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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Keywords Bioinorganic Chemistry, Metalloproteins, Sensor Protein

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

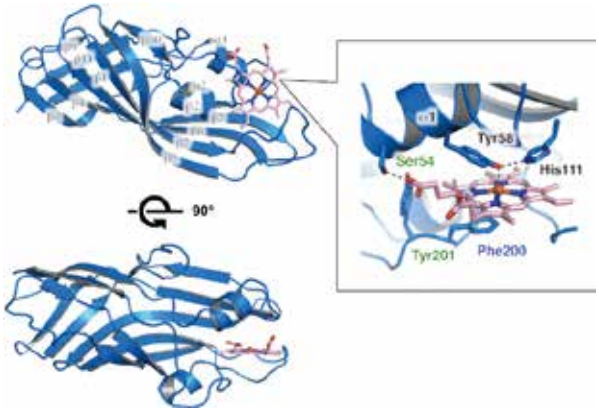

Figure 1. The crystal structure of CgHtaA-N and the close-up view of heme binding site in CgHtaA-N.

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*

As iron is an essential trace element for most of organisms, they develop sophisticated iron acquisition systems. Pathogenic bacteria can use heme as an iron source partly because heme is the most abundant iron species in their host. However, there is little free heme molecule as most of heme molecules are tightly bound to hemoproteins as a prosthetic group. Therefore, some heme acquisition system is required to use heme in hemoproteins as an iron source.

In Gram-negative bacteria, hemophores that are secreted to the extracellular medium acquire heme from hemoproteins and transport it to a specific outer membrane receptor. The outer membrane receptor transports heme across the outer membrane to the periplasmic space, where a periplasmic heme-binding protein binds heme to transport it to an ABC-type heme transporter. On the other hand, in Gram-positive bacteria, heme uptake occurs by direct interaction between hemoproteins or heme and the membrane anchored proteins responsible for heme binding and transport. In a Gram-positive bacterium *Corynebacterium glutamicum*, heme is captured by the membrane anchored heme binding proteins, HtaA and HtaB proteins, and then heme is transferred to HmuT, which is a heme-binding protein for the ABC-type heme transporter HmuUV. Heme is transported into cytoplasm by this ABC transporter. While this heme uptake process is proposed based on the genetic and microbiological studies, the molecular mechanisms of heme uptake/transport are not obvious mainly due to a lack of structural information of these proteins. We have characterized HtaA, HtaB, and HmuT from *Corynebacterium glutamicum* (CgHmuT) by X-ray crystallography to elucidate the molecular mechanism of heme transport and uptake.

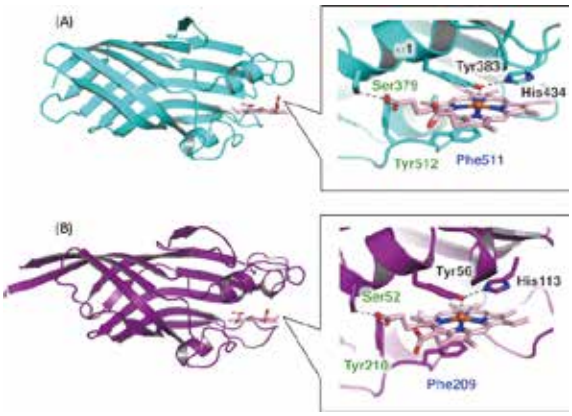

Figure 2. The crystal structures of (A) CgHtaA-C and (B) CgHtaB with the close-up view of their heme binding sites.

HtaA consists of two homologous domains, HtaA-N and HtaA-C. We have determined the structure of CgHtaA-N at a resolution of 2.0 Å (Figure 1). HtaA-N consists of 11 β-strands and 2 short α-helices and binds a heme molecule with Tyr58 as an axial ligand in a hydrophobic pocket. Tyr58 forms a hydrogen bond with His111, which may regulate the heme binding affinity of HtaA-N. Phe200 forms π-π stacking with heme and heme propionate forms a hydrogen bond with Ser54.

The crystal structures of CgHtaA-C and CgHtaB have also been determined as shown in Figure 2. The whole structures of CgHtaA-N, CgHtaA-C, and CgHtaB are superimposable and the heme environmental structures are highly conserved among them including the axial ligand, hydrogen bonding interaction between Tyr and His, and heme propionate and Ser, and π-π stacking of heme and Phe.

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

Vitamin B12 is well known as a cofactor for the B12-dependent enzymes that catalyze carbon skeleton rearrangement or elimination reactions, where Co–C bond hemolysis takes place to form the radical species as the reaction intermediate. Recently, a novel biological function of vitamin B12 has been reported: A photosensor protein CarH utilizes adenosylcobalamin (vitamin B12) as its senor unit for light sensing. We have determined the crystal structure of CarH from *Thermus thermophilus* to elucidate the molecular mechanisms of photosensing and signal transduction of CarH (Figure 3). CarH forms homo-tetramer and each subunit binds an adenosylcobalamin (AdoCbl). The protomer of CarH consists of the

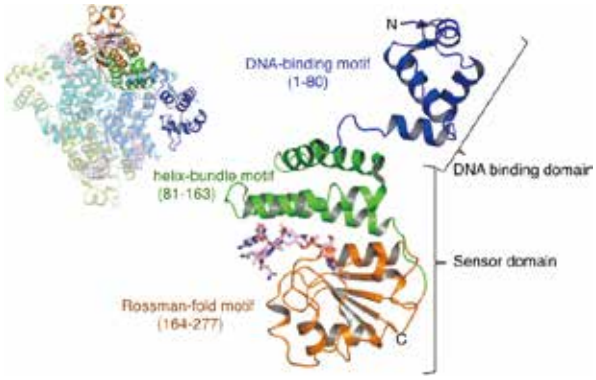

Figure 3. The crystal structures of AdoCbl-bound CarH.

DNA-binding and sensor domains as shown in Figure 3. The sensor domain consists of the helix-bundle motif and Rossman-fold motif, between of which AdoCbl is accommodated.

AdoCbl-bound CarH is photosensitive and dissociation of tetramer to monomer takes place upon photo irradiation. The adenosyl group is dissociated from Co ion upon photosensing, which is a trigger of the change of CarH quaternary structure.

* 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.

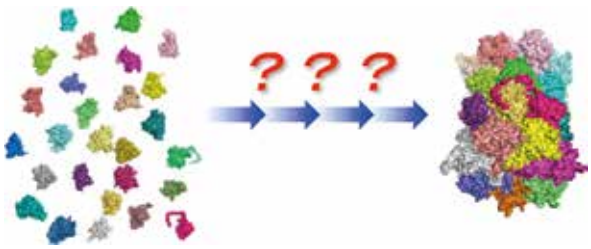

Figure 1. Formation of supramolecular machinery through dynamic assembly and disassembly of biomolecules.

Selected Publications

- M. Yagi-Utsumi, T. Yamaguchi, 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).
- Y. Zhang, T. Yamaguchi, M. Yagi-Utsumi, Y. Kamiya, Y. Sakae, Y. Okamoto and K. Kato, "Conformational Dynamics of Oligosaccharides Characterized by Paramagnetism-Assisted NMR Spectroscopy in Conjunction with Molecular Dynamics Simulation," in *Advances in Experimental Medicine and Biology*, Springer; Switzerland, **842**, pp. 217–230 (2015).
- T. Yamaguchi and K. Kato, "Paramagnetism-Assisted Nuclear Magnetic Resonance Analysis of Dynamic Conformations and Interactions of Oligosaccharides," in *Glycoscience: Biology and Medicine*, Springer; Japan, **1**, pp. 137–145 (2014).

1. Characterization of Dynamic Process of Protein Assembly and Disassembly

In our group, various physicochemical and biochemical approaches are integrated to characterize assembly and disassembly of proteins exemplified by formation of proteasomes and cargo receptor complexes. The core part of the eukaryotic proteasome contains heteroheptameric rings compose of α1-7 subunits. Among these homologous subunits, α7 is spontaneously assembled into a homotetrasecamer having a double ring structure as shown by our crystallographic analysis. Intriguingly, our native mass spectrometric (MS) data indicate that this double ring is disrupted upon addition of α6, suggesting that proteasome formation involves the disassembly of non-native oligomers, which are assembly intermediates.[1)] Furthermore, we characterized the pH-dependent coiled–coil interactions of yeast putative cargo receptors (Emp46p and Emp47p), identifying the key residue that controls this interaction.[2)] These results contribute toward understanding the molecular mechanisms underlying the dynamic cargo receptor assembly in the yeast secretory pathway. Our findings will provide a framework for designing molecular assembly and disassembly systems mediated by intermolecular interactions.

2. Structural Basis of Drug-Induced Conformational Change of HIV-1 Reverse Transcriptase

Human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) is an important target for antiviral therapy against acquired immunodeficiency syndrome. Using NMR and native MS methods, we characterized the interactions between the heterodimeric HIV-1 RT enzyme and non-nucleoside reverse transcriptase inhibitors with different inhibitory activities.[3,4)] We also applied a paramagnetism-assisted NMR technique for detecting the inhibitor-induced conformational change of HIV-1 RT, offering a strategy to identify allosteric inhibitors.[5)] Our approaches thus provide useful tools in protein-based drug screening in developing anti-HIV drugs.

3. Interactions of Amyloidogenic Proteins with Membranes and Molecular Chaperones

Lipid membranes provide active platform for dynamic interactions of a variety of biomolecules on cell surfaces. Our solid-state NMR data of amyloid β (Aβ) employing 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) vesicle as model membrane have elucidated the membrane-induced dichotomous conformation of Aβ, in which the disordered N-terminal segment is followed by the stable C-terminal β strand, providing an insight into the molecular processes of the conformational transition of Aβ coupled with its assembly into parallel β structures (Figure 2).[6)]

It has been proposed that molecular chaperones actively contribute to the suppression of toxic aggregate formation of various neurodegenerative disordered proteins. We identified a *chaperone-philic* binding motif of α-synuclein on the basis of NMR data and determined the crystal structure of its complex with the substrate-binding domains of protein disulfide isomerase (PDI) (Figure 2).[7)] Our findings provided a structural basis for the mechanism underlying the redox-dependent substrate binding of PDI.

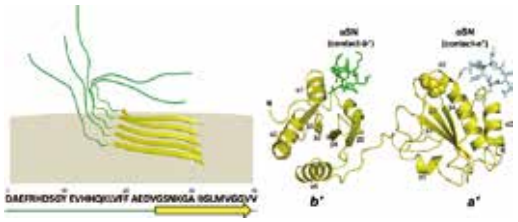

Figure 2. Structural model of Aβ(1–40) bound to DMPC bilayers characterized by solid-state NMR analyses (left). Crystal structure of the oxidized PDI *b'*–*a'* domains complexed with the αSN peptide (right).

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2) K. Ishii, H. Enda, M. Noda, M. Kajino, A. Kim, E. Kurimoto, K. Sato, A. Nakano, Y. Kobayashi, H. Yagi, S. Uchiyama and K. Kato, *PLoS One* **10**, e0140287 (2015).
3) R. Thammaporn, M. Yagi-Utsumi, T. Yamaguchi, P. Boonsri, P. Saparpakorn, K. Choowongkomon, S. Techasakul, K. Kato and S. Hannongbua, *Sci. Rep.* **5**, 15806 (2015).
4) R. Thammaporn, K. Ishii, M. Yagi-Utsumi, S. Uchiyama, S. Hannongbua and K. Kato, *Biol. Pharm. Bull.* **39**, 450–454 (2016).
5) S. Seetaha, M. Yagi-Utsumi, T. Yamaguchi, K. Ishii, S. Hannongbua, K. Choowongkomon and K. Kato, *ChemMedChem* **11**, 363–366 (2016).
6) M. Yagi-Utsumi, K. Kato and K. Nishimura, *PLoS One* **11**, e0146405 (2016).
7) M. Yagi-Utsumi, T. Satoh and K. Kato, *Sci. Rep.* **5**, 13909 (2015).

Awards

YOGO, Rina; Best Presentation Award, The Tokai Branch Meeting of the Pharmaceutical Society of Japan (2015).
TONG, Zhu; Poster Presentation Award, The 4th International Symposium of "Dynamical ordering of biomolecular systems for creation of integrated functions" (2015).
TONG, Zhu; Young Scientist Award, The 12th Forum of the Glycoscience base for Chubu (2015).
YANAKA, Saeko; The 32nd Inoue Research Award for Young Scientists (2016).
YANAKA, Saeko; Poster Award, The 80th Annual Meeting of Chubu Branch, the Japanese Biochemical Society (2016).

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Operation and Design Principles of Biological Molecular Machines

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Keywords Molecular Machines, Protein Engineering, Single-Molecule Analysis

Activity of life is supported by various molecular machines made of proteins. These biological molecular machines are tiny, but show high performance, and are superior to man-made machines in many aspects.

One of the representatives of the 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.

We will unveil operation principles of molecular motors with single-molecule functional analysis and advanced structural analysis. With the help of computer science, we will also engineer new, non-natural molecular machines 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

- S. Enoki, R. Iino, Y. Niitani, Y. Minagawa, M. Tomishige and H. Noji, "High-Speed Angle-Resolved Imaging of Single Gold Nanorod with Microsecond Temporal Resolution and One-Degree Angle Precision," *Anal. Chem.* **87**, 2079–2086 (2015).
- A. Yukawa, R. Iino, R. Watanabe, S. Hayashi and H. Noji, "Key Chemical Factors of Arginine Finger Catalysis of F_1-ATPase Clarified by an Unnatural Amino Acid Mutation," *Biochemistry* **54**, 472–480 (2015).
- R. Iino, H. Ueno, Y. Minagawa, K. Suzuki and T. Murata, "Rotational Mechanism of *Enterococcus hirae* V_1-ATPase by Crystal-Structure and Single-Molecule Analyses," *Curr. Opin. Struct. Biol.* **31**, 49–56 (2015).
- 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. Direct Observation of Intermediate States during the Stepping Motion of Kinesin-1[1)]

The dimeric motor protein kinesin-1 walks along microtubules by alternatingly hydrolyzing ATP and moving two motor domains ('heads'). Nanometer-precision single-molecule studies demonstrated that kinesin takes regular 8-nm steps upon hydrolysis of each ATP; however, the intermediate states between steps have not been directly visualized. Here, we employed high-temporal resolution dark-field microscopy to directly visualize the binding and unbinding of kinesin heads to or from microtubules during processive movement (Figure 2). Our observations revealed that upon unbinding from microtubules, the labeled heads were displaced rightward and underwent tethered diffusive movement. Structural and kinetic analyses of wild-type and mutant kinesins with altered neck linker lengths provided evidence that rebinding of the unbound head to the rear-binding site is prohibited by a tension increase in the neck linker and that ATP hydrolysis by the leading head is suppressed when both heads are bound to the microtubule, thereby explaining how the two heads coordinate to move in a hand-over-hand manner.

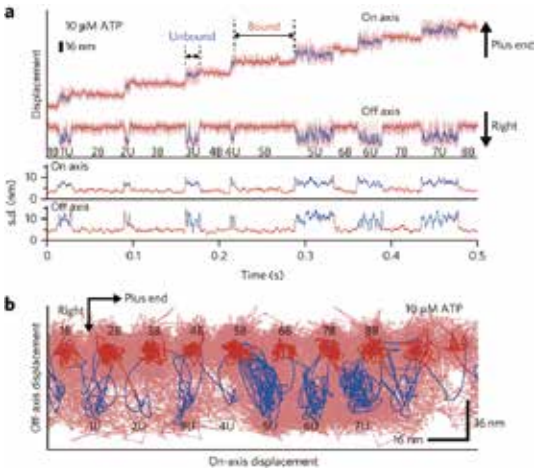

Figure 2. (a) Typical trace for the centroid position of the gold probe attached to a kinesin head (light red lines), toward the microtubule long axis (on axis) and perpendicular to the microtubule axis (off axis). Red and blue lines depict the median-filtered traces (window size of 51 frames) for the bound and unbound states, respectively. Lower panel shows the s.d. of on- and off-axis positions for each time frame t (calculated as [t–20, t+20]). (b) Two-dimensional plot of the gold probe shown in a. Numbers denote the temporal order of the bound (B) and unbound (U) states.

2. Direct Imaging of Binding, Dissociation, and Processive Movement of *Trichoderma reesei* Cel6A and Its Domains on Crystalline Cellulose[2)]

Trichoderma reesei Cel6A (*Tr*Cel6A) is a cellobiohydrolase that hydrolyzes crystalline cellulose into cellobiose. Here, we observed the binding, dissociation, and movement of single-molecule intact *Tr*Cel6A on a crystalline cellulose, in addition to isolated catalytic domain (CD), cellulose-binding module and linker (CBM-Linker), and CBM (Figure 3). The CBM-Linker had a binding rate constant almost half that of intact *Tr*Cel6A, whereas those of the CD and CBM were only one-tenth of intact *Tr*Cel6A. These results indicate that the linker region largely contributes to initial binding on crystalline cellulose. After binding, all samples showed slow and fast dissociations, likely caused by the two different bound states due to the heterogeneity of cellulose surface. The CBM showed much higher (12-times) specificity to the high-affinity site than to the low-affinity site, whereas the CD did not, suggesting that the CBM leads the CD to the hydrophobic surface of crystalline cellulose. The intact molecules showed slow, processive movements (8.8 ± 5.5 nm/s) in addition to fast diffusional movements (30–40 nm/s), whereas the CBM-Linker, the CD, and a full-length but catalytically inactive mutant showed only fast diffusional movements. These results suggest that in addition to direct binding, surface diffusion also contributes to the searching of the hydrolysable point of the cellulose chains. The duration time constant for the processive movement was 7.7 s. Our results reveal the role of each domain in the elementary steps of the reaction cycle and provide the first direct evidence of the processive movement of *Tr*Cel6A on crystalline cellulose.

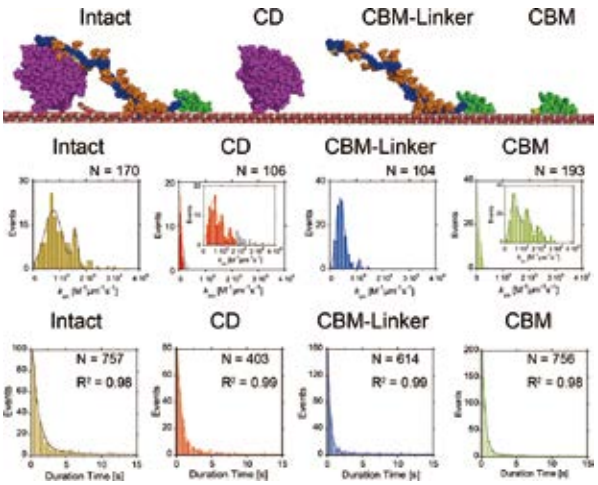

Figure 3. (Top) Domain structures of Intact, CD, CBM-Linker, and CBM of *Tr*Cel6A. (Middle) Distributions of the binding rate constant. (Bottom) Distributions of the duration time on cellulose.

References

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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.

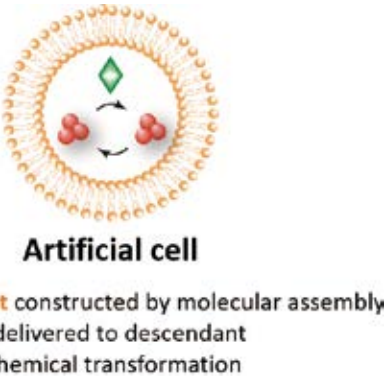

Figure 1. Artificial cell model. The replicating systems of compartment and the replicating system of information materials are combined. The reactions in the two replicating systems are accelerated by each proper catalysts.

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.

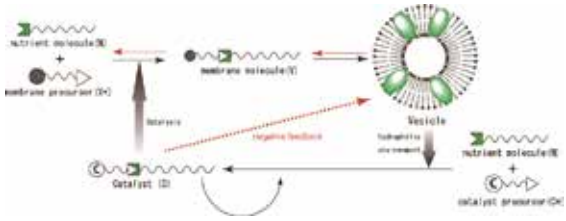

Figure 2. Scheme of our new artificial cellular system. The membrane molecules of the GV was synthesized by the catalyst produced in the GV.

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.

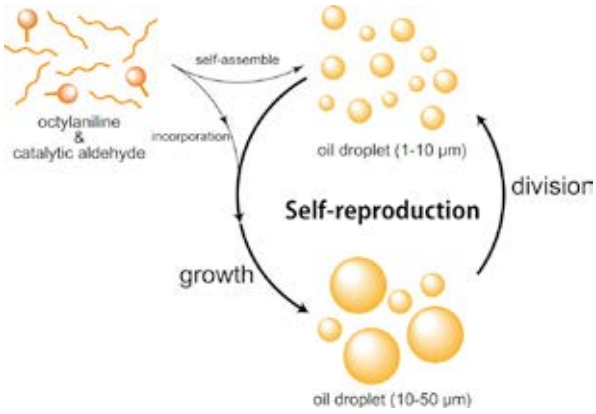

Figure 3. Scheme of the self-reproducing oil droplet (oil-in-water emulsion) system.

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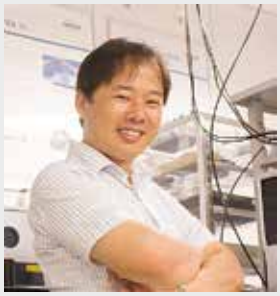
3) K. Takakura, T. Yamamoto, K. Kurihara, T. Toyota, K. Ohnuma and T. Sugawara, *Chem. Commun.* **50**, 2190–2192 (2014).
4) L. Sheng and K. Kurihara, *Chem. Lett.* **45**, 598–600 (2016).
5) L. Sheng and K. Kurihara, *Chem. Commun.* **52**, 7786–7789 (2016).

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.

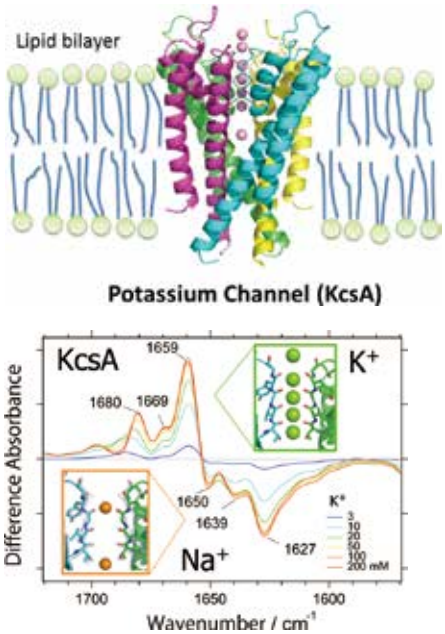

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

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).
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1. Molecular Characteristics of a Mammalian Photoreceptive Protein, Melanopsin for Non-Visual Function[1)]

Animals use external light signals not only for vision but also for "non-visual" functions such as regulation of biological clock. In particular, mammals including human receive ambient light through retina, leading to photoentrainment of the circadian clock and pupil responses. It had been thought that visual cells (rods and cones) are only photoreceptor cells in mammalian retina, but recent studies have shown that small population of retinal ganglion cells are also photoreceptive and play important roles in the non-visual photoreception. Since the intrinsically photoreceptive retinal ganglion cells (ipRGCs) show extremely low photosensitivity (less than 1/10,000-fold sensitivity of visual cells), mammals can detect condition of ambient light in a wide dynamic range by using ipRGCs as well as visual cells. Thus, lowering the photosensitivity of ipRGCs is important for non-visual photoreception in mammals.

ipRGCs express a photoreceptive protein melanopsin. Like visual pigments in rods and cones, melanopsin is a member of the opsin family, and it consists of a protein moiety with seven transmembrane α-helices and the chromophore retinal. Interestingly, the amino acid sequence of melanopsin is more similar to that of invertebrate visual pigment rather than to that of vertebrate visual pigment (Figure 2a). In this context, we speculated that mammalian melanopsins possess some molecular characteristics contributing to the low photosensitivity of ipRGCs. We thus compared biochemical, spectroscopic and electrophysiological properties of mouse and human melanopsins with those of closely related invertebrate melanopsin and visual pigment.

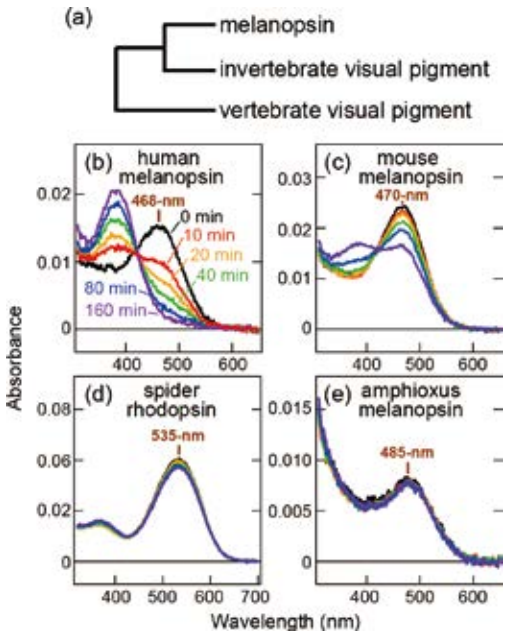

Figure 2. (a) Schematic representation of phylogenetic relationship of melanopsin, invertebrate visual pigment and vertebrate visual pigment. (b)–(e) Absorption spectra showing time-dependent loss of absorbance in the visible region for human melanopsin (b), mouse melanopsin (c), jumping spider rhodopsin, an invertebrate visual pigment (d), and an invertebrate amphioxus melanopsin (e). Panels (b)–(e) are adopted from ref. 1.

We expressed mouse and human melanopsin in mammalian cultured cells, and purified them through immuno-affinity chromatography. The purified proteins were kept at 37 °C in the dark, and subsequent spectral changes were recorded. During the incubation, the mammalian melanopsins showed time-dependent loss of absorbance at ~470-nm, indicating that they spontaneously hydrolyze the Schiff base linkage with the retinal (Figure 2, b and c). In contrast, such a hydrolysis was not observed in invertebrate melanopsin and visual pigment, both of which are closely related to mammalian melanopsins (Figure 2, d and e). Interestingly, human melanopsin showed the hydrolysis much faster (~10-fold) than mouse one whereas their sequences are very similar (~80% identity). Electrophysiological analyses using *Xenopus* oocytes expressing human or mouse melanopsin confirmed that the faster retinal release in human melanopsin occurs in cells, too.

These results indicate that mammalian melanopsins can loss their photoreceptive ability by spontaneous hydrolysis and release of the retinal chromophore. This characteristic would have an effect to lower photosensitivity of melanopsin-expressing cells (Figure 3). Our findings suggest that molecular characteristics of mammalian melanopsin are tuned for their non-visual photoreception. In addition, the further destabilized retinal attachment in human melanopsin would result in further lowered photosensitivity of human ipRGCs. This "enhanced" characteristic of human melanopsin might reflect adaptation to bright environment humans live.

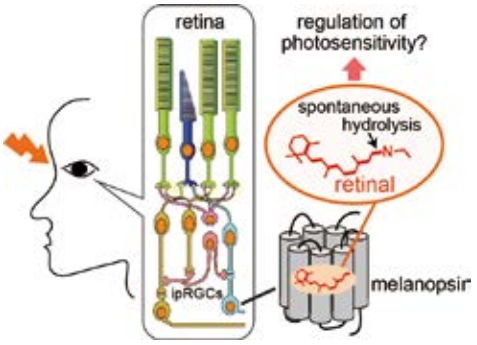

Figure 3. Our model illustrating how molecular characteristics of melanopsin contributes to non-visual photoreception in mammals. The spontaneous hydrolysis would play an important role in lowering photosensitivity of the cells.

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Development of Heterogeneous Catalysis toward Ideal Chemical Processes

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2014 Distinguished Professor, Three George University
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2008 Research Project Leader, NEDO Project (–2012)
2011 Deputy Research Project Leader, JST CREST (–2016)
2014 Research Project Leader, JST ACCEL Project (–2019)

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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.

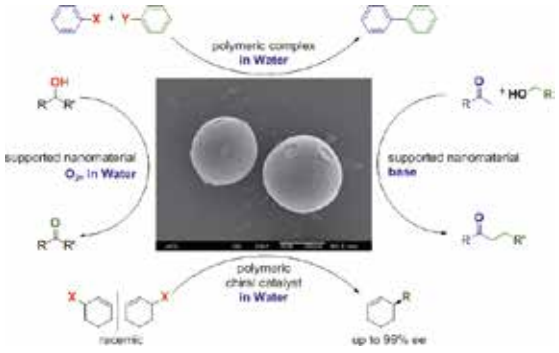

Figure 1. Typical Examples of Heterogeneous Aquacatalyses using Amphiphilic Polymer-Supported Metal Complexes and Metal Nanoparticles.

Selected Publications

- 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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1. A Vesicular Self-Assembled Amphiphilic Palladium NNC-Pincer Complex-Catalyzed Allylic Arylation of Allyl Acetates with Sodium Tetraarylborates in Water[1)]

The allylic arylation of various allyl acetates with sodium tetraarylborates proceeded in water in the presence of a vesicular self-assembled amphiphilic palladium NNC-pincer complex to give the corresponding arylated products in high yield, whereas the same complex as an amorphous powder did not promote the reaction efficiently. The formation of a vesicular structure was therefore shown to be essential for efficient promotion of the reaction.

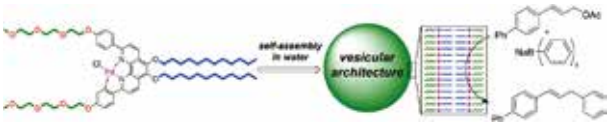

Figure 2. Allylic arylation reaction in water in the presence of a self-assembled vesicular amphiphilic palladium NNC-pincer complex.

2. Organoborane-Catalyzed Hydrogenation of Unactivated Aldehydes with a Hantzsch Ester as a Synthetic NAD(P)H Analogue[2)]

We have developed a method for the hydrogenation of unactivated aldehydes, using a Hantzsch ester as a NAD(P)H analogue in the presence of an electron-deficient triarylborane as a Lewis acid catalyst. Thus, tris[3,5-bis(trifluoromethyl) phenyl]borane efficiently catalyzes the hydrogenation of aliphatic aldehydes with a Hantzsch ester in 1,4-dioxane at 100 °C to give the corresponding aliphatic primary alcohols in up to 97% yield. Aromatic aldehydes also undergo the hydrogenation, even at 25 °C, to furnish the corresponding aromatic primary alcohols in up to 100% yield.

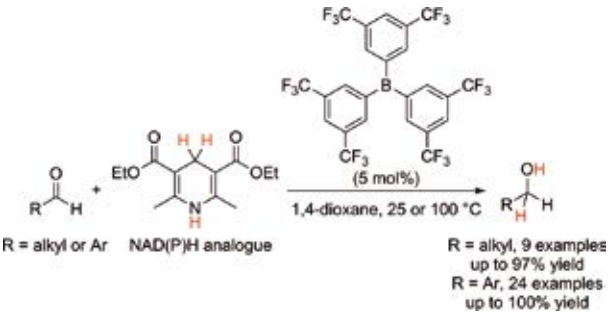

Figure 3. Organoborane-catalyzed hydrogenation of aldehydes with a Hantzsch ester.

3. Recyclable Polystyrene-Supported Copper Catalysts for the Aerobic Oxidative Homocoupling of Terminal Alkynes[3)]

Polystyrene-supported copper(II) *N*,*N*,*N*',*N*'-tetraethyldiethylenetriamine [Cu(II)–TEDETA] complexes were prepared by immobilization of TEDETA onto crosslinked polystyrene resin, followed by complexation with copper salts. The polystyrene-immobilized $CuSO_4$–TEDETA complex efficiently catalyzed the oxidative homocoupling of terminal alkynes under air to give the corresponding 1,3-diynes in up to 99% yield. The catalyst was easily recovered by simple filtration and reused eight times without significant loss of catalytic activity.

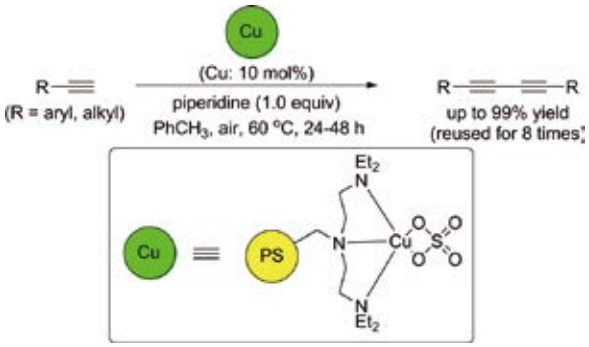

Figure 4. Homocoupling of terminal alkynes in the presence of a polystyrene-supported copper catalyst.

4. Instantaneous Click Chemistry by a Copper-Containing Polymeric-Membrane-Installed Microflow Catalytic Reactor[4)]

Instantaneous Huisgen cycloaddition has been achieved by developing a novel catalytic dinuclear-copper-complex-containing polymeric-membrane-installed microflow device. The microflow device instantaneously promotes the click reaction with a variety of alkynes and organic azides to afford the corresponding triazoles in quantitative yield.

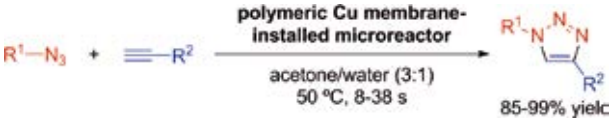

Figure 5. Homocoupling of terminal alkynes in the presence of a polystyrene-supported copper catalyst.

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2) G. Hamasaka, H. Tsuji and Y. Uozumi, *Synlett* **26**, 2037–2041 (2015).
3) S. Yan, S. Pan, T. Osako and Y. Uozumi, *Synlett* **27**, 1232–1236 (2016).
4) Y. M. A. Yamada, A. Ohno, T. Sato and Y. Uozumi, *Chem. –Eur. J.* **21**, 17269–17273 (2015).

Design and Synthesis of Chiral Organic Molecules for Asymmetric Synthesis

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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.

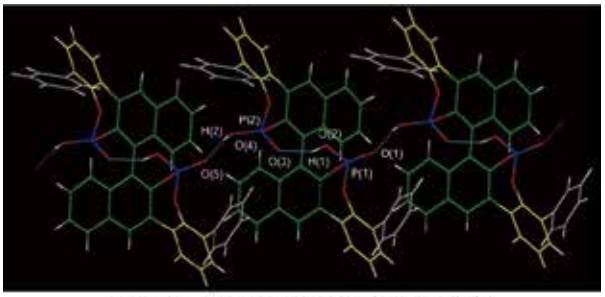

Figure 1. Hydrogen bonding network in chiral bis-phosphoric acid catalyst derived from (*R*)-3,3'-di(2-hydroxy-3 -arylphenyl)binaphthol. Hydrogen bond acts as activation unit for the substrate in asymmetric reaction space and controls atropisomeric behavior in naphthyl–phenyl axis.

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- T. P. Yoon and E. N. Jacobsen, *Science* **299**, 1691–1693 (2003).
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- 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.*, in press (2016). DOI: 10.1021/jacs.6b07150

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)]

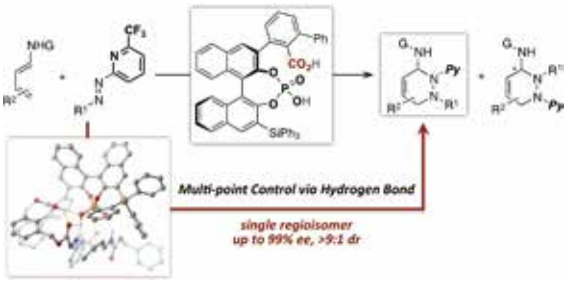

Figure 2. Chiral carboxylic acid–phosphoric acid-catalyzed azo-hetero-Diels–Alder reaction.

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 to give the corresponding product in good yield.[8)]

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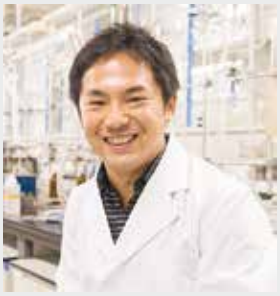
Development of Functional Metal Complexes for Artificial Photosynthesis

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



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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.

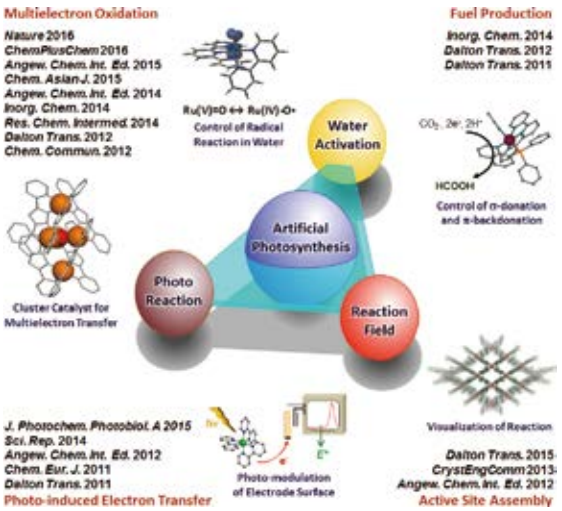

Figure 1. An overview of our work.

Selected Publications

- M. 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).
- T. Itoh, M. Kondo, H. Sakamoto, K. Wakabayashi, M. Kanaike, K. Itami and S. Masaoka, "Porous Frameworks Constructed by Non-Covalent Linking of Substitution-Inert Metal Complexes," *Dalton Trans.* **44**, 15334–15342 (2015).
- A. Fukatsu, M. Kondo, Y. Okabe and S. Masaoka, "Electrochemical Analysis of Iron-Porphyrin-Catalyzed CO_2 Reduction under Photoirradiation," *J. Photochem. Photobiol. A* **313**, 143–148 (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. A Pentanuclear Iron Catalyst Designed for Water Oxidation[1)]

Water oxidation ($2H_2O \rightarrow O_2 + 4H^+ + 4e^-$) is considered the main bottleneck in the production of chemical fuels from sunlight and/or electricity. In nature, the oxidation of water is efficiently catalysed by the oxygen-evolving complex (OEC) in photosystem II (PSII). Because extraction of the OEC is extremely difficult, various synthetic molecular catalysts have been investigated over the last decades. However, the development of efficient, robust and abundant metal-based molecular catalysts remains a challenge. In this work, we show a water oxidation reaction catalysed by a pentanuclear iron complex. Electrochemical analysis revealed that the pentairon complex exhibits rich redox flexibility with six different oxidation states between Fe^{II}_5 and Fe^{III}_5, in which the Fe^{III}_5 state is the active species for oxidising water. A computational investigation indicated that the O–O bond formation proceeds from the mixed-valence $Fe^{II}_2Fe^{III}(Fe^{IV}{=}O)_2$ intermediate with a reaction barrier of less than 10 kcal mol^{-1}. The turnover frequency of the water oxidation catalyst was determined to be 1,900 s^{-1}, which is considerably greater than that of the OEC (100–400 s^{-1}). Our findings indicate that efficient water oxidation catalysts can be created based on multinuclear iron complexes with redox flexibility and adjacent water-activation sites.

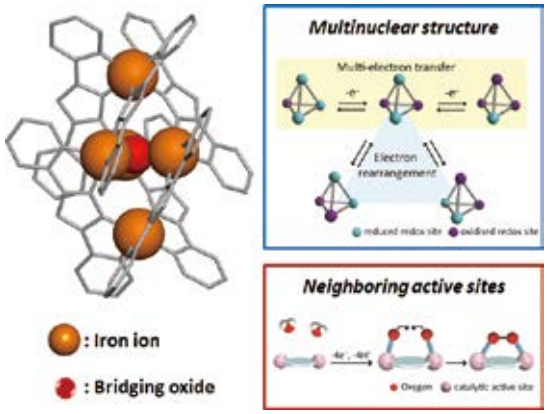

Figure 2. Structure (a) and characteristics (b) of the Fe_5 catalyst.

2. Electrochemical Analysis of Iron-Porphyrin-Catalyzed CO_2 Reduction under Photoirradiation[2)]

To understand the mechanisms of solar-to-fuel conversion reactions, electrochemical responses of catalysts should be investigated under photoirradiation because the electrochemical process proceeds subsequent to the photochemical process. However, in general, the electrochemical and photochemical properties of molecular catalysts are separately evaluated using different experimental setups. In this study, the photochemical reaction of a metal-complex-based catalyst was analyzed by electrochemical measurements. A well-known catalyst for the CO_2 reduction reaction, *meso*-tetraphenylporphyrin iron(III) chloride (**Fe(tpp)Cl**), was selected as the target analyte. Although the analysis of the electrochemical response of **Fe(tpp)Cl** under photoirradiation with conventional cyclic voltammetry (CV) was not allowed, the adaptation of thin layer cyclic voltammetry (TLCV) enabled us to detect the photochemical reaction of **Fe(tpp)Cl**. The influence of photoirradiation on the electrochemical property of **Fe(tpp)Cl** was investigated both under Ar and CO_2 atmospheres. Although the thin layer cyclic voltammograms of **Fe(tpp)Cl** upon photoirradiation under an Ar atmosphere were almost the same as those measured in the dark, the measurements under a CO_2 atmosphere clearly indicated the change of the electrochemical response upon photoirradiation. The detailed analysis of this phenomenon revealed that the photoinduced decarbonylation reaction regenerates the original [Fe^{II}(tpp)] complex under photoirradiation.

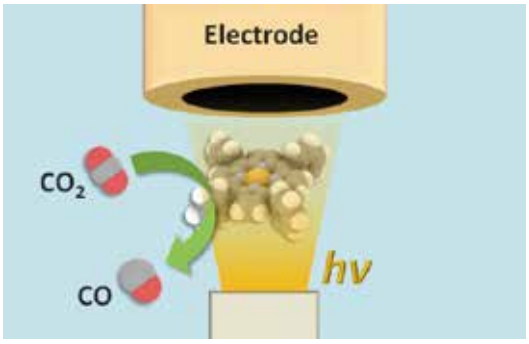

Figure 3. Electrochemical CO_2 reduction under photoirradiation.

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2) A. Fukatsu, M. Kondo, Y. Okabe and S. Masaoka, *J. Photochem. Photobiol. A* **313**, 143–148 (2015).

Awards

ENOMOTO, Takafumi; Outstanding Student Prize, Tokai Branch of the Chemical Society of Japan (2016).
LEE, Sze Koon; Poster Award, The Winter School of Asian-Core Program (2016).
ENOMOTO, Takafumi; Poster Award, The 27th Symposium on Photochemistry and Photophysics of Coordination Compounds (2016).
FUKATSU, Arisa; IZU, Hitoshi; ENOMOTO, Takafumi; Presentation Award, SOKENDAI Physical Science Student Seminar (2016).
ENOMOTO, Takafumi; Adobe Award (the excellence award), SOKENDAI Physical Science Student Seminar (2016).

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, transient active species were prepared at low temperature under inert atmosphere. Electronic structures of these active species were investigated with various techniques including absorption, ^{1}H and ^{2}H NMR, EPR, IR resonance Raman spectroscopy and magnetic susceptibility measurement. Correlations between electronic structures and electron transfer ability are investigated in detail.

1. Reductive Manganese Species Related to Dioxygen Activation

The previous study has shown that a manganese(III) salen complex mediates O_2 activation in the presence of OH^- from 2 M KOH aqueous solution.[1)] This study investigated an active species that is responsible for dioxygen activation in this reaction. Then, the reaction of a manganese(III) salen complex with Bu_4NOH was carefully carried out at low temperature (–80 °C). It was found that a new species with characteristic visible absorptions is generated. Cyclic voltammetry shows that the Mn(III)/Mn(IV) redox of the new species appears at –0.31 V *vs* Fc/Fc^+, which is drastically lower than that of the starting manganese(III) complex by 1.13 V. According to the ^{1}H and ^{2}H NMR spectra, the new species is assigned as a six-coordinate anionic $[Mn^{III}(salen)(OH)_2]^-$ complex. Magnetic susceptibility measurements show the spin state is changed from $S = 2$ for the starting complex to $S = 1$ for the new species.

Reference

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Visiting Professors

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

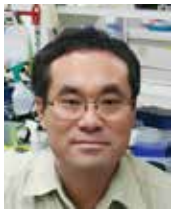
Transformation of Unsaturated Carbonyl Compounds via Nickelacycles

Chemists no longer doubt the importance of a methodology that could activate and utilize aldehydes in organic syntheses since many products prepared from them support our daily life. Tremendous effort has been devoted to the development of these methods using main-group elements and transition metals. Thus, many organic chemists have used an activator–(aldehyde oxygen) interaction, namely, η^1 coordination, whereby a Lewis or Brønsted acid activates an aldehyde. In the field of coordination chemistry, η^2 coordination of aldehydes to transition metals by coordination of a carbon–oxygen double bond has been well-studied; this activation mode, however, is rarely found in transition-metal catalysis. In view of the distinctive reactivity of an η^2-aldehyde complex, unprecedented reactions via this intermediate are a distinct possibility. We have been focusing on the formation of an η^2-aldehyde complex and its application to catalytic reactions. The key to success is efficient formation of oxa-nickelacycles generated by oxidative cyclization with carbon–carbon unsaturated bond. These nickelacycle allow us to develop new transformation of unsaturated carbonyl compounds.

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

Development of Novel Biocatalysts Based on Substrate Misrecognition of Enzymes

Gaseous alkanes such as methane and ethane are important fuels and potential chemical feedstock, but the selective hydroxylation of gaseous alkanes is a long-standing challenge and a current topic of interest considering increasing industrial and economic requirements. Although cytochrome P450s (P450s) are capable of breaking strong C–H bonds of hydrocarbons, the substrate specificity of cytochrome P450s makes them unsuitable for the hydroxylation of gaseous small alkanes, because P450s, especially those isolated from bacteria, recognize their specific substrates by intermolecular interactions to ensure their specificity and efficiency. We focused on the substrate misrecognition of P450s induced by inert dummy substrates (decoy molecules) that have a structural similarity to their natural substrates. We have demonstrated that even wild-type P450BM3 can catalyze the hydroxylation of gaseous alkanes such as ethane and propane by the addition perfluorinated carboxylic acids as decoy molecules. We believe that the catalytic turnover rate and coupling efficiency for hydroxylation of non-native substrates would be further improved by optimizing the structure of decoy molecules based on the crystal structure of P450BM3 with 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 question, we focus on microbial denitrification, a form of anaerobic respiration, in which nitrate is reduced to dinitrogen through nitrite, NO and nitrous oxide. In denitrification, cytotoxic NO is produced as an intermediate product, but denitrifying bacteria can grow without any damage from NO, suggesting that there is a system for effective NO elimination. As a possible system, we recently found that NO-generating nitrite reductase (NiR) forms a complex with NO-decomposing nitric oxide reductase (NOR) to suppress the diffusion of NO. On the basis of this result, we further analyze the structure and function of the NOR:NiR complex by X-ray crystallography, mutagenesis, and time-resolved spectroscopic methods.