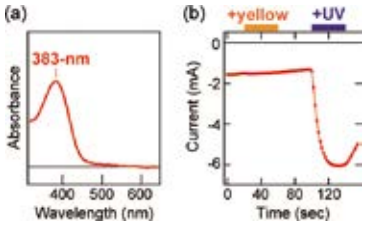

Figure 3. UV sensing ability of *Platynereis* c-opsin.
(a) Absorption spectrum of purified *Platynereis* c-opsin. (b) Photoresponses of a *Xenopus* oocyte expressing *Platynereis* c-opsin with a potassium channel GIRK1/GIRK2.

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Award

FURUTANI, Yuji; The 3rd Biophysics and Physicobiology Editors' Choice Award (2016).

Development of Heterogeneous Catalysis toward Ideal Chemical Processes

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1990 Assistant Professor, Hokkaido University
1994 Research Associate, Columbia University
1995 Lecturer, Kyoto University
1997 Professor, Nagoya City University
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Professor, The Graduate University for Advanced Studies
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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)
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Awards
1991 Eisai Award, Synthetic Organic Chemistry
1998 The Pharmaceutical Society of Japan Award for Young Scientist
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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.

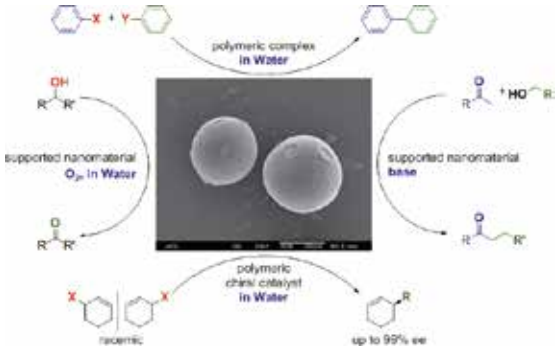

Figure 1. Typical Examples of Heterogeneous Aquacatalyses using Amphiphilic Polymer-Supported Metal Complexes and Metal Nanoparticles.

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- Y. M. A. Yamada, S. M. Sarkar and Y. Uozumi, "Amphiphilic Self-Assembled Polymeric Copper Catalyst to Parts per Million Levels: Click Chemistry," *J. Am. Chem. Soc.* **134**, 9285–9290 (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. The Development of a Vesicular Self-Assembled Amphiphilic Platinum NCN-Pincer Complex and Its Catalytic Application to Hydrosilylation of Alkenes in Water[1)]

An amphiphilic platinum NCN-pincer complex bearing hydrophilic tri(ethylene glycol) and hydrophobic dodecyl chains was designed and synthesized for use as a new aqua-catalytic system. The complex self-assembled in aqueous medium to form bilayer vesicles that catalyzed the hydrosilylation of alkenes by dimethyl(phenyl)silane in water to give the hydrosilylated products in good yields. In contrast, the complex in its amorphous form did not promote the reaction efficiently, and thus, the formation of a vesicular structure was essential to promote the reaction.

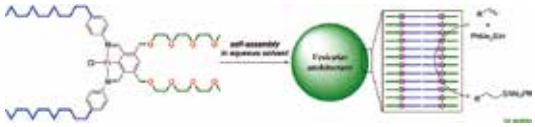

Figure 2. Hydrosilylation of styrene with dimethyl(phenyl)silane in water in the presence of a self-assembled vesicular amphiphilic platinum NCN-pincer complex.

2. Detailed Structural Analysis of a Self-Assembled Vesicular Amphiphilic NCN-Pincer Palladium Complex by Using Wide-Angle X-Ray Scattering and Molecular Dynamics Calculations[2)]

Wide-angle X-ray scattering experiments and all-atomistic molecular dynamics calculations were performed to elucidate the detailed structure of bilayer vesicles constructed by self-assembly of an amphiphilic palladium NCN-pincer complex. We found an excellent agreement between the experimental and calculated X-ray spectra and between the membrane thickness determined from a TEM image and that calculated from an electron-density profile, indicating that the calculated structure was highly reliable. The analysis of the simulated bilayer structure showed that the bilayer membrane could act as a nanoreactor. The self-assembled vesicles were shown to be catalytically active in a Miyaura–Michael reaction in water.

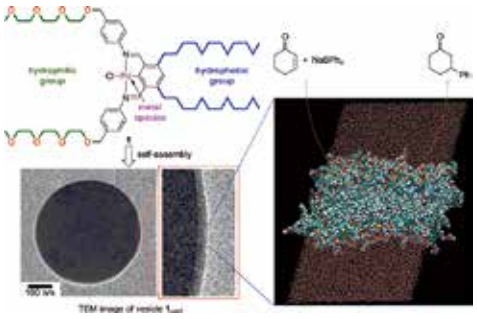

Figure 3. A self-assembled vesicular palladium NCN-pincer complex.

3. Palladium-Catalyzed Asymmetric Suzuki–Miyaura Cross Coupling with Homochiral Phosphine Ligands Having Tetrahydro-1*H*-imidazo[1,5-*a*]indole Backbone[3)]

Amphiphilic polystyrene-poly(ethylene glycol) resin-supported chiral imidazoindole phosphines (PS-PEG-L*) bearing PPh_2, P(*t*-Bu)$_2$, and P(*c*-Hex)$_2$ groups were designed and prepared with a view toward using them in aqueous heterogeneous asymmetric Suzuki–Miyaura biaryl coupling. The asymmetric coupling of 2-substituted 1-iodonaphthalenes and 2-substituted naphthalen-1-ylboronic acid took place in water under heterogeneous conditions in the presence of 10 mol% palladium of PS-PEG-L*-Pd complexes to give up to 94% ee of (*S*)-2,2'-disubstituted 1,1'-binaphthyls.

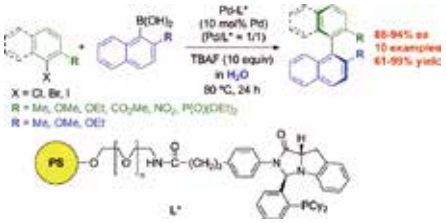

Figure 4. Palladium-catalyzed asymmetric biaryl coupling in water with a PS-PEG resin-supported imidazoindolephosphine.

4. Huisgen Cycloaddition with Acetylene Gas by Using an Amphiphilic Self-Assembled Polymeric Copper Catalyst[4)]

A copper-mediated Huisgen cycloaddition of flammable acetylene gas and a variety of organic azides proceeded smoothly by using our amphiphilic self-assembled polymeric copper catalyst MPPI-Cu to give the corresponding triazoles in high yield. MPPI-Cu was readily reused without loss of catalytic activity.

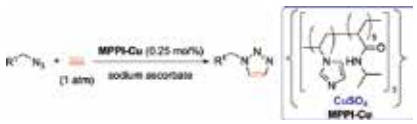

Figure 5. Huisgen cycloaddition of azides and acetylene in the presence of self-assembled polymeric copper catalyst.

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3) Y. Uozumi, Y. Matsuura, T. Suzuka, T. Arakawa and Y. M. A. Yamada, *Synthesis* **49**, 59–68 (2017).
4) Y. M. A. Yamada, H. Yoshida, A. Ohno, T. Sato, T. Mase and Y. Uozumi, *Heterocycles* **95**, 715–721 (2017).

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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
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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.

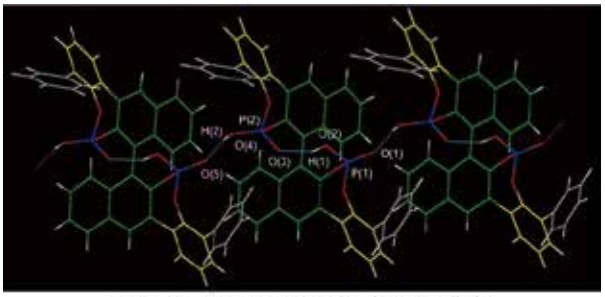

Figure 1. Hydrogen bonding network in chiral bis-phosphoric acid catalyst derived from (*R*)-3,3'-di(2-hydroxy-3 -arylphenyl)binaphthol. Hydrogen bond acts as activation unit for the substrate in asymmetric reaction space and controls atropisomeric behavior in naphthyl–phenyl axis.

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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)]

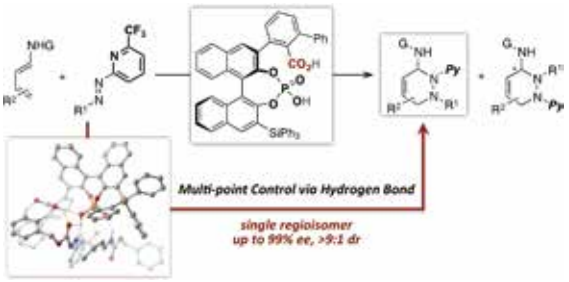

Figure 2. Chiral carboxylic acid–phosphoric acid-catalyzed azo-hetero-Diels–Alder reaction.

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 pentafluoroiodebenzene was able to catalyze the allylation reaction of isoquinolines, quinolones, and pyridines with allylsilatrane, crotylsilatrane, and prenyl silatrane to give the corresponding product in good yield.[8)]

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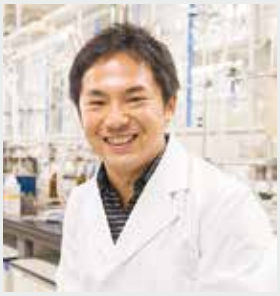
Development of Functional Metal Complexes for Artificial Photosynthesis

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



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2002 JSPS Research Fellow (DC2)
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Keywords Metal Complex, Water Oxidation, 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 energy. Our group studies the development of functional metal complexes toward the realization of artificial photosynthesis. Specific areas of research include (i) synthesis of ruthenium-based molecular catalysts for water oxidation and carbon dioxide reduction, (ii) creation of cluster catalysts for multi-electron transfer reactions, (iii) mechanistic investigation into water oxidation catalyzed by metal complexes, (iv) application of proton-coupled electron transfer toward multi-electron transfer reactions, (v) electrochemical evaluation of the activity of molecular catalysts for water oxidation and carbon dioxide reduction, (vi) electrochemical measurement of metal complexes in homogeneous solutions under photoirradiation, and (vii) development of reaction fields via self-assembly of molecular catalysts.

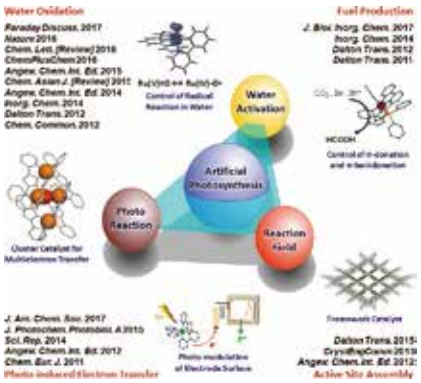

Figure 1. An overview of our work.

Selected Publications

- M. Yoshida, M. Kondo, M. Okamura, M. Kanaike, S. Haesuwannakij, H. Sakurai and S. Masaoka, "Fe, Ru, and Os Complexes with the Same Molecular Framework: Comparison of Structures, Properties and Catalytic Activities," *Faraday Discuss.* **198**, 181–196 (2017).
- V. K. K. Praneeth, M. Kondo, P.-M. Woi, M. Okamura and S. Masaoka, "Electrocatalytic Water Oxidation by a Tetranuclear Copper Complex," *ChemPlusChem* **81**, 1123–1128 (2016).
- 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. Fe, Ru, and Os Complexes with the Same Molecular Framework: Comparison of Structures, Properties and Catalytic Activities[1)]

Water oxidation ($2H_2O \rightarrow O_2 + 4H^+ + 4e^-$) is a key reaction in energy conversion in natural and artificial photosynthesis. The development of artificial water oxidation catalysts (WOCs) has attracted growing interest in recent years due to the urgent need to solve the world's energy problems. Metal complexes containing group 8 metal centres (Fe, Ru, or Os) and water coordination sites can be regarded as the most attractive candidates for molecular WOCs, because such metal-aqua species can generate high-valent metal-oxo species, which are the key intermediates triggering the formation of the O–O bond, via stepwise electron removal involving concomitant proton loss.

In this study, we present the syntheses, crystal structures, spectroscopic and electrochemical properties, and water oxidation activities of a series of Fe(II), Ru(II), and Os(II) complexes bearing a pentadentate ligand and a monodentate ligand. The nature of the metal ions are extracted and discussed by comparing the difference of the structure, properties and reactivities among a series of group 8 metal complexes with the same molecular framework. The results will provide new insight into the design and development of group 8 metal-based molecular catalysts for water oxidation.

Figure 2. Crystal structures of the Fe, Ru, and Os Complexes.

2. Electrocatalytic Water Oxidation by a Tetranuclear Copper Complex[2)]

Cu-based WOCs have attracted much interest among researchers due to the low cost and biological relevance of Cu.[3)] One of the most attractive targets in this field of research can be considered to be discrete multinuclear Cu complexes due to their homogeneity and multinuclearity. However, there are only a few reports on water oxidation catalysis by discrete multinuclear Cu complexes.

In this study, a novel tetranuclear copper-based water oxidation catalyst was designed and synthesized by using a new multinucleating ligand containing two proton dissociation sites, 1,3-bis(6-hydroxy-2-pyridyl)-1*H*-pyrazole. The copper complex showed electrocatalytic activity for water oxidation reactions under aqueous basic conditions (pH 12.5) with an overpotential of approximately 500 mV. UV/Vis absorption and energy-dispersive X-ray (EDX) spectroscopic techniques coupled with electrochemical analyses of the catalyst system strongly suggest that the tetranuclear copper complex works as a homogeneous system under the conditions used. The results demonstrate the utility of a discrete tetranuclear copper complex in water oxidation reactions.

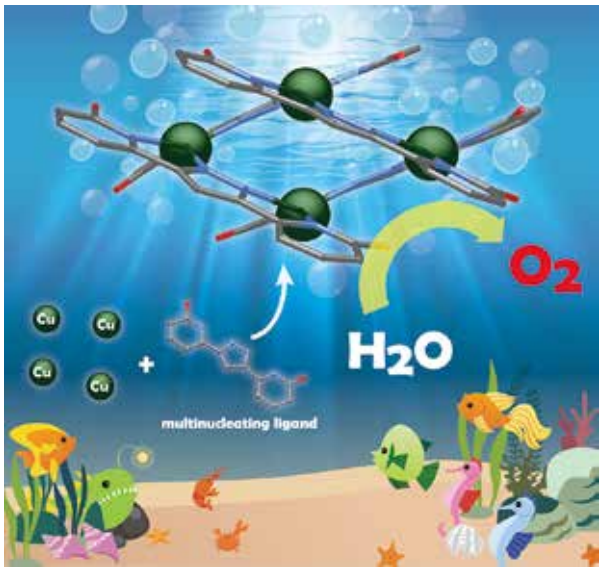

Figure 3. Water oxidation by the tetranuclear copper complex.

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Awards

MASAOKA, Shigeyuki; The 13th (FY 2016) JSPS Prize (2017).
KONDO, Mio; Morita Science Research Award (2017).
CHINAPANG, Pondchanok; Excellent Poster Award, International Conference on Artificial Photosynthesis (2017).
IZU, Hitoshi; Excellent Poster Award, International Conference on Artificial Photosynthesis (2017).
ENOMOTO, Takafumi; Poster Award, 6th CSJ Chemistry Festa (2016).
OKAMURA, Masaya; KONDO, Mio; MASAOKA, Shigeyuki; Special Prize of the Nature Industry Award (2016).
ENOMOTO, Takafumi; Poster Prize, the 66th JSCC Symposium (2016).
ENOMOTO, Takafumi; Dalton Transactions Award (2016).

Control of Electron Transfer for Efficient Oxygenation Reactions

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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.

1. Design and Synthesis of Photoactive Salen-Type Ligands

Salen-type ligands are well known as the most versatile framework for catalysts, because of the simple structure that is suitable for large-scale preparations and chemical modifications. The present study investigated a new method to incorporate excellent photochemical properties without sacrificing structural advantages of salen-type ligands.

The key concept is tautomerization of a salicylidene ring of a salen-type ligand, which converts a phenolate structure to a quinoidal structure. The resulting quinoidal salen-type ligand is expected to show better photochemical properties, because quinones having a quinoidal structure are excellent photocatalysts.

To compare photochemical properties of salen-type ligands in a quantitative manner, fluorescence properties of salen-type ligands with redox-inactive zinc(II) ion were investigated. One of the achievements is a finding that the steric bulk of a substituent on salicylidene rings in addition to the molecular space around the diamine moiety plays a critical role for high fluorescence emission efficiency. Another point is an electron-donating or electron-withdrawing substituent on salicylidene rings, which systematically alters an absorption/fluorescence wavelength.

Visiting Professors

Visiting Professor
OGOSHI, Sensuke *(from Osaka University)*

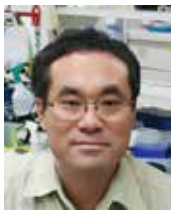
Transformation of Tetrafluoroethylene via Oxycupration

Organofluorine compounds have attracted much attention, mostly on account of their applications in a variety of research areas, including pharmaceutical, agrochemical, and materials science, and consequently substantial efforts have been devoted to the development of novel strategies for the construction of fluorinated organic compounds. Among these, fluoroalkyl ethers such as Ar–OCF_2CF_2–Ar' (Ar/Ar' = aryl) have garnered special attention, as they represent key structures in insecticides and lubricants. In addition, this structural motif is fascinating with respect to perfluoroalkoxylation of aromatic compounds. Most of practical approaches to the construction of Ar–OCF_2CF_2–Ar' moieties have to use the fluorinated starting materials that have very high greenhouse gas effect. Under these circumstances, we have to develop new reactions to allow us to produce those chemicals without using such starting materials. We have been focusing on the synthesis of a variety of fluorinated compounds by using tetrafluoroethylene (TFE) of which greenhouse gas effect is almost zero. So far, we have reported the transformation of TFE by Palladium catalyzed coupling reaction with aryl metals (Zn, B, Si) to give α,β,β-trifluorostyrene and nickel-catalyzed co-trimerization with ethylene and aldehyde. Although the introduction of both oxygen and carbon into TFE is the one of the most difficult reactions, the oxycupration of TFE allows us to the construction of Ar–OCF_2CF_2–Ar' moieties.

Visiting Associate Professor
SHOJI, Osami *(from Nagoya University)*

Gaseous Alkane and Benzene Hydroxylation Catalyzed by Cytochrome P450BM3 with the Assistance of Decoy Molecules

Cytochrome P450BM3 (P450BM3) is one of the most promising P450s for construction of biocatalysts because of its high monooxygenase activity. Because the substrate binding is crucial for the generation of active species of P450BM3 (Compound I), substrates whose structures are largely different from that of its native substrates (long-alkyl-chain fatty acids) cannot be hydroxylated by P450BM3. However, we found that P450BM3 starts to catalyze hydroxylation of nonnative substrates in the presence of perfluorinated carboxylic acids (PFCs) as inert dummy substrates (decoy molecules). Recently, we have succeeded in developing the next generation of decoy molecules by modifying the carboxylate of PFCs with amino acids and succeeded in enhancing the catalytic activity for gaseous alkanes. Furthermore, we have succeeded in crystallizing the *N*-perfluorononanoyl-*L*-tryptophan (PFC9-*L*-Trp)-bound form of P450BM3. The crystal structure analysis of PFC9-*L*-Trp-bound form of P450BM3 showed that the terminal of alkyl chain does not reach to the active site owing to the multiple hydrogen bonding interactions between the carboxyl and carbonyl groups of PFC9-*L*-Trp and amino acids located at the entrance of P450BM3. More recently, we have demonstrated that various carboxylic acids modified with amino acids (*N*-acyl amino acids) as well as amino acid dimers having a completely different structure from fatty acids can serve as decoy molecules. Benzene was more efficiently hydroxylated in the presence of these decoy molecules. Furthermore, we have succeeded in controlling the enantioselectivity of benzylic hydroxylation using these decoy molecules.



Visiting Associate Professor
TOSHA, Takehiko *(from RIKEN SPring-8 Center)*

Elucidation of Mechanism for Effective Chemical Reactions by Supracomplex Formation

Nitric Oxide (NO) plays diverse and significant roles in biological processes such as signal transduction, vasodilation and memory consolidation, despite its high cytotoxicity, raising the essential question of how biological systems control the action of NO to minimize its cytotoxic effect in cells. To answer this, we focus on microbial denitrification in which cytotoxic NO is produced as an intermediate product. However, denitrifying bacteria can grow without any damage from NO, suggesting that there is a system for effective NO elimination. As a possible system, we found from X-ray crystallography, mutagenesis and molecular dynamics simulation that NO-generating nitrite reductase (NiR) forms a complex with NO-decomposing nitric oxide reductase (NOR) to suppress the diffusion of NO. To further elucidate how the proteins involved in denitrification effectively catalyze the consecutive chemical reactions, we explore the possibility of their supracomplex formation using cryo-electron microscopic technique.